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Identification of a Novel *MAPK1::BCR* Fusion Gene/t(9;22)(q34;q11) in a Case of Acute Promyelocytic Leukemia

Akut Promiyelositik Lösemi Hastasında Yeni Bir *MAPK1::BCR* Füzyon Geni/t(9;22)(q34;q11)'in Tanımlanması

Qian Wang[1,2]*, Ling-Ji Zeng[3]*, Man Wang[1,2], Jian-Yu Weng[3], Jin-Lan Pan[1,2]

[1]*National Clinical Research Center for Hematological Diseases, Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, China*
[2]*Institute of Blood and Marrow Transplantation, Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China*
[3]*Guangdong Provincial People's Hospital, Department of Hematology, Guangzhou, China*

To the Editor,

Acute promyelocytic leukemia (APL) is characterized by *PML::RARA* rearrangement, resulting from the t(15;17)(q24;q21) translocation. The t(9;22)(q34;q11) translocation, which results in the *BCR::ABL1* fusion gene, has been identified in several hematological malignancies, such as chronic myeloid leukemia, mixed-phenotype acute leukemia, and acute lymphoblastic leukemia. The occurrence of both t(15;17)(q24;q21) and t(9;22)(q34;q11) in APL patients is extremely rare. In this report, we describe the identification and treatment of a case of APL with the coexistence of both t(15;17) and t(9;22) with a novel *MAPK1::BCR* fusion gene.

A 64-year-old woman was admitted to the hospital on June 4, 2022, due to scattered petechial hemorrhages and ecchymosis on the abdominal skin for 3 days. Complete peripheral blood counts showed a white blood cell count of 6.86×10^9/L, hemoglobin level of 90 g/L, and platelet count of 70×10^9/L. Fibrinogen and D-dimer levels were 1.99 g/L (reference range: 1.90-4.00 g/L) and 20 µg/mL (reference range: 0.00-0.50 µg/mL), respectively. A bone marrow (BM) smear revealed hypercellular marrow with 94.5% hypergranular promyelocytes with Auer rods. Multiparameter flow cytometry revealed that the blasts expressed CD13, CD33, CD117, and MPO; weakly expressed CD4, CD15, and CD38; and did not express CD19, CD34, cCD3, cCD79a, TDT, and HLA-DR. Karyotype analysis at diagnosis revealed t(9;22)(q34;q11) in addition to t(15;17)(q24;q21) in 15 of 20 cells (Figure 1A). Fluorescence in situ hybridization (FISH) analysis revealed evidence of *PML::RARA* rearrangement (Figure 1B). FISH analysis using the *BCR::ABL1* dual-color dual-fusion probe revealed two normal-size red (*ABL1*) signals and two reduced-size and one normal-size green (*BCR*) signals in 95% of the cells analyzed (Figure 1C). It is noteworthy that among five separate signals (3G2R), one small green signal was always adjacent to one large red signal in addition to three other separate signals (2G1R), suggesting that the translocation breakpoint was in the *BCR* gene and ruling out *BCR::ABL1* rearrangement.

To characterize the rearrangement involving the *BCR* gene, we performed RNA sequencing of the BM samples with the NextSeq 550system (Illumina, San Diego, CA, USA) and found *PML::RARA* fusion and novel *MAPK1::BCR* fusion (Figures 1D and 1E). For validation of the novel fusion, reverse transcription-polymerase chain reaction (RT-PCR) and Sanger sequencing were performed and the *MAPK1::BCR* transcript was confirmed, but a reciprocal *BCR::MAPK1* transcript was not detected (Figure 1F). *MAPK1* exon 1 was fused to exon 2 of *BCR* out of frame, which led to a premature stop codon near the fusion breakpoint (Figure 1G). This fusion transcript encodes a protein of 40 amino acids, which does not lead to a fusion protein. A total of 136 known or putative mutational gene targets in hematological neoplasms were sequenced using next-generation sequencing (Table 1). *ASXL1*-p.Gly643Val, *CALR*-p.Gly65fs, *KRAS*-p.Gly12Arg, and *NOTCH1*-p.Arg1672His variations were identified in this patient (Table 2).

