

## **DEK-NUP214-Fusion Identified by RNA-Sequencing of an Acute Myeloid Leukemia with t(9;12)(q34;q15)**

IOANNIS PANAGOPOULOS<sup>1</sup>, LUDMILA GORUNOVA<sup>1</sup>, SYNNE TORKILDSEN<sup>1,2</sup>,  
GEIR E. TJØNNFIJORD<sup>2,3</sup>, FRANCESCA MICCI<sup>1</sup> and SVERRE HEIM<sup>1,3</sup>

<sup>1</sup>Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics,  
The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;

<sup>2</sup>Department of Haematology, Oslo University Hospital, Oslo, Norway;

<sup>3</sup>Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway

**Abstract.** *Background/Aim:* Given the diagnostic, prognostic, biologic, and even therapeutic impact of leukemia-associated translocations and fusion genes, it is important to detect cryptic genomic rearrangements that may exist in hematological malignancies. *Case Report:* RNA-sequencing was performed on an acute myeloid leukemia case with the bone marrow karyotype 45,X,-Y,t(9;12)(q34;q15)[16]. *Results:* The DEK-NUP214 and PRRC2B-DEK fusion genes were found. Reverse transcriptase polymerase chain reaction together with direct sequencing verified the presence of both. Fluorescence *in situ* hybridization showed that the DEK-NUP214 fusion gene was located on the 6p22 band of a seemingly normal chromosome 6. *Conclusion:* RNA-sequencing proved to be a valuable tool for the detection of a fusion of genes DEK and NUP214 in a leukemia that showed cryptic cytogenetic rearrangement of chromosome band 9q34.

Acquired genetic abnormalities are present in the leukemic bone marrow cells in hematological malignancies, including acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (1-4). Microscopic studies have shown that these aberrations are often visible as balanced chromosomal changes such as translocations and inversions, as well as

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*Correspondence to:* Ioannis Panagopoulos, Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Montebello, PO Box 4954 Nydalen, NO-0424 Oslo 0310, Norway. Tel: +47 22782363, e-mail: ioannis.panagopoulos@rr-research.no

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unbalanced anomalies such as deletions, monosomies, duplications, and trisomies (1). Leukemia-associated translocations and inversions often result in fusions at the breakpoints through melting together parts of two genes, one in each breakpoint. The chromosomal rearrangements giving rise to fusion genes are often seen as sole aberrations at cytogenetic analysis, are considered to be primary tumorigenic events, and are of pathogenetic, diagnostic, and prognostic importance (1).

Sometimes, cytogenetic analyses fail to detect chromosomal rearrangements because the regions involved are of similar size and have similar banding patterns, because of poor chromosome morphology and spreading, because too few metaphase cells could be analyzed, or only normal metaphases derived from non-leukemic cells are found after culturing. The application of molecular cytogenetic techniques such as fluorescence *in situ* hybridization (FISH), multicolor FISH, array comparative genome hybridization (aCGH), and single-nucleotide polymorphism (SNP) arrays may then be particularly valuable, alone or together with more exclusively molecular methods based on the polymerase chain reaction (PCR), and may improve significantly the rate at which genetic changes are found in hematological malignancies (5-12).

Not surprisingly, some chromosome aberrations and their corresponding fusion genes can be detected only with molecular methods. One of them is the t(12;21)(p13;q22)/ETV6-RUNX1 which is one of the most common genetic rearrangements in pediatric B-lineage acute lymphoblastic leukemia (ALL) and defines a subgroup of patients with excellent prognosis (13, 14). Another example is the del(4)(q12q12)/FIP1L1-PDGFRα characteristic of hypereosinophilic syndrome. Patients with del(4)(q12q12)/FIP1L1-PDGFRα have an excellent prognosis when treated with imatinib (15-17).

Given the diagnostic, prognostic, biologic, and even therapeutic impact of leukemia-associated translocations and fusion genes, it is important to detect also cryptic genomic rearrangements that may be present in hematologic

malignancies. High-throughput sequencing has been proven to be a powerful method for detecting fusion genes in cases with normal karyotype or with aberrations seemingly not involving the chromosome regions where the fusion gene partners map (18-20).

