



Case Report

RUNX1 deletion/amplification in therapy-related acute myeloid leukemia: A case report and review of the literature ☆,☆☆

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ABSTRACT

Intrachromosomal amplification of chromosome 21 (*iAMP21*) is a rare occurrence in acute myeloid leukemia (AML). We describe here a case of AML with apparent amplification of *RUNX1* by cytogenetics and FISH. A 39-year-old female in remission from stage IIIa breast cancer was diagnosed with therapy-related AML (t-AML). The patient's bone marrow was hypocellular for her age (30–40%) with 25% blasts. Cytogenetic analyses revealed a complex karyotype, characterized by rearrangements in chromosomes 1, 5, 17, 20, an additional unidentified marker chromosome, and apparent amplification of chromosome 21. *Fluorescence in situ hybridization* detected deletions of *CKS1B*, *EGR1*, *TP53*, and apparent amplification of *RUNX1* (6–8 signals). Array comparative genomic hybridization (array-CGH) detected amplification of the 5' non-coding region of *RUNX1* and deletion of the 3' coding region of *RUNX1*. These results show that this is not a true case of *iAMP21* and suggest that *RUNX1* is not the primary target of amplification.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematologic group of disorders characterized by the overproduction of abnormal immature myeloid cells (blasts). In the United States, it is the most common form of leukemia found in adults with a median age of diagnosis of 67 years and a five year survival rate of 27.4% [1]. AML arises from a series of genetic mutations in the hematopoietic stem cell precursor. These mutations alter normal hematopoietic development, differentiation, and self-renewal. They lead to the accumulation of blasts in the bone marrow, peripheral blood, and sometimes other organs. The direct clinical consequence of AML is typically a combination of leukocytosis and signs of bone mar-

row failure; as production of normal blood cells drastically decrease and/or malignant, poorly differentiated myeloid cells accumulate, AML patients may show signs of neutropenia, anemia, thrombocytopenia, splenomegaly, hepatomegaly, fatigue, weight loss, and pallor [2,3].

Due to the higher incidence of leukemia in Down syndrome (DS) patients [4], there has been much investigation into critical genes involved in leukemogenesis. *RUNX1* (*AML1*, *runt-related transcription factor 1*) has been a particular gene of interest due to its involvement in myeloid and lymphoid malignancies and other neoplasms. *RUNX1* is located at chromosome 21q22.12 and encodes a protein (core binding factor alpha, *CBFα*). Among the reported chromosomal aberrations, intrachromosomal amplification of chromosome 21 (*iAMP21*) is a rare occurrence in AML. Historically, formal cases of *iAMP21* was originally defined as multiple copies (> 3) of *RUNX1* on a single structurally abnormal chromosome 21 [5–8]. *iAMP21* is found in 2% of pediatric B-cell acute lymphoblastic leukemia (B-cell ALL) cases which lack the *ETV6/RUNX1* fusion [6]. Mechanistically, it is hypothesized that *iAMP21* formation occurs through breakage-fusion-bridge cycles followed by chromothripsis and other complex structural rearrangements [9–12].

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In this paper, we describe a patient who developed therapy-related AML (t-AML) and whose bone marrow showed a complex karyotype with amplification and deletion of the *RUNX1* gene. We discuss the patient's specific aberrations, focusing on *RUNX1* and other potential leukemogenic genes (*APP*, *ERG*, *ETS2*) and their prognostic implications in AML as well a review of the literature for amplifications/deletions of *RUNX1*.

Materials and methods case history

Case history

A 39-year-old female in remission from stage IIIa breast cancer presented to Tulane University Medical Center (TUMC) for t-AML. She had previously received 7+3 chemotherapy with cytarabine+idarubicin at an outside hospital. At day 14 her bone marrow demonstrated that she was refractory to treatment. At TUMC, her bone marrow was hypocellular for her age (30–40%) with 25% blasts and suppressed background trilineage maturation, with a white blood cell count of 0.8L, 6% monocytes, 0.0% eosinophils, 4.0% segmented neutrophils, 0.0% basophils, and 90% lymphocytes. Blasts were identified by surface markers using flow cytometric immunophenotyping CD45 gating, which showed an aberrant myeloid phenotype with blasts positive for CD13 (100.0%), CD33 (97.0%), CD34 (86.0%), CD38 (100.0%), CD117 (69.0%), and HLA-DR (84.0%). According to the World Health Organization (WHO) 2016 guidelines [19], this patient was diagnosed with primary refractory t-AML and considered for allogeneic bone marrow transplantation.

