Supplemental clinical information

Clinical and genetic characterization

The first four individuals, three sisters and their father, were part of a previously reported family with a germline *RUNX1* Y260X mutation in isoform RUNX1b (p.Y287X in RUNX1c) present in at least 18 members.[1] Each of these relatives (Table 1, A1-A4) had easy bruising and abnormal platelet function testing with prolonged collagen/epinephrine occlusion times. The father (A1) and the youngest daughter (A4) also had prolonged collagen/adenosine diphosphate (ADP) occlusion times. The father developed myelodysplastic syndrome at the age of 49, characterized by worsening thrombocytopenia, megakaryocyte dysplasia and an abnormal del(11)(q14q25) karyotype, confirmed by fluorescence *in situ* hybridization (FISH) for *KMT2A* (*MLL*) deletion in 7% of cells. He had no increase in blasts by morphology or flow cytometry and his CBC at diagnosis demonstrated thrombocytopenia, normal red blood cell indices and a slightly low white blood cell count. At the age of 50, he underwent hematopoietic stem cell transplantation (HSCT). The youngest daughter (A4) first presented at the age of 12 years with fevers, hypotension, diffuse lymphadenopathy, and splenomegaly. Her CBC revealed an elevated white blood count (46.7 K/□L) with 84% blasts, neutropenia, thrombocytopenia (PLT = 43 K/□L), and anemia (Hgb = 8.2 g/dL). She was diagnosed with acute myeloid leukemia, with her karyotype identifying 46,XX,t(2;11)(q31;p15)[20], presumed to correspond with a fusion of the *NUP98* gene (chr. 11p15) to either the *HOXD11* or *HOXD13* gene (chr. 2q31). Within 5.5 months of her diagnosis, she underwent a mismatched unrelated donor cord blood transplant, and now, 7.5 years later, is doing well with normal platelet counts. The oldest daughter (A2) had mildly low platelets (ranging from 123 K/□L to 168 K/□L), while the middle daughter (A3) had platelets at the low end of the normal range (fluctuating from 173 K/□L to 193 K/□L). Both

daughters are followed with quarterly complete blood counts (CBCs) and bi-yearly or yearly bone marrow evaluations. White blood cell counts have been normal in both sisters, though the older sister had iron deficiency anemia at the time of her first bone marrow examination.

Individual B1, a boy, had a microarray performed as a neonate as a part of an evaluation for global developmental delay, dysmorphic features, and seizures, that identified a 4.6 megabase (Mb) deletion on chromosome 21q22.11-q22.2, encompassing *RUNX1*. Due to thrombocytopenia as an infant (platelets as low as 52 K/□L), he was brought to the attention of hematology/oncology, and undergoes yearly bone marrow evaluations. CBC values other than platelets have been normal. He was also noted to have an eczematous rash in infancy.

Individual C1 came to medical attention at the age of 11 when he was noted to have thrombocytopenia and neutropenia in the setting of a chronic idiopathic elevation in creatine kinase and myopathy of unclear etiology. His thrombocytopenia could be traced back to the newborn period (platelets = 29-90K/□L). He was adopted at 4 months of age, so his family history was unknown. His first bone marrow aspirate at age 11 had an abnormal 46,XY,?del(13)(q14q22)[2]/46,XY[18] karyotype, though FISH did not confirm the del(13q). Morphological examination demonstrated megakaryocytic hypoplasia and dysplasia, leading to the diagnostic consideration of a myelodysplastic syndrome. He was followed with serial bone marrow examinations, the third of which, at age 13, demonstrated del(5q) in 10% of cells by karyotype. Evaluation of his neutropenia, thrombocytopenia, and clonal abnormalities included chromosomal breakage studies for Fanconi anemia, telomere length analysis for dyskeratosis congenita, Fragile X *FMR1* and *FMR2* repeat expansion analysis, microarray comparative genomic hybridization, *SBDS* sequencing for Shwachman-Diamond Syndrome, and *GATA2* gene sequencing, all of which were normal. Three years after his initial presentation, sequencing of

the *RUNX1* gene was performed, identifying the mutation c.567C>G, p.Y189X (NM_001754), presumed to be germline. Due to the progressive increase in the del(5q) clone, he underwent a mismatched unrelated double cord HSCT, and now, four years later, he is doing well with normal platelet counts.