We here report an AML case carrying a t(9;12)(q34;q15) chromosomal translocation as the sole cytogenetic anomaly. Using RNA-sequencing, a cryptic *DEK-NUP214* (formerly *DEK-CAN*) fusion gene as well as a *PRRC2B-DEK* fusion were found.

## Materials and Methods

**Ethics statement.** The study was approved by the Regional Committee for Medical and Health Research Ethics, South-East Norway (REK Sør-Øst; <http://helseforskning.etikkom.no>) and written informed consent was obtained from the patient. The ethics committee's approval included a review of the consent procedure. All patient information has been anonymized.

**Case report.** A 20-year-old male was transferred to our institution with a preliminary diagnosis of AML. He had for two weeks experienced flu-like symptoms with general malaise and strikingly reduced physical stamina. A few days prior to hospitalization, he had also noticed stationary dark spots in his visual field.

Clinical examination was unremarkable. Of note, no gingival hyperplasia was found. There was significant anemia (6.9 g/dl (13.4-17.0)), thrombocytopenia ( $26 \times 10^9/l$  (165-387)), and leukocytosis ( $46.3 \times 10^9/l$  (3.6-9.3), monocytes  $9.7 \times 10^9/l$  (0.3-0.9)). Elevated LDH (724 U/l (<205)) and lysozyme (42 mg/l (5-15)) were also found. A blood smear disclosed numerous blasts and monocytosis. A bone marrow smear revealed a pleomorphic "blastoid" population with cells of variable size, some of which had irregular nuclei with many nucleoli and "dustlike" granules, whereas others had rounded nuclei and abundant blue-grey cytoplasm without granules. Some atypical monocytes and more mature granulocytes with atypical nucleus segmentation were also seen. The blasts displayed the immunophenotype CD34<sup>+</sup>MPO<sup>-</sup>CD64<sup>-/+</sup>CD38<sup>+</sup>CD33<sup>+</sup>CD13<sup>+</sup>HLA-DR<sup>+</sup>. A diagnosis of acute monoblastic leukemia was made.

Molecular genetic analyses were negative for *RUNX1-RUNX1T1*, *CBFB-MYH11*, *MLLT-MLL*, *AFF1-MLL*, and *MLL-PTD* fusion transcripts as well as for *NPM1*, but *FLT3* mutation (TKD) was detected.

Complete hematologic remission was accomplished following induction treatment with daunorubicin and cytarabine. Subsequently, he received two cycles of high dose cytarabine and an allogeneic stem cell transplant from a matched unrelated donor following myeloablative conditioning. At follow up five years post transplantation, he was in complete remission with no signs of graft-versus-host disease (GVHD).

**G-banding analysis.** Bone marrow cells aspirated at diagnosis were cytogenetically investigated (21, 22). Chromosome preparations were made from metaphase cells of a 24-h culture, G-banded using Leishman stain, and karyotyped according to ISCN 2016 guidelines (23).

**Fluorescence in situ hybridization (FISH).** BAC clones were retrieved from the Human "32K" BAC Re-Array library (BACPAC Resources, <https://bacpacresources.org/home.htm>). They had been

selected according to physical and genetic mapping data on chromosomes 6 and 9 (see below) as reported on the Human Genome Browser at the University of California, Santa Cruz website (May 2004, <http://genome.ucsc.edu/>). FISH mapping of the clones on normal controls was performed to confirm their chromosomal location. For the *DEK* locus on chromosome 6, the clones were CTD-2300B02 (Position: chr6:18199501-18330748; Band: 6p22.3; UCSC Genome Browser on Human May 2004 NCBI35/hg17 Assembly) and RP11-758O07 (Position: chr6:18301067-18524918; Band: 6p22.3); they were labelled red. For the *NUP214* locus on chromosome 9, the clones were RP11-723C24 (chr9:130882132-131078602; Bands: 9q34.12 - 9q34.13), RP11-68C20 (chr9:131050288-131215735; Band: 9q34.13), and RP11-178K16 (Position: chr9:131131824-131308655; Band: 9q34.13); they were labelled green.

