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Constitutional chromosome rearrangements that mimic the 2017 world health organization "acute myeloid leukemia with recurrent genetic abnormalities": A study of three cases and review of the literature

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Abstract

Objectives: To identify and characterize constitutional chromosomal rearrangements that mimic recurrent genetic abnormalities in acute myeloid leukemia (AML).

Methods: Bone marrow and blood chromosome studies were reviewed to identify constitutional rearrangements that resemble those designated by the 2017 revised World Health Organization (WHO) "*AML with recurrent genetic abnormalities*". Mate-pair sequencing (MPseq) was performed on cases with constitutional chromosome mimics of recurrent AML abnormalities to further define the rearrangement breakpoints.

Results: Three cases with constitutional rearrangements were identified, including *t*(6;9)(p23;q34), inv(16)(p13.1q22), and *t*(9;22)(q34.1;q12.2). Two cases were bone marrow specimens being evaluated for hematologic neoplasms, while one case was a blood specimen being evaluated for primary ovarian insufficiency. MPseq provided high-resolution and precise rearrangement breakpoints, and resolved the atypical FISH results generated with each rearrangement.

Conclusions: Our findings illustrate that constitutional rearrangements can mimic recurrent genetic abnormalities observed in AML, and we emphasize the importance of correlating genetic data with clinical and hematopathologic information.

Keywords Acute myeloid leukemia, Constitutional translocation mimics, Mate-pair sequencing (MPseq), Fluorescence *in situ* hybridization (FISH).

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Introduction

Constitutional (germline) chromosomal rearrangements, including translocations and inversions, are well recognized for their potential to impact fertility and miscarriage risk [1,2]. Conventional chromosome studies are routinely performed on adult patients with histories of infertility or recurrent miscarriages, and it is under these circumstances when constitutional rearrangements are often revealed [1,2]. However, in this context, carriers of constitutional rearrangements are often phenotypically and cognitively normal (assuming the rearrangements are truly balanced and genes of clinical significance are intact) and these rearrangements will remain undetected without a clinical indication for chromosome studies. Conventional chromosome and fluorescence *in situ* hybridization (FISH) studies are routinely performed on bone marrow and blood specimens to detect acquired (somatic) rearrangements which provide diagnostic, prognostic, and/or guidance for therapy-related decisions in acute myeloid leukemia (AML) [3,4]. Several recurrent acquired genetic abnormalities associated with characteristic chromosome rearrangements are observed in AML and are recognized as distinct diagnostic subgroups by the WHO, including *DEK-NUP214* [*t*(6;9)(p23;q34.1)] and *CBFB-MYH11* [inv(16)(p13.1q22) or *t*(16;16)(p13.1;q22)] fusions [3,4]. Importantly, *BCR-ABL1* fusion associated with *t*(9;22)(q34;q11.2) is now a provisional entity in the 2017 revised World Health Organization (WHO) classification of AML with recurrent genetic abnormalities [3].

While rare, balanced constitutional rearrangements that mimic acquired rearrangements observed in AML have been documented in the literature [5–11]. Of the seven cases described to date, all were revealed by chromosome studies performed on products of conception or for abnormal congenital phenotypes such as intellectual disability [5–11]. We describe three cases that appeared to harbor recurrent genetic abnormalities observed in AML, only to reveal a constitutional origin. In addition, two of the three specimens were bone marrow samples sent for evaluation of a hematologic neoplasm. These cases highlight the importance of integrating refined cytogenetic data with clinical history, bone marrow evaluation, and flow cytometry studies. In addition, mate-pair sequencing (MPseq), a novel next-generation sequencing-based technology, was utilized to confirm the breakpoints in each case with significantly higher resolution and precision compared to chromosome and FISH studies. Herein, we share our experience in the detection of constitutional chromosomal rearrangements that mimic recurrent genetic abnormalities observed in AML.

Materials and methods

Patient selection

Following institutional review-board approval, a retrospective review of the Mayo Clinic cytogenetic database was performed on bone marrow and peripheral blood specimens to identify chromosome studies with constitutional rearrangements [indicated by "[c]" in the karyotype reporting field for the International System for Chromosome Nomenclature (ISCN)] that closely resemble AML subtypes with acquired rearrangements as designated by the 2017 revised World Health Organization (WHO) "*AML with recurrent genetic abnormalities*", including: *t*(8;21)(q22;q22.1), inv(16)(p13.1q22) or *t*(16;16)(p13.1;q22), *t*(15;17)(q24.1;q21.2), *t*(9;11)(p21.3;q23.3), *t*(6;9)(p23;q34.1), inv(3)(q21.3q26.2) or *t*(3;3)(q21.3;q26.2), *t*(1;22)(p13.3;q13.1), and *t*(9;22)(q34.1;q11.2) [3]. Using these search criteria, three cases were identified. Two specimens were bone marrow aspirates and one specimen was peripheral blood.

Conventional chromosome analysis

Cells were cultured, harvested, and banded utilizing standard cytogenetic techniques according to specimen-specific protocols. At least twenty metaphases were analyzed per case by qualified clinical cytogenetic technologists and interpreted by a board-certified clinical cytogeneticist [American Board of Medical Genetics and Genomics (ABMGG)].

Fluorescence *in situ* hybridization (FISH)

Commercial and "laboratory-developed" (LD) dual-color dual-fusion FISH probes (D-FISH) were utilized to detect *DEK-NUP214* (LD), *CBFB-MYH11* (LD), and *BCR-ABL1* (Abbott Molecular, Des Plaines, IL) fusions (abnormal cutoff: $\geq$0.6% of 500 interphase cell analysis per D-FISH probe set). All specimens were subjected to standard FISH pretreatment, hybridization, and fluorescence microscopy according to specimen-specific laboratory protocols. FISH analysis was performed by qualified clinical cytogenetic technologists and 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 on the Illumina HiSeq 2500. Data was aligned to the reference genome (GRCh38) using BIMAv3, and abnormalities were identified and visualized using SVAtools and Ingenium, both in-house developed bioinformatics tools. Detailed protocols and additional information regarding MPseq technology and bioinformatics tools can be found in Drucker et al. and Johnson et al. [12,13].

