A case of *KMT2A–SEPT9* fusion–associated acute megakaryoblastic leukemia

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Abstract Acute megakaryoblastic leukemia (AMKL) constitutes ∼5%–15% of cases of non–Down syndrome AML in children, and in the majority of cases, chimeric oncogenes resulting from recurrent gene rearrangements are identified. Based on these rearrangements, several molecular subsets have been characterized providing important prognostic information. One such subset includes a group of patients with translocations involving the *KMT2A* gene, which has been associated with various fusion partners in patients with AMKL. Here we report the molecular findings of a 2-yr-old girl with AMKL and t(11;17)(q23;25) found to have a *KMT2A–SEPT9* fusion identified through targeted RNA sequencing. A *KMT2A–SEPT9* fusion in this subset of patients has not previously been reported.

[Supplemental material is available for this article.]

CASE PRESENTATION

A previously healthy 2-yr-old female was initially seen for recurrent fevers and decreased appetite. She was treated for suspected otitis media but continued to experience a poor appetite and fevers. A complete blood count showed anemia (Hb 7.2 g/dl) and thrombocytopenia (plt 60 k/µl), with a white blood cell count in the normal range (6.54 k/µl, ANC 2500/ml). A bone marrow biopsy and aspiration were performed that demonstrated a normocellular marrow with left-shifted granulopoiesis, progressive erythroid maturation, and atypical, hypolobulated megakaryocytes. Reticulin staining demonstrated variable increase in reticulin fibrosis from mild to marked (1–3+). The specimen contained an expanded blast population (30%), with blasts that were variable in size and had round nuclei, fine chromatin, variable nucleoli, and agranular cytoplasm. Some blasts showed cytoplasmic blebs with some of the larger blasts having vacuolated cytoplasm. Flow-cytometric analysis showed the blast population had expression of CD4, CD33, CD38, CD41, CD45, CD61, CD71, CD117, and CD123. The findings were consistent with a diagnosis of AMKL.

Cytogenetic analysis showed 46, XX, t(11;17)(q23;q25) in nine of 20 metaphase cells and the presence of a rearrangement involving *KMT2A* was confirmed by FISH in 8% of 300 cells. Targeted RNA sequencing identified a corresponding KMT2A–SEPT9 fusion transcript.

The patient received induction chemotherapy consisting of daunorubicin, cytarabine, and etoposide (ADE) in combination with gemtuzumab ozogamicin. Repeat bone marrow

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analysis at the end of induction demonstrated an MRD-negative complete remission. Because of the poor outcomes associated with *KMT2A* rearrangements in pediatric patients with AMKL, the decision was made to proceed to bone marrow transplantation in first complete remission. After completing two additional cycles of consolidative therapy, she received an allogeneic bone marrow transplant from an HLA-matched unrelated donor and has no evidence of disease more than 2 months after her transplant.

TECHNICAL ANALYSIS

The presence of the translocation involving Chromosomes 11 and 17 was identified by standard karyotype analysis and confirmed by a break-apart FISH probe (Abbott Molecular) (Fig. 1A,B). Targeted RNA sequencing using a customized 199-gene Archer FusionPlex panel identified the KMT2A–SEPT9 transcript involving exon 7 and exon 2 of *KMT2A* and *SEPT9*, respectively (Table 1; Fig. 1C).

