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Germline *SAMD9* Mutation in Siblings with Monosomy 7 and Myelodysplastic Syndrome

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Myelodysplastic syndromes (MDS) are poorly understood and rare hematologic malignancies in children. In recent years several germline mutations have been implicated in a subset of familial cases of MDS, other myeloid neoplasms, or bone marrow failure syndromes, including mutations in *GATA2*, *ETV6*, and *DDX41* (1, 2). However, for some families, such as those first reported nearly 30 years ago (3, 4), who present with MDS and monosomy 7 (OMIM 252270), the predisposing genetic alteration has remained elusive. Here we report our findings on a family without historical suggestions of MDS/AML predisposition but with three siblings with monosomy 7 and MDS.

A 4-year-old male (proband/SJ015856/Sibling 1) was found to have severe neutropenia, macrocytosis, and thrombocytopenia (ANC=400/mm³, MCV=101.7fL, platelets=111×10⁹/L). Bone marrow evaluation revealed hypocellularity with trilineage dysplasia and 1% blasts (Figure 1A). His 3-year-old sister (SJ015855/Sibling 2) also had low peripheral cell counts and bone marrow dysplasia, while his youngest brother (14-months-old/SJ018228/Sibling 3) had normal peripheral counts and a hypocellular bone marrow with megakaryocytic dysplasia. Cytogenetic analysis was significant for monosomy 7 in all siblings (Figure 2 & Supplemental Figure 1) and thus all 3 were diagnosed with refractory cytopenia of childhood. All three siblings had a transient thrombocytopenic phase

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Conflict of Interest Statement

The authors have nothing to disclose.

at birth requiring a platelet transfusion, but had otherwise normal developmental histories and stature-for-age (at or above the 75th percentile). The proband was born with severe hypospadias and a bifid scrotum requiring multiple corrective surgeries without any bleeding complications. A karyotype during the neonatal period demonstrated a normal male karyotype. No other congenital abnormalities were noted in the siblings. Chromosome breakage studies for Fanconi Anemia were negative. The two eldest siblings have been treated with matched, unrelated donor BM transplants while the youngest sibling remains asymptomatic with normal peripheral blood counts and is monitored annually.

Bone marrow samples from the 3 siblings were banked for research after informed consent was obtained. All studies were approved by the Institutional Review Board at St. Jude Children's Research Hospital. Cryopreserved marrow samples were flow-sorted into lymphocyte (source of "germline" DNA), as an alternate source of germline DNA was not available (5), and bulk myeloid populations. WES was performed on paired tumor/normal samples from the three siblings. WGS was also performed on the paired samples from Sibling 2 and only the normal samples from Siblings 1 and 3. Genomic data have been deposited in the European Genome-Phenome Archive (EGAS00001002202).

The loss of chromosome 7 was confirmed in the myeloid cells of all three siblings using WES data (6), with a clear subclonal loss in Sibling 3 (Figure 1B). No copy number loss was observed in the lymphocyte samples; however, a copy-number neutral loss of heterozygosity (CN-LOH) event was noted at 7q (chr7:78Mb–159Mb, q21.11–q36.3) in Sibling 3 (Figure 1C). A total of 19 non-synonymous shared high-confidence germline variants were present when intersecting the WES and WGS data from all three siblings, 10 of which were predicted to functionally impact the gene product by either SIFT or Polyphen2 (Supplemental Figure 2 & Supplemental Table 1). These analyses pointed to a heterozygous missense mutation (c.3406G>C) in *SAMD9* (NM_017654), resulting in a p.E1136Q mutation (NP_010124; CADD score:19.7), as a potential causal lesion for MDS and monosomy 7 in this family (7). This mutation affects an evolutionarily conserved position and is not present in the ExAC or in-house databases. Sanger sequencing of genomic DNA isolated from the peripheral blood of the parents observed this mutation in the clinically asymptomatic mother, confirming this as an inherited variant (Figure 2A & B). Furthermore, a CLIA-approved laboratory confirmed the presence of the *SAMD9* p.E1136Q in a buccal swab sample obtained from the proband. Similar *SAMD9* missense mutations have recently been reported in patients with MIRAGE syndrome, a multisystem disorder associated with MDS and monosomy 7 (8).

