

# MLL: The Gene and Its Translocation Partners. 2 Cases with a t(11;17) Involving MLL and Mimicking the Variant t(11;17) RARA Translocation

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## Abstract

The MLL gene or KMT2A as it is now called is located at 11q23 and was first identified in 1992 by Rowley, et. al., as a “gene involved in human leukemia”. Since then, the “myeloid/lymphoid leukemia” or “mixed-lineage leukemia” gene has been one of the most extensively studied and characterized genes. MLL is implicated in just over 10% of all acute leukemias including myeloid (AML), lymphoid (ALL), bi-phenotypic. In virtually all cases when MLL is abnormal, the prognosis is poor.

MLL abnormalities including deletion, amplification and gene rearrangements have been reported in hundreds of peer-reviewed journal articles. Of these various abnormalities, MLL gene rearrangement is perhaps the best known and reported. To-date MLL has been identified as one of the genes involved in 85 separate, recurrent translocations earning it a reputation as (one of) the most promiscuous and mutable genes in the human genome.

We report on two cases where acute leukemia was suspected and a t(11;17)(q23;q21) was identified via cytogenetic testing. The translocation certainly looked like the “classic” t(11;17) RARA variant translocation but FISH analysis for both cases utilizing the t(15;17) dual-color, dual-fusion probe set (PML 15q22 red, RARA 17q21 green) and the RARA break-apart probe set were normal with two green (RARA) signals and two fusion signals respectively, in all interphase nuclei. Metaphase FISH analysis on both cases with the RARA break-apart probe set showed a RARA fusion signal on one of the number 17 chromosomes and a second fusion on one of the number 11 chromosomes as identified through a reverse banding technique to help identify the respective chromosomes involved in the translocation. Interphase FISH analysis of both cases with the MLL break-apart probe set showed the classic 1R1G1F abnormal signal pattern in the majority of interphase nuclei. Metaphase FISH analysis via reverse banding on both cases with this same MLL probe set showed one red signal on the derivative 11 chromosome and one green signal on the derivative 17 chromosome.

Four of the MLL translocation partners are located at 17q21 where the hematopoietic gene RARA (retinoic acid receptor alpha) is located. The t(11;17)(q23;q21) is a well-known RARA variant translocation and is important to identify when acute promyelocytic leukemia (APL) is suspected. Per the current version of the WHO classification, this variant t(11;17) RARA translocation would be diagnostic of APL regardless of blast count in the appropriate clinical setting. However, in our two cases, the t(11;17)(q23;q21) and t(11;17)(q23;q22) did not involve RARA at 17q21-22 but involved one of the other MLL gene rearrangement partners in the q21-q22 region. A negative result for either the t(15;17) probe set (this probe set eliminates the 15 as a translocation partner) or the RARA break-apart probe set and a positive (abnormal) result for the MLL break-apart will help differentiate a true RARA variant translocation from one that cytogenetically looks the same. Being aware of these MLL translocations with any of its partner genes located at 17q21 can help prevent erroneous interpretation as a variant APL translocation.

## Introduction

Translocations involving the chromosome band 11q23 occur frequently in hematologic cancers, affecting approximately 7 to 10 percent of acute lymphoblastic leukemias (ALLs), and 5 to 6 percent of AMLs [3]. Translocations involving 11q23 are the most common cytogenetic abnormality in infants with acute leukemia, regardless of the phenotype [3]. They account for approximately 70 percent of all cases of both AML and ALL in infants [3]. These translocations are also observed in therapy-related leukemias, especially in patients previously treated with inhibitors of topoisomerase II [3].

The t(11;17) involving MLL rearrangement without RARA rearrangement has shown clinically to be a very rare translocation. A total of 4 cases with t(11;17) have been reported and all 4 of these cases have molecular breakpoints determined to be proximal to RARA in all 4 reported cases [1]. A lack of RARA involvement was also seen in our two cases. One previously reported study utilized a BAC clone that was labeled by FISH probes covering all of the MLL(11q23) gene and a gene on 17q21 proximal to RARA identified by University of California Santa Cruz as AF17 [1]. This study revealed MLL/AF17 fusions on both the der(11) and der(17) chromosomes [1]. Specific disease identification for 3 of these previous t(11;17) cases from the Mitelman Database of Chromosomal Aberrations in Cancer have reported an AML diagnosis [1]. Future work to help identify a common t(11;17) translocation partner will help determine clinical characteristics and, possibly, genetically driven therapy.