DNA was extracted and probes were labelled and hybridized according to Abbott Molecular recommendations (<http://www.abbottmolecular.com/home.html>). Chromosome preparations were counterstained with 0.2 µg/ml DAPI and overlaid with a 24×50 mm<sup>2</sup> coverslip. Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

**RNA-sequencing.** Three µg of total RNA extracted from the patient's bone marrow at the time of diagnosis were sent for high-throughput paired-end RNA-sequencing at the Norwegian Sequencing Centre, Ullevål Hospital (<http://www.sequencing.uio.no/>). Detailed information about the procedure was given previously (24). The software FusionCatcher was used to discover fusion transcripts (25) (<https://github.com/ndaniel/fusioncatcher>).

**PCR analyses.** The procedures of reverse transcriptase-Polymerase Chain Reaction (RT-PCR) and direct sequencing of the PCR products were previously described (20, 24). For the amplification of the *DEK-NUP214* fusion transcript, two primer sets were used: 1) the forward DEK-1060F1 (CACCAAGAAGAATCAAAACAGTTCCA) together with the reverse NUP214-2767R1 (TGAAGACTATCCACCA GGTGATTACAG) and 2) the forward DEK-1060F1 together with NUP214-2709R1 (TGTTGGCTAGGGTGTTAAA CAGTGTC). For amplification of the *PRRC2B-DEK* fusion transcript, the forward primer PRRC2B-2072-F1 (AGGTGGATGATGATGCCTTCCTAC) together with the reverse primer DEK-1328-R1 (GGGAACGAGTCA TCTTCTCTGTCC) were used.

## Results

The G-banding analysis yielded the karyotype 45,X,-Y,t(9;12)(q34;q15)[16] (Figure 1).

Using the FusionCatcher software with the fastq files obtained from the Norwegian Sequencing Centre, 9 fusion genes were found (Table I), among them *DEK-NUP214* and *PRRC2B-DEK*. We chose to focus exclusively on these two fusions partly because they were the only ones corresponding to an interchange of material between chromosome bands 9q34, known to be rearranged by chromosome analysis, and another genomic site, but also because *DEK-NUP214* is a known leukemia-specific fusion gene.

PCR and direct sequencing verified the presence of both *DEK-NUP214* and *PRRC2B-DEK* chimeric transcripts