Although present in the patients' lymphocytes, the mutant allele was less common in the myeloid cell fraction (see Figure 2B), suggesting a preferential loss of the copy of chromosome 7 that harbors the *SAMD9* variant. A similar decrease in VAF was reported in children with MIRAGE syndrome, presumably through a mechanism referred to as "adaptation by aneuploidy," to deal with the growth-restrictive properties of *SAMD9* mutant proteins (8). Similar to those mutations present in MIRAGE syndrome, the p.E1136Q mutation results in decreased induction of ERK phosphorylation (Figure 2C) after serum stimulation. Additional sibling-specific alterations involving *SAMD9* (Supplemental Figure 3 & 4) were also observed in the lymphocyte fractions: a p.R221* (c.661C>T) in Sibling 1,

p.F583I (c.1747C>A) in Sibling 2, and the aforementioned CN-LOH event in Sibling 3. The p.R221* and p.F583I were confirmed to be in *cis* with the p.E1136Q (Supplemental Figure 3C). These additional mutations are likely acquired rather than germline variants. Unlike the germline *SAMD9* p.E1136Q, the p.R221* mutation was not present in DNA isolated from the buccal swab sample from Sibling 1. Further, the p.F583I in Sibling 2 and the CN-LOH event in Sibling 3 are subclonal events in the lymphocyte fraction. Sibling 2 also harbored a somatic mutation in the myeloid cells in *ETV6* (NM_001987;p.R369W) at a codon previously observed in MDS (9). No somatic mutations recurrently reported in MDS were observed in the other two siblings.