Results

Case 1

A 49-year-old female with a reported history of cervical and ovarian cancer had a bone marrow evaluation to "rule out a myeloproliferative neoplasm (MPN)" and a bone marrow aspirate was received for conventional cytogenetic analysis. Hematopathologic review demonstrated a slightly hypercellular bone marrow with slight panhyperplasia and no morphologic features of involvement by an MPN, myelodysplastic syndrome (MDS), metastatic carcinoma, or other primary malignancies. Flow cytometry on the bone marrow aspirate did

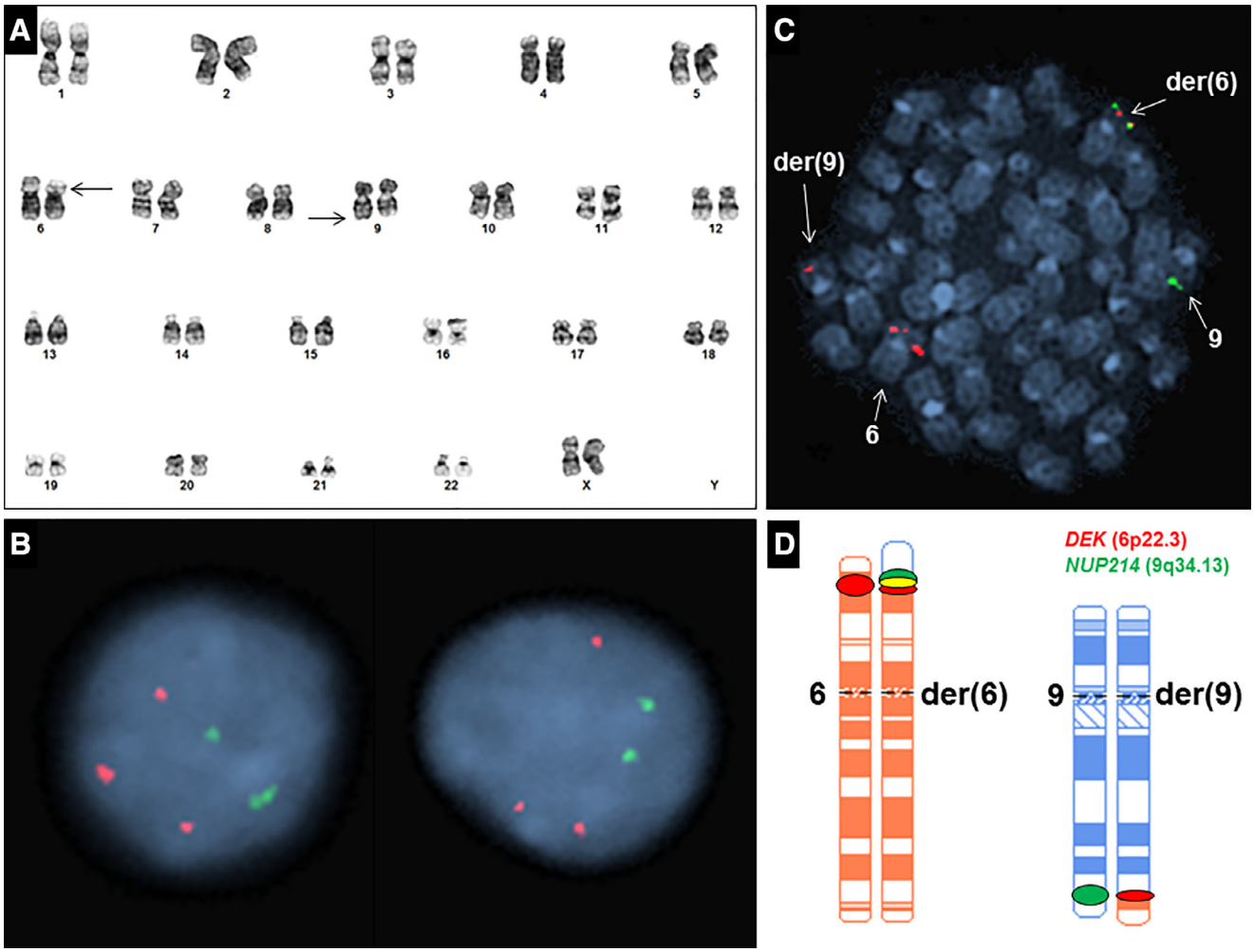

Fig. 1 Conventional chromosome and FISH results for Case 1. (A) Representative bone marrow karyogram showing *t*(6;9)(p23;q34). (B) Representative interphase cells showing three *DEK* signals (red) and two *NUP214* signals (green). (C) Sequential FISH analysis of a representative metaphase cell demonstrating an apparent single fusion signal (yellow) located on the short arm of the der(6), a single *DEK* signal (red) located on the long arm of the der(9), and one signal each for *DEK* and *NUP214* located on each normal copy of chromosomes 6 and 9. (D) Ideogram (400-band level; created by CyDAS [22]) of chromosomes 6 and 9 illustrating the observed abnormal FISH signal pattern for *DEK* and *NUP214*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

not identify any monotypic B- or T-cell populations, increase in blasts, or aberrant patterns of myeloid maturation. In addition, molecular studies to detect *JAK2* V617F, *JAK2* exons 12–15, *CALR* exon 9, and *MPL* exon 10 mutations from a peripheral blood specimen were negative. FISH for *BCR-ABL1* fusion was also negative.

Conventional chromosome studies on the bone marrow indicated all 20 metaphases had a *t*(6;9)(p23;q34), appearing identical to the recurrent *t*(6;9) observed in AML and MDS [3] (Fig. 1A); no normal metaphases were observed. FISH studies using a *DEK* (6p23) and *NUP214* (9q34) D-FISH probe set was performed and 93.2% of 500 interphase cells contained an additional diminished *DEK* signal (Fig. 1B) with no *DEK-NUP214* fusion signal. To determine the location of the additional *DEK* signal, sequential FISH analysis was performed on the abnormal metaphases. A single *DEK-NUP214* fusion signal was located on the derivative 6 chromosome, a single *DEK* signal was located on the derivative 9 chromosome, and one signal each for *DEK* and *NUP214* was located on each normal copy of chromosomes 6 and 9 (Fig. 1C, D). Due to the apparent discrepancy of the cytogenetic and FISH results with the essentially normal hematopathologic review (bone marrow biopsy and other testing), suspicion for a constitutional translocation was raised. A peripheral blood specimen was obtained from the patient to perform a phytohemagglutinin (PHA)-stimulated (T-lymphocyte mitogen) chromosome study *versus* the bone marrow myeloid chromosome study. All 20 metaphases from the T-cell stimulated chromosome study also revealed a *t*(6;9)(p23;q34), thus verifying this translocation in a second cell lineage and confirming a constitutional origin. To further characterize this translocation, MPseq was performed and revealed a rearrangement that did not disrupt either the *DEK* or *NUP214* genes. Instead, a single translocation event involving the *KDM1B* gene at 6p22.3 and an intergenic chromosomal region at 9q33.3 was identified

