Journal of Hematopathology (2019) 12:99–104
https://doi.org/10.1007/s12308-019-00355-x

CASE REPORT

Characterization of a cryptic *KMT2A/AFF1* gene fusion by mate-pair sequencing (MPseq) in a young adult with newly diagnosed B-lymphoblastic leukemia

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Received: 6 March 2019 / Accepted: 22 April 2019 / Published online: 3 May 2019

Abstract

The detection of recurrent chromosomal rearrangements in B-lymphoblastic leukemia/lymphoma (B-ALL/LBL) is critical for patient management decisions. We present a newly diagnosed case of B-ALL in a young adult with a cryptic *KMT2A/AFF1* fusion that was unappreciable by conventional chromosome and fluorescence in situ hybridization (FISH) *KMT2A* break-apart probe studies. To further characterize this abnormality, a next-generation sequencing strategy, mate-pair sequencing (MPseq) was performed and characterized a cryptic, insertional rearrangement that created *KMT2A/AFF1* gene fusion. This case highlights the superior precision and resolution capabilities of NGS when compared to traditional cytogenetic methodologies, including conventional chromosome and FISH studies.

Keywords *KMT2A*(*MLL*) · *AFF1*(*AF4*) · B-lymphoblastic leukemia/lymphoma (B-ALL/LBL) · Next-generation sequencing (NGS) · Mate-pair sequencing (MPseq)

Introduction

The *AFF1* gene (4q21.3-q22.1) (also known as *AF4*) is the most common *KMT2A* gene (11q23.3) fusion partner observed in all age groups of B-lymphoblastic leukemia/lymphoma (B-ALL/LBL) and is associated with an unfavorable prognosis [1–6]. The detection of *KMT2A* rearrangements in B-ALL/LBL and other acute leukemia subtypes mainly relies upon conventional chromosome and/or fluorescence in situ hybridization (FISH) studies, including *KMT2A* break-apart probes (BAP) and dual-color, dual-fusion (D-FISH) probe sets that can detect *KMT2A* rearrangements or specific *KMT2A* gene fusion partners, respectively. Based on the experience from our genomics laboratory, the most commonly reported *KMT2A* gene partners (*AFF1*, *AFDN*, *MLLT3*, *ELL*, *MLLT1*), with the exception of *MLLT10*, usually create balanced rearrangements as indicated by conventional chromosome and/or D-FISH studies [7, 8].

Herein, we present a 25-year-old female with newly diagnosed B-ALL with normal conventional chromosome and *KMT2A* BAP studies. However, chromosomal microarray analysis (CMA) revealed an ~584 kb heterozygous deletion that spanned exons 1–4 of the *AFF1* gene region, suggesting a potential rearrangement that was subsequently confirmed as "cryptic" *KMT2A/AFF1* fusion by our *KMT2A/AFF1* D-FISH probe set. To further characterize this cryptic rearrangement, we utilized mate-pair sequencing (MPseq), a next-generation sequencing (NGS) strategy that can resolve structural abnormalities with greater resolution and precision compared to conventional chromosome and FISH methodologies [9–11].