APL was diagnosed and treated with all-trans retinoic acid (ATRA). After 6 weeks of induction treatment, the patient achieved complete hematological remission. A BM smear showed normocellularity with 1.0% promyelocytes. The fusion transcript of *PML::RARA* was detected by quantitative RT-PCR at a ratio of 0.084%. *PML::RARA* was not detected after consolidation therapies. The normal *BCR* and *ABL1* signals were also observed by FISH. The patient has been in molecular remission for more than 12 months since diagnosis.

APL is characterized by a balanced translocation of chromosomes 15 and 17. However, additional chromosomal abnormalities (ACAs) besides t(15;17) can be detected and are also reported in

about 40% of cases. The presence of t(15;17) along with other recurrent genetic rearrangements such as t(9;22) is rare in APL. To date, the chromosomal abnormality of t(9;22) has been reported in several APL cases, and both *BCR::ABL1* and *PML::RARA* can be detected in these cases (Table 3) [1,2,3,4,5,6,7]. Interestingly, we described a case of APL in which t(15;17)(q24;q21) and t(9;22)(q34;q11) were detected in the same clone at diagnosis by R bands. However, the *BCR::ABL1* fusion gene was not detected, while a novel *MAPK1::BCR* fusion gene was detected by RNA sequencing. To the best of our knowledge, this is the first reported APL case with both *PML::RARA* and *MAPK1::BCR*. Both *MAPK1* and *BCR* genes are located on chromosome 22q11. Based on the results of karyotyping, FISH, and RNA sequencing, we inferred that the occurrence of t(9;22)/*MAPK1::BCR* arises from a two-step mechanism as shown in Figure 2.