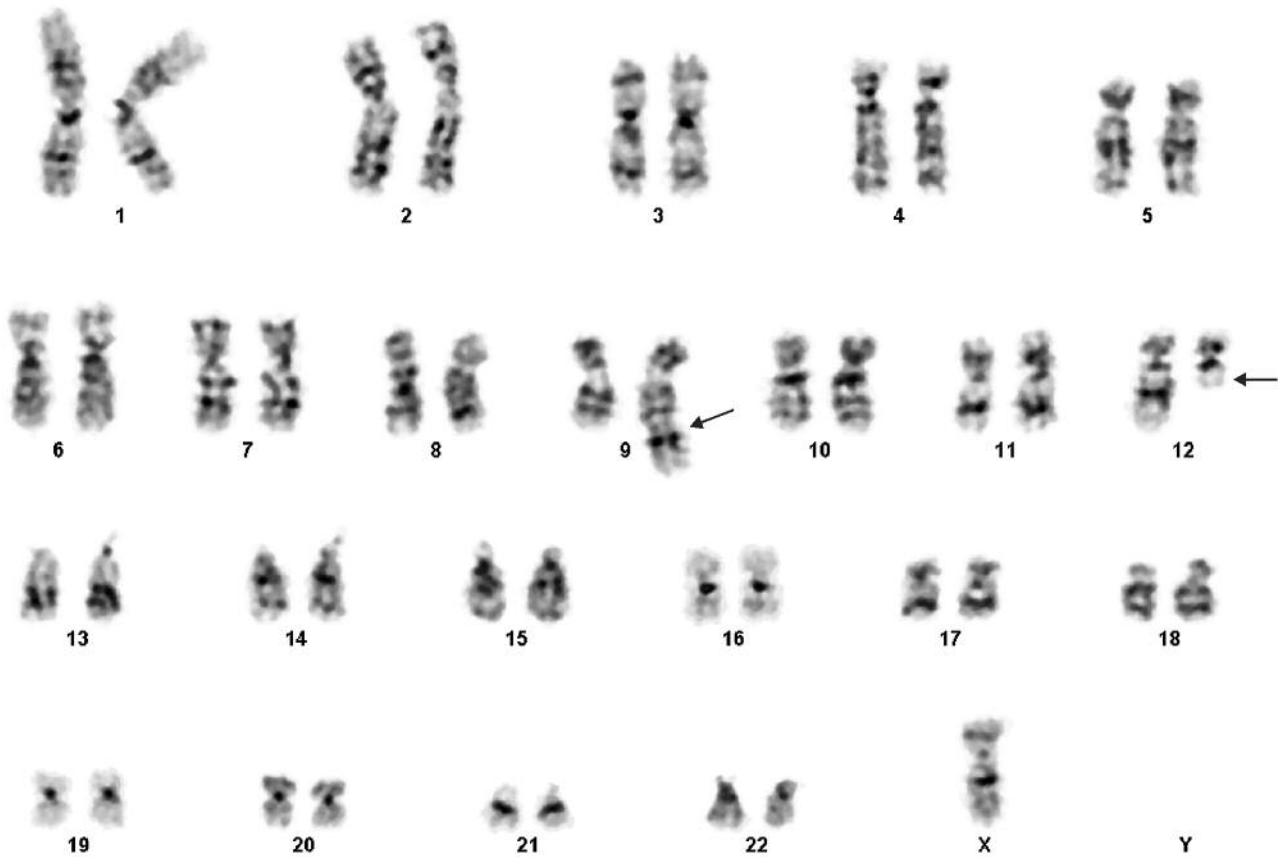


Figure 1. G-banding analysis of the bone marrow cells of the AML patient. Karyotype showing the chromosome aberration of leukemic cells: 45,X,-Y,t(9;12)(q34;q15). Arrows indicate breakpoints.

Table I. Fusion genes detected using FusionCatcher.

Gene 1 (5'-partner)	Gene 2 (3'-partner)	Fusion description	Fusion_sequence
<i>PRRC2B</i>	<i>DEK</i>	Oncogene	TACCCCTCCGCCCTGCATCCCTCAG*GTCTATGAAAATTATCCTACTTATG
<i>AL122127.25</i>	<i>KIAA0125</i>	Readthrough	CCCCTTCTCCCAGGAGGGAGGCAC*AGCCTGGAGACACAGGCCCATCCT
<i>DEK</i>	<i>NUP214</i>	Oncogene	CACAATGAAACAGATTGCAAAAAG*GAAATTCCGGCGCCTTCATCAGTATG
<i>CTBS</i>	<i>GNG5</i>	Readthrough	GCGGGCTCCTTATTATAACTATAAA*GTTTCCCAGGCAGCTGCAGACTTGA
<i>DHRS1</i>	<i>RABGGTA</i>	Readthrough	GCAAAGGAGGAGGTCTGCAGGATC*CTCTTGTGGAACGTGCAAAGCCTGT
<i>C20ORF197</i>	<i>MIR646HG</i>	Readthrough	GTGACTGCAACACCCAGCAGCGCG*AAAGGGACCAGCTTGACGAAGCTTC
<i>CRELD1</i>	<i>PRRT3-AS1</i>	Readthrough	ATCTGTGTGAAGGAGCAGATCCCAG*TGTTGGTTGGCAAGTCATTTAACTTT
<i>SMG5</i>	<i>PAQR6</i>	Readthrough	AGGGGCTGGCTTTGGAGTGTGAG*GTCAACGTGGAGGTACCAGGCCACC
<i>POLA2</i>	<i>CDC42EP2</i>	Readthrough	GTCAGAGCTGAGGTACTTCGTGAAG*CTCCTCAGCCCTGGACCGGGACAA

(Figure 2A-D). In the *DEK-NUP214* transcript, exon 9 of *DEK* (nt 1240 in sequence with accession number NM\_003472 version 3) was fused in frame to exon 18 of *NUP214* (nt 2581 in NM\_005085 version 3) (Figure 2A and B). In the *PRRC2B-DEK* transcript, exon 13 of *PRRC2B*

(nt 2162 in sequence with accession number NM\_013318 version 3) was fused out of frame to exon 10 of *DEK* (nt 1241 in NM\_003472 version 3) (Figure 2C and D).