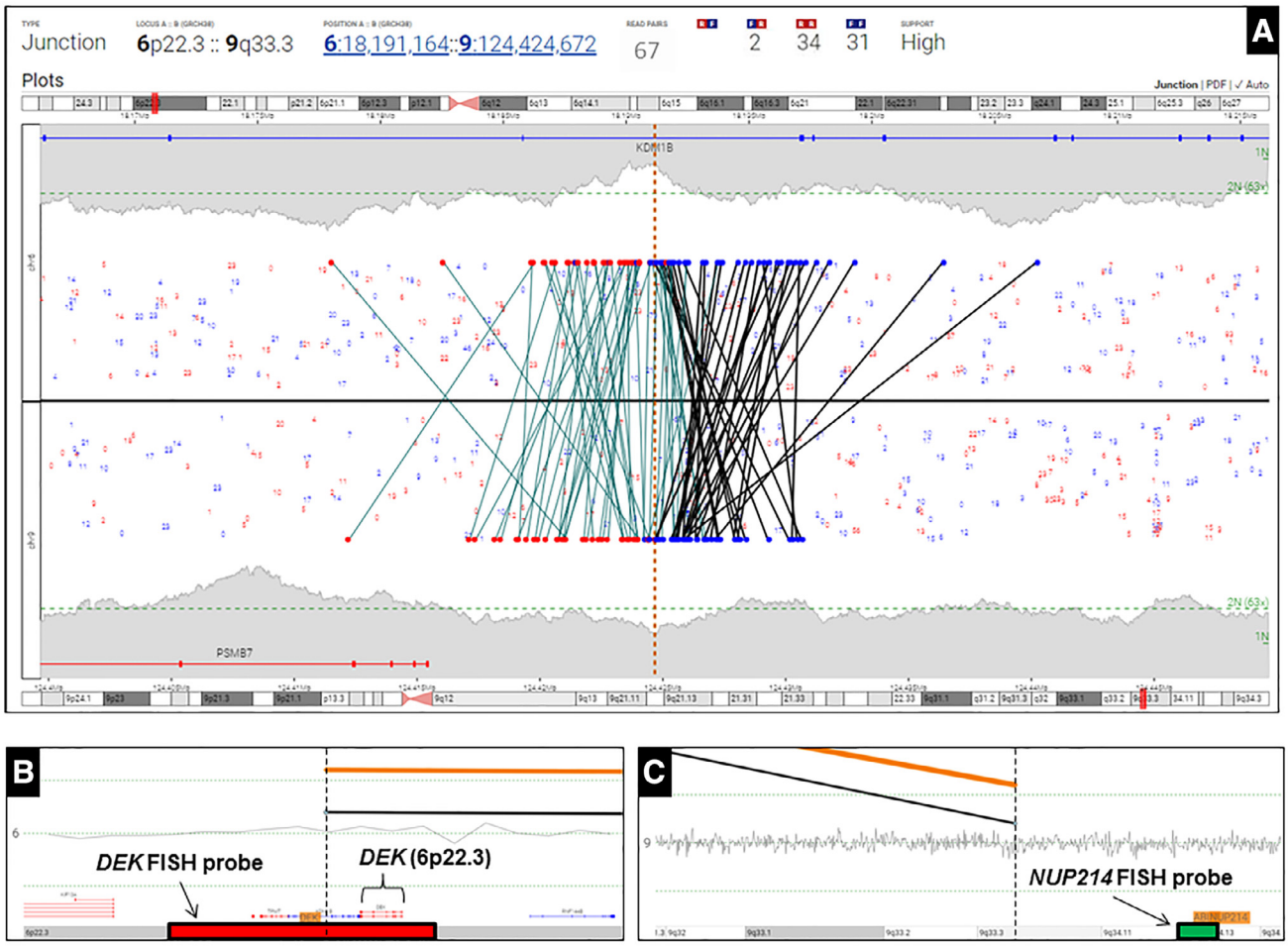

Fig. 2 Mate-pair sequencing (MPseq) results for Case 1 visualized in Ingenium. (A) Junction plot demonstrating a translocation between the *KDM1B* gene (intron 8; NM_153,042) at 6p22.3 and an intergenic chromosomal region at 9q33.3. (B) Focused view of the 6p22.3 breakpoint region (vertical dashed line) in relation to the *DEK* gene and the *DEK* FISH probe (red bar). While the *DEK* gene was not disrupted, the breakpoint did disrupt the *DEK* FISH probe region. This breakpoint location accounts for the additional *DEK* signal observed by interphase FISH analysis. (C) Focused view of the 9q33.3 breakpoint region (vertical dashed line) in relation to the *NUP214* gene and the *NUP214* FISH probe (green bar). Neither the *NUP214* gene nor *NUP214* FISH probe region were disrupted. The breakpoint mapped proximally (centromeric) from the *NUP214* locus, explaining the localization of the *NUP214* FISH signal on the derivative chromosome 6 in metaphase FISH studies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resulting in two derivative chromosomes: seq[GRCh38] *t*(6;9)6qter→6p22.3(18,191,164)::9q33.3(124,424,672)→9qter;6pter→6p22.3(18,191,567)::9q33.3(124,424,611)→9pter (Fig. 2).

Case 2

A 46-year-old male with a reported history of Marfan syndrome had a bone marrow evaluation for evaluation of "cytopenias" and a bone marrow aspirate was received for conventional chromosome analysis. Hematopathologic review revealed a normocellular marrow with mild erythroid hyperplasia and adequate storage iron with no evidence of a hematologic neoplasm. Flow cytometry on the bone marrow aspirate did not identify any monotypic plasma-, B-, or T-cell populations. *JAK2* V617F mutation analysis performed on the peripheral blood specimen was negative. Additional clinical information included splenomegaly and a surgical history significant for prosthetic aortic valve placement.

