

Short communication

Cryptic $\text{ins}(4;11)(q21;q23q23)$ detected by fluorescence
in situ hybridization: a variant of $\text{t}(4;11)(q21;q23)$ in an infant
with a precursor B-cell acute lymphoblastic leukemia
report of a second case

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Abstract

We report the chromosomal findings in a 4-year-old female with precursor B-cell acute lymphoblastic leukemia (ALL). The diagnostic karyotype showed an isochromosome 7q, $\text{i}(7)(q10)$, as well as questionable rearrangements on 9p and 11q. Fluorescence in situ hybridization (FISH) studies on both interphase and metaphase cells using the *MLL* “break-apart” and the centromeric chromosome 4 probes were instrumental in the characterization of an *MLL* gene rearrangement, which was cryptic by conventional cytogenetic analysis. Specifically, the FISH pattern was consistent with an insertion of the 5' region of the *MLL* gene into chromosome 4 at band q21, most likely a variant $\text{t}(4;11)(q21;q23)$. This is the second case of FISH detection of an $\text{ins}(4;11)$ in ALL. Our case exemplifies the importance of FISH in the further characterization of precursor B-cell ALL cases without any apparent prognostically significant chromosomal abnormalities. © 2007 Elsevier Inc. All rights reserved.

1. Introduction

The *MLL* gene encodes a protein that contains regions with homology to the *Drosophila* trithorax gene [1] and it has been shown to be involved in nonrandom translocations with a number of different partner chromosomes in acute leukemia [2]. One of the most recurrent rearrangements involving 11q23 is the $\text{t}(4;11)(q21;q23)$, which results in the fusion of the *MLL* gene at 11q23 with the *AF4* gene at 4q21, and is characteristic of precursor B-cell acute lymphoblastic leukemia (ALL) [2]. This translocation is usually associated with an unfavorable prognosis and has been reported primarily in very young children (1–2 years of age; 50% under 4 years), but has also been observed in adults.

To our knowledge, this is the second case of an insertion of the *MLL* gene into chromosome 4 at 4q21 detected by fluorescence in situ hybridization (FISH) analysis [3]. We assume that this cryptic insertion, as results in the fusion of the 5' portion of the *MLL* gene with the *AF4* gene at 4q21, as is seen in the typical $\text{t}(4;11)(q21;q23)$. The translocation between the *MLL* and *AF4* may disrupt functional activities altering expression of genes regulated by *MLL* and *AF4*, or may create fusion proteins with transcriptional regulatory activity [3]. Unfortunately, a fresh sample from the diagnostic bone marrow in our patient was not longer available to perform molecular studies to further detect the presence of an *MLL/AF4* transcript. However, there is only another translocation involving chromosomes 4 and 11, which leads to a *SEPT11/MLL* fusion. According to published data, this fusion does not appear to be involved in leukemogenesis [4].

The case we report herein demonstrates that complementing conventional cytogenetics with FISH provides

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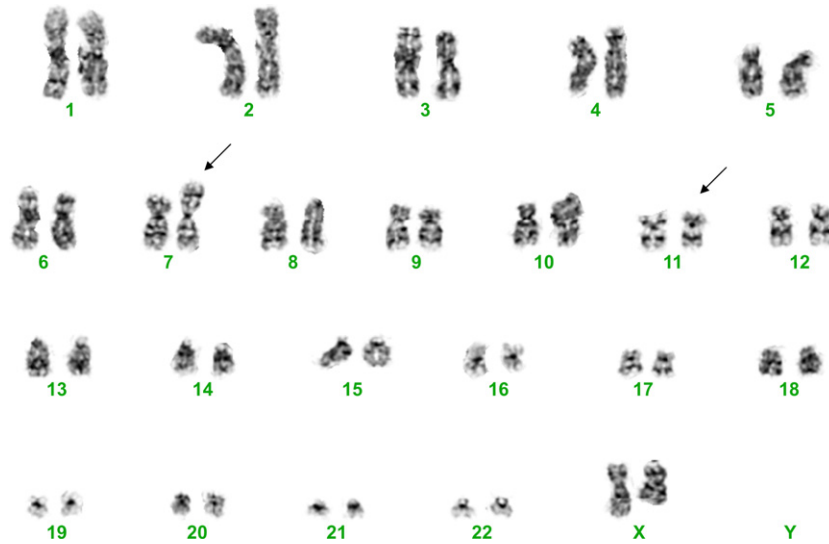