FISH on metaphase spreads with probes for *DEK* (Figure 3A and B; red signal) and *NUP214* (Figure 3C and D; green

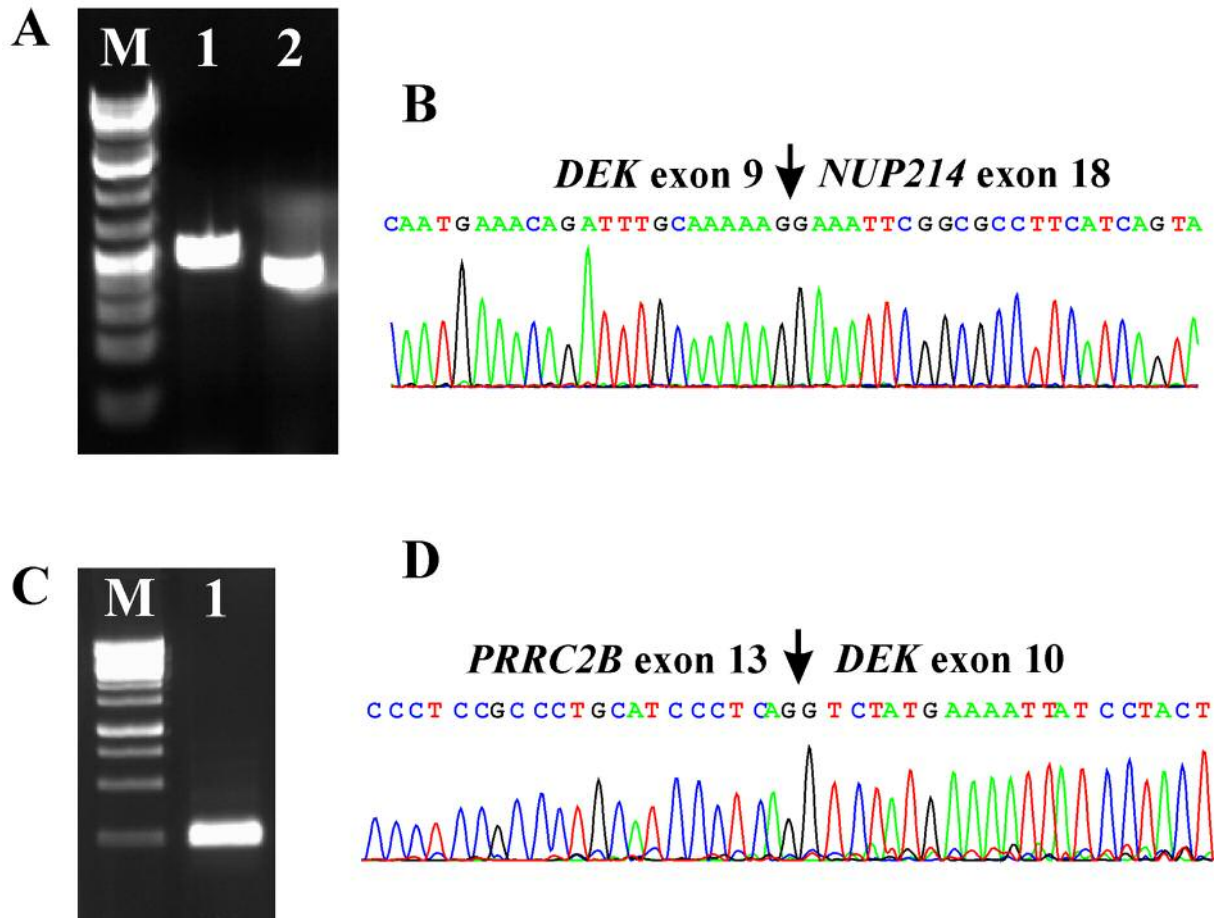


Figure 2. Molecular genetic analysis of bone marrow cells from the AML patient. (A) Gel electrophoresis showing the amplified cDNA *DEK-NUP214* fragments using two primer sets. Lane 1, amplification with the primers *DEK-1060F1* and *NUP214-2767R1*. Lane 2, amplification with the primers *DEK-1060F1* and *NUP214-2709R1*. M, 1 Kb DNA ladder (GeneRuler, ThermoFisher). (B) Partial sequence chromatogram of the amplified cDNA fragment showing the junction point of the *DEK* and *NUP214* genes. (C) Gel electrophoresis showing the amplified cDNA *PRRC2B-DEK* fragment using the primers *PRRC2B-2072-F1* and *DEK-1328-R1* (lane 1). M, 1kb DNA ladder. (D) Partial sequence chromatogram of the amplified cDNA fragment showing the junction point of the *PRRC2B* and *DEK* genes.

signal) showed a red signal on one chromosome 6, two green signals, on the normal chromosome 9 and on the der(9), and a yellow fusion signal on the other chromosome 6, although both copies of chromosome 6 appeared cytogenetically normal by G-banding. Thus, FISH showed that the *DEK-NUP214* fusion gene was located on the 6p22 band (Figure 3E).