## Materials and Methods

For our first patient study we completed a full 20 metaphase cytogenetics study (24EB, 48EB hour cultures), note: Ethidium Bromide was added to cytogenetic cultures in order to act as an intercalating or lengthening agent and is abbreviated (EB), a FISH AML Standard panel (24EB hour cytogenetics culture) and a RARA break apart interphase/metaphase FISH confirmation study (24EB hour cytogenetics culture). For our second patient we also completed a full 20 metaphase study and interphase/metaphase FISH t(11;17) confirmation testing was done using MLL break apart (see figure 6 for the probe map) and RARA break apart (see figure 13 for the probe map) probes. All testing for both FISH and cytogenetics was completed on a bone marrow aspirate specimen for both patients. Our Cytogenetics Department uses the following instruments on all tests both ordered and internal confirmation studies: A Hanabi PIII Harvester for use of an automated harvesting technique, a Thermotron CDS-5 slide dropping chamber for specimen dropping and drying purposes. Cytogenetic tech analysis for all cytogenetics testing was completed using Ikaros Imaging Systems. Our Cytogenetics Department uses a GTG banding technique. FISH co-hybridization for all FISH testing was achieved using a Thermobrite instrument. FISH image capturing is automated using a Bioview Duet-3 or manually using a MetaSystems Isis station. FISH analysis was completed with a Bioview Solo Workstation or manually on the Fluorescent microscope. Our FISH Department utilized a reverse DAPI feature for the RARA break apart metaphase/interphase on patients 1 and 2 to help eliminate RARA 17q21 involvement. Our FISH Department uses DAPI II as the counterstain for all interphase/metaphase FISH studies.

## Acknowledgements

The authors would like to thank the NeoGenomics FISH and Cytogenetics teams for their hard work in helping to accurately diagnosis these two patients.

## Results and Discussion

The cytogenetics study done on Patient 1 revealed a translocation involving the 11 and 17 chromosomes in all 20 cells with the following karyotype result: 46,XY,t(11;17)(q23;q21)[20]. Two karyograms of the translocation abnormality as seen in patient 1 are added below, one from the 24 hour culture (see figure 2) and one from the 48 hour culture (see figure 1). The FISH testing showed: nuc ish(MLLx2)(5’MLL sep 3’MLLx1) [138/200]. A RARA break apart probe test was ordered to confirm or refute that the RARA gene at 17q21 was involvement. Metaphases were analyzed (see figure 7) and reverse DAPI images showed two normal unbroken RARA signals which signifies a negative result for RARA involvement (figure 8). All 78 metaphase FISH cells and 200 interphase cells showed normal results for RARA in our confirmation study of patient 1.

The cytogenetics study done on Patient 2 shows a similar t(11;17) translocation with the breakpoint on the 17 slightly more distal on the q arm. The karyotype result was: 46,XY,t(11;17)(q23;q22)[19]/46,XY[1]. Figure 3 shows an abnormal cell with the t(11;17) karyogram from the 24EB Culture of patient 2 and figure 4 shows an abnormal cell with the t(11;17) karyogram from the 48EB Culture of patient 2. A metaphase FISH image with RARA(17q21) break apart probe can be seen in (Figure 9). Note that no RARA rearrangement is seen. The reverse DAPI image of the same metaphase can be seen in figure 10. MLL testing with the FISH break apart probe shows a FISH MLL metaphase, with a normal 11 with the red/green fusion and the translocated derivative 11 showing the red signal of MLL and the translocated derivative 17 showing the green signal of MLL. Figure 12 shows the reverse DAPI of the same metaphase confirming the localization of the 11 and 17 rearrangements.

