



Pediatric M5 acute myeloid leukemia with *MLL-SEPT6* fusion and a favorable outcome

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ABSTRACT

Acute myeloid leukemia (AML) patients with *MLL-SEPT6* fusion represent a small subset of AML. The uncommon *MLL-SEPT6* rearrangement results from t(X;11) or other variants like ins(X;11), and it is usually associated with complex cytogenetic abnormalities. We herein report a case of AML-M5-infant with ins(X;11)(q24;q23q13) and *MLL-SEPT6*. The one-year-old boy presented with leukocytosis, anemia and thrombocytopenia. He had a favorable response to chemotherapy according to ELAM02 protocol and is currently in complete remission. We here, highlight the occurrence of *MLL-SEPT6* as the sole abnormality in a pediatric-AML-M5 case, discuss the prognostic implication of this genetic variant, while reviewing previously reported AML-*MLL-SEPT6* cases.

1. Introduction

Abnormalities within 11q23 chromosomal region, resulting in a rearranged mixed lineage leukemia (*MLL*) gene, are frequently observed in both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) [1,2]. The *MLL* gene encodes a multidomain protein that regulates class I homeobox (*HOX*) gene expression [3]. *HOX* genes appear to play a key role in modulating hematopoietic development. *MLL* rearrangements result in an altered *MLL* activity causing abnormal *HOX* gene expression in hematopoietic stem cells (HSCs), resulting in a halt of HSCs maturation, thus promoting leukemogenesis [4].

To date, more than 80 *MLL* fusion partners have been reported and characterized [5], including the evolutionarily conserved family of genes coding for Septins, *SEPT2*, *SEPT5*, *SEPT6*, *SEPT9* and *SEPT11*, that are involved in diverse functions such as cytokinesis, cell polarity and oncogenesis [6–8]. To our knowledge, the *MLL-SEPT6* fusion has so far only been described in 17 AML patients [8–19]. We herein present clinical and molecular analyses of an AML-M5 patient with an *MLL-SEPT6* rearrangement, along with a concise review of all previously described *MLL-SEPT6* AML cases.

2. Case presentation

A one-year old boy presented to the hospital in November 2020 with a high-grade fever lasting more than 4 days, and a CBC showing anemia (8.9 g/dl), leukocytosis (WBC 75,350/mm³) and thrombocytopenia (67,000/mm³). He suffered general pain and irritability. Physical examination upon admission showed absence of adenopathy, spleen/liver enlargement or cutaneous lesions. Neurological assessment was normal. After admission, follow up blood tests confirmed the presence of anemia, thrombocytopenia and leukocytosis with a WBC of 106,600/mm³ and 86% of blasts, as well as an elevated LDH (629 U/l). A chest X-Ray was also performed showing a normal result.

The patient was admitted to the hospital with suspicion of leukemia. Work up including flow cytometry on bone marrow aspirate revealed the presence of blasts expressing CD13+, Cd33+, HLADR+, CD117+, CD11c+, CD11b+, CD15+ and intracellular MPO+. Cytochemistry analysis reported more than 90% of blast cells being positive for Sudan black B, with a morphology suggestive of acute myeloblastic leukemia FAB M5. Cerebrospinal fluid analysis was normal.

A karyotype on bone marrow aspirate was performed. Two short term cell cultures were set up in RPMI 1640 medium supplemented with 20% FBS followed by RHG banding. Fifteen R-banded metaphases were karyotyped and analyzed according to the International System for

