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Case study

Use of mate-pair sequencing to characterize a complex cryptic *BCR/ABL1* rearrangement observed in a newly diagnosed case of chronic myeloid leukemia

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Summary Chronic myeloid leukemia is characterized by a t(9;22)(q34;q11.2) resulting in *BCR/ABL1* fusion located on the derivative chromosome 22, also known as the Philadelphia chromosome. We present the first case, to our knowledge, of chronic myeloid leukemia with 2 cryptic insertional events resulting in *BCR/ABL1* fusion on the derivative chromosome 9 and *FNBP1/BCR* fusion on the derivative chromosome 22. These insertional events were misinterpreted as a typical balanced *BCR/ABL1* translocation by interphase fluorescence in situ hybridization studies and were cryptic by conventional chromosome analysis, resulting in a "normal" karyotype. Mate-pair sequencing, a novel next-generation sequencing technology that can detect and characterize structural variants with significantly higher resolution and precision compared with traditional cytogenetic methodologies, identified 2 insertional events and resolved the seemingly discrepant chromosome and fluorescence in situ hybridization results. This case demonstrates the complexities of genetic abnormalities unappreciable by traditional cytogenetic methodologies and highlights the clinical utility of mate-pair sequencing.

1. Introduction

Approximately 90% to 95% of chronic myeloid leukemia (CML) cases harbor the t(9;22)(q34;q11.2) resulting in *BCR/ABL1* fusion, whereas the remaining 5% to 10% of CML cases have more complex rearrangements involving additional chromosomes along with 9 and 22, or cryptic rearrangements

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resulting in *BCR/ABL1* fusion [1]. Despite the varying mechanisms that produce *BCR/ABL1* fusions, such as balanced variant translocations or cryptic insertional events, the phenotypic and prognostic impact remains unchanged when compared with CML with a standard Philadelphia chromosome [2]. Limited by ~5- to 10-Mb resolution, conventional chromosome analysis cannot detect all variant *BCR/ABL1* rearrangements, whereas fluorescence in situ hybridization (FISH) is a highly reliable technique used to identify both typical and atypical rearrangements resulting in *BCR/ABL1* fusions [3-6].

The advent of mate-pair sequencing (MPseq), a next-generation sequencing (NGS)–based technology, has ushered in a new era of cytogenetic testing that enables the characterization of chromosomal rearrangements with significantly higher resolution and precision when compared with chromosome and FISH methodologies [7,8]. Herein, we present a unique case of CML with complex cryptic rearrangements resulting in *BCR/ABL1* fusion that required characterization by MPseq, demonstrating the clinical utility of this NGS-based methodology.

2. Materials and methods

2.1. Case presentation

A 68-year-old man with a suspected diagnosis of CML had a bone marrow aspirate specimen sent to the Mayo Clinic genomics laboratory for *BCR/ABL1* FISH studies and conventional chromosome analysis. Approximately 3 weeks earlier, a peripheral blood smear from the patient revealed basophilia, absolute neutrophilia with circulating myeloid precursor cells, and thrombocytosis. Qualitative and quantitative reverse-transcription polymerase chain reaction (RT-PCR) was performed and identified a *BCR/ABL1* e14/a2 (p210) messenger RNA transcript estimated to represent 69% total *ABL1* (% *BCR/ABL1* [p210]:*ABL1*). A quantitative, allele-specific PCR assay to detect the *JAK2* V617F mutation was negative. Flow cytometry performed on the bone marrow aspirate specimen did not reveal an increase in blasts or a monotypic B- or T-cell population.

2.2. Conventional chromosome analysis

Cells from the bone marrow aspirate specimen were cultured, harvested, and banded using standard cytogenetic techniques according to specimen-specific protocol. Twenty metaphases were analyzed by 2 qualified clinical cytogenetic technologists and interpreted by a board-certified clinical cytogeneticist (American Board of Medical Genetics and Genomics).