SUMMARY

KMT2A–SEPT9 fusions are rare events that have been most commonly described in various myeloid leukemias exhibiting monocytic differentiation (Taki et al. 1999; Yamamoto et al. 2002; Shih et al. 2006; Strehl et al. 2006; Kurosu et al. 2008). They have infrequently been described in M0/M1/M2 AML, t-AML, and de novo myelodysplastic syndrome (Supplemental Table 1; Osaka et al. 1999; Strehl et al. 2006; Kreuziger et al. 2007; Saito et al. 2010;
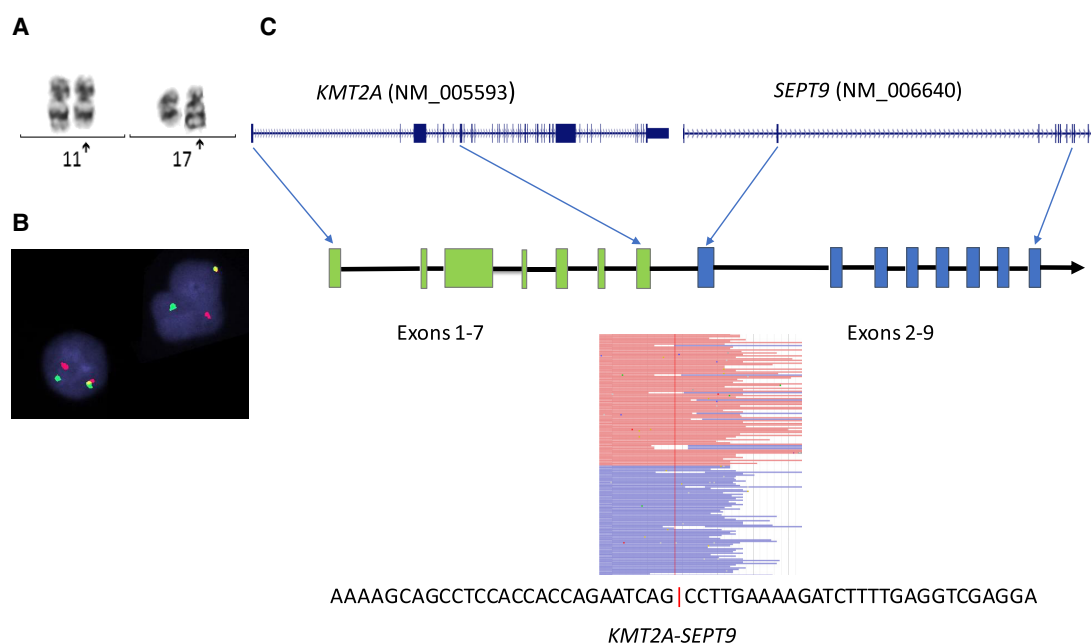

Figure 1. (*A*) Chromosome analysis reveals a balanced chromosome translocation between Chromosomes 11 and 17—that is, t(11;17)(q23;q25) (arrows). (*B*) FISH analysis with a *KMT2A* break-part probe set (Abbott Molecular) shows a split *KMT2A* signal pattern—that is, t(11;17). The 5′ *KMT2A* and 3′ *KMT2A* were labeled with green and orange, respectively. (*C*) Schematic illustration of the protein structure, bidirectional RNA sequencing reads, and transcript sequence of the *KMT2A* (NM_005593)–*SEPT9* (NM_006640) in-frame fusion product detected by Archer FusionPlex with exons 1–7 of *KMT2A* fused to exons 2–9 of *SEPT9*.

Table 1. *KMT2A–SEPT9* fusion detected in patient

Gene 1	Gene 2	Position 1	Position 2	Transcript 1	Exon number 1	Transcript strand 1	Transcript 2	Exon number 2	Transcript strand 2	Fusion junction sequence	Frameshift class
KMT2A	*SEPT9*	Chr 11: 118482092	Chr 17: 77402059	NM_005933	7	+	NM_006640	2	+	AAAAGCAGCCTCCA CCACCAGAATCAG| *CCTTGAAAAGATCT TTTGAGGTCGAGGA*	In-frame

Santos et al. 2010). To our knowledge, this is the first report of *KMT2A–SEPT9* fusion–associated AMKL, as well as the first reported occurrence of any *KMT2A–SEPTIN* fusion occurring in AMKL (Cerveira et al. 2011). In this case the fusion is located at the intron 7 breakpoint. *KMT2A–SEPT9* fusions may have a propensity to involve the intron 7 or 8 breakpoint, as the majority of reported cases involve this region. This contrasts with more common *KMT2A* fusion partner genes, which most frequently involve the region between exon 9 and intron 11 (Meyer et al. 2018). However, the limited number of cases prevents any definitive conclusions.

AMKL is a subtype of AML with bimodal age distribution, with peaks occurring in early childhood before the age of 3 and later in adulthood (Tallman et al. 2000; Athale et al. 2001). In patients with Down syndrome (DS), AMKL is the most frequently occurring form of AML and is characterized by the presence of mutations involving *GATA1* (Wechsler et al. 2002). In patients with non-DS pediatric AMKL, several molecular subsets have recently been characterized and provide valuable prognostic information (de Rooij et al. 2016, 2017; Hara et al. 2017). Commonly reoccurring rearrangements include *RBM15–MKL1*, *CBF2T3–GLIS2*, *NUP98–KDM5A*, and *KMT2A* (de Rooij et al. 2016, 2017; Hara et al. 2017). Patients with fusions involving *KMT2A* make up 7%–17.4% of pediatric patients with non-DS AMKL (de Rooij et al. 2016, 2017; Hara et al. 2017). Numerous *KMT2A* fusion partners have been identified in children with AMKL such as *MLLT1*, *MLLT3*, *MLLT6*, *MLLT9*, and *MLLT10* (de Rooij et al. 2016, 2017; Hara et al. 2017). Although little is known about the prognostic implications of the various *KMT2A* fusion partners, collectively it appears the presence of these rearrangements is a high-risk feature associated with a greater risk of relapse and worse overall survival, indicating a role for allogeneic transplantation in first remission, which was recommended for the patient discussed in this case (de Rooij et al. 2016, 2017).