Patient sample, culture initiation and harvest

Standard procedures were used for unstimulated bone marrow culture initiation, harvest, slide making, and G-banding [13]. Slides were scanned and analyzed using Leica Biosystems CytoVision®. Karyotypic nomenclature was described according to the International System for Human Cytogenomic Nomenclature (ISCN) 2016 [14].

Fluorescence in situ hybridization (FISH)

Standard procedures were used for FISH hybridization and washing [13]. The following probe sets were utilized: the *CKS1B* (1q21.3) and *CDKN2C* (1p32.3) amplification/deletion probe; *D5S630/D5S2064* (5p15.3) and *EGR1* (5q31.2) deletion probe; *TP53* (17p13) and *D17Z1* (17 centromere) deletion probe; and the *RUNX1* (AML1-21q22.1) and *RUNX1T1* (ETO-8q21.3) dual color-dual fusion translocation probe (CytoCell Inc.). Slides were analyzed using Leica Biosystems CytoVision®. FISH nomenclature was described according to the ISCN 2016 [14].

Array comparative genomic hybridization (aCGH)

Standard procedures were used for DNA isolation, quantification, labeling, hybridization, and washing according to the Agilent

Table 1

Summary of aCGH results for chromosomes 1, 17, 19, 20 and 21.

Other findings	
arr[GRCh37]	1p32.2p31.1(57165062_78013912)x1
arr[GRCh37]	1q21.3q22(153671910_155154990)x1
arr[GRCh37]	1q23.1q23.3(157652504_162137058)x1
arr[GRCh37]	1q32.1q32.2(205677777_209978460)x1
arr[GRCh37]	1q42.2q42.3(234467812_235963691)x1
arr[GRCh37]	5q13.2q35.3(70386541_180696806)x1
arr[GRCh37]	17p13.3p13.1(76263_8842895)x1
arr[GRCh37]	17q11.2(26006133_30694239)x1
arr[GRCh37]	19q13.42q13.43(54949183_59095418)x3
arr[GRCh37]	20q13.12q13.13(45187113_48148886)x1
Summary of aCGH results for chromosome 21 only.	
Chromosome 21 findings	
arr[GRCh37]	21q21.1(22729579_23891677)x3~4
arr[GRCh37]	21q21.1q21.2(23968489_26151041)x6~8
arr[GRCh37]	21q21.2q21.3(26538484_26950020)x3
arr[GRCh37]	21q21.3(23968489_26151041)x3
arr[GRCh37]	21q21.3q22.11(29041743_32166339)x8
arr[GRCh37]	21q22.11(32217286_33757836)x1
arr[GRCh37]	21q22.11(33796012_34192162)x7~8
arr[GRCh37]	21q22.11q22.12(34223471_36289908)x1
arr[GRCh37]	21q22.12q22.3(36311151_48090317)x6~8

*GRCh37 (hg19) - human reference genome February 2009.

Oligonucleotide Array-Based CGH for Genomic DNA Analysis protocol on the Agilent high-resolution microarray [15]. The array image was captured by a GenePix 4000B Array Scanner. Microarray data was analyzed by the software package of Feature Extraction and DNA Analytics from Agilent. Reported copy number variants (CNVs) information was extracted from the Database of Genomic Variants at <http://dgv.tcag.ca/dgv/app/home>. CGH array nomenclature was described according to the ISCN 2016 [14].

Results

Cytogenetics and FISH results

Chromosome analysis of the patient's bone marrow cells showed a composite complex karyotype of 45,XX,-1,psu dic(5;17)(q11.2;p11.2),der(20)t(1;20)(p31;q13.2), der(21)add(21)(q22)trp(21)(q11.2q22),+mar[cp19]/46,XX[1].