2.3. Fluorescence in situ hybridization

A commercial (Abbott Molecular, Des Plaines, IL) dual-color dual-fusion FISH probe kit (D-FISH) was used to detect *BCR/ABL1* fusion (abnormal cutoff, ≥0.6% of 500 interphase cell analysis). The bone marrow aspirate specimen was subjected to standard FISH pretreatment, hybridization, and fluorescence microscopy according to specimen-specific laboratory protocols. FISH analysis was performed by 2 qualified clinical cytogenetic technologists and interpreted by a board-certified clinical cytogeneticist (American Board of Medical Genetics and Genomics).

2.4. Mate-pair sequencing

DNA was extracted using the Autopure LS (Qiagen, Hilden, Germany). From the DNA extract, 1 μg was used for MPseq library preparation and processed using the Illumina Nextera Mate Pair library kit (Illumina, San Diego, CA) and the Illumina TruSeq DNA library prep kit (Illumina). Library preparation consisted of tagmentation to simultaneously shear and biotinylate the genomic DNA, strand displacement to fill any gaps left by the tagmentation step, and overnight circularization (16-20 hours) to produce stabile 2- to 5-kb DNA fragments. MPseq libraries were multiplexed at 2 samples per lane to be sequenced on the Illumina HiSeq 2500 in rapid run mode. On both ends of each mate-pair fragment, 101 base pairs were sequenced to a bridged coverage of 43× and a base coverage of 3×. Data were aligned to the reference genome (GRCh38) using BIMAv3, and abnormalities were identified and visualized using SVAtools and Ingenium, both in-house developed bioinformatics tools. Additional information on MPseq technology and bioinformatics tools can be found in Drucker et al [7] and Johnson et al [8].

2.5. Sanger sequencing

Reference DNA sequences spanning the minimal 5′ and maximal 3′ positions of MPseq approximate breakpoints were used for primer design using Primer3Plus. End-point PCR was performed on patient DNA with a 50% 2X Paq5000 Hotstart PCR Master Mix (Agilent, Santa Clara, CA) on a touchdown PCR program. Results were visualized on a 2% agarose gel in a UV light box, and amplicon sizes were estimated. Selected amplicons were purified with Exo-SAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA). Sanger sequencing was performed on a 3730*xl* DNA Analyzer (Thermo Fisher Scientific). The resulting sequences were analyzed using Sequencher DNA Sequence Analysis Software (Gene Codes Corporation, Ann Arbor, MI) and mapped to the GRCh38 genome using the BLAT function in the UCSC genome browser to determine precise breakpoints in each rearrangement.

3. Results

3.1. Conventional chromosome analysis

All 20 metaphases appeared normal (46,XY) (Fig. 1A). No chromosomal evidence of a t(9;22)(q34;q11.2) was observed.

