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Case Report

Intrachromosomal Amplification of Chromosome 21 in Childhood Acute Lymphoblastic Leukemia: Study of 3 Cases

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Keywords

Intrachromosomal amplification of chromosome 21 · Acute lymphoblastic leukemia · Fluorescence in situ hybridization · Single-nucleotide polymorphismarray

Abstract

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood. The presence or absence of a characteristic genetic abnormality usually observed in childhood ALL plays a very important role in determining the prognosis and stratification for treatment. Intrachromosomal amplification of chromosome 21 (iAMP21) is an uncommon high-risk chromosomal abnormality than can occur only in 2% of childhood B-cell precursor lymphoblastic leukemia. Molecular genetic analysis and the fluorescence in situ hybridization (FISH) technique are the basic methods used to detect the presence of the most common genetic abnormalities, the presence or absence of which has an impact on the patient's classification into the appropriate risk group. This work presents 3 BCP-ALL iAMP21-positive patients who were detected during routine genetic diagnostics using the FISH method and microarray test.
iAMP21 is associated with a poor prognosis and high risk for relapse. Children with B-cell precursor lymphoblastic leukemia with this genetic entity are associated with a delayed treatment response. The FISH method and single-nucleotide polymorphism array provides a useful method to detect characteristic genetic changes.

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Introduction

The most common childhood malignancy is acute lymphoblastic leukemia (ALL). About 80–85% of cases are B-cell precursor ALL (BCP-ALL), while 15–20% are T-cell leukemias. Diagnostic tests of bone marrow blast cells allow the analysis of characteristic genetic abnormalities, usually determining prognosis and stratification for treatment in childhood ALL [1, 2]. Intrachromosomal amplification of chromosome 21 (iAMP21) is associated with a poor outcome and high relapse risk when the patients were treated by a standard therapy. Detection of iAMP21 is very significant to the choice of appropriate treatment [3–6]. Potentially, iAMP21 will be detected during routine tests by fluorescence in situ hybridization (FISH) if *ETV6/RUNX1* probes are used. The interphase nuclei show extra signals from the *RUNX1* probe, but metaphases show that signals are located on an abnormal chromosome 21. Some laboratories in routine diagnostics use the break-apart probe to detect only *ETV6* gene rearrangement. An important point is that the iAMP21 can be detected by the FISH method exclusively using the *ETV6/RUNX1* translocation probe or by performing a microarray test [2, 7–9]. We present 3 cases of BCP-ALL with amplification of *RUNX1* detected by FISH and microarray.

Case Presentation

Case One

A 14-year-old girl was admitted to the Department of Pediatric Hematology, Oncology, and Transplantology, Medical University of Lublin in Poland due to an anemia and weakness. Lymphadenopathy, hepatosplenomegaly, and the infiltration of the central nervous system were not found. The laboratory results were as follows: white blood cell (WBC) 1,950/μL, platelet count 120,000/μL, and hemoglobin level 10.8 g/dL. Evaluation of the bone marrow showed 73% blast cells. The girl was diagnosed with preB common positive ALL in May 2018 and she was treated according to the ALL Intercontinental-Berlin-Frankfurt-Münster 2009 (ALL IC-BFM 2009) protocol for the intermediate risk group.

Cytogenetic analysis was performed on a bone marrow sample. G-banded chromosome analysis revealed a female karyotype 47,XX,+mar/46,XX. Poor quality of the metaphase chromosomes made them impossible to evaluate. FISH was performed with use of the commercially available probes: *BCR/ABL1*, *KMT2A*, *ETV6/RUNX1* (Vysis, Abbott Molecular, Des Plaines, IL, USA). No rearrangements were found in *BCR/ABL1*, *KMT2A*, or *ETV6/RUNX1* tests. FISH analysis revealed between 6 and 9 signals of *RUNX1* in 80.6% of the analyzed nuclei. Metaphases showed that extra signals were clustered together and located on abnormal chromosomes difficult to identify (Fig. 1A). CytoScan HD array (Thermo Fisher Scientific, Waltham, MA, USA) was performed on the same sample of bone marrow. This method confirmed iAMP21 arr[GRCh37] 21q21.3q22.3(28054448_43559902)x4. The size of amplification was 15.5 Mb and contained the following genes: *DYRK1A*, *TMPRSS2*, *OLIG2*, *RUNX1*, *ADAMTS1*, *TIAM1*, *ETS2*, and *ERG*. Apart from this, analysis revealed duplication of a fragment of the long arm of chromosome 1, deletion within 4–7 exons of the *IKZF1* gene, deletion within chromosome 13 including the *RB1* gene (20–27 exon biallelic deletion), and deletion of band 21q22.3 (*PTTG1IP*, *S100B*, *TFF3*, *U2AF1*, *TMPRSS3*, *TFF1*, *TFF2*, *CSTB*). Loss of heterozygosity of the entire chromosome 22 was also demonstrated (Fig. 1B). FISH analysis revealed that additional signals were located on a large, hard to identify chromosome. Microarray analysis suggested translocation between chromosome 21 and a fragment of the long arm of chromosome 1. Performing a FISH test with whole chromosome painting probes for chromosomes 1 and 21 (Cytocell Ltd, Oxford Gene Technology, Cambridge, UK) confirmed translocation. Additional *RUNX1* signals were clustered together on this translocated chromosome.