We believe this to be the first report of *SAMD9* mutations in isolated familial MDS. Although the *in vitro* impact on ERK phosphorylation appears similar to that reported in MIRAGE syndrome, clearly the p.E1136Q results in a milder phenotype as these siblings lack the other clinical manifestations of that syndrome. In addition, this family demonstrates a maternal pattern of inheritance yet the mother has no documented history of cytopenias. The reason for this variable penetrance is unclear, but could be explained by monoallelic gene expression, which has been observed in some families with MDS and *GATA2* mutations (10). Of note, mutations in the related gene *SAMD9L* have recently been reported in Ataxia-Pancytopenia Syndrome (11, 12) and in some families with MDS (13), and loss of *SAMD9L* can result in myelodysplasia in mice (14). Tesi and colleagues described a phenomenon of somatic revertant mosaicism in which additional *SAMD9L* mutations could relieve the deleterious impact of the initial germline mutation (13), which may explain the additional *SAMD9* variants we observed in these siblings. Collectively, these findings establish *SAMD9/SAMD9L* mutations as a new class of germline lesions with variable clinical phenotypes, including familial MDS. It is likely that the MDS observed in these cases is not due to the *SAMD9/SAMD9L* mutations per se. Rather, the MDS likely is a direct consequence of haploinsufficiency of multiple genes on chromosome 7 (15) and this genetic loss may represent a cellular adaptation to these deleterious germline *SAMD9/SAMD9L* mutations. Future studies to explore the hematopoietic impact of these mutations and to determine their recurrency in larger cohorts of families with MDS are clearly warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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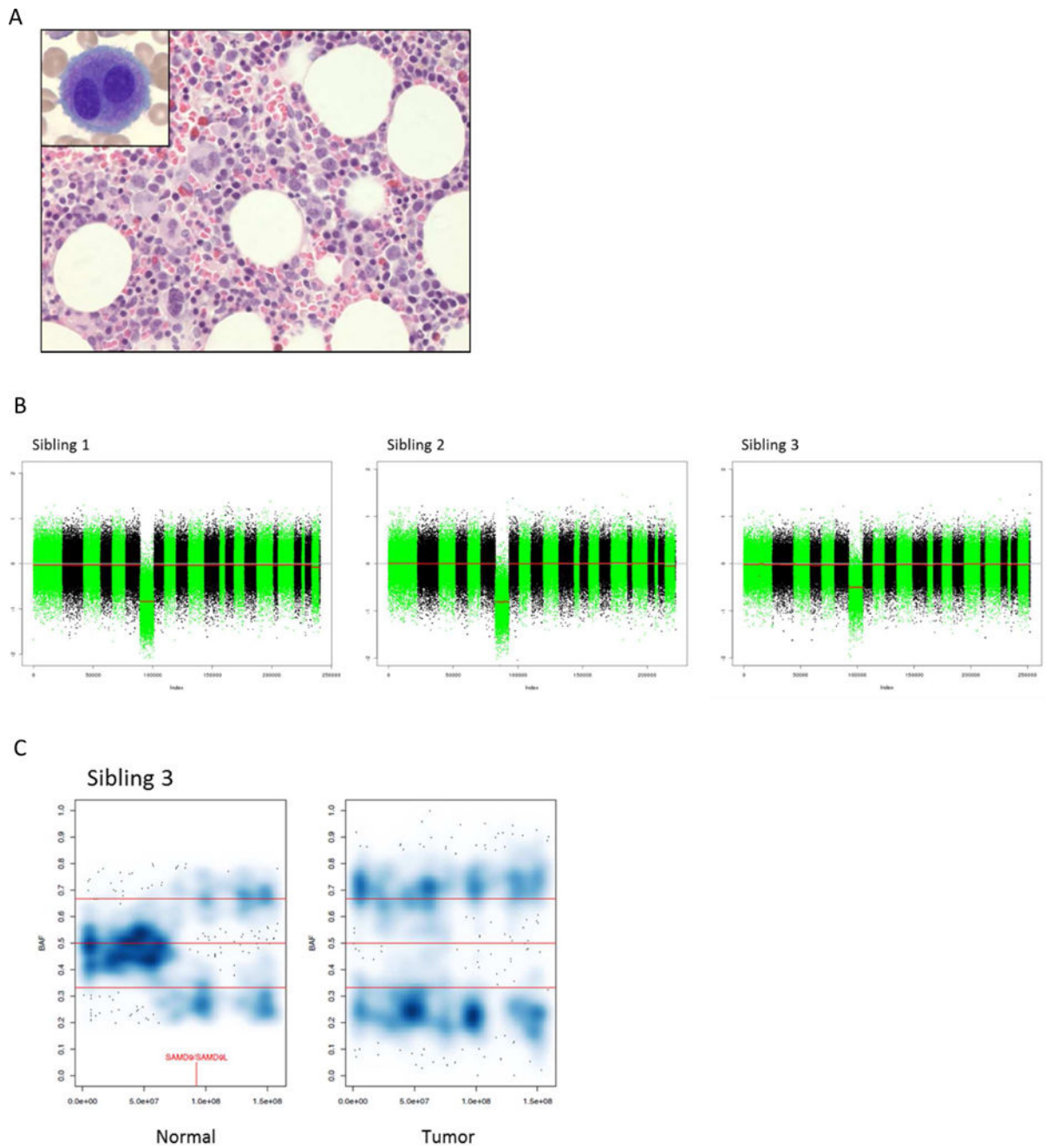