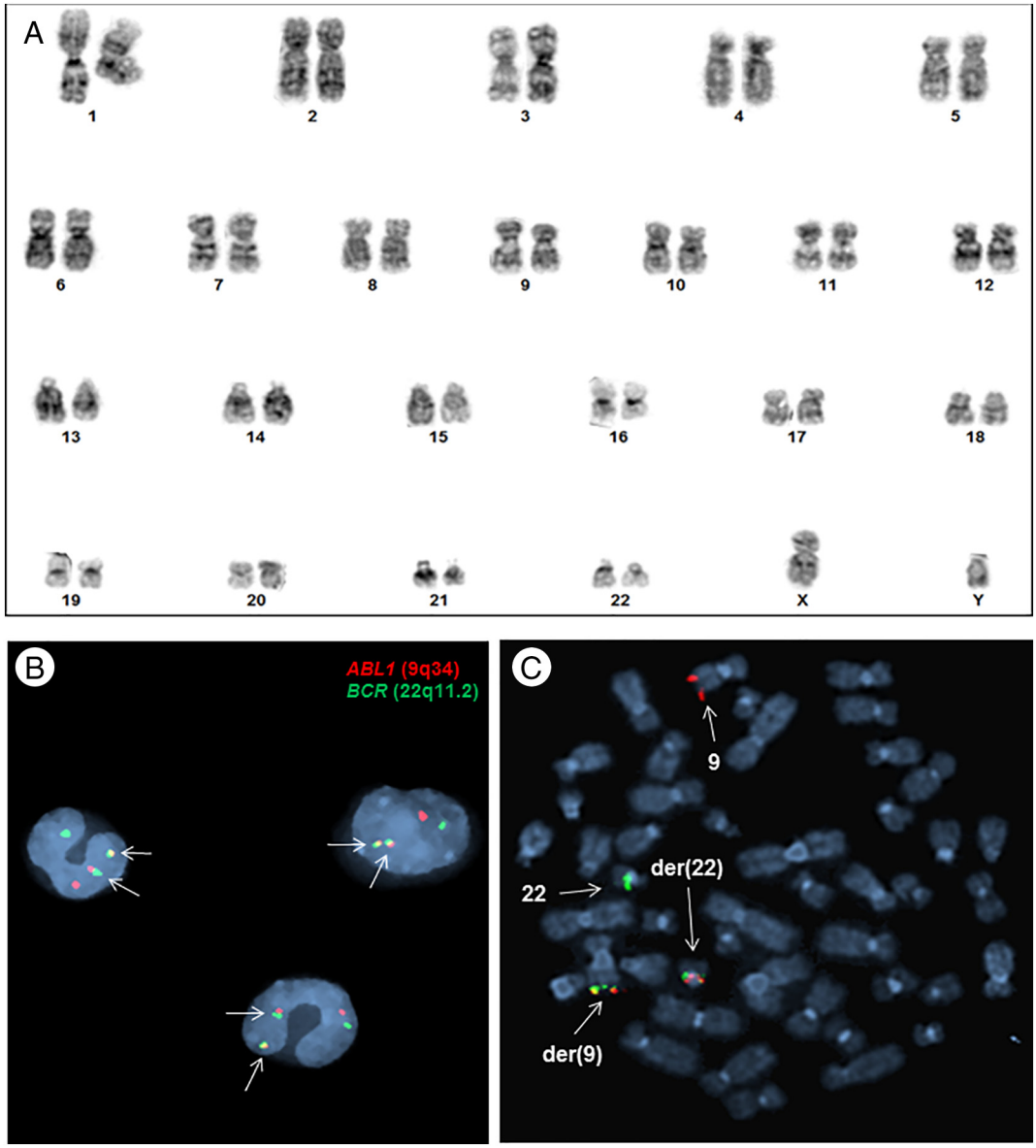

Fig. 1 A, Representative karyogram showing no evidence of the t(9;22)(q34;q11.2). B, Representative interphase cells demonstrating 2 fusion signals (arrows), indicating an apparently balanced translocation between the *BCR* and *ABL1* gene regions. Single *ABL1* (red) and *BCR* (green) signals represent the normal copies of chromosomes 9 and 22. C, Representative metaphase FISH study demonstrating fusion signals near the expected derivative chromosome regions on 9q34 and 22q11.2. Single *ABL1* and *BCR* signals were observed on the normal copies of chromosomes 9 and 22.

3.2. Fluorescence in situ hybridization

FISH analysis using the *BCR/ABL1* D-FISH probe set demonstrated an abnormal result in 89.4% of 500 interphase cells as indicated by the presence of 1 *ABL1* signal, 1 *BCR* signal, and 2 *BCR/ABL1* fusion signals (Fig. 1B), which is the expected abnormal signal pattern for a typical t(9;22)(q34;q11.2). Metaphase FISH analysis using the *BCR/ABL1* D-FISH probe set was performed on "normal" metaphase cells and revealed a *BCR/ABL1* fusion near the expected chromosome regions on 9q34 and 22q11.2 (Fig. 1C). The single *ABL1* and *BCR* signals were also verified on the normal locations of chromosomes 9 and 22 by metaphase FISH analysis.