G-banding analysis revealed complex structural rearrangements involving chromosomes 1, 5, 17, and 20, along with an additional unidentified marker chromosome (Fig. 1). Chromosome 21 appeared "zebra-like", with similar morphology to *iAMP21* cases reported in pediatric B-cell ALL. FISH detected deletions of *CKS1B*, *EGR1*, and *TP53*. Apparent *RUNX1* amplification was detected with 6–8 signals (Fig. 2).

aCGH results

aCGH was performed for further characterization of the structural and numerical alterations (Table 1). aCGH detected five deletions on chromosome 1, ranging from 1.483 Mb to 20.849 Mb. One of these deletions included the *CDKN2C* gene. There was a

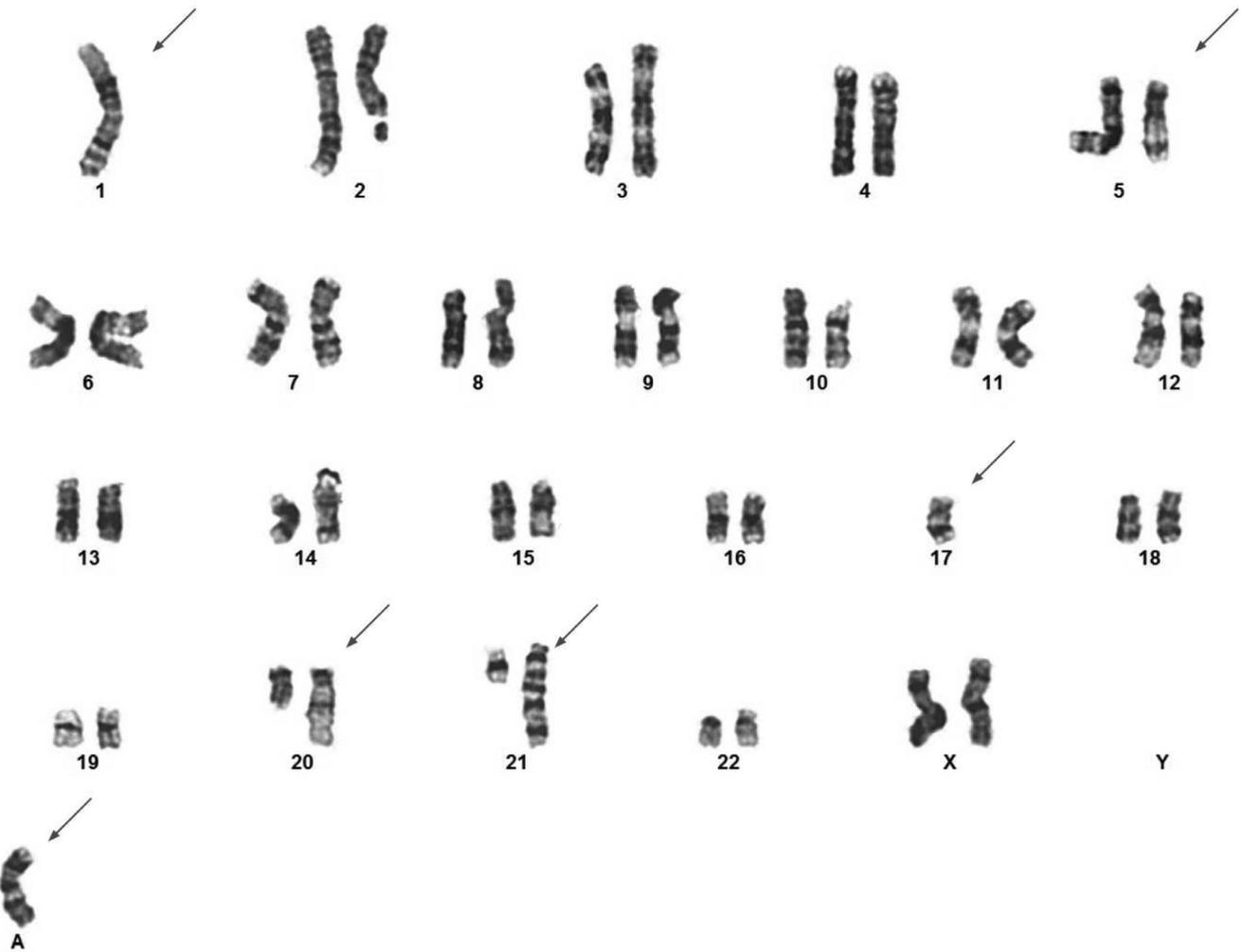


Fig. 1. G-banded karyogram of bone marrow cells before re-induction chemotherapy. The karyotype is 45,XX,-1,psu dic (5;17) (q11.2;p11.2), der (20) t (1;20) (p31;q13.2),der (21) add (21) (q22) trp (21) (q11.2q22). Arrows indicate abnormal chromosomes.

110.31 Mb deletion in the long arm of chromosome 5 from q13.2 to q35.3, which confirmed cytogenetic and *FISH* findings of a deletion in the 5q and *EGR1*, respectively. Two deletions were revealed on chromosome 17: a 4.688 Mb deletion at 17q11.2 and an 8.767 Mb deletion from p13.3 to p13.1. This confirmed the *TP53* deletion found by *FISH*. aCGH also detected the segmental amplifications and deletions across the long arm of chromosome 21 (Table 1). The regions of amplification ranged from 0.412 Mb to 11.79 Mb, with the largest area of amplification between q22.12 and q22.3. Two smaller deletions of 1.541 Mb and 2.066 Mb were detected between q22.11 and q22.12. Gains of the 5' part of *RUNX1* were accompanied by losses in the 3' part (Fig. 3). These segmental 21q amplifications and deletions suggest chromothripsis, a one-step genome-shattering and rearranging event.

Discussion

We identified a complex karyotype with apparent amplification of chromosome 21 in a patient with t-AML. While *FISH* exhibited

apparent *RUNX1* amplification (>3 signals), interestingly, aCGH further characterized the 21q alterations as the amplification of the *RUNX1* 5' non-coding region and loss of the remaining gene (including the entire *RUNX1* coding region). These alterations within *RUNX1* most likely lead to a lack of gene expression and suggest that this is not the same *iAMP21* as seen in pediatric B-cell ALL cases. Unfortunately, the functional consequence of this genetic aberration could not be confirmed due to the lack of additional patient sample. These segmental gains and loss of *RUNX1* have been hypothesized to occur through breakage-fusion-bridge cycles following chromothripsis [9–12].

Chromothripsis is a one-step cataclysmic mechanism of genomic instability. It may occur through DNA replication timing errors. These errors lead to sustained cell cycle arrest and production of micronuclei, which favor the acquisition of structural rearrangements [16]. An alternative theory suggests that chromothripsis is a new form of cellular apoptosis, arising as an unfinished event of chromosome fragmentation [17]. Chromothripsis has been correlated to abnormal DNA double-strand break response, gains of *EGFR*, *MDM2*, and *MDM4*, losses of *CDKN2A*