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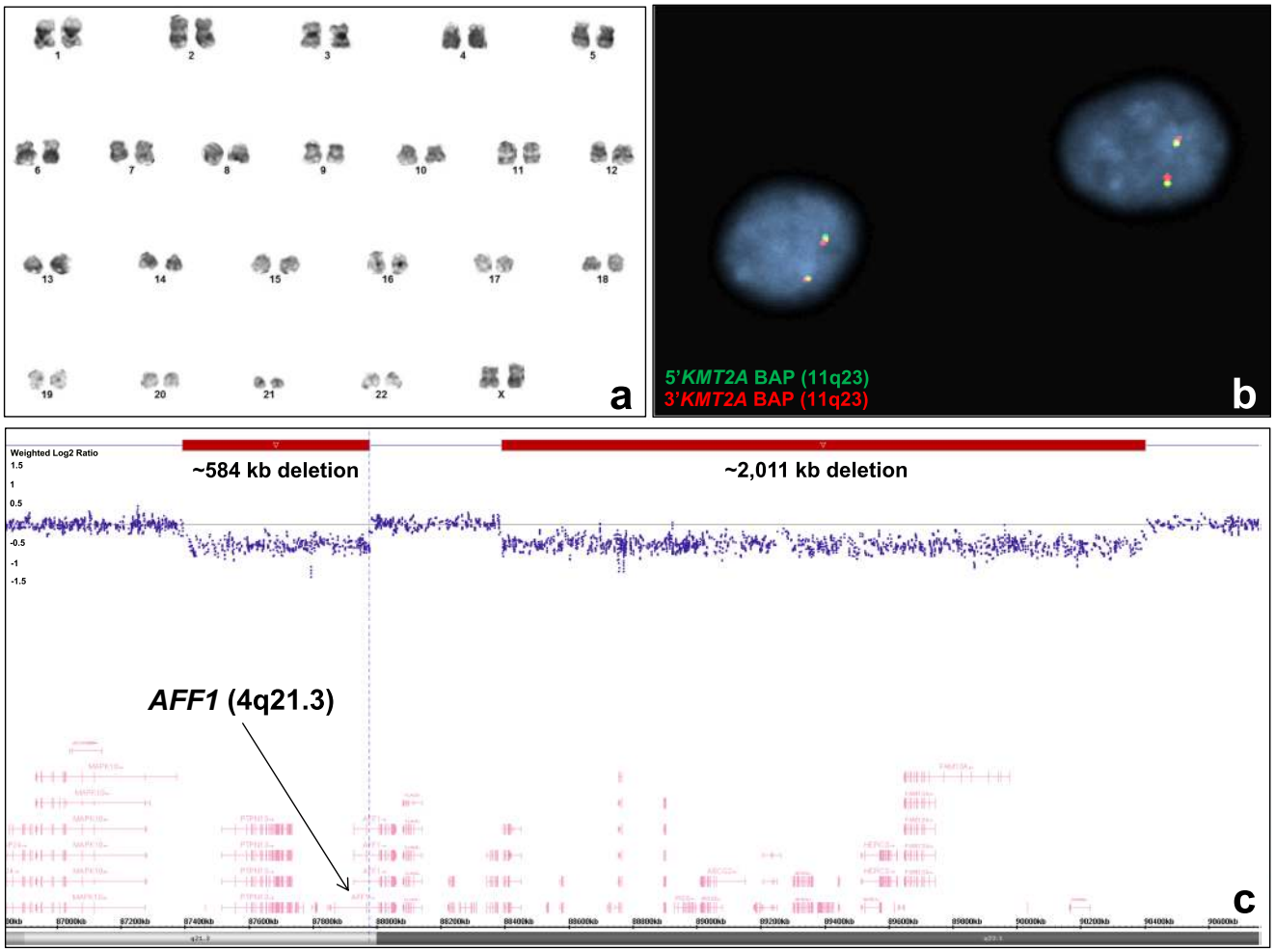

Fig. 1 **a** Representative normal female karoygram (46,XX). No structural or numerical abnormalities were observed in 20 metaphases. **b** Representative interphase nuclei demonstrating a normal result of two intact *KMT2A* fusion signals (yellow) using the *KMT2A* FISH BAP set. This signal pattern is observed when the *KMT2A* gene regions are not disrupted (negative). **c** Microarray analysis demonstrating an ~584 kb heterozygous deletion (indicated by the left horizontal red rectangle) that spans the 5′*AFF1* gene region at 4q21.3 (vertical dashed blue line). This finding indicated a potential *AFF1* gene rearrangement. In addition, an ~2011 kb heterozygous deletion was observed telemetric to the ~584 deletion that was separated by an ~409 kb gap with a normal copy number

Clinical history

Hematopathology evaluation

A 25-year-old female presented with marked leukocytosis with 75% of circulating blasts, normocytic anemia, and thrombocytopenia. A subsequent bone marrow evaluation demonstrated marrow replacement by lymphoblasts. Flow cytometric analysis of the bone marrow aspirate demonstrated an immature population of B cells, expressing CD19, CD10 (very dim), TdT, CD34, and HLA-DR, while lacking expression for kappa or lambda light chains, CD20, CD22, cytoplasmic myeloperoxidase, and cytoplasmic CD3. Immunohistochemical stains performed and blasts were positive for CD19 and CD79a, and negative for myeloperoxidase. Taken together, the morphologic and immunophenotypic findings were consistent with a diagnosis of B-ALL/LBL.