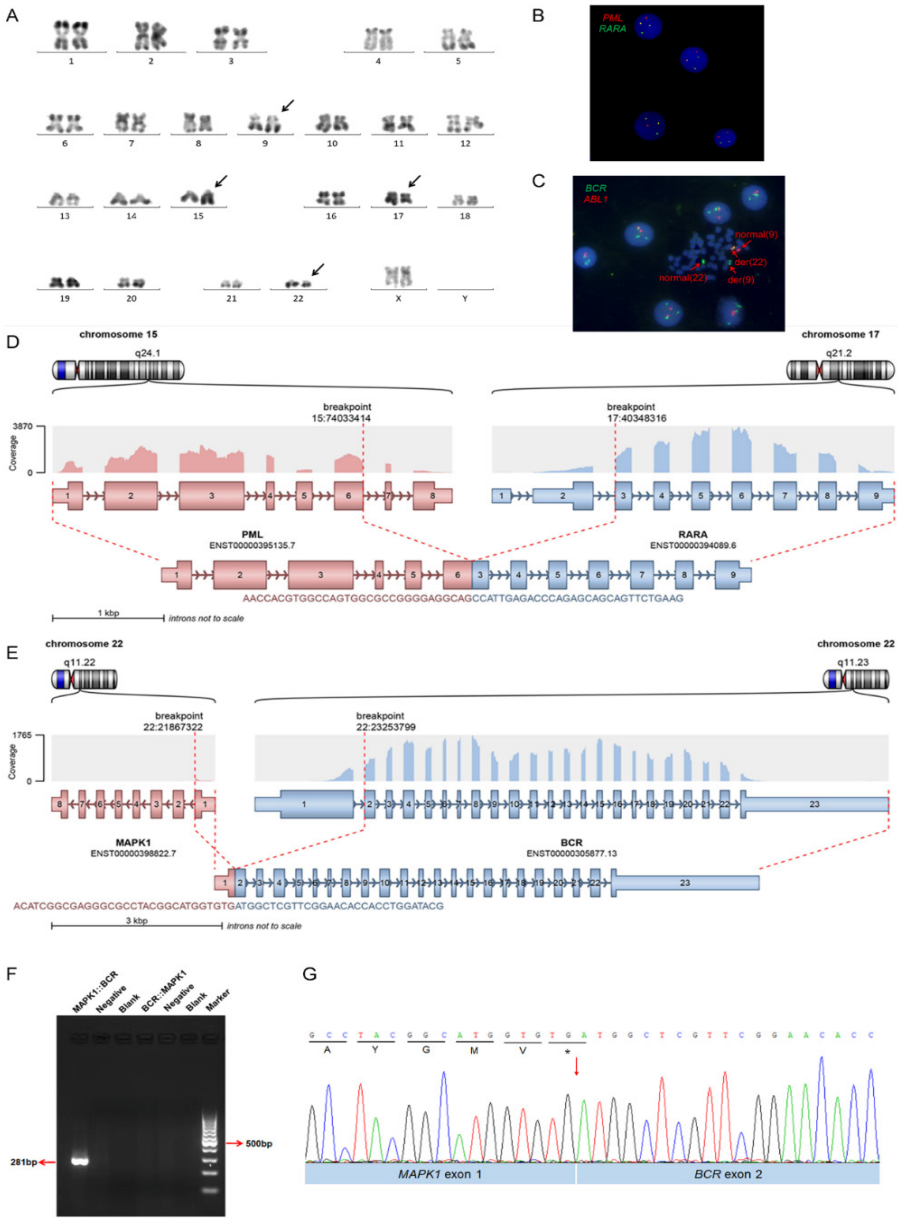

Figure 1. Laboratory characteristics of the case with acute promyelocytic leukemia with *MAPK1::BCR* fusion. (A) Karyotype analysis at diagnosis. R-banding analysis using a bone marrow sample revealed a karyotype of 46,XX,t(9;22)(q34;q11),t(15;17)(q24;q21)[15]/46,XX[5]. (B) Fluorescence in situ hybridization (FISH) analysis using the *PML::RARA* dual-color dual-fusion probe revealed evidence of *PML::RARA* rearrangement (two yellow fusion signals, one red signal, and one green signal). (C) FISH analysis using the *BCR::ABL1* dual-color dual-fusion probe revealed two red (*ABL1*) signals and three green (*BCR*) signals in 95% of the cells analyzed. Metaphase showed a yellow fusion signal on chromosome 22. (D) RNA sequencing analysis revealed that exon 6 of the *PML* gene was fused with exon 3 of the *RARA* gene. (E) RNA sequencing analysis revealed that exon 1 of the *MAPK1* gene was fused with exon 2 of the *BCR* gene. (F) A product of 281 bp was detected by reverse transcription-polymerase chain reaction in the sample at diagnosis. Marker: GeneRuler 100 bp DNA ladder. (G) Sequence alignment of the amplified product revealed breakpoints between exon 1 of *MAPK1* and exon 2 of *BCR*.

Table 1. Panel of 136 genes detected by next-generation sequencing.							
ABL1	*ANKRD26*	*ARID1A*	*ARID1B*	*ASXL1*	*ASXL2*	*ASXL3*	*ATG2B*
ATM	*ATRX*	*BCOR*	*BCORL1*	*BLM*	*BRAF*	*BRCA1*	*BRCA2*
BRINP3	*CALR*	*CARD11*	*CBL*	*CCND1*	*CCND2*	*CD28*	*CDKN2A*
CEBPA	*CELA2A*	*CHD1*	*CHD4*	*COL12A1*	*CREBBP*	*CRLF2*	*CROCC*
CSF3R	*CSMD1*	*CUX1*	*DDX11*	*DDX41*	*DIS3*	*DKC1*	*DNMT3A*
DOT1L	*EP300*	*EPPK1*	*ETNK1*	*ETV6*	*EZH2*	*FAT1*	*FBXW7*
FLT3	*GATA1*	*GATA2*	*GFI1*	*GNAS*	*GSKIP*	*HAX1*	*HRAS*
IDH1	*IDH2*	*IKZF1*	*IL7R*	*JAK1*	*JAK2*	*JAK3*	*KDM5A*
KDM6A	*KIT*	*KMT2A*	*KMT2C*	*KMT2D*	*KMT2E*	*KMT6A*	*KRAS*
MECOM	*MPL*	*MUC16*	*MYC*	*MYD88*	*NCOR1*	*NCOR2*	*NF1*
NFE2	*NOTCH1*	*NOTCH2*	*NOTCH3*	*NPM1*	*NRAS*	*NT5C2*	*PAX5*
PBRM1	*PCLO*	*PDGFRA*	*PHF6*	*PIGA*	*PML*	*PPM1D*	*PRPF40B*
PRPF8	*PTEN*	*PTPN11*	*PTPRT*	*RAD21*	*RB1*	*ROBO1*	*ROBO2*
RUNX1	*RUNX2*	*SBDS*	*SETBP1*	*SF1*	*SF3B1*	*SH2B3*	*SMARCA2*
SMC1A	*SMC3*	*SMN1*	*SPN*	*SRP72*	*SRSF2*	*STAG1*	*STAG2*
STAT3	*STAT5B*	*SUZ12*	*TERC*	*TERT*	*TET2*	*TP53*	*TTN*
TYK2	*U2AF1*	*U2AF2*	*WAC*	*WT1*	*ZMYM3*	*ZNF608*	*ZRSR2*