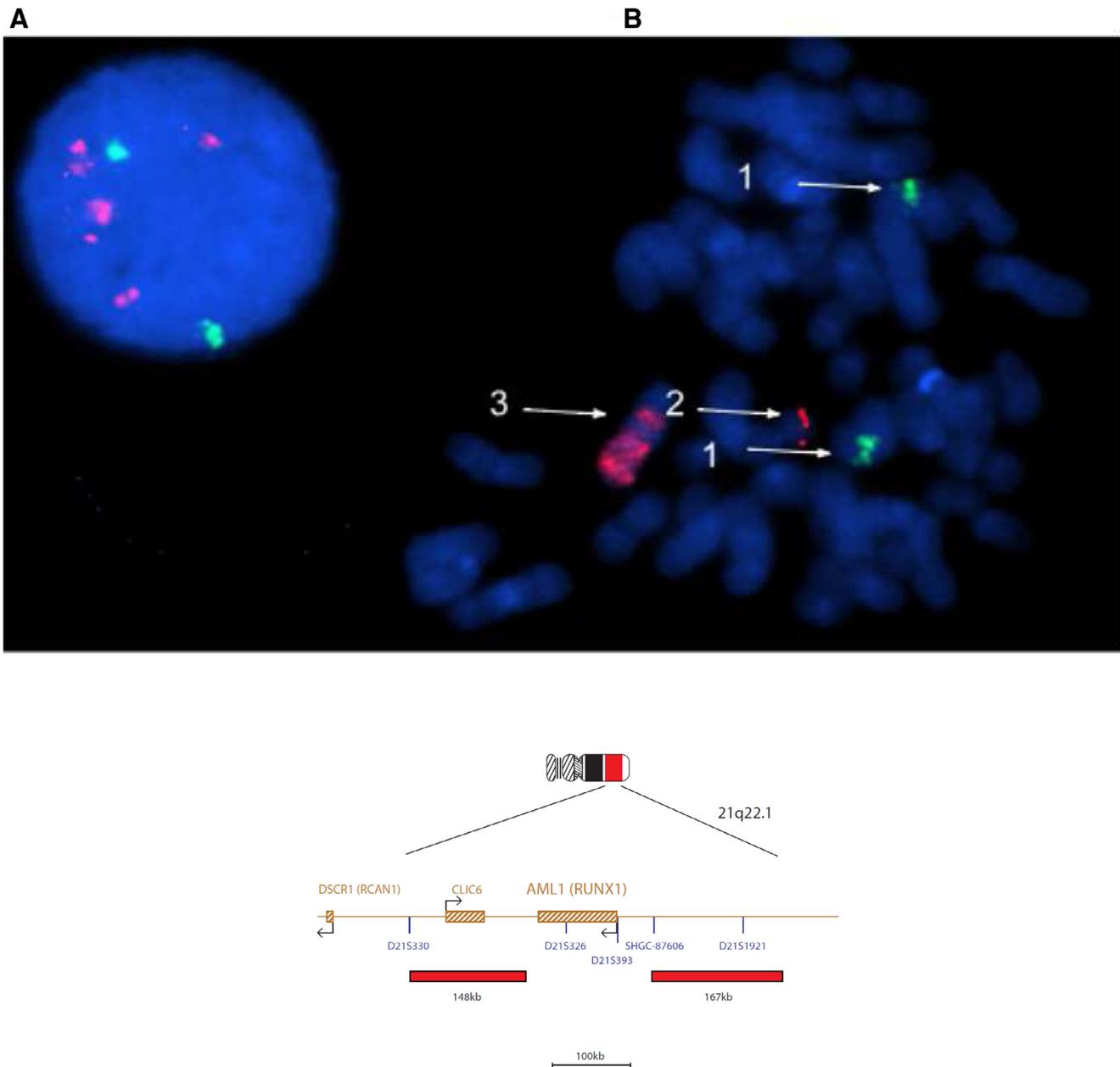


Fig. 2. FISH analyses with probes for *RUNX1/RUNX1T1* on an (A) interphase nucleus and (B) a metaphase spread. (A) Two *RUNX1T1* signals (green) and > 5 *RUNX1* signals (red) are detected. (B) Arrows indicate (1) *RUNX1T1* signals (green) on normal chromosome 8, (2) *RUNX1* signal (red) on normal chromosome 21, and (3) *RUNX1* amplified signal (red) on the der(21). Probe map of *RUNX1 (AML1)*. The *AML1* probe mix, labeled in red, consists of a 148 kb probe centromeric to the *RUNX1* gene, including the *CLIC6* gene and a 167 kb probe covering the telomeric end of the *RUNX1* gene and extending beyond the marker *D21S1921* [40].

and *PTEN*, *ATM* and *TP53* mutations, and complex karyotypes [18]. Moreover, it has been shown that chromothripsis can be used as an independent prognostic indicator in AML, conferring both a poor prognosis and decreased overall survival rate [18–20].

Taken together, the patient's cytogenetic and molecular genetic findings demonstrate chromothripsis resulting from genomic instability. In particular, the patient exhibited an additional unidentified marker chromosome, and loss of both *TP53* and 5q, which are find-

ings that are more frequently present in chromothripsis-positive AML patients with complex karyotypes [18–20]. These alterations have been linked to an adverse prognosis, chemoresistance, and an early relapse rate [18–20].