Materials and methods

Conventional chromosome and fluorescence in situ hybridization (FISH) studies

All genomic studies were performed on the diagnostic bone marrow aspirate specimen. For conventional chromosome studies, G-banding by trypsin using Leishman stain was performed on bone marrow cells that were cultured and harvested as per protocol. A total of 20 metaphases were fully analyzed. The bone marrow aspirate specimen was processed for FISH according to

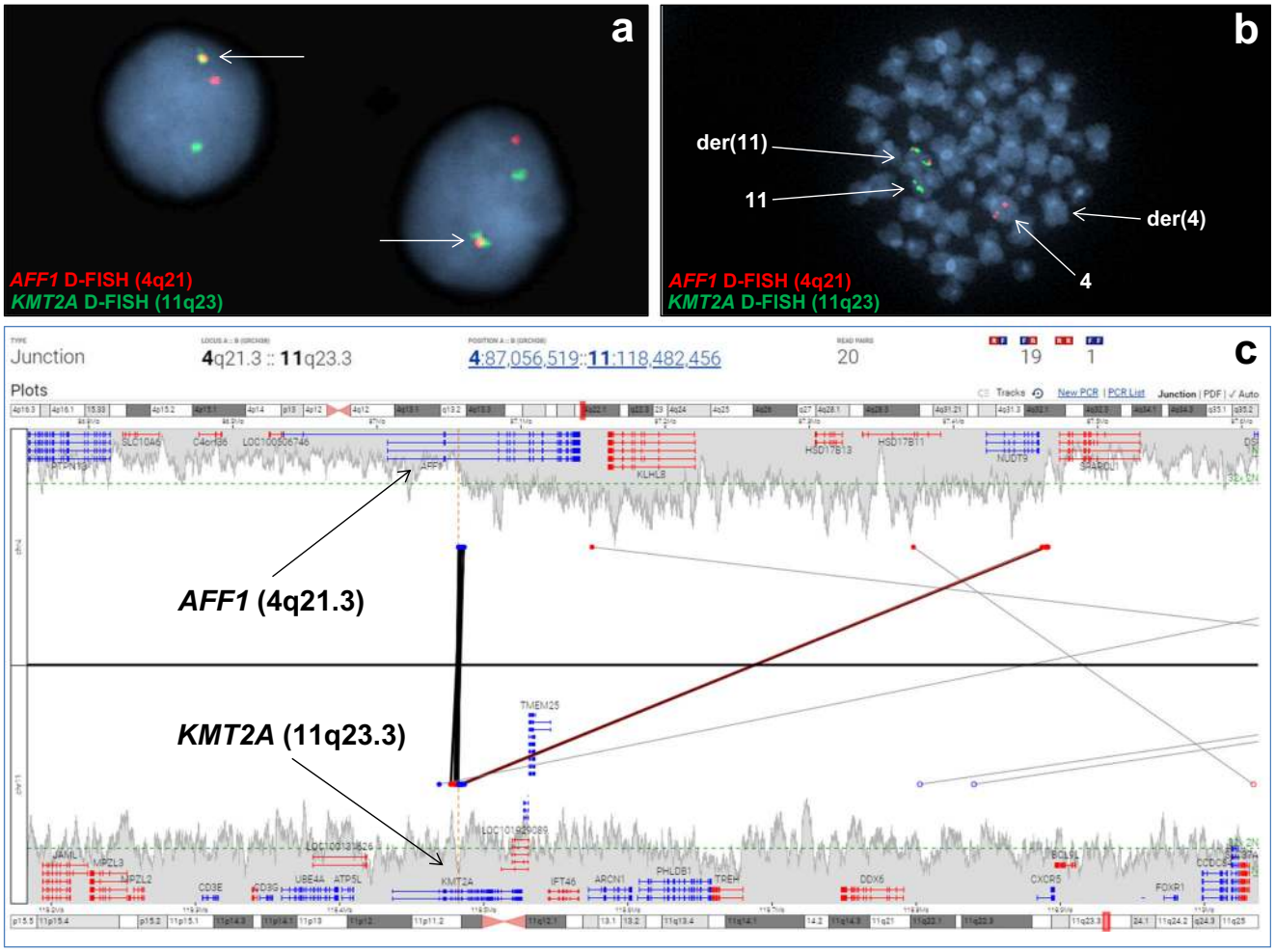

Fig. 2 **a** Representative interphase nuclei using the *KMT2A/AFF1* D-FISH probe set demonstrating a single yellow fusion signal (arrow) indicating *KMT2A/AFF2* fusion. This signal pattern was observed in 480 of 500 (96%) interphase nuclei. **b** Representative sequential FISH result performed on a "normal" metaphase using the *KMT2A/AFF1* D-FISH probe set. A single *KMT2A/AFF1* fusion signal was detected on the distal long arm of chromosome 11. **c** Mate-pair sequencing (MPseq) results. Junction plot demonstrating the insertion of a segment from chromosome 4q into chromosomal region 11q23.3. This insertional event resulted in *KMT2A/AFF1* fusion