3.3. Mate-pair sequencing

MPseq revealed a complex rearrangement involving 2 insertional translocation events (Figs. 2 and 3). The 5′*BCR* gene region (exons 1-14; intron 14; NM_004327.3) was

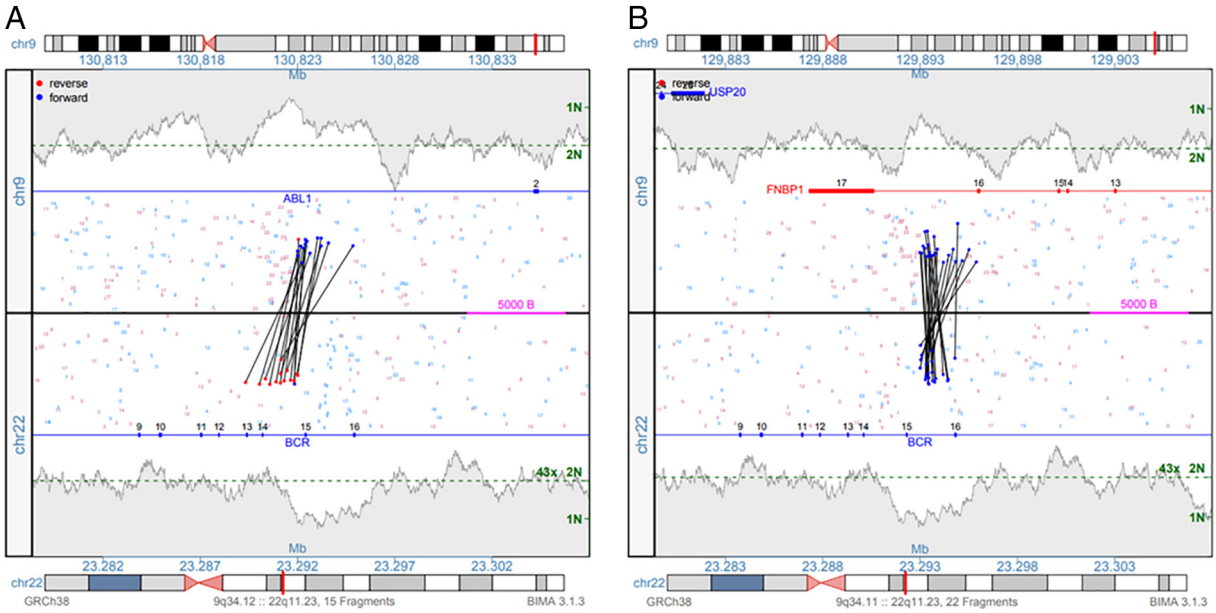

Fig. 2 MPseq results visualized in Ingenium. A, Junction plot demonstrating a translocation between the *ABL1* gene (intron 1; NM_007313.2) at 9q34.12 and the *BCR* gene (intron 14; NM_004327.3) at 22q11.23. B, Junction plot demonstrating a translocation between the *FNBP1* gene (intron 16; NM_015033.2) at 9q34.11 and the *BCR* gene (intron 15; NM_004327.3) at 22q11.23.