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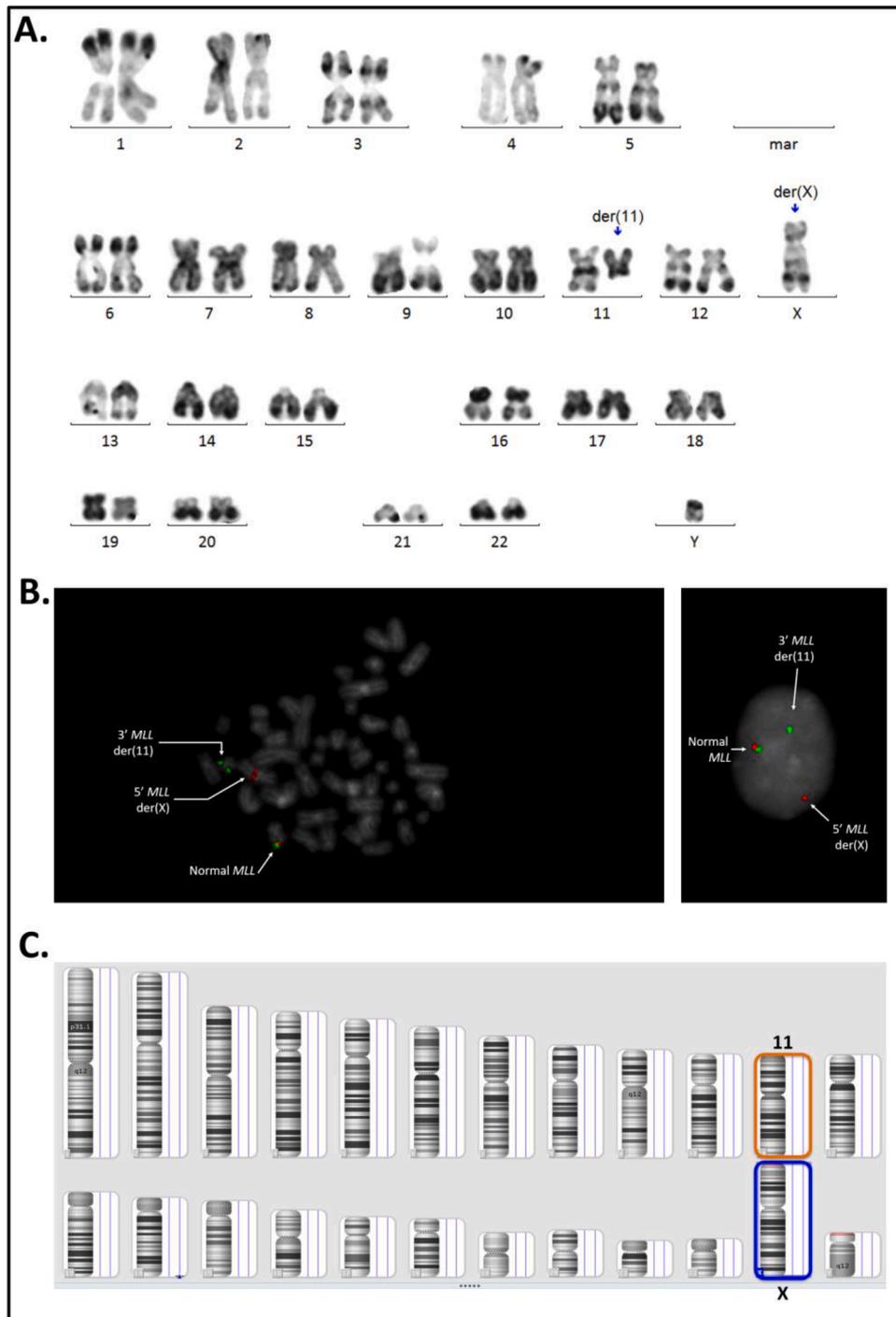


Fig. 1. (A) Bone marrow karyotype showing $\text{ins}(X;11)(q24;q23q13)$. (B) Metaphase and interphase FISH showing one intact *MLL* signal (Red and Green fusion) on normal chromosome 11, one split *MLL* signal with red signal on abnormal chromosome X ($\text{der}(X)$: derivative chromosome X) and green signal on abnormal chromosome 11 ($\text{der}(11)$: derivative chromosome 11). (C) SNP array result confirming the balanced status of the $\text{ins}(X;11)(q24;q23q13)$.