specimen-specific laboratory protocols and subjected to standard pretreatment, hybridization, and fluorescence microscopy. A commercially available *KMT2A* BAP set (Abbott Molecular, Des Plaines, IL) and "laboratory developed" *KMT2A/AFF1* D-FISH probes set were utilized. Conventional chromosome and FISH results were interpreted by a board-certified clinical cytogeneticist (ABMGG).

Mate-pair sequencing (MPseq)

DNA was processed using Illumina Nextera Mate Pair library kit (Illumina, San Diego, CA), multiplexed at two samples per lane, and sequenced (Rapid Run mod) on the Illumina HiSeq 2500 using 101-basepair reads and paired-end sequencing. Data were aligned to the reference genome (GRCh38) using BIMAv3, and abnormalities were characterized using SVAtools and Ingenium, both in-house developed bioinformatics tools [9, 10].

Results

All genomic studies were performed on a submitted bone marrow aspirate specimen. Twenty metaphases were fully analyzed and indicated an apparently normal female karyotype (46,XX) (Fig. 1a). A comprehensive B-ALL FISH panel, including the *KMT2A* BAP set (Fig. 1b), was performed and all results were normal.

Based on the normal conventional chromosome and FISH studies, chromosomal microarray analysis was performed to identify potential abnormalities of clinical significance that were unappreciable by traditional cytogenetic methodologies. Microarray analysis revealed an ~ 584 kb heterozygous deletion including exons 1–4 of the *AFF1* gene region