Table 2. Gene variations detected by next-generation sequencing.							
Gene	**Transcript ID**	**Chromosome**	**Exon**	**DNA change**	**AA change**	**VAF**	**Type of variation**
ASXL1	NM_015338.5	chr20	12	c.1928G>T	p.Gly643Val	0.5344	Missense
CALR	NM_004343.3	chr19	2	c.191_192dupAA	p.Gly65fs	0.3466	Frameshift
KRAS	NM_004985.4	chr12	2	c.34G>C	p.Gly12Arg	0.3849	Missense
NOTCH1	NM_017617.4	chr9	26	c.5015G>A	p.Arg1672His	0.4339	Missense
AA: Amino acid; VAF: variant allele frequency.							

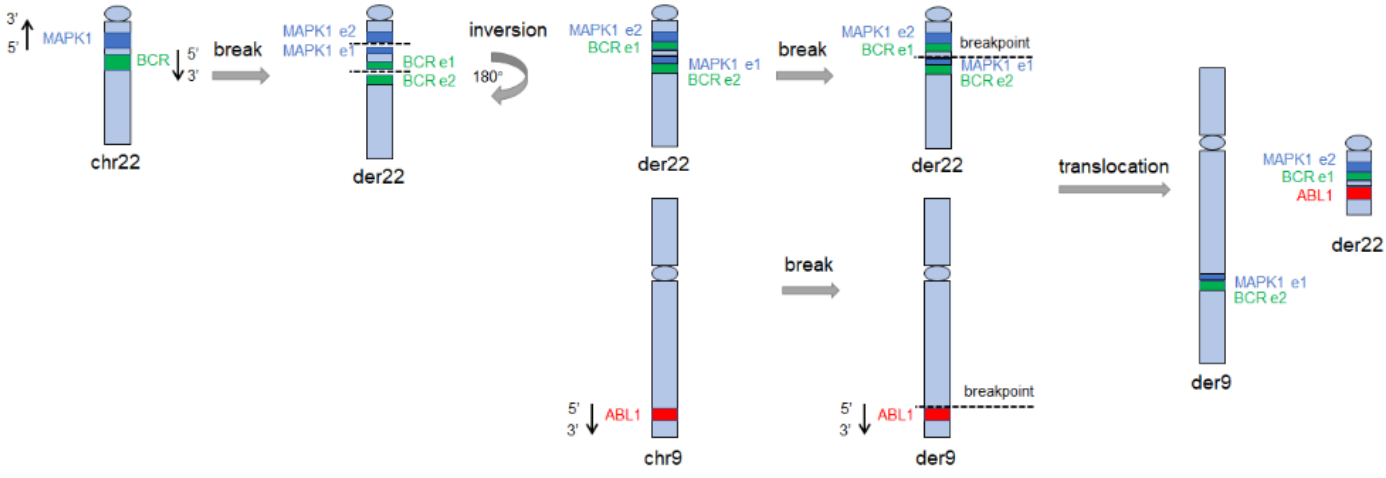

Figure 2. Schematic illustration of the occurrence of t(9;22)(q34;q11)/*MAPK1::BCR*.