Human Cytogenomic Nomenclature (ISCN) 2016. Karyotype analysis showed in both cultures an apparently balanced insertion of an 11q segment into the long arm of chromosome X: $46,Y,\text{ins}(X;11)(q24;q23q13)[12]/46,XY[3]$ (Fig. 1A). In light of the clinical diagnosis and the cytogenetic result, FISH analysis using the XL *MLL* plus probe (Break-apart probe, Metasystems, Germany) was carried out. Two hundred interphase nuclei were analyzed, and showed a split *MLL* signal indicating the involvement of *MLL* gene in the $\text{ins}(X;11)$ rearrangement (Fig. 1B). Molecular analysis using the Leukemia Fusion Genes (Q30) Screening Kit (QuanDx, USA) detected the presence of an *MLL-SEPT6*

fusion transcript in the processed bone marrow sample.

Furthermore, in order to rule out any chromosomal imbalance not detected by the conventional technique, a microarray analysis using SNP array CytoScan 750 K (Affymetrix, USA) was performed. SNP data analysis confirmed the balanced status of the $\text{ins}(X;11)$, and showed no other chromosome gain or loss (Fig. 1C).

Based on all these findings, the diagnosis of *MLL*-rearranged AML with no neurological involvement was established. The treatment was initiated according to the recommended ELAM02 regimen [20]. The patient received, in accordance with his age, Aracytine $150 \text{ mg/m}^2/\text{day}$

Table 1
Summary of *MLL-SEPT6* cases in the literature and the present case.

Case	Age	Sex	FAB	Karyotype	Reference
1	6 m	F	M2	46,X,ins(X;11)(q24;q23)	[10]
2	20 m	F	M4	47,X,der(X)t(X;11)(q22;q23)t(3;11)(p21;q12),der(3)t(3;11)(p21;q23)t(X;11)(q22;q25),+6,der(11)del(11)(q12?qter)	[11]
3	10 m	M	M2	46,Y,t(X;11)(q22;q23)[25]/46,XY[5]	[11]
4	3 m	F	M2	46,XX,t(5;11)(q13;q23)[6]/46,sl,add(X)(q22)[12]/47,sdl,+add(X)[1]/46,XX[1]	[9]
5	7 m	M	M2	46,XY[20]46,XY,ins(X;11)(q22-q24;q23)	[9]
6	6 m	F	M1	46,X,add(X)(q2?),del(11q?)[20]	[9]
7	29 m	M	M5	46,Y,ins(X;11)(q24;q23q13)[13]/46,XY[7]	[12]
8	8 m	M	M4	46,XY	[13]
9	13 m	M	M4	46,Y,ins(11;X)(q23;q24q22)[14]/46, idem,i(10)(q10)[6]	[14]
10	26 m	F	M4	46,XX,t(11;17)(q23;q25)[20]	[15]
11	17 m	F	M2	47,X,add(X)(p11),+6,add(11)(q23)[20]	[8]
12	12 m	M	NA	46,Y,t(X;11)(q24;q23)[11]/46,XY[9]	[8]
13	0 m	M	NA	46,Y,ins(X;11)(q24;q13q23)[11]	[8]
14	43 y	M	M5	47,Y,ins(X;11)(q24-q25;q23),+10[20]/46,XY[1]	[17,18]
15	5 y	F	M5	49,X,ins(X;11)(q24;q13q23),+4,+8,+20	[16]
16	1 y	M	M5	46,Y,der(X)t(X;11),der(11)t(X;11)(?;?p14)t(X;11)(?;q23)t(X;12)(?;q?13),del(12)(q?22),der(12)t(X;12)/47, idem,+19	[16]
17	10y	M	M2	46,Y,-X,del(6)(q23),add(11)(q23),add(22)(q13),+mar[13]/46,XY[7]	[19]
18	12 m	M	M5	46,Y,ins(X;11)(q24;q23q13)[12]/46,XY[3]	(Preprint) Present study

*m=months, y=years, M=male, F=female, NA=not available.

for 7 days, Mitoxantron 9 mg/m²/day for 5 days, IT (intrathecal) triples on day 1 and day 7, prophylactic Bactrim 3 times/week, Zyloric 500 mg/m²/day, along with an optimal continuous hydration.