In cases of pediatric B-cell ALL and apparent amplification of chromosome 21, is *RUNX1* the true target of amplification or is it a bystander amplified with other genes? In 18 pediatric B-cell ALL cases, Rand et al. observed that recurrent aberrations affecting key pathways, involving *RB1*, *PAX5*, *ETV6*, *IKZF1*, and *CDKN2A/B*,

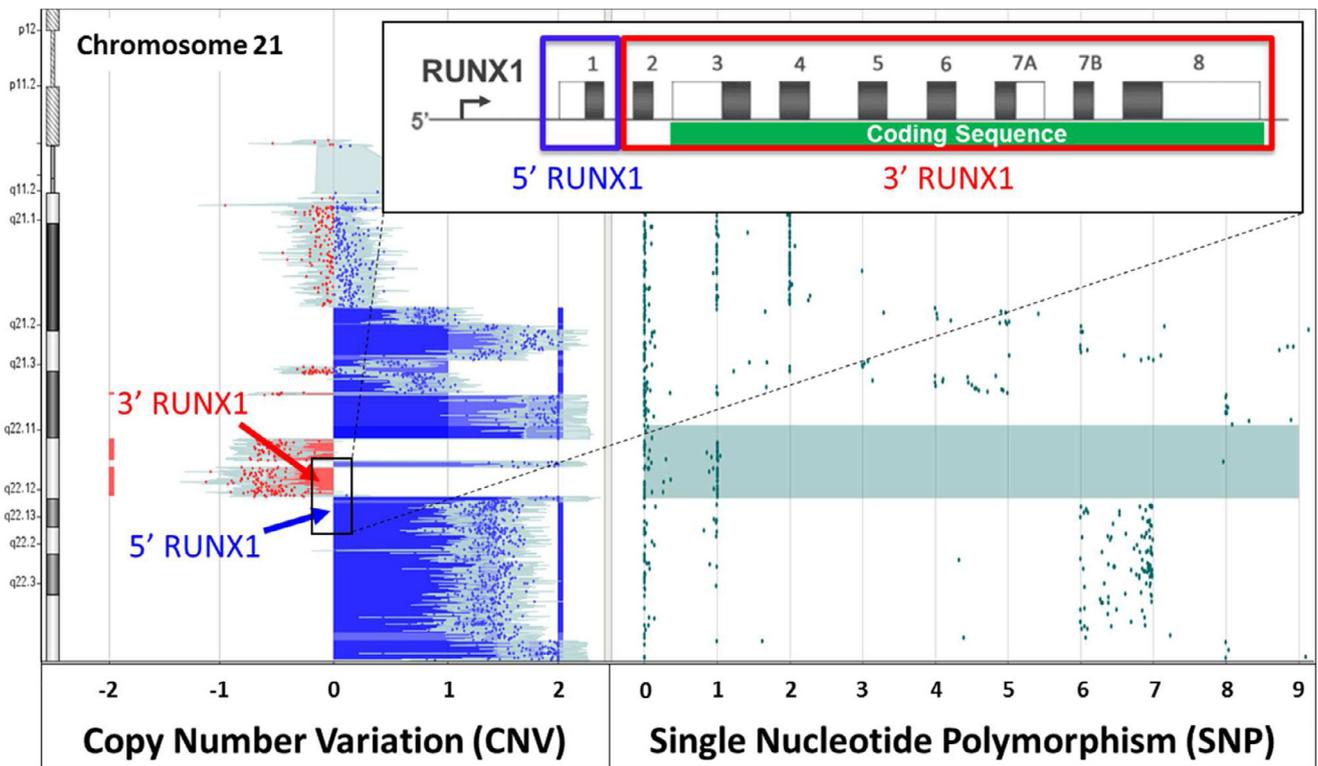


Fig. 3. CGH array showing chromosome 21 positioned vertically, with the centromeric to telomeric positions running top to bottom. Deleted regions are in red and amplified regions are in blue. Arrows indicate the location of 5' *RUNX1* amplification (blue) and 3' *RUNX1* deletion (red). *RUNX1* exons and introns shown along the top.

were secondary to chromosome 21 rearrangements [8]. Furthermore, *RUNX1* did not show significant overexpression using transcriptome analysis, thus Rand et al. inferred that *RUNX1* did not appear to be the likely target of amplification [8]. Similar findings were presented by Tsuchiya et al. in their *iAMP21* pediatric B-cell ALL case, *RUNX1* was not located within the highest region of amplification [12], thus concluding that *RUNX1* did not appear to be the primary target of amplification on 21q.