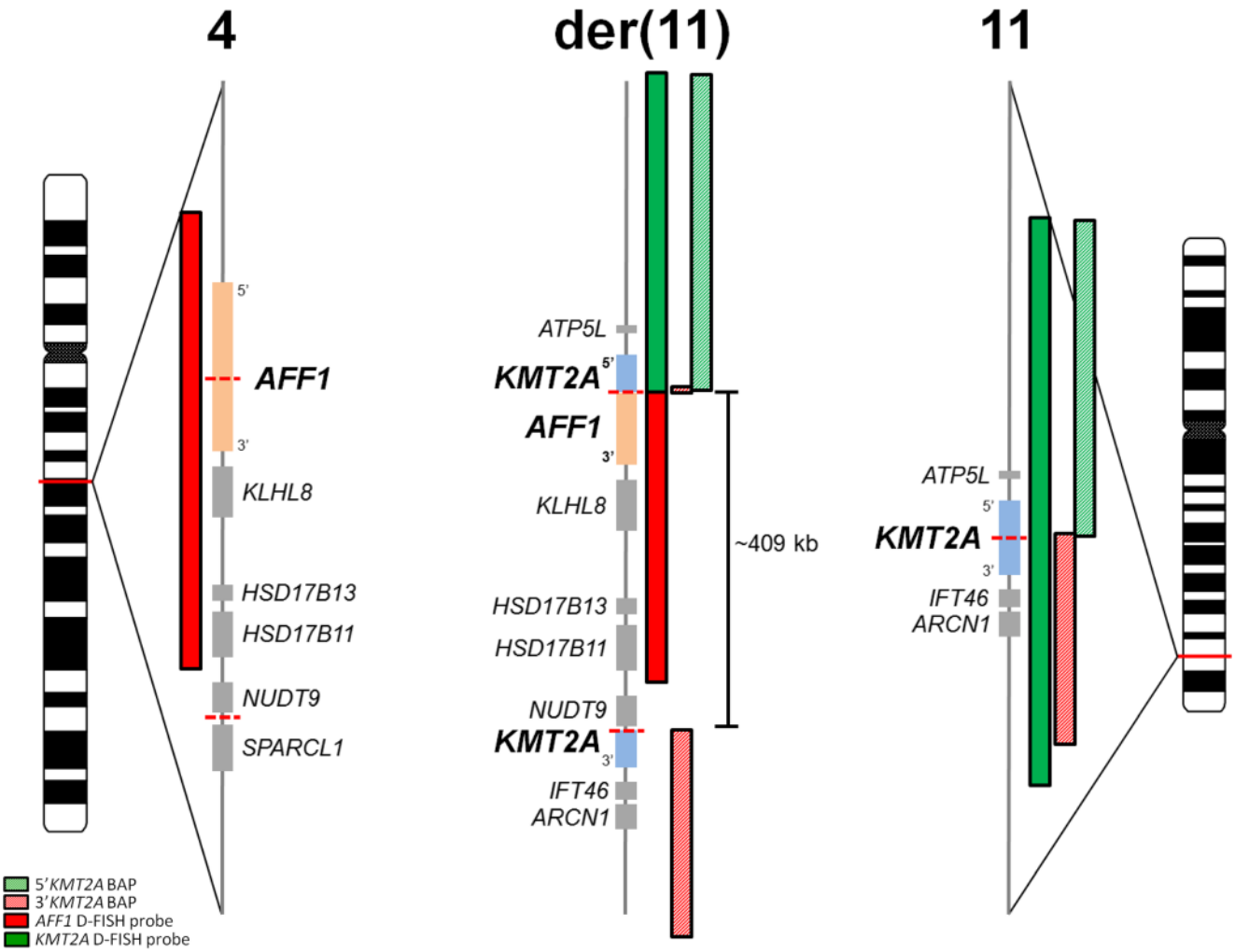

Fig. 3 A focused view of the *AFF1* and *KMT2A* gene regions on each normal copy of chromosome 4 and 11, in addition to the derivative chromosome 11. Horizontal dashed red lines indicate breakpoints. Vertical rectangles with green and red stripes indicate the 5′*KMT2A* and 3′*KMT2A* break-apart probe (BAP) footprints, respectively. Solid red and green vertical rectangles indicate the *AFF1* and *KMT2A* dual-color, dual-fusion (D-FISH) probe footprints, respectively. An ~409 kb segment of 4q21.3-q22.1 was inserted into 11q23.3, resulting in a 5′*KMT2A* (exons 1–8, NM_005933) and 3′*AFF1* (exons 5–21, NM_001116693) gene fusion. The ~409 kb insertion is not sufficient to disrupt the *KMT2A* BAP probe, thus resulting in a false-negative result. However, the juxtaposition of the *AFF1* and *KMT2A* D-FISH footprints generates a single fusion signal as observed in 96% of 500 interphase nuclei

(NM_001166693) (Fig. 1c). In addition, an ~2011 kb heterozygous deletion was observed telemetric to the ~584 deletion separated by an ~409 kb intact portion of DNA which included a normal copy number for the remaining exons 5–21 of the *AFF1* gene (Fig. 1c). Suspicious for a potential cryptic *KMT2A/AFF1* fusion, we performed our *KMT2A/AFF1* D-FISH probe set that is usually reserved for reflex testing following a positive *KMT2A* BAP study. A single *KMT2A/AFF1* fusion signal was detected in 480 of 500 (96%) interphase nuclei (Fig. 2a). Subsequently, serial FISH studies performed on apparently "normal" metaphases demonstrated the single *KMT2A/AFF1* fusion signal located on the distal long arm of an apparently normal chromosome 11 (Fig. 2b).

To further characterize the cryptic *KMT2A/AFF1* fusion, MPseq was performed and confirmed an ~409 kb segment of 4q21.3-q22.1, including exons 5–21 of the *AFF1* gene, and was inserted into chromosomal region 11q23.3, resulting in a 5′*KMT2A* (exons 1–8, NM_005933) and 3′*AFF1* (exons 5–21, NM_001166693) gene fusion (Fig. 2c). Sanger sequencing subsequently confirmed the *KMT2A/AFF1* fusion identified by MPseq.