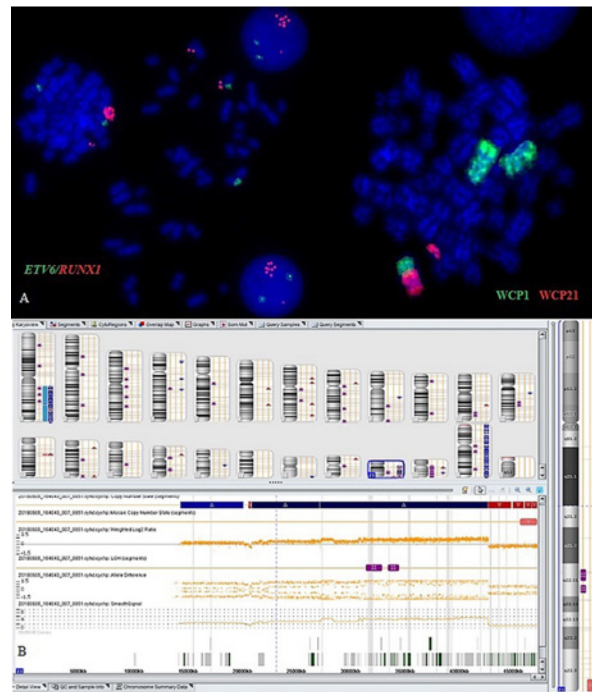

Fig. 1. Case 1. **A** FISH analysis on metaphase and interphase with an LSI *ETV6/RUNX1* ES Dual Color Translocation Probe Set (Vysis). **B** SNP array revealed the highest level of amplification located within the RUNX1 locus.

Case Two

The second patient was a 13-year-old boy who was admitted to the hospital because of recurrent infections and petechiae. Lymphadenopathy, hepatosplenomegaly, and the infiltration of the central nervous system were not observed. A diagnostic test revealed: WBC 1,800/μL, platelet count 21,000/μL, hemoglobin level 4.1 g/dL, and 90% leukemic cells in the bone marrow. The boy was diagnosed with BCP-ALL in August 2017. He presented a good treatment response to steroids, but was FMC-MRD-positive on day 15. Therefore, he was classified as high-risk group. According to the ALL IC-BFM 2009 protocol cytogenetic analysis and FISH were performed on a bone marrow sample. GTG band staining revealed an abnormal male karyotype with a marker chromosome probably derived from chromosome 21. The FISH study showed no rearrangements in *BCR/ABL1*, *KMT2A*, or *ETV6/RUNX1*. However, FISH revealed 8–9 signals of *RUNX1* in 91% interphase cells (Fig. 2A). CytoScan HD array revealed amplification of arr[GRCh37] 21q11.2q21.1(15006457_22491098)x4, including *NRIP1* and *MIR125B2* genes and arr[GRCh37] 21q21.3q22.3(28054448_44101079)x4 containing 12 genes: *TIAM1*, *OLIG2*, *RUNX1*, *DYRK1A*, *ERG*, *ETS2*, *TMPRSS2*, *TFF3*, *ADAMTS1*, *TFF1*, *TFF2*, and *TMPRSS3*. The size of amplification was 16.5 Mb. In addition, the test also showed deletion of band 21q22.3 (*PTTG1IP*, *S100B*, *CSTB*, *U2AF1*), loss of heterozygosity within the long arm of chromosome 12, and a mosaic loss of heterozygosity within the long arm of chromosome 1 (Fig. 2B). The patient qualified for matched unrelated donor bone marrow transplantation.

