

A NEW PEDIATRIC ACUTE MYELOID LEUKEMIA CASE INVOLVING *KMT2A-MLLT6* FUSION WITH A HETEROZYGOUS *RARA* GENE DISPLACEMENT: CYTOGENOMICS AND CLINICAL DATA EVALUATION

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Objectives

In pediatric acute leukemias, chromosomal rearrangements frequently generate gene fusions involving the lysine (K)-specific methyltransferase 2A gene (*KMT2A*, also known as *MLL*).

Specific *KMT2A* fusion partners are associated with the disease phenotype (lymphoblastic vs. myeloid), and different prognosis can be observed depending on the type of *KMT2A* rearrangement. So far, 80 different direct *KMT2A* fusion partners and 120 different reciprocal *KMT2A* fusion variants have been reported in acute leukemia. In this sense, recent studies have reported that the follow-up of patients during treatment and therapy adjustment based on minimal residual disease (MRD) monitoring has a very strong impact on the outcome.

However, the *KMT2A* partner gene cannot always be identified by banding karyotyping and other conventional approaches. To overcome this matter, different scientific groups have combined efforts to establish a network to study the cytogenomic landscape of the *KMT2A* breakpoints in patients.

Moreover, verify the *KMT2A* fusion to precisely confirm the diagnosis, monitoring the MRD, and uncover new partners implicated in childhood leukemogenesis. Here we present rare cytogenomic and clinical data from a pediatric acute myeloid leukemia patient with *KMT2A-MLLT6* fusion and a heterozygous *RARA* gene displacement.

Material and Methods

Bone marrow sample from a 15-year-old girl was referred to the Laboratory of Cytogenetics, INCA. Immunophenotyping showed 85% of blast cells, and a profile compatible with acute monocytic leukemia, being treated under the AML-BFM-2004 protocol. She evolved with disseminated intravascular coagulation (DIC), pancytopenia, and intense bleeding. The patient presented 21% of blast cells in the first assessment (induction phase, day 21). The girl did not experience remission after the first cycle of chemotherapy, and due to the presence of a rare cytogenetic alteration, she was referred to high-risk protocol, and indicated to allogeneic bone marrow transplantation. Cytogenetic analysis was performed on bone marrow under standard protocols. Long-distance inverse polymerase chain reaction (LDI-PCR) assays were used to identify the *KMT2A* partner genes and their corresponding breakpoints.

Results

Cytogenetic studies showed the karyotype: 50,XX,+4,+8,t(11;17)(q23;q12-21),+18,+19 (Figure 1). The FISH analysis revealed *KMT2A* gene rearrangement and a *RARA* gene displacement (Figure 2). The LDI-PCR sequencing revealed the fusion *KMT2A-MLLT6*.

Discussion

The t(11;17)(q23;q12-21) / *KMT2A-MLLT6* is rare in childhood AML. By G-banding and FISH we observed that the patient presented a t(11;17) involving 11q23 e *KMT2A-r*. Although, she was tested for PML-RARA because there was a hypothesis of a variant PML-RARA, as suggested by the pediatrician, and due to a severe DIC, but it was negative.

So far, three *MLL* fusion partners, namely *LASP1*, *ACACA*, and *MLLT6* have been identified in 17q12-21; *MLLT6* (*MLLT6*, PHD Finger Containing) is a Protein Coding gene.

These translocations cannot be distinguished cytogenetically and the accurate detection of the specific fusion gene requires refined FISH analysis and/or cytogenomic assays.