In cases of AML/MDS with complex karyotypes, Haferlach et al. concluded that gene amplifications are rare events and *KMT2A* (*MLL*) is the most frequently amplified gene [21]. Similarly, Mrózek et al. demonstrated that 11q, 21q, and 22q are regions that are typically overrepresented in AML [7]. In our patient, while no structural or numerical aberrations were detected in the 11q or 22q, aCGH detected a 11.779 Mb amplification at 21q22.1q22.3. Within this amplified region are three genes of interest that may play a role in myeloid leukemogenesis: *APP*, *ERG*, and *ETS2*. While *APP* has primarily been linked to Alzheimer's disease and dementia in adults with Down syndrome, *ETS2* and *ERG* are proto-oncogenic, transcription factors that are involved in cell cycle development and regulation. In their *RUNX1* amplification AML study, Baldus

et al. observed amplification of *ERG*, *APP*, and *ETS2* genes, with substantial mRNA expression of the *APP* and *ETS2* genes [5]. Similarly, Weber et al. observed higher genomic expression values of *ERG* in their *RUNX1* AML cohort [22]. In this patient, *ERG* and *ETS2* were found to be significantly amplified.

Alternatively, *RUNX1* may play a role in leukemogenesis through other mechanisms, such as fusion events. Aside from the *RUNX1-ETV6* rearrangement, the majority of *RUNX1* translocations that lead to a product will retain the N-terminal domain of *RUNX1* (5' sequence) with the C-terminal domain (3' sequence) of a gene partner [23]. The 5' sequence of *RUNX1* contains two alternate promoters (P1, P2) and the runt homology domain (RHD). Currently, 55 *RUNX1* fusion partner genes have been described, with only 21 gene partner translocations identified at the molecular level [24,25]. In this patient, the 5' sequence of *RUNX1* was amplified while the remaining gene was deleted. While this particular *RUNX1* aberration may not lead to expression of *RUNX1*, amplification of the promoter regions of *RUNX1* may favor expression of other genes through oncogenic fusion events.

Research into the prognostic impact of *RUNX1* alterations in AML is still ongoing. Haferlach et al. [26,27] concluded *RUNX1* gains and losses are more frequent in patients with adverse cytogenetics and *RUNX1* deletions are associated with a poor prognosis [21,22]. These findings are concordant with the patients' unfavorable cytogenetics: 5q deletion with a complex karyotype, *TP53* deletion, and amplification/deletion of *RUNX1*. Or *RUNX1* may be a red herring and overexpression of other genes such as *ERG* and *ETS2* may be the real culprit. In three different studies with a large cohort, it was validated that overexpression of *ETS2* and *ERG* are predictors of poor outcome in cytogenetically normal AML (CN-AML) patients [28–30]. Alternatively, 21q alterations that include *RUNX1* in complex karyotypes may not matter in AML; Canzonetta et al. found lack of *RUNX1*, *ETS2*, and *ERG* involvement in an AML patient with Down syndrome and a constitutionally normal individual [31]. Further studies are needed to better elucidate the link between 21q abnormalities and AML pathogenesis.

In this case report, we used an integrative approach to better characterize our patient's t-AML chromosomal alterations. For future hematologic studies involving apparent *iAMP21*, the *RUNX1* break-apart probe set should be utilized in conjunction with molecular studies to better detect cryptic *RUNX1* aberrations. To our knowledge, this particular AML *RUNX1* alteration, which includes a simultaneous amplification of the 5' non-coding region and loss of the *RUNX1* coding region has been reported previously by Haferlach et al. [21,22,26,27]. While not performed for this patient, it would be of particular interest to perform gene expression studies on future patients that develop AML with 21q intrachromosomal amplifications/deletions to identify potential genes involved in leukemogenesis. Such aims are in the hopes of discovering effective biomarkers to guide treatment options.

Declaration of Competing Interest

All the authors declare no conflict of interest in this work.

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