Fig. 1. G-banded karyogram of the patient interpreted according to FISH and chromosome results: 46,XX,ins(4;11)(q21;q23q23),i?(q10),?add(9)(p22). Although a rearrangement of 11q23 was suspected, the ins(4;11) was detected only by FISH.

a more complete characterization of ALL, which may have prognostic implications, and alternative therapeutic approaches may be necessary.

2. Clinical presentation

A 4-year-old female presented with bruises distributed over the trunk and extremities. The spleen was 3 cm below the rib margin at the midclavicular line, but there was no significant adenopathy or palpable hepatomegaly. The remainder of the physical exam was nonspecific.

Blood tests revealed thrombocytopenia (47×10^3 platelets/ μL), a white blood cell count of $12 \times 10^4/\mu\text{L}$, hemoglobin of 10 g/dL, and a hematocrit of 29%. Flow cytometry identified 95% blasts that were CD10⁻, CD19⁺, HLA-DR⁺, CD15⁺, and partial dim for terminal deoxynucleotidyl transferase. These results are consistent with a precursor B-cell lineage acute lymphoblastic leukemia. In particular, the CD10⁻, CD19⁺ finding is typical of ALL with an *MLL* rearrangement.

Induction chemotherapy, consisting of dexamethasone, vincristine, daunorubicin, peg-asparaginase, and intrathecal ara-c, was initiated. The repeat bone marrow aspirate on day 8 of therapy revealed no identifiable blasts remaining. However, screening of siblings to identify a human leukocyte antigen-identical donor for bone marrow transplantation is proceeding.

3. Material and methods

3.1. Chromosome analysis

A G-banded chromosome study was performed using standard cytogenetic techniques. Briefly, two unstimulated cultures were set up in RPMI 1640 medium enriched with

20% fetal calf serum, giant cell tumor conditioning media, L-glutamine, and antibiotics (penicillin and streptomycin). The cells were cultured for 24 and 48 hours in a humidified environment with 5% CO₂ in a 37°C incubator until harvest.

Before harvesting, the cultures were treated with Colcemid (25 μL) for 16–18 hours. Soon after, the cells were exposed to hypotonic solution (0.075 mol/L KCl) and fixed with methanol/acetic acid (3:1). The slides were prepared and stained using a G-banding (Trypsin-Giemsa-Wright) technique. Twenty-two metaphases were analyzed and karyograms were prepared using the CytoVision computer-assisted karyotyping system (Applied Imaging, Santa Clara, CA, USA). The karyotypes were described according to the International System for Human Cytogenetics Nomenclature (ISCN 2005) [5].

3.2. FISH analysis

The FISH procedure was performed on interphase and metaphase cells following the manufacturer's guidelines with minor modifications. The LSI *MLL* Dual Color, Break Apart Rearrangement probe (Abbott Molecular, Inc., Des Plaines, IL, USA) was used to determine the origin of a questionable rearrangement involving 11q23 which was seen during chromosome analysis. In addition, the CEP 4 SpectrumGreen probe (Abbott Molecular) was used to confirm that the other chromosome involved in the insertion was in fact chromosome 4. FISH studies with the p16 (9p21)/CEP9 probe (Abbott Molecular) were also performed to detect the presence of a rearrangement on 9p21.