Conventional chromosome studies on the bone marrow indicated all 20 metaphases had an inv(16)(p13.1q22), appearing identical to the recurrent inv(16) observed in AML [3] (Fig. 3A); no normal metaphases were observed. To confirm the presence of a classically associated gene fusion, FISH using the *CBFB* (16p13.11) and *MYH11* (16q22.1) D-FISH probe set was performed. Of 500 interphase cells, greater than 90% contained either an additional "diminished" *CBFB* FISH signal or a single, apparent fusion signal (Fig. 3B). Sequential FISH analysis was performed on the abnormal metaphases to determine the location of the additional diminished *CBFB* or single fusion signals. The metaphase FISH results showed a single fusion signal located on the long arm of the derivative chromosome 16, a single *CBFB*

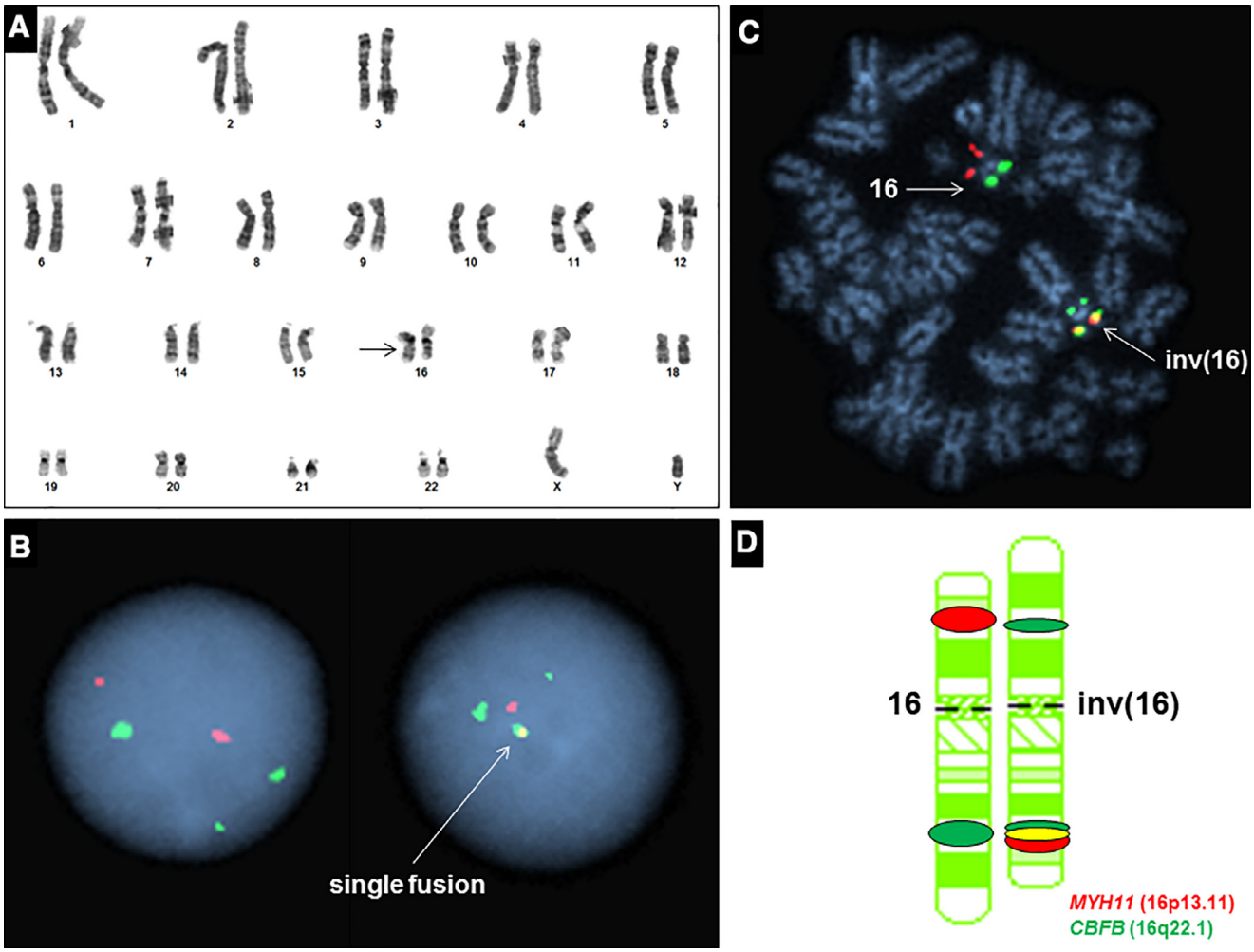

Fig. 3 Conventional chromosome and FISH results for Case 2. (A) Representative bone marrow karyogram showing an inv(16)(p13.1q22). (B) Representative interphase cells showing the two different FISH signal patterns observed: three *CBFB* signals (green) and two *MYH11* signals (red), or two *CBFB* signals (green), one *MYH11* signal (red), and one fusion signal (yellow). (C) Sequential FISH analysis of a representative metaphase cell showing a single fusion signal (yellow) located on the long arm of the inv(16), a single *CBFB* signal (green) located on the short arm of the inv(16), and one signal each for *MYH11* and *CBFB* located on the normal copy of chromosome 16. (D) Ideogram (400-band level; created by CyDAS [22]) of chromosomes 16 illustrating the observed abnormal metaphase FISH signal pattern for *MYH11* and *CBFB*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

signal located on the short arm of the derivative chromosome 16, and one signal each for *CBFB* and *MYH11* located on the normal copy of chromosome 16 (Fig. 3C, D). Although recommended, a PHA-stimulated T-cell chromosome study was not performed on this patient. To further characterize this translocation, MPseq was performed and revealed a rearrangement that did not disrupt the *CBFB* or *MYH11* genes. Instead, a complex rearrangement involving two inversion events was revealed. The larger inversion event involved the *SNX29* gene at 16p13.12 and an intergenic chromosomal region at 16q22.1 (Fig. 4). Nested within the larger inversion event was a smaller inversion event involving the *PDP2* gene at 16q22.1. The resulting complex chromosome 16 can be described as: seq[GRCh38] inv(16)16pter→16p13.12(12,541,370)::16q22.1(66,880,415)→16q22.1 (66,897,286)::16q22.1(66,889,420)→16p13.12(12,531,422)::16q22.1(66,897,286)→16qter.

Case 3

A 41-year-old female with a history of primary ovarian insufficiency (POI) had a peripheral blood specimen submitted for conventional chromosome analysis. Laboratory studies included a complete blood count (CBC) which revealed a normal white blood cell count, hemoglobin, and platelet count. No evidence of a leukemic process was identified.