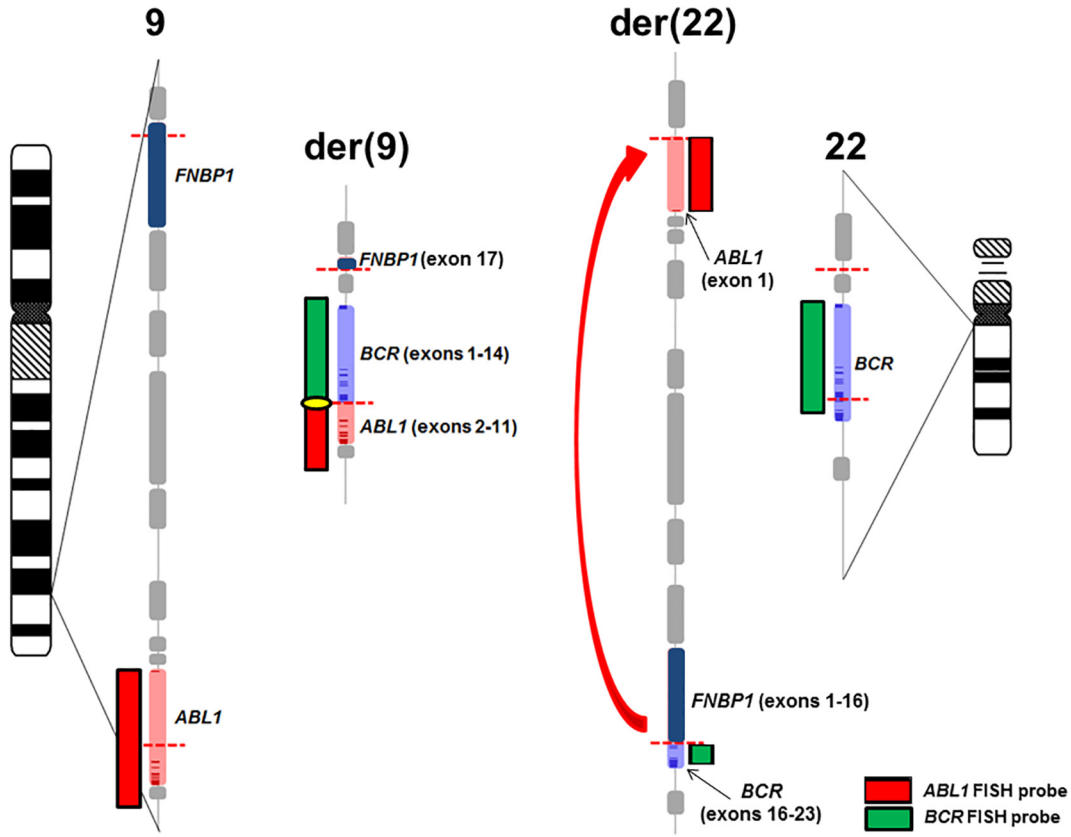

Fig. 3 A focused view of the *ABL1* and *BCR* gene regions on the derivative chromosomes 9 and 22 and normal chromosomes 9 and 22. Horizontal dashed red lines indicate the breakpoints on the derivative chromosomes 9 and 22 in relation to the normal chromosomes 9 and 22. The solid vertical red arrow indicates the inverted orientation of the ~930-kb chromosomal region inserted into the derivative chromosome 22.

Table MPseq and Sanger sequencing results for each insertional translocation

Junction	MPseq breakpoints (hg38)	Sanger breakpoints (hg38)	Primer sequence	Gene	No. of supporting fragments
1	chr9:129892905	chr9:129892913	CTATCCGATGACAAACTCATTTCCAG	*FNBP1*	22
	chr22:23293275	chr22:23293195	GATCAAAGAGTAGTGGATGCTGAGAAAG	*BCR*	
2	chr9:130823123	chr9:130823023	TATACCTGATAAAGAACTTGGAAGGCC	*ABL1*	15
	chr22:23292172	chr22:23292230	CATCAGTGAGGCTTCTTAGTCATCTC	*BCR*	

translocated and inserted proximally to the *ABL1* gene region (exons 2-11; intron 1; NM_007313.2) on the derivative chromosome 9 (Fig. 2A). The 5′*FNBP1* gene region (exons 1-16; intron 16; NM_015033.2) was translocated and inserted in an inverted orientation proximally to the *BCR* gene region (exons 16-23; intron 15; NM_004327.3) on the derivative chromosome 22 (Fig. 2B).

3.4. Sanger sequencing

Sanger sequencing confirmed the presence of both fusions identified by MPseq. Detailed information is provided in the Table.