## Discussion

The present case of AML had a t(9;12)(q34;q15) as the sole aberration at cytogenetic analysis. This translocation was therefore assumed to be a primary leukemogenic rearrangement which might have led to generation of a pathogenetically-essential fusion gene, and we decided to perform RNA-sequencing to find it. We compared karyotyping and

sequencing data looking specifically for suggested fusions corresponding to rearrangements between the chromosomal breakpoints, *i.e.*, 9q34 and 12q15. However, none of the 9 fusion genes suggested by the RNA-sequencing data using FusionCather corresponded to the t(9;12)(q34;q15) (Table I). Instead, the analysis showed generation of *DEK-NUP214* and *PRRC2B-DEK* fusion genes and revealed a submicroscopic cytogenetic aberration involving chromosome band 6p22 (where *DEK* maps) and 9q34 (where the *NUP214* and *PRRC2B* genes map). Subsequently, the presence of both fusion transcripts was confirmed with RT-PCR/direct sequencing methodology. Further FISH investigation showed that both *DEK-NUP214* and *PRRC2B-DEK* were generated by insertion of a fragment from 9q34 into the 6p22 band of a seemingly normal chromosome 6 (Figure 1). Since all three

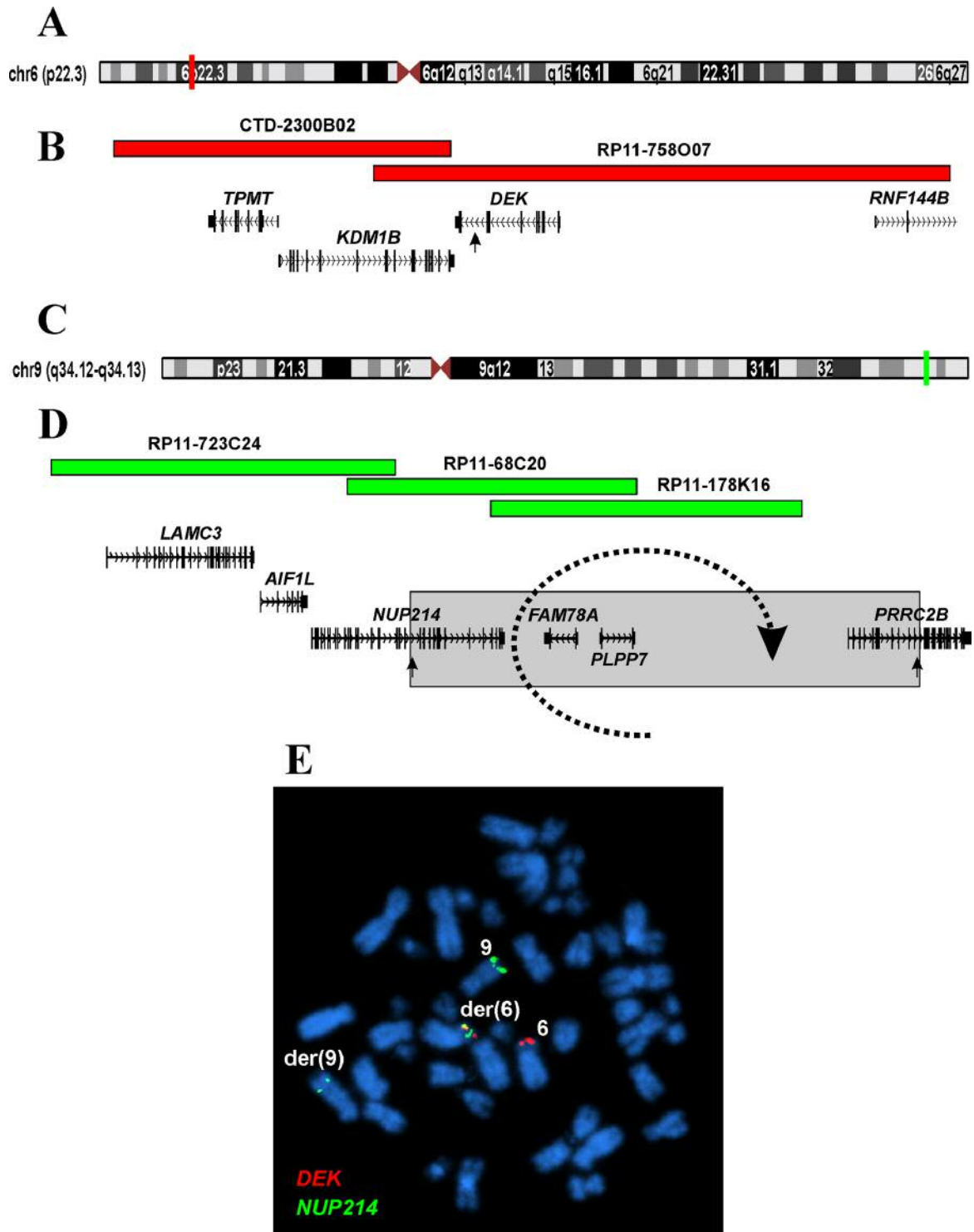


Figure 3. Fluorescence in situ hybridization (FISH) of bone marrow cells from the AML patient. (A) Ideogram of chromosome 6 showing the mapping position of the *DEK* gene (vertical red line). (B) Diagram showing the FISH probe for *DEK*. Additional genes in this region are also shown. (C) Ideogram of chromosome 9 showing the mapping position of the *NUP214* and *PRCC2B* genes (vertical green line). (D) Diagram showing the FISH probe for *NUP214*. Additional genes in this region are also shown. The region between *NUP214* and *PRCC2B* genes which was inverted and inserted in chromosome 6 is marked as grey box. (E) FISH on metaphase with the *DEK* (red signal) and *NUP214* (green signal) probes showing a red signal on chromosome 6, two green signals, one on normal chromosome 9 and one on *der(9)*, and a yellow fusion signal on the second chromosome 6, which was seen as cytogenetically normal. The *DEK-NUP214* fusion gene was located on the 6p22 band.