After overnight hybridization and subsequent washing, interphase and metaphase cells were analyzed using a BX51/BX52 Olympus fluorescence microscope (Exfo America; Olympus, Richardson, TX, USA). Selected

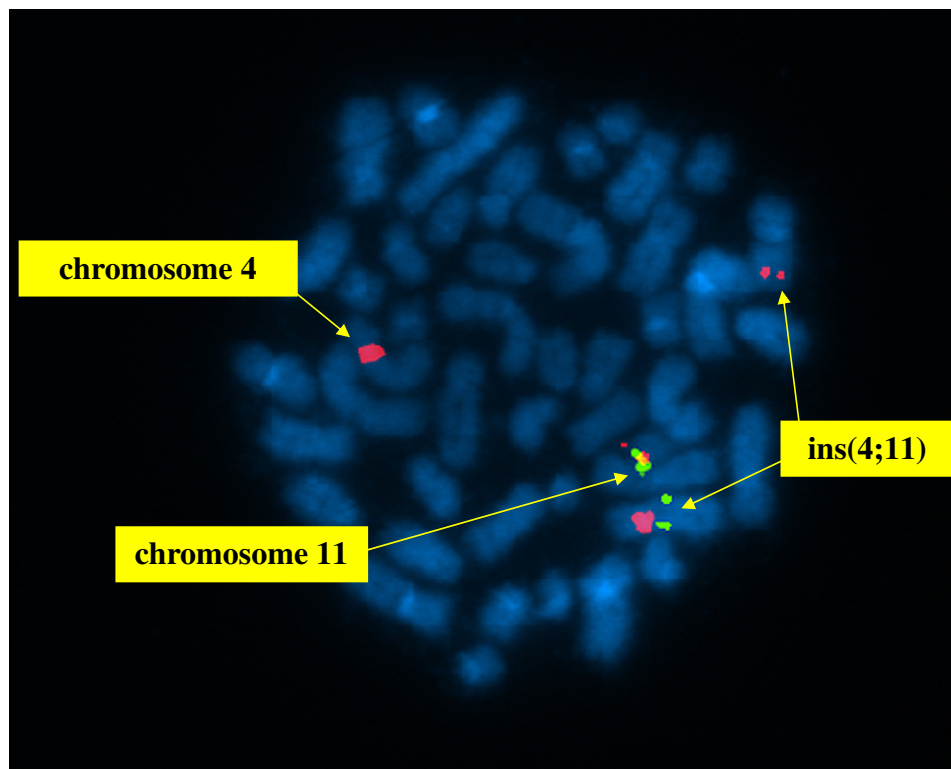


Fig. 2. FISH on metaphase cells showing the cryptic insertion of the proximal (5' region; green signal) of the *MLL* gene into chromosome 4 (large red signal on chromosome 4 centromere). The small red signal is still present on the rearranged chromosome 11, whereas the fusion indicates the normal chromosome 11.

images were captured using a CCD camera (Sensys; Photometrics, Tucson, AZ, USA) and CytoVision (Applied Imaging).

4. Results

4.1. Chromosome analysis

Routine chromosome analysis detected an abnormal female chromosome complement in 5/22 (23%) metaphases. The karyotype showed an *i*(7)(q10) as well as a possible loss of material from 11q and a questionable rearrangement on 9p. However, FISH detected a cryptic insertion involving chromosomes 4 and 11. Based on cytogenetic and FISH analyses, the karyotype was interpreted as: 46,XX,ins(4;11)(q21;q23q23),*i*(7)(q10),?add(9)(p22)[5]/46,XX[17]. Fig. 1 shows the *i*(7)(q10) and the questionable rearrangements on 9p and 11q.

4.2. FISH analysis

The observed FISH pattern was consistent with insertion of the 5' region of the *MLL* gene into one chromosome 4 at band q21. The centromeric probe for chromosome 4 confirmed that the insertion of the 5' *MLL* gene region involved this chromosome, leading to our interpretation of an insertion (4;11)(q21;q23q23) (Figs. 2 and 3). FISH did not detect a deletion of the 9p21 region. However, the question

of a possible rearrangement on 9p, possibly at band 9p22, still remains.