Table 3. Summary of published cases of co-expression of t(15;17)/*PML::RARA* and t(9;22)/*BCR::ABL1*, and the present case.

No.	Age (years)	Sex	WBC ($x10^9$/L)	Hb (g/L)	PLT ($x10^9$/L)	Diagnosis	BM morphology	Cytogenetics
1	39	Female	242.2	88	20	APL	Promyelocytic cell population	46,XX,t(9;22)
2	38	Female	1.8	61	12	APL	73% promyelocytes	46,XX,t(9;22)(q34;q11),t(15;17)(q22;q21)[4]/46,XX[16]
3	50	Male	0.45	73	3	APL	38% blasts and 20% promyelocytes	46,XY,t(15;17)(q22;q12)[9]/46,XY,del(6)(q?),t(9;22)(q34;q11.2)[1]/46,XY[10]
4	48	Male	1.15	86	15	APL	1% myeloblasts and 91.5% promyelocytes	46,XY,t(9;22)(q34;q11),t(15;17)(q22;q21)[10]/47,idem,+8[4]/46,idem,der(14)t(9;14)(q10;q10)[6]
5	51	Female	287.83	74	116	APL	8.5% myeloblasts and 43.5% promyelocytes	46,XX,t(9;22)(q34;q11),t(15;17)(q22;q21)
6	69	Female	1.17	118	79	APL	5.2% blasts and 48% abnormal promyelocytes	46,XX,t(15;17)(q24.1;q21.1)[8]/46,XX[16]
7	49	Female	6.48	69	27	APL	10% blasts and 80% abnormal promyelocytes	46,XX,der(6)t(6;8)(p23;q13),t(15;17)(q24;q21)[20]
8	56	Female	6.86	90	70	APL	94.5% hypergranular promyelocytes	46,XX,t(9;22)(q34;q11),t(15;17)(q24;q21)[15]/46,XX[5]

Table 3. Continued.

No.	Immunophenotype	PCR	Treatment	CR	Outcome, months	References
1	Positive for CD33 (72%), CD13 (88%), CD2 (4%); negative for HLA-DR and CD34	*PML::RARA BCR::ABL1*	Idarubicin + Ara-C	Yes	Death, 5	[1]
2	Positive for CD33 (98.69%), CD117 (35.65%), MPO (31.27%), and CD13 (23.64%); negative for CD7, CD10, CD19, HLA-DR, CD34, CD61, CD64, CD14, CD35, CD56, CD15, CD11b, and CD65s	*PML::RARA BCR::ABL1*	ATRA + arsenic trioxide	No	Death, <1	[2]
3	Not available	*PML::RARA BCR::ABL1*	ATRA	Yes	Survivor, >2	[3]
4	Positive for CD13, CD33, CD38, CD64, MPO, CD4, CD11b, and CD117; negative for CD34 and HLA-DR	*PML::RARA BCR::ABL1*	ATRA + arsenic trioxide + imatinib	Yes	Survivor, 18	[4]
5	Positive for MPO, CD13, CD33, and CD34; negative for HLA-DR	*PML::RARA BCR::ABL1*	ATRA + DA	Yes	Survivor, 11	[5]
6	Positive for CD13, CD33, cytoplasmic MPO, CD11c, and CD10; negative for HLA-DR	*PML::RARA BCR::ABL1*	ATRA + idarubicin	Yes	Death, 6	[6]
7	Positive for MPO, CD13, CD33, CD117, CD34, CD2, and CD56; negative for HLA-DR	*PML::RARA BCR::ABL1*	ATRA + idarubicin	Yes	Survivor, 10	[7]
8	Positive for CD13, CD33, CD117, and MPO; negative for CD34 and HLA-DR	*PML::RARA MAPK1::BCR*	ATRA	Yes	Survivor, >12	Present case

WBC: White blood cell count; Hb: hemoglobin; PLT: platelet count; APL: acute promyelocytic leukemia; BM: bone marrow; PCR: polymerase chain reaction; Ara-C: cytarabine; ATRA: all-trans retinoic acid; DA: daunorubicin and cytarabine; CR: complete remission.