Individual D1 also came from a family with many individuals affected with FPDMM, including a paternal uncle, paternal aunt, paternal cousin, and paternal grandfather. As a result of her sister's diagnosis of AML, genetic testing was performed, and a RUNX1 p.R201X mutation (NM_001754) was identified in D1, her sister, and her father. Individual D1 had a history of petechiae, epistaxis, and thrombocytopenia, ranging from 60 K/□L to 80 K/□L, which worsened with viral illnesses; she had normal white blood cell counts and red blood cell indices, though hemoglobin was mildly elevated. Two bone marrow evaluations have been performed.

Individuals E1-E4 are from a large pedigree with multiple individuals with leukemia/MDS and a *RUNX1* p.Y414X mutation. The proband (E1) is a 42 year old woman with a history of "immune thrombocytopenic purpura" (ITP) who was treated with steroids and splenectomy with unknown response. She was diagnosed with acute myeloid leukemia at 42 years of age and was treated with chemotherapy and an HLA-matched sibling peripheral blood stem cell transplant. Her unaffected sister served as the donor. Targeted capture NGS identified the p.Y414X mutation. Two of the proband's nieces (E2 and E3) also tested positive for the p.Y414X mutation; both have a history of easy bruising and mild intermittent thrombocytopenia, and E2 has a history of severe menorrhagia requiring dilation and curettage and transfusion which has been further complicated by longstanding iron deficiency anemia. Individual E4, the proband's great nephew and son of E2, presented at the age of 5 with a history of easy bruising, epistaxis, and mild chronic thrombocytopenia, noted since the age of 2. Thought possibly to be

ITP, he was re-evaluated at the age of 7 after his maternal great aunt was found to have an *RUNX1* mutation. At the time of his bone marrow evaluation, he had a mild normocytic anemia, but normal neutrophil counts.

Individuals F1 and G1 also carry the RUNX1 p.Y414X mutation (relatedness to E1-4 has not been established by clinical history). Individual F1 presented at the age of 5 years with a history of intermittent neutropenia, persistent moderate thrombocytopenia, and recurrent infections including bacterial skin abscesses and viral infections. She had a known constitutional 47,XXX karyotype. Due to her cytopenias and infections, genetic testing for *HAX1*, *ELANE*, and *SBDS* were performed with no identified mutations. Telomere length and chromosome breakage analyses were also normal. A bone marrow failure NGS panel identified the *RUNX1* mutation p.Y414X (NM_001754), which was confirmed to be of germline origin by fibroblast testing. Yearly bone marrow evaluations are performed on her for MDS surveillance.

Individual G1 was diagnosed with T cell lymphoblastic lymphoma at 5 years of age and was treated on a pediatric ALL chemotherapy protocol and achieved remission. He subsequently was diagnosed at 25 years of age with an acute leukemia, felt consistent with either an acute undifferentiated leukemia versus an acute myeloid leukemia with minimal differentiation (M0 by French-American-British Classification) and characterized by a blast population negative for CD3, CD19, CD79a, and MPO. Targeted capture NGS of skin fibroblast DNA identified a heterozygous RUNX1 p.Y414X mutation. He is currently receiving chemotherapy in preparation for an unrelated donor peripheral blood stem cell transplantation.

Lastly, individual H1 presented at 3 years of age with a history of easy bruising and was found to have microthrombocytopenia with a platelet count in the 30-50 K/□L range and a normal WBC count and hemoglobin. His history was also notable for eczema and frequent

upper respiratory tract infections. Due to the triad of microthrombocytopenia, eczema, and infections, testing for Wiskott-Aldrich Syndrome was performed and was negative. Additionally, chromosomal breakage analysis was normal. A bone marrow failure NGS panel identified a 117 base pair deletion in *RUNX1* (c.1208_1322del, p.Y403Cfs*153, NM_001754), predicted to result in a truncated protein. The mutation was also present in DNA isolated from skin fibroblasts, but not in the DNA either parent, confirming that it was germline and *de novo*.

Reference

1. Michaud J, Wu F, Osato M, et al. In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. *Blood*. 2002;99:1364-1372.