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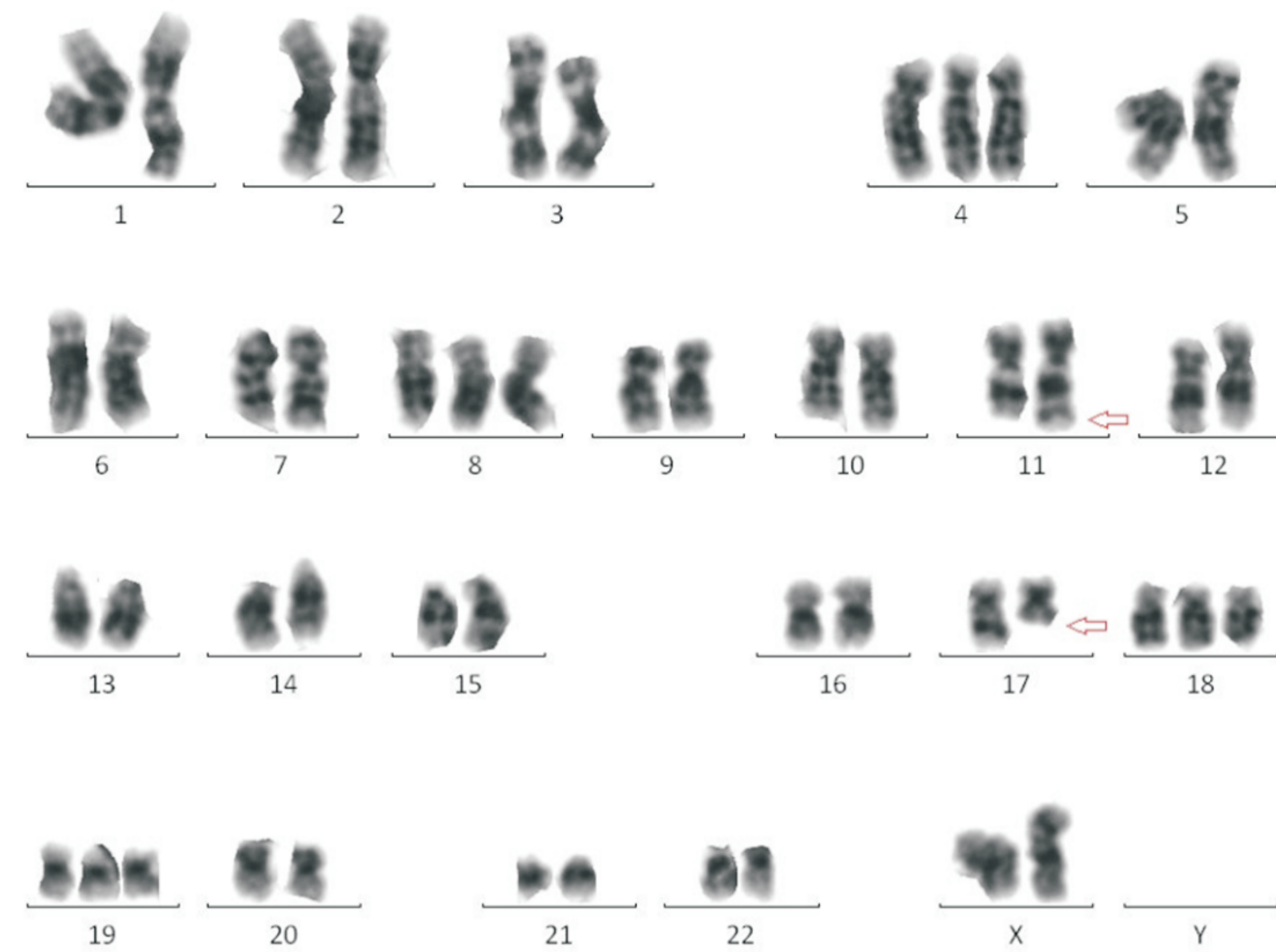


Figure 1: G-banding showed a karyotype 50,XX with trisomy of chromosomes 4, 8, 18 and 19, besides t(11;17)(q23;q12-21) as the red arrows