At day 2 of treatment, WBC count dropped to 1000/mm³ with no metabolic manifestation observed (no evidence for tumor lysis syndrome) and no blasts detected on peripheral blood. At day 4 of chemotherapy, the child had no signs of irritability or general pain. Bone marrow evaluation at day 15 showed poor cellularity with no blasts. At day 30, bone marrow aspirate showed normal aspect with no blasts, a normal karyotype and an undetectable *MLL-SEPT6* fusion transcript by PCR were found as well. Subsequently, consolidation treatment was initiated. The patient had no HLA compatible donor in the event of a potential transplantation procedure. He has been in complete remission for more than 12 months since the initiation of his treatment.

3. Discussion

Cytogenetic abnormalities play a prominent role in risk stratification of AML cases. t(X;11)(q24;q23)-associated AML represents a subset of pediatric leukemia [9,21]. In 2001, Borkhardt *et al.* reported on the *SEPT6* gene (*Septin 6*, *KIAA0128*) as a new partner for the *MLL* gene in the context of an ins(X;11)(q24;q23) in an infant with AML-M2 [10]. Chromosomal rearrangements involving 11q23 and Xq24 resulting in *MLL-SEPT6* fusion are often complex and sometimes cryptic [21].

We reviewed the literature in Mitelman database for chromosomal aberration and gene fusions (mitelmandatabase.isb-cgc.org), Atlas of genetics and cytogenetics in oncology and hematology (atlas-geneticsoncology.org), as well as in PubMed. They all feature few AML cases mainly in infants and young children. The *MLL-SEPT6* fusion has

so far only been reported in 17 AML patients [8–19]. With the exception of one adult case (43 years old), all patients described in the literature, in addition to our patient, are children (age range: 0months – 10years), presenting with AML classified as FAB M1 (1 patient), M2 (6 patients), M4 (4 patients) and M5 (5 patients including our case) (table 1).

It is well established that the majority of pediatric cases with *MLL* rearrangements have ALL, however it was hypothesized that the *SEPT6* domains of the *MLL-SEPT6* chimeric protein contribute to myeloblastic leukemogenesis in children [6]. Indeed, the proteins from fusion between other Septins and the *MLL* gene tend to take part in the myeloblastic rather than the lymphoblastic leukemogenesis [6,8,12,22].

The only AML-M5 (monocytic) case reported with *MLL-SEPT6* as a sole genetic abnormality was a 29-month-old boy (Table1: case number 7) presenting with pancytopenia and blasts in peripheral blood [12]. Our patient was younger at diagnosis (12 months), he had bicytopenia (anemia and thrombocytopenia) and leukocytosis, in addition to peripheral blasts, along with a cytogenetic rearrangement resulting in an insertion (X;11) and a rearranged *MLL* confirmed by FISH. Three other AML-M5 cases were also reported, however all of them had complex karyotypes with additional chromosomal abnormalities such as +4, +8, +10, +19, +20 and del 12q [16–18].

As for the treatment, the previously published AML-M5 case, showing similar cytogenetic and molecular characteristics as in our patient, was treated with idarubicin, 1-β-D-arabinofuranosylcytosine, and thioguanine in line with the BHAC regimen, while he had complete remission at 4 weeks of treatment, he later received cord blood transplant with a reported survival at 8 months upon diagnosis. Our patient on the other hand, was treated according to the recommended ELAM 02 regimen and has been in complete remission for over 12 months now.

In conclusion, we report a rare case of ins(X;11)(q24;q23q13) associated with *MLL-SEPT6* fusion as a sole acquired abnormality in an infant with AML-M5. This case provides additional data both at the clinical and genetic aspects of the unusual *MLL-SEPT6* rearrangement in infants. As opposed to what has been previously reported with regard to the “rather poor” prognosis and outcome [21], our case suggests that the prognostic value of *MLL-SEPT6* fusion might be conditional on other associated genomic abnormalities. Additional cases as well as long-term follow-up data would help in further outlining the prognostic relevance of *MLL-SEPT6*.

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Declaration of Competing Interest

The authors declare no potential conflicts of interest.

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