Conventional chromosome studies on the peripheral blood indicated all 20 metaphases had an apparent *t*(9;22)(q34.1;q11.2), appearing identical to the recurrent *t*(9;22) observed in AML, B-lymphoblastic leukemia/lymphoma (B-ALL/LBL), CML, and mixed-phenotype acute leukemia (MPAL) [3] (Fig. 5A); no normal metaphases were observed. FISH studies using an *ABL1* (9q34.1) and *BCR* (22q11.23) D-FISH probe set were performed and no abnormal signal patterns were

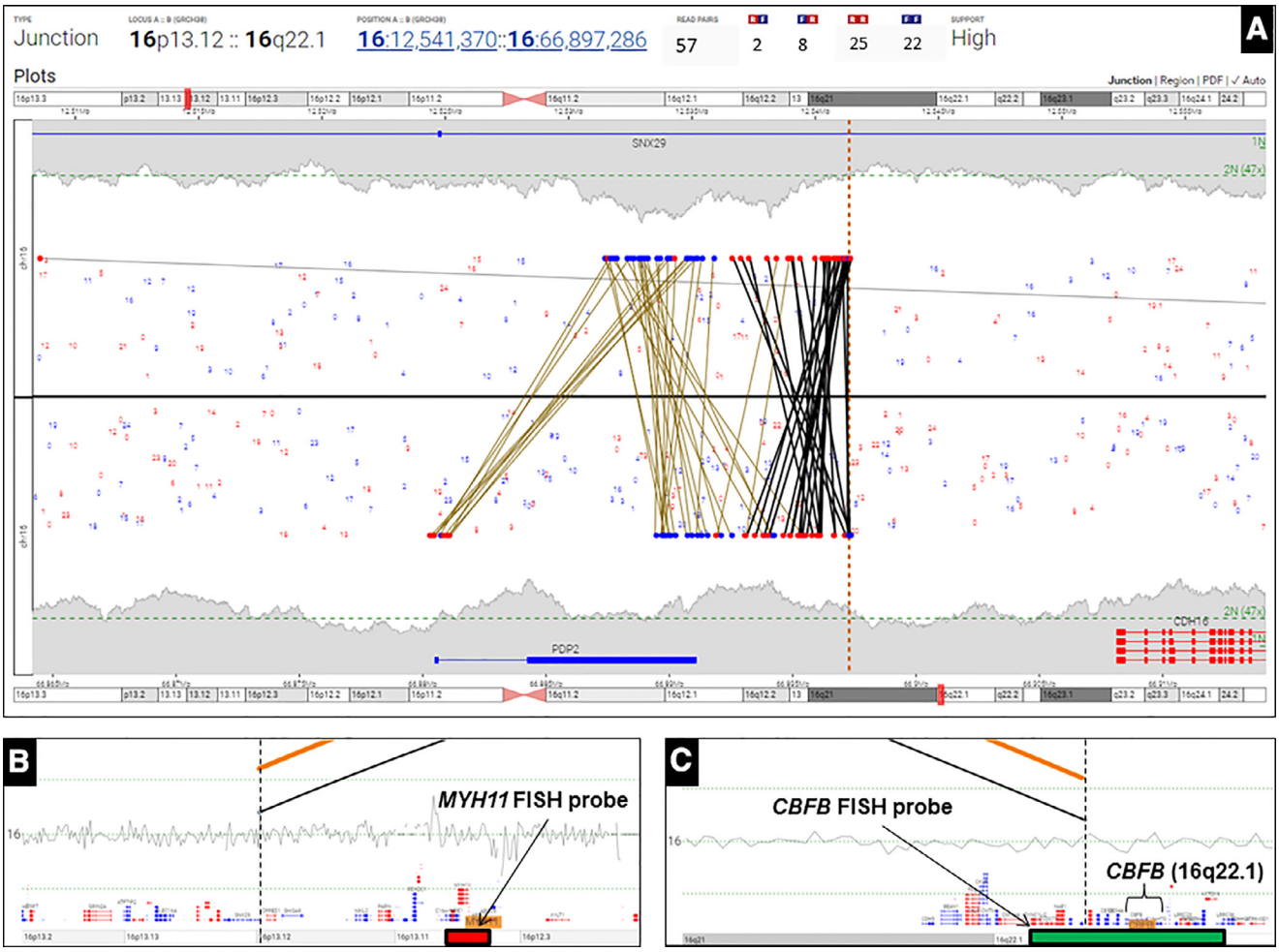

Fig. 4 Mate-pair sequencing (MPseq) results for Case 2 visualized in Ingenium. (A) Junction plot demonstrating an inversion between the *SNX29* gene (intron 20; NM_032167) at 16p13.12 and an intergenic chromosomal region at 16q22.1 (vertical dashed line). In addition, a nested inversion (located within the previously described inversion) involving the *PDP2* gene was observed. (B) Focused view of the 16p13.12 breakpoint region (vertical dashed line) in relation to the *MYH11* FISH probe (red bar). Neither the *MYH11* gene nor *MYH11* FISH probe region were disrupted. (C) Focused view of the 16q22.1 breakpoint region (vertical dashed line) in relation to the *CBFB* gene and the *CBFB* FISH probe (green bar). While the *CBFB* gene was not disrupted, the breakpoint did disrupt the *CBFB* FISH probe region. This breakpoint location accounts for the additional *CBFB* signal observed by interphase FISH analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

observed in 500 interphase cells (Fig. 5B). Sequential FISH analysis was performed on metaphases and confirmed two signals each for *ABL1* and *BCR*, each located in the correct chromosomal regions on both copies of chromosomes 9 and 22 (Fig. 5C, D). The final karyotype reassigned a more telomeric breakpoint on chromosome 22q based on FISH results to 46,XX,*t*(9;22)(q34.1;q12.2). To further characterize this abnormality, MPseq was performed and revealed a rearrangement that did not disrupt either the *ABL1* or *BCR* genes. Instead, a single apparently balanced translocation event involving the *RALGDS* gene at 9q34.2 and the *CBY1* gene at 22q13.1 was identified, resulting in two derivative chromosomes: seq[GRCh38] *t*(9;22)9pter→9q34.2(133,106,680)::22q13.1 (38,667,143) →22qter; 22pter→22q13.1(38,667,143)::9q34.2(133,106, 680)→9qter (Fig. 6).