Case Three

The third patient was a 14-year-old boy who was admitted to hospital due to a fever that had lasted for 5 days, and pain of the lower limbs. Lymphadenopathy, hepatosplenomegaly, and the infiltration of the central nervous system were not observed, but the WBC count was 3,900/μL, platelet count was 359,000/μL, and hemoglobin level was 9.5 g/dL. Bone marrow evaluation showed 69% blasts. The boy was diagnosed with B-cell precursor common positive

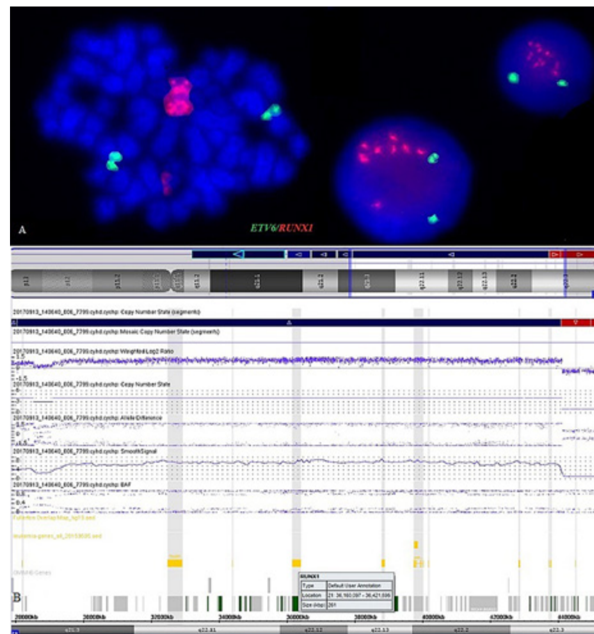

Fig. 2. Case 2. **A** FISH analysis on metaphase and interphase with an LSI *ETV6/RUNX1* ES Dual Color Translocation Probe Set (Vysis). **B** SNP array revealed the highest level of amplification located within the RUNX1 locus.

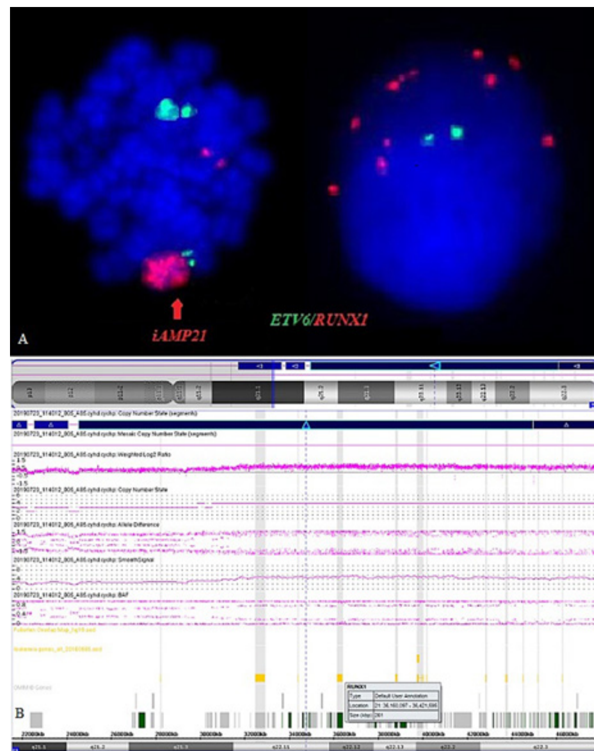

Fig. 3. Case 3. **A** FISH analysis on metaphase and interphase with an LSI *ETV6/RUNX1* ES Dual Color Translocation Probe Set (Vysis). **B** SNP array revealed the highest level of amplification located within the RUNX1 locus.

ALL and chemotherapy was started in July 2019, according to the AIEOP-BFM ALL 2017 protocol. He was classified into the early non-high-risk group. According to the protocol cytogenetic analysis and FISH were performed on a bone marrow sample. G-banded chromosome analysis revealed a male karyotype 45,XY,–21,+mar/46,XY. The FISH study revealed no rearrangements in *BCR/ABL1*, *KMT2A*, or *ETV6/RUNX1*, but revealed 8–10 signals of *RUNX1* in 24.4% of interphase cells (Fig. 3A). CytoScan HD array revealed amplification of arr[GRCh37] 21q22.11q22.3(31846769_47960084)x4. The size of amplification was 16.1 Mb and included

following genes: *OLIG2*, *TIAM1*, *ERG*, *ETS2*, *TMPRSS3*, *TFF2*, *TFF3*, *TFF1*, *DYRK1A*, *U2AF1*, *RUNX1*, *TMPRSS2*, *CSTB*, and *PTTG1IP*. Furthermore, analysis showed duplication of the entire long arm of chromosome 9 and deletion within chromosome 14q23.2-q31.3 (Fig. 3B).