genes - *DEK*, *NUP214*, and *PRRC2B* - are transcribed from centromere to telomere, we conclude that the fragment from 9q34 was inverted and inserted into the *DEK* gene (Figure 3D).

*DEK-NUP214* is usually the result of a chromosomal translocation, t(6;9)(p22;q34). It encodes a 165 kDa protein (26, 27). The exact role of *DEK-NUP214* in leukemogenesis is unknown, but the *DEK-NUP214* protein was shown to increase protein synthesis in myeloid cells and the expression of *DEK-NUP214* correlates with increased phosphorylation of the translation initiation protein, EIF4E (28). Studies of cell lines carrying a *DEK-NUP214* fusion gene have revealed increased cellular proliferation presumably brought about by upregulation of mTOR (29). In addition, *DEK-NUP214* induces leukemia in mice (30). The *PRRC2B* gene codes for a 2229 amino acid residue protein. Neither the expression of *PRRC2B* nor the function of its protein product is known (<https://www.ncbi.nlm.nih.gov/gene/84726>). The gene has no known role in leukemogenesis.

In the 2008 World Health Organization classification, AML with t(6;9)(p22;q34)/*DEK-NUP214* is recognized as a distinct entity (31) corresponding to approximately 1% of all AMLs. Patients with t(6;9)(p22;q34)/*DEK-NUP214* are often young adults (median age 35 years) or children (median age 13 years). The bone marrow shows distinctive morphologic features, there is a high frequency of *FLT3* mutations, the disease is chemotherapy resistant, and the prognosis is very poor (31); however, hematopoietic stem cell transplantation improves the outcome in these patients (32-34). Thus, identification of t(6;9)(p22;q34) or the *DEK-NUP214* fusion gene is crucial in choosing the right treatment. RNA-sequencing, as reported here, proved valuable for the detection of a *DEK-NUP214* fusion in the leukemic cells whose only cytogenetic clue to being carriers of this leukemogenic change was that they showed rearrangement of chromosomal band 9q34.

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