Discussion

We identified three cases with constitutional chromosomal rearrangements that mimic recurrent genetic abnormalities observed in AML [3]. Cases 1 and 2 with *t*(6;9)(p23;q34) and inv(16)(p13.1q22), respectively, were identified in bone marrow specimens undergoing evaluation for a clonal hematologic neoplasm, while Case 3 with a *t*(9;22)(q34.1;q12.2) was identified in a blood specimen being evaluated for POI. Following the identification of recurrent genetic abnormalities observed in AML by chromosome analysis in Cases 1 and 2, FISH studies were performed on interphase cells and revealed atypical signal patterns. The *DEK-NUP214* D-FISH studies from Case 1 revealed a single additional *DEK* signal in greater than 90% of both myeloid and lymphoid cells based on interphase cell morphology, with no fusion signals

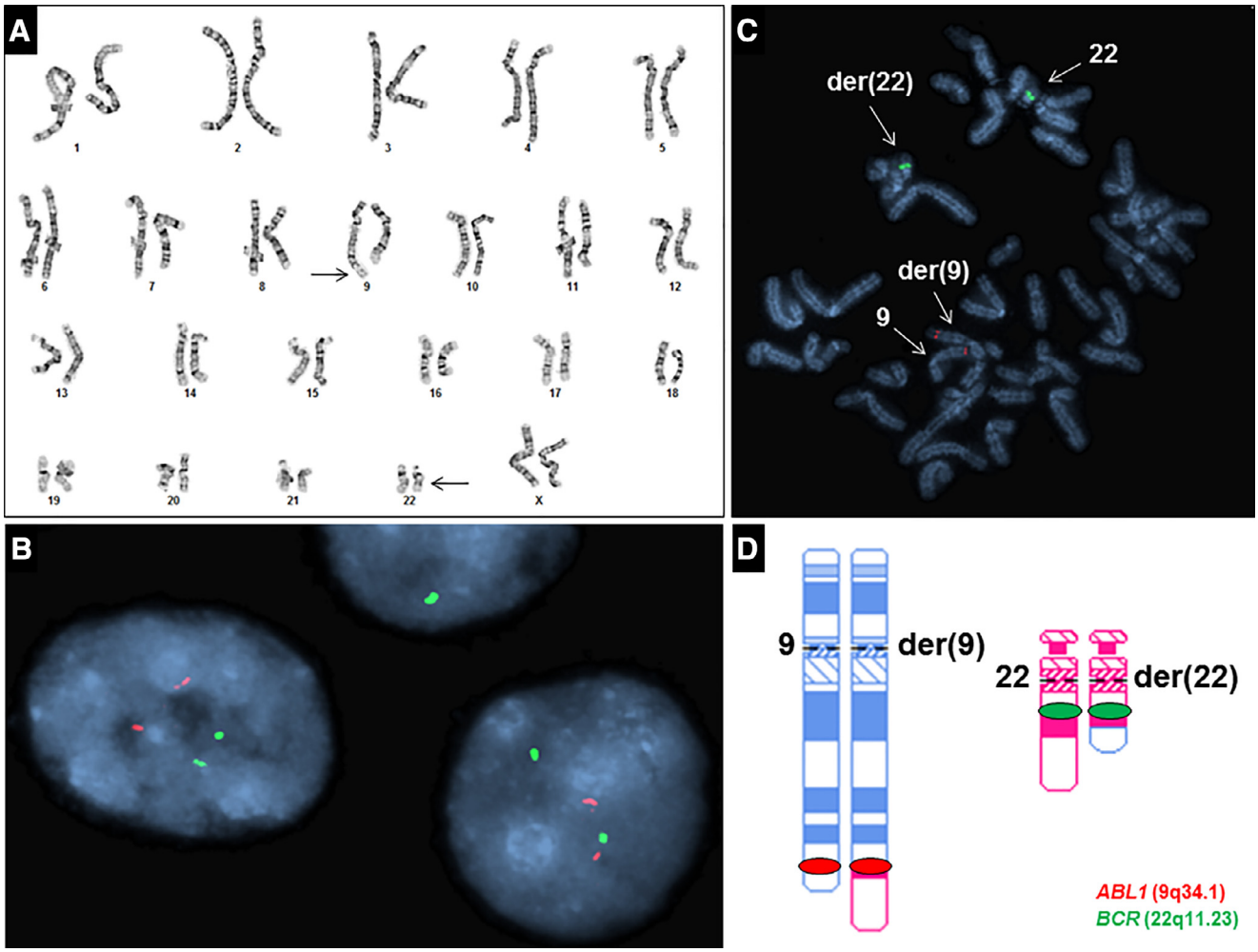

Fig. 5 Conventional chromosome and FISH results for Case 3. (A) Representative peripheral blood karyogram showing the *t*(9;22)(q34.1;q12.2). (B) Representative interphase cells showing normal results with two *ABL1* signals (red) and two *BCR* signals (green). (C) Sequential FISH analysis of a representative metaphase cell showing single *ABL1* signals (red) located on the long arms of the der(9) and the normal chromosome 9, and single *BCR* signals (green) located on the long arms of the der(22) and the normal chromosome 22. (D) Ideogram (400-band level; created by CyDAS [22]) of chromosomes 9 and 22 illustrating the normal metaphase FISH signal patterns for *ABL1* and *BCR*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

evident. Similarly, the *CBFB-MYH11* D-FISH studies from Case 2 revealed either a single additional *CBFB* signal, or a single fusion signal in greater than 90% of both myeloid and lymphoid cells. However, sequential FISH analysis performed on metaphase cells revealed only a single fusion signal in each case. No morphologic or immunophenotypic evidence of a hematologic neoplasm was observed by bone marrow or peripheral blood analysis in either case. Together, these genetic and hematopathologic findings suggest constitutional rearrangements that partially disrupt the *DEK* or *CBFB* FISH probe regions. While single extra *DEK* and *CBFB* signals are observed by interphase cell analysis when chromosomes are decondensed, single "pseudo" fusions are created by metaphase cell analysis when rearrangements bring the *NUP214* probe in close proximity to the *DEK* probe and the *MYH11* probe in close proximity to the *CBFB* probe. Furthermore, single *DEK* and *CBFB* FISH signals are observed on the derivative chromosome 9 and short arm of the derivative chromosome 16, which is consistent with disruption of the *DEK* and *CBFB* FISH probe regions.