Discussion

iAMP21 defines a distinct cytogenetic subgroup of childhood BCP-ALL which was identified in 2003 [8]. It was detected by performing FISH for the presence of the *ETV6/RUNX1* fusion. This entity is a rare chromosomal abnormality that can occur in approximately 2% of pediatric patients with BCP-ALL. Patients with BCP-ALL and iAMP21 are characterized by older age and a low WBC count, similar to our presented patients [3, 5, 7, 8, 10]. Moorman et al. [5] observed that the presence of this abnormality is associated with an increased risk of relapse. Overall survival and event-free survival are inferior in patients with iAMP21, as was observed by Heerema et al. [4]. Our patients are in a stable and continuous state of remission, but the observation time is too short. The 5.1-Mb region, which included the *RUNX1* gene, miR-802, and genes mapping to the Down syndrome critical region, is the most common region of amplification on chromosome 21 [3, 10]. In our cases, CytoScan HD array revealed size of amplification from 15.5 to 16.5 Mb. In addition, some patients present a deletion at the telomeric end of chromosome 21 [2, 10]. Microarray analysis revealed a deletion of chromosome 21 at the telomeric end in 2 of our 3 patients.

The most common method to identify this entity is FISH. iAMP21 is defined as a presence of three or more extra copies of *RUNX1* on a structurally abnormal chromosome 21 or a total of five or more *RUNX1* signals per cell. The *RUNX1* gene is used as a marker, although there is no evidence that this gene is the target of this abnormality [2, 11]. Recent studies have shown that the most common genetic abnormality among patients with iAMP21 is chromosomal instability of chromosome 21, which suggests that it is a primary genetic event [3, 10].

RUNX1 is a transcription factor which plays a very important role in regulating the development of hematopoiesis and hematopoietic stem cell homeostasis in mammals. *RUNX1* regulates the expression of the hematopoiesis-specific genes, including cytokines such as IL-3 and GM-CSF, cytokine receptors, for example M-CSFR, T and B receptors, and megakaryocyte-specific genes such as PF4. Furthermore, *RUNX1* is the most frequently mutated gene of hematological malignancies, such as acute myeloid leukemia, ALL, chronic myelomonocytic leukemia, and myelodysplastic syndrome. In childhood ALL, the most common chromosome abnormality is t(12; 21), which is present among 17% of young patients. The fusion is generated by t(12; 21) between *ETV6* on chromosome 12 and *RUNX1* on chromosome 21 (*ETV6/RUNX1*). This abnormality leads to loss or impairment RUNX1 function [10, 11].

In routine diagnostics, detection of iAMP21 is possible only with a probe that detects *ETV6/RUNX1* translocations. In this way we can detect one of the most common changes t(12; 21) and one of the rare changes: iAMP21. Conventional G-banding karyotype and the FISH technique is a standard method for detecting the most common structural abnormalities observed in BCP-ALL (*BCR/ABL1*, *KMT2A*, *ETV6/RUNX1*, *TCF3*). However, cytogenetic analysis sometimes has a diagnostic problem because of cell culture failure or poor quality of GTG staining. FISH analysis is mostly aberration specific and requires a large number of unique probes [12]. For this reason, our laboratory performs molecular karyotype tests as a standard for patients with BCP-ALL. Single-nucleotide polymorphism (SNP) array is a powerful tool to complete a conventional cytogenetic (CC) analysis and FISH method. This method is able to detect numerical and structural chromosomal rearrangements, gains and deletions of genomic regions (copy number variations), and copy-neutral loss-of-heterozygosity which cannot be detected by CC and FISH. Chromosomal microarray analysis enables detection of chromo-

somal aberrations at the exon level [12–14]. This method helps identify recurrent genetic abnormalities occurring in ALL: *IKZF1*, *CDKN2A/B*, *PAX5*, *ETV6*, *ERG*, and *RB1* [15]. According to the new AIEOP-BFM ALL 2017 protocol the SNP microarray is recommended for determining the status of the IKZF^{plus} group in children with BCP-ALL [12, 14]. The poor quality of metaphase chromosomes in our patients made them difficult to evaluate. Genetic changes were identified by molecular karyotyping.

Analysis of SNP microarray revealed that among our patients they all had *ERG* gene amplification, one additionally had deletion in the *IKZF1* and *RB1* genes. Furthermore, for our first patient SNP microarray helped to determine the origin of the marker chromosome.

iAMP21 is an uncommon abnormality which can be identified in the routine process of searching for *ETV6/RUNX1* fusion. iAMP21 has been associated with a poor prognosis and this genetic entity is important for classification into the appropriate risk group. Using the SNP microarray method, we observed other abnormalities which cannot be observed in cytogenetic analysis, the presence of which may be relevant to patient stratification into the relevant risk group.

Statement of Ethics

This study was approved by the ethics committee of the Medical University of Lublin, Poland (reference No. KE-0254/222/2012). Written informed consent was obtained from the parents of the patients for publication of the case report and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.L. was responsible for the conception and design of the study. M.L. and A.M. conducted the laboratory work. M.L. and A.M. were responsible for the interpretation of data. A.M. prepared the final manuscript for publication. All authors read and approved the final manuscript.

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