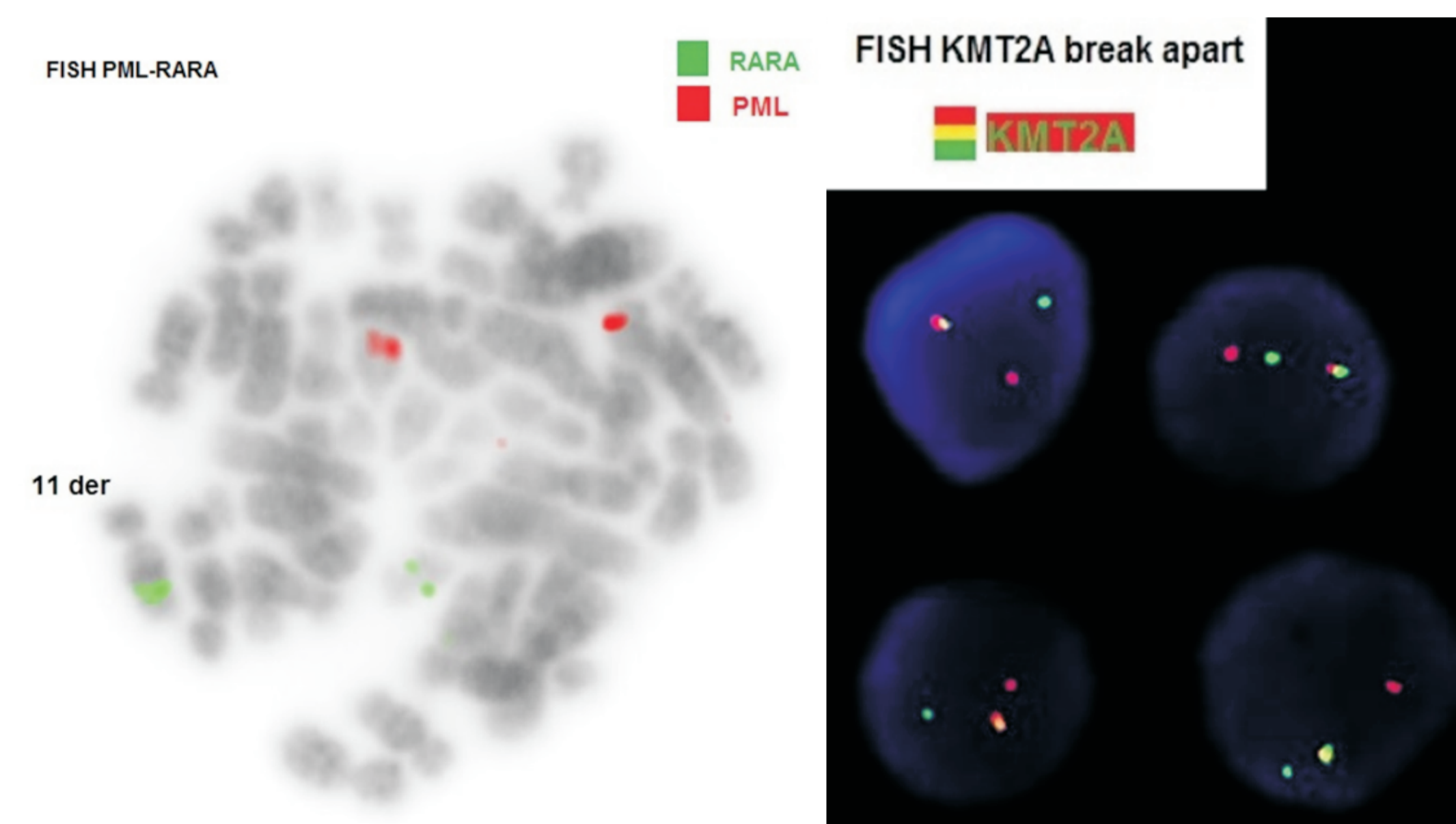


Figure 2: FISH analysis revealed *KMT2A* gene rearrangement and a *RARA* gene displacement.

MLL-MLLT6

MLL: Intron 8
MLLT6: Intron 12

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TGCTGCAGGAACATGAGAGTGCAGATATCTCTTTGATATACTGATTTCCCTTTATTTGG
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TAAAGCTCAGATTTCTCTCTGACTCCAGCCAGTGTCTTCCAGGCTGAGCGTGTCT
TCCCTTGGCTTCGTAGTGTGCTTTGGGATCAGCGCTTACGGAGTCTCAACCTCTCCG
GTATTTACAGTAAAGGGGCGGTCACAAAGCCCGCCCTCTCCCTGGAGAGTCAGCC
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GAGTTTATGTTTGCCTCCTGATAAGGATCTGATGAATGTAAGGATCATACAGGCTA
ATATGATAATAGCTCAGTCATTAAGGGCTTGTACATGCTAGCTAAACCTTCAGGTA
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Figure 3: Results of LDI-PCR assay. The LDI-PCR sequencing identified the *KMT2A-MLLT6* fusion gene, and the corresponding breakpoints.

Conclusion

Currently, further genomic analyses are being conducted to elucidate the role of the *KMT2A-MLLT6* fusion in this case and the *RARA* gene displacement prognostic implication. In our case the patient has been on remission for 4 months. However, it is not enough to conclude the role of this new translocation. Additional cases are needed to delineate the true epidemiology of this rare entity.