The *KMT2A* gene located on Chromosome 11 band q23 is a frequent target of translocation events with more than 100 recurrent rearrangements having been identified (Meyer et al. 2018). *KMT2A* rearrangements are commonly seen in both adult and pediatric acute leukemias but have particularly strong associations with infant ALL (Meyer et al. 2018), M4/M5 AML (Cimino et al. 1995; Schoch et al. 2003; Meyer et al. 2018), and therapy-related AML (t-AML) (Smith et al. 1994; Meyer et al. 2018), where it typically is found in patients exposed to topoisomerase II inhibitors. The *KMT2A* gene product is a DNA-binding protein capable of positively regulating gene expression, including the *Hox* family of genes, which play an important role in hematopoiesis and lymphoid cell development (Caslini et al. 2000; Milne et al. 2002). Chimeric proteins resulting from *KMT2A* rearrangements can efficiently transform hematopoietic precursors into leukemic stem cells (Krivtsov and Armstrong 2007). However, the fusion partner appears to play an important role in transformation because simply enhancing *KMT2A* promoter activity is not sufficient to induce leukemogenesis (Corral et al. 1996).

The septin family of genes is an evolutionarily conserved GTP-binding, filament-forming protein believed to be involved in polarity determination, cytoskeletal reorganization,

membrane dynamics, vesicle trafficking, and exocytosis (Kartmann and Roth 2001). Aside from *SEPT9*, several human septin genes have been identified as partners for translocation events with *KMT2A* including *SEPT5*, *SEPT6*, and *SEPT11* (Hall and Russell 2004). The role of *SEPT9* in leukemogenesis has not been clearly elucidated. However, studies have shown that variants of SEPT9 interact with both α and ɣ tubulin, and cells with enhanced expression of *SEPT9* experienced defects in both cytokinesis and mitotic spindle defects, contributing to genomic instability (Peterson et al. 2011). The role of *SEPT9* in malignant transformation may not be restricted to AML/MDS, as alterations in expression or deletion of *SEPT9* are frequently observed in breast and ovarian cancer, indicating its potential role as a tumor suppressor (Kalikin et al. 2000; Burrows et al. 2003).

The mechanism by which the *KMT2A–SEPT9* fusion drives leukemogenesis has not been firmly established. For many *KMT2A* fusion partner genes, it is believed that rearrangement events result in the fusion of transcriptional activation domains to *KMT2A* and are capable of driving leukemogenesis (So and Cleary 2003; Zeisig et al. 2003). However, as is the case with SEPT9, several *KMT2A* partners are localized to the cytoplasm and unlikely to have nuclear function. In a number of these partners dimerization of fusion oncoproteins has been identified as an alternative mechanism of transcriptional activation of *KMT2A* (Martin et al. 2003; So et al. 2003). Likewise, homo-oligomerization of *KMT2A–SEPT6* fusion products proved to be capable of immortalizing stem cell progenitors (Ono et al. 2005). Drawing from sequence homology across the septin family of proteins and the ability of SEPT9 to form homodimers, it is reasonable to hypothesize that KMT2A–SEPT9 fusion protein dimerization is a key step in leukemic transformation (Abbey et al. 2016).

In summary, this report describes the first documented case of *KMT2A–SEPT9* fusion–associated AMKL and is also the first report of any *KMT2A–SEPTIN* fusion occurring in AMKL. This rearrangement was first detected by conventional cytogenetics and confirmed by targeted RNA sequencing. Despite the numerous documented cases of the *KMT2A–SEPT9* fusion, the mechanism of its role in leukemic transformation and its prognostic impact are unclear.

ADDITIONAL INFORMATION

Data Deposition and Access
The variant described in this manuscript was deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and assigned the accession number SCV000852004.

Ethics Statement
Informed and signed consent was obtained for the research performed and publication of the results. The patient was enrolled in the Memorial Sloan Kettering Cancer Center (MSKCC) targeted gene sequencing research study (Genomic profiling in cancer patients; NCT01775072) with approval from the MSKCC Institutional Review Board under protocol IRB# 12-245.

Author Contributions
C.J.F., N.S., Y.Z., and M.R. conceived the study. Y.Z., J.Y., and R.B. provided figures and associated legends. All authors reviewed and drafted the manuscript.

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