4. Discussion

MPseq is an NGS-based technology that can be used to detect and characterize structural variants with significantly higher precision and resolution compared with conventional cytogenetic methodologies, typically narrowing the breakpoint to the precise intron, exon, or flanking region of the involved gene [7,8]. The library preparation of MPseq involves circularizing 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. The clinical application of this novel NGS-based technology is powerful, as MPseq can resolve both simple and complex chromosomal abnormalities throughout the genome and can also identify cryptic rearrangements unappreciable by chromosome and FISH studies. Furthermore, the detection of targeted abnormalities is not limited to a disease-specific panel of FISH probes. For example, MPseq could be used to detect all recurrent translocations observed in B-lymphoblastic leukemia, in addition to the growing list of kinase gene rearrangements observed in Philadelphia chromosome–like B-lymphoblastic leukemia [9,10].

In the current CML case, MPseq revealed 2 insertional events that could not be characterized by conventional cytogenetic methodologies. Although *BCR/ABL1* fusion was identified by FISH and RT-PCR analysis, the *FNBP1/BCR* fusion was misinterpreted by the D-FISH probe as an *ABL1/BCR* fusion owing to the close proximity (~930 kb) of residual *ABL1* and *BCR* FISH signals (Fig. 3). This explains the apparent balanced translocation signal pattern (2 fusions) by interphase and metaphase FISH analysis. Because the insertional events were cryptic, they could not be observed by chromosome analysis.

5. Conclusions

This case demonstrates a complex *BCR/ABL1* rearrangement that was unappreciable by traditional cytogenetic methodologies. MPseq was critical in resolving discordant results that were generated by all 3 techniques used to evaluate this patient's CML clone. The apparently normal chromosome study was incorrect. The *BCR/ABL1* D-FISH probe result was partially correct, only identifying one in-frame fusion of clinical relevance (*BCR/ABL1*). Ultimately, MPseq was necessary to clarify and unify all the results, which were both simple and complex, highlighting the clinical utility of this new technology.

References

[1] Vardiman JW, Melo JV, Baccarani M, Radich JP, Kvasnicka HM. Chronic myeloid leukaemia, BCR-ABL1–positive. In: Swerdlow SH, Campo E, Harris NL, et al, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2017. p. 30-6.

[2] Johansson B, Fioretos T, Mitelman F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. 2002;107:76-94.

[3] Primo D, Tabernero MD, Rasillo A, et al. Patterns of BCR/ABL gene rearrangements by interphase fluorescence in situ hybridization (FISH) in BCR/ABL+ leukemias: incidence and underlying genetic abnormalities. Leukemia 2003;17:1124-9.

[4] Vargas MT, Portero MA, Rodríguez A, Reyes J, Fernández-Novoa C. Cytogenetic, fluorescence in situ hybridization, and molecular characterization of chronic myeloid leukemia in chronic phase with four BCR/ABL1 fusion signals: a case report. Cancer Genet Cytogenet 2009;195:71-4.

[5] Shao L, Miller S, Keller-Ramey J, Zhang Y, Roulston D. Cytogenetic, fluorescence in situ hybridization, and genomic array characterization of chronic myeloid leukemia with cryptic BCR-ABL1 fusions. Cancer Genet 2015;208:396-403.

[6] Duan MH, Li H, Cai H. A rare e13a3 (b2a3) BCR-ABL1 fusion transcript with normal karyotype in chronic myeloid leukemia: the challenges in diagnosis and monitoring minimal residual disease (MRD). Leuk Res 2017;59:8-11.

[7] Drucker TM, Johnson SH, Murphy SJ, Cradic KW, Therneau TM, Vasmatzis G. BIMA V3: an aligner customized for mate pair library sequencing. Bioinformatics 2014;30:1627-9.
[8] Johnson SH, Smadbeck JB, Smoley SA, et al. SVAtools for junction detection of genome-wide chromosomal rearrangements by mate-pair sequencing (MPseq). Cancer Genet 2018;221:1-18.
[9] Borowitz MJ, Chan JKC, Downing JR, et al. B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2017. p. 203-9.
[10] Pui CH, Roberts KG, Mullighan CG. Philadelphia chromosome–like acute lymphoblastic leukemia. Clin Lymphoma Myeloma Leuk 2017;17:464-70.