Figure 1. Diagnostic work up of 3 siblings with MDS and monosomy 7

A.) Bone marrow core biopsy and aspirate (inset) showing dysplastic megakaryocytes in Sibling 1. B.) Copy number analysis demonstrating monosomy 7 in myeloid cells all three siblings. C.) Smoothed color density plots of chromosome 7 B-allele frequency (BAF) of lymphocyte (normal) and myeloid (tumor) DNA in Sibling 3 showing a subclonal population with allelic imbalance, despite the lack of a copy number change, consistent with CN-LOH in the lymphocytes. Darker blue signifies a higher data point (SNP alternative allele frequencies) density. Red vertical line indicates genomic location of SAMD9 and SAMD9L.

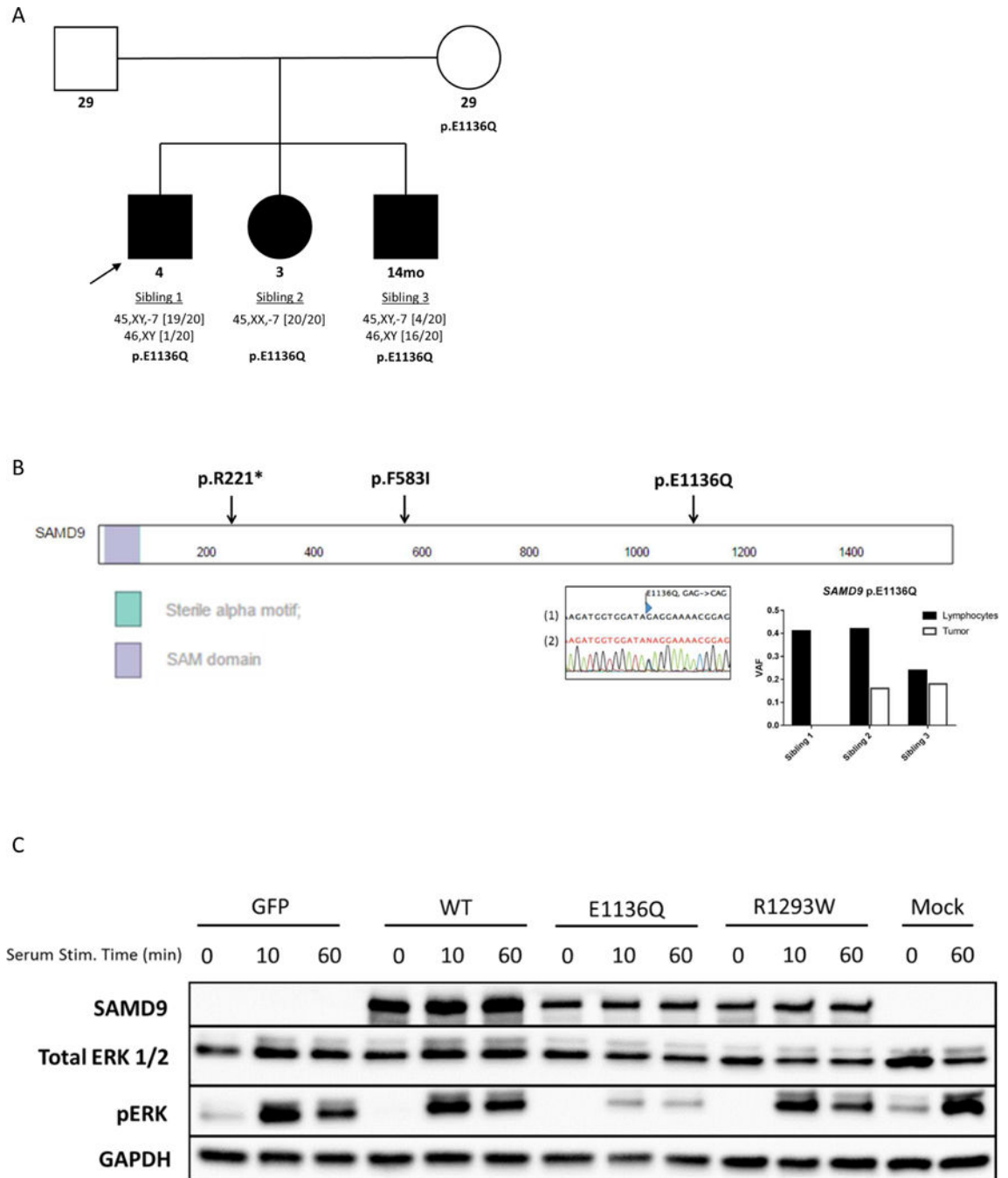


Figure 2. Sequencing validation and biologic characterization of the *SAMD9* p.E1136Q mutation

A.) Family pedigree identifying those with a *SAMD9* mutation and their monosomy 7 status (filled: monosomy 7; open: untested karyotype). Ages at diagnosis are below pedigree symbols, bracketed numbers adjacent to karyotype below indicate fraction of metaphases affected. B.) Locations of mutations within the *SAMD9* protein, a Sanger sequencing electropherogram (1-Reference, 2-Reverse primer) confirming the *SAMD9* p.E1136Q mutation in the mother, and tumor/normal variant allele frequency (VAF) values for the p.E1136Q mutation. C.) Western blot (representative of multiple biological replicates)

showing less ERK phosphorylation in 293T cells transiently expressing the mutant GFP-SAMD9 protein when compared to the wild-type at 0, 10, and 60 minutes following serum stimulation. The R1293W mutation was identified in some patients with MIRAGE syndrome (8). GFP indicates the empty GFP-fusion vector. The following antibodies were used: SAMD9: abcam (ab180575), From Cell Signaling Technologies: Total ERK (4695S), Phos-ERK (9101S), and GAPDH (2118S).

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