## Conclusion

There always seems to be one exception to every rule. We wanted to report this one cytogenetic caveat when AML is suspected. A rare but widely recognized variant RARA translocation, t(11;17), has been shown to have a fusion of RARA with ZBTB16 at 11q24, with distinctly worse prognosis than M3 AML with t(15;17), mainly because the patients fail to respond to the ATRA [4]. This report shows the utility of investigating RARA rearrangement whenever a t(11;17) translocation is identified by cytogenetics in AML and the morphology or flow cytometric diagnosis is not typical for APL.

## References

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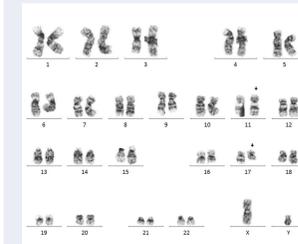


Figure 1. Patient 1 Karyogram from 48EB hr culture representing 46,XY,t(11;17)(q23;q21)[20]

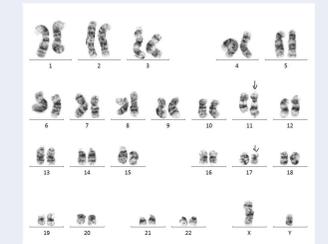


Figure 2. Patient 1 Karyogram from 24EB hr culture representing 46,XY,t(11;17)(q23;q21)[20]

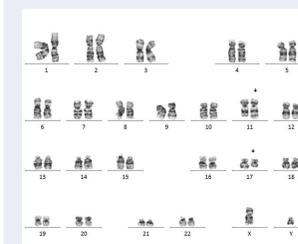


Figure 3. Patient 2 Karyogram from 24EB hr culture representing 46,XY,t(11;17)(q23;q22)[19]/46,XY[1]

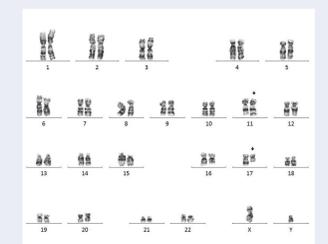


Figure 4. Patient 2 Karyogram from 48EB hr culture representing 46,XY,t(11;17)(q23;q22)[19]/46,XY[1]

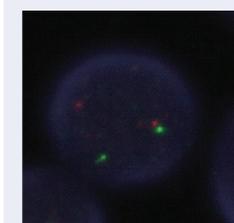


Figure 5. Patient 1 Abnormal MLL interphase representing nuc ish(MLLx2)(5' MLL sep 3' MLLx1)[138/200]

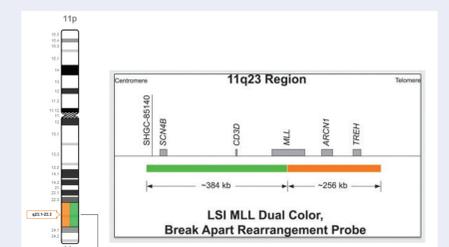


Figure 6. Representative MLL probe map with labeled 11q23.1-23.3 region

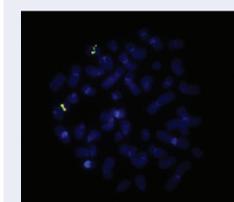


Figure 7. Patient 1 DAPI II RARA 17q21 labeled metaphase



Figure 9. Patient 2 DAPI II RARA 17q21 labeled metaphase

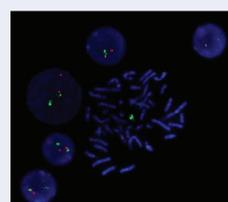


Figure 11. Patient 2 DAPI II MLL 11q23 labeled metaphase



Figure 8. Patient 1 Reverse DAPI RARA 17q21 labeled metaphase



Figure 10. Patient 2 Reverse DAPI RARA 17q21 labeled metaphase

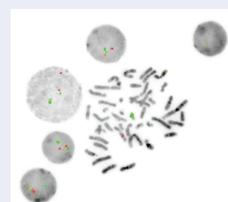


Figure 12. Patient 2 Reverse DAPI MLL 11q23 labeled metaphase

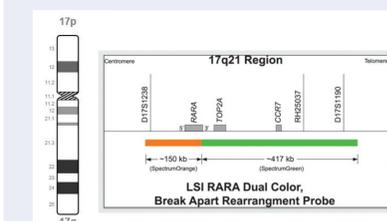


Figure 13. Representative RARA probe map with labeled 17q21 region