5. Discussion

To our knowledge, only one case of *MLL* gene insertion into chromosome 4, detected by FISH, has been reported to date [3]. Von Berg and colleagues described a 30-year-old male who presented with asthenia, anorexia, dyspnea, fever, and violent occipital headache. Conventional bone marrow cytogenetic analysis showed an apparently normal karyotype. However, FISH confirmed the presence of an insertion of the *MLL* gene into chromosome 4, and molecular studies confirmed an *MLL-AF4* transcript [3].

We assume that the cryptic insertion in our case also results in the fusion of the 5' portion of the *MLL* gene with the *AF4* gene at 4q21, as seen in the typical *t*(4;11)(q21;q23). The presence of the *i*(7q), which is an additional abnormality seen in approximately 10% of ALL cases with *t*(4;11), strengthens our assumption that this insertion is a variant of the typical *t*(4;11)(q21;q23). Unfortunately, there was no leftover diagnostic bone marrow in our patient to perform molecular studies to confirm the presence of an *MLL/AF4* transcript. However, there is only another translocation involving chromosomes 4 and 11, which leads to a *SEPT11/MLL* fusion. According to

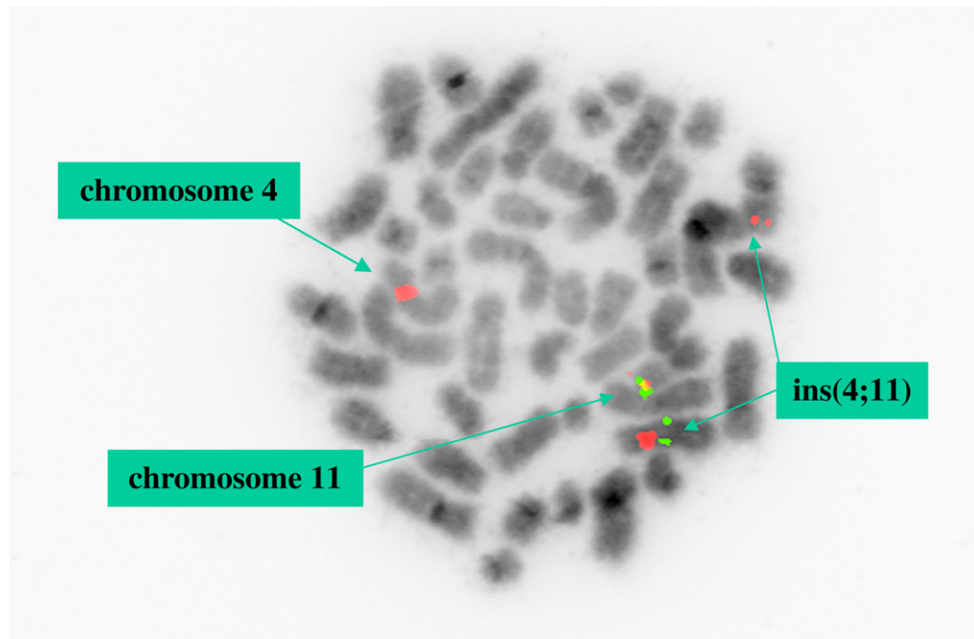


Fig. 3. Reverse DAPI image of the metaphase depicted in Fig. 2.

published data, this fusion does not appear to be involved in leukemogenesis [4].

Other cryptic insertions of *MLL* have been reported, including insertions into 10p and 5q [6–8]. Insertions are not unique to *MLL*, however. Cryptic rearrangements due to insertion have been described to involve chromosomes 15 and 17 in acute promyelocytic leukemia [9] and chromosomes 9 and 22 in chronic myeloid leukemia [10], as indicated also by Von Bergh et al. [3].

Chen et al. [2] showed a strong correlation between the presence of 11q23 molecular defects and prognostically adverse clinical factors, including young age, a CD10 negative immunophenotype, and a high leukocyte count. Their data also suggest that the outcome of infants with ALL may be strongly influenced by the presence or absence of 11q23 rearrangements.

In conclusion, this report exemplifies the importance of complementing conventional cytogenetics with FISH to better characterize chromosomal abnormalities in pre B-cell ALL, for the purpose of designing the best therapeutic approach in these leukemic patients.

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