To confirm our suspicions of constitutional rearrangements juxtaposing the FISH probes in metaphase cells, MPseq was performed on Cases 1 and 2 and revealed rearrangements that explained the chromosome and atypical FISH patterns observed. The rearrangement in Case 1 involved the *KDM1B* gene (intron 8; NM_153,042) at 6p22.3 and an intergenic chromosomal region at 9q33.3, while the rearrangement in Case 2 was actually a pericentric inversion involving the *SNX29* gene (intron 20; NM_032167) at 16p13.12 and an intergenic chromosomal region at 16q22.1. Curiously, a second nested inversion involving the *PDP2* gene was also revealed in Case 2. When comparing chromosomal breakpoints identified by MPseq to the d-FISH probe maps, the *DEK* and *CBFB* FISH probes would be expected to demonstrate disruptions, thus confirming our observed FISH results. In addition, the MPseq results verify the close proximity of the chromosomal breakpoints in relation to the *NUP214* and *MYH11* FISH probes. Importantly however, MPseq confirmed that *DEK* and *NUP214* in Case 1, and *CBFB* and *MYH11* in Case 2 were, in fact, not disrupted.

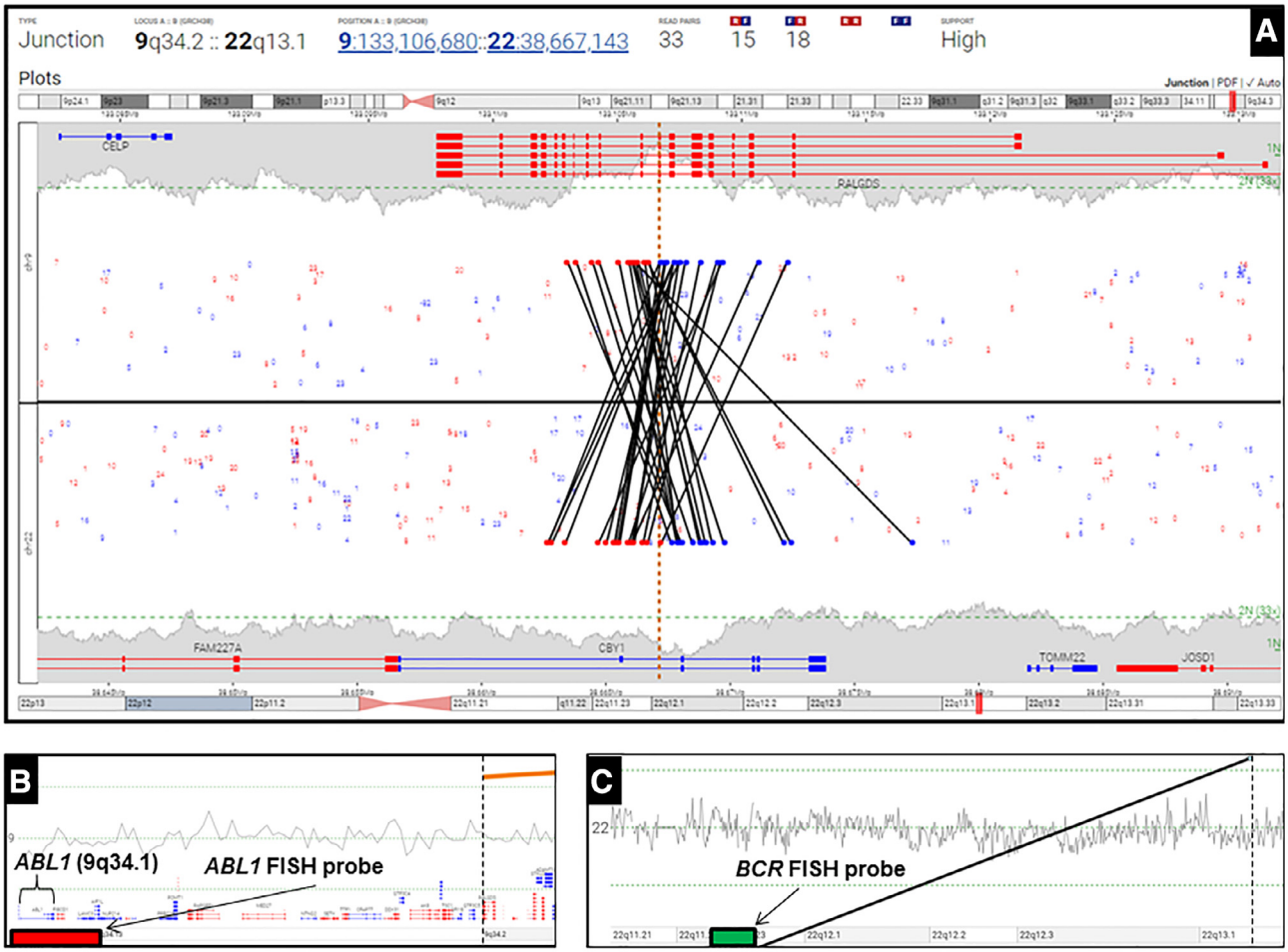

Fig. 6 Mate-pair sequencing (MPseq) results for Case 3 visualized in Ingenium. (A) Junction plot demonstrating a translocation between the *RALGDS* gene (intron 8 or exon 8; NM_006266) at 9q34.2 and the *CBY1* gene (intron 1; NM_015373) at 22q13.1. (B) Focused view of the 9q34.2 breakpoint region (vertical dashed line) in relation to the *ABL1* gene and *ABL1* FISH probe (red bar). Neither the *ABL1* gene nor *ABL1* FISH probe region were disrupted. (C) Focused view of the 22q13.1 breakpoint region (vertical dashed line) in relation to the *BCR* FISH probe (green bar). Neither the *BCR* gene nor *BCR* FISH probe region were disrupted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PHA-stimulated peripheral blood chromosome studies for Case 3 were initiated for the evaluation of POI and revealed what resembled a *t*(9;22)(q34.1;q11.2) in all 20 metaphases, a recurrent genetic abnormality observed in AML, B-ALL/LBL, CML, and MPAL [3]. However, FISH studies using *BCR* and *ABL1* probes were performed and revealed normal signal patterns in all interphase and metaphase cells analyzed and no evidence of a *BCR-ABL1* fusion event was observed. Additional clinical information revealed a normal CBC with no evidence of a leukemic process. Based on the additional clinical information and normal FISH results, the karyotype designation was reassigned as *t*(9;22)(q34.1;q12.2) and the translocation was determined to be constitutional in origin. MPseq revealed a rearrangement involving the *RALGDS* gene (exon 8 or intron 8; NM_006266) at 9q34.2 and the *CBY1* gene (intron 1; NM_015373) at 22q13.1. As both *ABL1* and *BCR* genes and their FISH probes were undisrupted by the translocation breakpoints assigned by MPseq, this result explains the normal FISH signal patterns observed by interphase and metaphase cell analysis.