Discussion

At our institution, microarray analysis is performed to detect unbalanced chromosomal gains and losses of diagnostic and/or prognostic significance in cases of B-ALL/LBL in the absence of a clear primary abnormality as identified by conventional chromosome and FISH studies. Since our

patient had normal chromosome and FISH studies, including the *KMT2A* BAP, chromosomal microarray was performed and revealed an ~ 584 kb heterozygous deletion that spanned exons 1–4 of the *AFF1* gene region. Considering the diagnosis of B-ALL and the known *AFF1* gene fusion association with *KMT2A*, we pursued additional testing to evaluate for a potential cryptic *KMT2A/AFF1* fusion. Using our *KMT2A/AFF1* D-FISH probe set, we confirmed a *KMT2A/AFF1* fusion in 96% (480 of 500) of interphase cells. This result was also confirmed by metaphase FISH studies revealing that the cryptic *KMT2A/AFF1* fusion was located on the distal long arm of chromosome 11. Based on the results collected thus far, we predicted an ~ 409 kb insertion of chromosome 4q21.3-q22.1 into the 11q23.3 chromosomal region that resulted in the *KMT2A/AFF1* fusion; however, the precise molecular characterization of this fusion could not be achieved using existing FISH studies. MPseq confirmed an ~ 409 kb insertion of 4q21.3-q22.1 into the 11q23.3 chromosomal region resulting in a 5′ *KMT2A* (exons 1–8, NM_005933) and 3′*AFF1* (exons 5–21, NM_001166693) gene fusion (Fig. 3). Taken together, CMA identified the initial clue to the *KMT2A/AFF1* fusion and MPseq provided the mechanism and explanation for the normal chromosome and *KMT2A* BAP studies due to the minute ~ 409 kb 4q insertion that was too small to be appreciated by either methodology.

The detection of recurrent chromosomal rearrangements in B-ALL is critical for prognostic and treatment-related decisions and most genomics laboratories rely on conventional chromosome and/or FISH studies for their detection [1, 3, 4]. While conventional chromosome analysis enables a low resolution (limit of detection for structural abnormalities: ~ 10 Mb) genome-wide view of individual neoplastic cells, cryptic or subtle rearrangements are undetectable without ancillary studies, most commonly interrogated by FISH. While targeted to specific genomic regions, FISH studies provide a significantly higher resolution and are capable of detecting balanced, unbalanced, and cryptic rearrangements that are unappreciable by conventional chromosome studies; although, as demonstrated in this case, some insertions may be too subtle for detection using BAP FISH strategies. However, NGS strategies like MPseq have now surpassed the resolution and precision capabilities of FISH as illustrated by this cryptic B-ALL clone. MPseq is a whole-genome sequencing method that has been developed for characterizing structural variants that often go undetected by short-read paired-end sequencing methods. The generation of 2–5 kb fragments with 101 base pairs sequenced on each fragment end is optimized during library preparation, resulting in a lower read depth necessary to detect structural variants throughout the genome. NGS-based strategies like MPseq are emerging during an opportune time, as chromosomal rearrangements of diagnostic, prognostic, and/or theranostic importance continue to be discovered [12, 13]. This new approach to cytogenetic diagnostics will enable the detection of both chromosomally visible and cryptic rearrangements throughout the genome, while alleviating the need to develop FISH probes for a rapidly growing list of important cytogenetic abnormalities that require interrogation.

In conclusion, we present a 25-year-old female with newly diagnosed B-ALL harboring a cryptic *KMT2A/AFF1* fusion that was unappreciable by conventional chromosome and *KMT2A* BAP FISH studies. While microarray analysis revealed an initial clue to this abnormality by detection of a heterozygous deletion that spanned the 5′*AFF1* gene region, MPseq was required to characterize the mechanism resulting in *KMT2A/AFF1* fusion. This B-ALL case demonstrates the clinical utility of MPseq in the detection of clinically significant abnormalities that may be unappreciable by traditional cytogenetic methodologies.

Compliance with ethical standards

Conflict of interest JFP, SAS, IML, BAP, CSR, JCBD, JBS, TY, PTG, RPK, and LBB declare that they have no conflict of interest. GV: Algorithms described in this manuscript for mate-pair sequencing are licensed to WholeGenome LLC owned by GV.

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