The first step is an inversion between exon 1 of *MAPK1* and exon 1 of *BCR* within der(22), theoretically resulting in the *MAPK1* exon 1::*BCR* exon 2 and *BCR* exon 1::*MAPK1* exon 2 fusion genes. A fragment of 281bp (*MAPK1*::*BCR*) was detected by RT-PCR, whereas *BCR*::*MAPK1* was not detected. The second step is a translocation between chromosome 9 with the 9q34 breakpoint mapped upstream of the *ABL1* gene and der(22) with a breakpoint located between *BCR* exon 1 and *MAPK1* exon 1. The abnormality of t(9;22)(q34;q11) was very similar to the standard t(9;22)/*BCR::ABL1*, which masked complex rearrangements. Therefore, the *BCR::ABL1* fusion gene should be detected by FISH, RT-PCR, or RNA sequencing when R/G banding karyotypes indicate concurrent t(9;22)(q34;q11) and t(15;17)(q24;q21) in APL.

The most common additional somatic mutations in APL mainly include the *FLT3*, *WT1*, and *NRAS* genes, whereas the variations detected in our case, including *ASXL1*, *CALR*, *KRAS*, and *NOTCH1*, are rare [8]. The clinical significance of these variations is unclear and needs to be confirmed in larger samples. There are conflicting data in the literature concerning the prognostic relevance of ACAs present at diagnosis in APL. Epstein-Peterson et al. [9] showed no influence of ACAs [≥1 cytogenetic abnormalities besides t(15;17)] on event-free survival (EFS), but complex karyotypes [≥2 cytogenetic abnormalities besides t(15;17)] conferred inferior EFS. Zeng et al. [10] reported that ACAs were an independent risk factor for adverse EFS. The majority of APL patients with t(9;22)/*BCR::ABL1* achieved complete remission after ATRA-based inductive treatment irrespective of whether it was combined with tyrosine kinase inhibitors [4,7]. Our patient was treated with ATRA for induction chemotherapy, followed by consolidation therapy including ATRA. At the time of writing, the patient has remained in complete remission for 12 months. This report suggests that the presence of an additional t(9;22)/*MAPK1::BCR* abnormality might not reduce the sensitivity to ATRA and did not affect the clinical outcome of this patient's treatment.

In conclusion, we have reported the first case of *MAPK1::BCR* in an adult APL patient with concurrent t(9;22)(q34;q11) and t(15;17)(q24;q21). The *BCR::ABL1* fusion gene should be detected by FISH, RT-PCR, or RNA sequencing when t(9;22)(q34;q11) is detected by conventional karyotyping in cases of APL with t(15;17)(q24;q21). The *MAPK1::BCR* fusion gene appears to have no influence on the clinical outcome of our APL patient treated with ATRA. Further molecular studies involving larger series of cases are needed to understand the mechanism of *MAPK1::BCR* in leukemogenesis.

Keywords: Acute promyelocytic leukemia, *PML::RARA*, *MAPK1::BCR*, t(9;22)(q34;q11), RNA sequencing

Anahtar Sözcükler: Akut promiyelositik lösemi, P*ML::RARA, MAPK1::BCR*, t(9;22)(q34;q11), RNA dizileme

Ethics

Informed Consent: Informed consent was obtained from the patient.

Authorship Contributions

Concept: J-L.P.; Design: J-L.P., J-Y.W.; Data Collection or Processing: Q.W., L-J.Z.; Analysis or Interpretation: M.W.; Literature Search: Q.W., L-J.Z.; Writing: Q.W., J-L.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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Address for Correspondence/Yazışma Adresi: Jin-Lan Pan, PhD, National Clinical Research Center for Hematological Diseases, Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University; Institute of Blood and Marrow Transplantation, Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China
Phone : +86-512-67780441
E-mail : jinlanpan@aliyun.com ORCID: orcid.org/0009-0000-7723-4989
***These authors contributed equally to this work.**

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