The use of MPseq to further define these three constitutional rearrangements proved invaluable. While chromosome studies have a ~5–10 Mb resolution, FISH can detect targeted gene rearrangement breakpoints with less subjectivity. However, as we observed in Cases 1 and 2, rearrangements that disrupt FISH probes without specifically involving the targeted gene can produce false positive results. MPseq is a next-generation sequencing-based technology that can be utilized to detect and characterize structural variants with significantly higher resolution and precision compared to chromosomes and FISH, typically narrowing the breakpoint to the precise intron or exon involved in a rearrangement. MPseq library preparation involves the circularization of long DNA fragments (~2–5 kb), followed by traditional paired end sequencing of the mate pair fragments. The long insert sequences are inferred from short paired end reads rather than direct sequencing, thus allowing for the robust detection of structural variants without extensive sequencing. Since MPseq leverages inferred sequence in between reads to achieve “bridged coverage”, the amount of sequencing

Table 1 Published cases (including ours) with constitutional chromosomal rearrangements that mimic recurrent genetic abnormalities observed in acute myeloid leukemia and other hematologic neoplasms.

Case	*Karyotype*	*Associated hematologic neoplasm(s)*
1	*t*(6;9)(p23;q34)c	AML, MDS
2	inv(16)(p13.1q22)c	AML
Bianchi et al.	inv(16)(p13q22)c	AML
3	*t*(9;22)(q34.1;q12.2)c	AML, B-ALL/LBL, CML, MPAL
McGoey et al.	*t*(9;22)(q34.3;q11.2)c	AML, B-ALL/LBL, CML, MPAL
Czuchlewski et al.	*t*(9;22)(q34;q11.2)c	AML, B-ALL/LBL, CML, MPAL
Blank et al.	*t*(9;22)(q13;q11)c	AML, B-ALL/LBL, CML, MPAL
Pivnick et al.	*t*(9;22)(q22;q11.2)c	AML, B-ALL/LBL, CML, MPAL
el-Fouly et al.	*t*(9;22)(q21.13;q12.1)c	AML, B-ALL/LBL, CML, MPAL
Komatsu et al.	*t*(9;22)(q12;q11.23)c	AML, B-ALL/LBL, CML, MPAL

c, constitutional; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; B-ALL/LBL, B-lymphoblastic leukemia/lymphoma; CML, chronic myeloid leukemia; MPAL, mixed-phenotype acute leukemia.

needed to gather evidence for structural variants and the resulting cost are much less than traditional paired end sequencing across the whole genome. Importantly, MPseq is not widely available as a clinical test and is certainly not necessary to confirm constitutional rearrangements that mimic those observed in AML. For example, real-time PCR is a commonly utilized methodology that could readily distinguish constitutional rearrangements from recurrent gene fusions observed in AML [14,15].

In Case 1, a translocation between the *KDM1B* gene at 6p22.3 and an intergenic region at 9q33.3 was revealed. The *KDM1B* gene (OMIM#: 613081) appears to regulate histone lysine methylation, an epigenetic mark that regulates gene expression and chromatin function and is required to establish maternal genomic imprints [16,17]. Considering this patient has a reported history of cervical and ovarian cancer, the *KDM1B* gene could be of potential clinical interest. Case 2 had two inversion events, one between the *SNX29* gene at 16p13.12 and an intergenic region at 16q22.1, and the second involving the *PDP2* gene at 16q22.1. The *SNX29* gene (not designated by OMIM) is one of approximately 30 members of the sorting nexins (SNX) gene family involved in regulating membrane trafficking in the endocytic pathway, while the *PDP2* gene (OMIM#: 615499) encodes mitochondrial serine phosphatases that dephosphorylate and activate the inactive phosphorylated pyruvate dehydrogenase complex [18,19]. Both genes do not appear to be associated with the patient's reported diagnosis of Marfan syndrome. Lastly, case 3 had a translocation between the *RALGDS* gene at 9q34.2 and the *CBY1* gene at 22q13.1. The *RALGDS* gene (OMIM#: 601619) encodes Ras-related GTPases that participate in signaling for a variety of cellular processes, while the *CBY1* gene (OMIM#: 607757) encodes a protein that localizes to centrioles/basal bodies and plays a crucial role in the formation and function of cilia [20,21]. The disruption of these genes and their involvement in POI are currently unclear.

To date, seven other constitutional rearrangements that mimic recurrent genetic abnormalities in AML have been published (Table 1) [5–11]. Bianchi et al. described an inv(16) with similar breakpoints to our case, while six cases with 9;22 translocations with varying breakpoints have been reported [5–11]. Similar to Case 3, the seven previously reported cases were all revealed by constitutional chromosome studies and not during the workup of a potential hematologic neoplasm. Context is critical when reviewing chromosome studies, and while any one of these recurrent rearrangements can represent AML, the likelihood of a constitutional origin is much greater in a patient with a provided history of recurrent miscarriages or offspring with unbalanced rearrangements. Particularly concerning with regard to AML were the *t*(6;9) and inv(16) results from Cases 1 and 2. In the absence of bone marrow and flow cytometry reports, both cases could have been erroneously reported as representative of a recurrent chromosomal rearrangement associated with AML. This is particularly important for establishing a diagnosis of AML, as inv(16)/*t*(16;16), *t*(8;21), and *t*(15;17) are AML defining genetic abnormalities, regardless of the blast count [3].

In conclusion, we describe three cases with constitutional rearrangements that mimic recurrent genetic abnormalities in AML. Our findings emphasize the importance of correlating genetic data with clinical and hematopathologic information when available, and the necessity to perform confirmatory FISH testing for recurrent chromosomal rearrangements. If the FISH is normal or atypical signal patterns are generated, concern should be raised that the chromosome rearrangement may not represent a classical abnormality associated with AML. We have also illustrated the utility of MPseq as a valuable next-generation sequencing-based technology that can detect and resolve with significantly higher resolution and precision the translocations and inversions that may ultimately represent a "constitutional chromosomal mimic" of a classic AML rearrangement.

Conflict of interest

SVA tools algorithms utilized for junction detection by mate-pair sequencing (MPseq) is licensed to WholeGenome LLC owned by George Vasmatzis.

The remaining authors have no conflicts of interest to declare.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2018.11.005.

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