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A Cryptic *ETV6/ABL1* Rearrangement Represents a Unique Fluorescence *In Situ* Hybridization Signal Pattern in a Patient with B Acute Lymphoblastic Leukemia

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Although rearrangements involving each *ETV6* and *ABL1* gene are some of the most commonly observed chromosomal translocations in hematological malignancies [1, 2], an *ETV6/ABL1* rearrangement is very rare, and only 29 such cases have been reported to date [3]. This fusion has been associated with a wide range of hematological malignancies, including 11 cases of atypical chronic myeloid leukemia (*BCR/ABL1* negative), 3 cases of myeloproliferative neoplasm (MPN), 5 cases of acute myeloid leukemia, and 10 cases of ALL. The rarity of this rearrangement is related to the inverse transcriptional orientation of the 2 genes relative to the centromeres, because it would involve at least 2 events to form an in-frame fusion transcript [3]. Here, we report a case of B-ALL with *ETV6/ABL1* rearrangement.

A 30-yr-old woman, 12 weeks pregnant with her second child, visited the Samsung Medical Center emergency room because of a complete blood count (CBC) abnormality detected at a referring hospital. She experienced cough and sore throat for 3 weeks prior to her visit, and headache, dyspnea, and tender gums occurred several days before presentation. A peripheral blood count revealed white blood cell counts 227.47×10^9/L with 85% blasts, hemoglobin 9.3 g/dL, platelet counts 39×10^9/L, and serum lactate dehydrogenase 2,316 U/L. Bone marrow aspirate smear revealed diffuse infiltration by lymphoblasts (75%), which expressed CD19, CD10, CD20, cCD22, cCD79a, HLA-DR, CD34, terminal deoxynucleotidyltransferase (TdT), and aberrant CD33; thus, the patient was diagnosed as having B-ALL. Conventional chromosome analysis of bone marrow cells indicated trisomy 5 as the sole abnormality in 19 of 20 metaphases analyzed. Multiplex reverse transcription (RT)-PCR indicated positivity for the 1,141-bp *ETV6/ABL1* type B fusion transcript (exons 1-5 of *ETV6* fused to exon 2 of *ABL1*) and also weak positivity for the type A fusion transcript (exons 1-4 of *ETV6* fused to exon 2 of *ABL1*). Sequence analysis of fusion transcripts using the Sanger method confirmed the fusion of *ETV6* exon 5 to *ABL1* exon 2, and the existence of 2 different transcripts, type A and B, was evidence for alternative splicing (Fig. 1G).

Since HemaVision (DNA Technology, Aarhus, Denmark) and Sanger sequencing had already revealed the *ETV6/ABL1* rearrangement, we performed FISH analysis using the following commercially available locus-specific identifiers (LSI): *ETV6/RUNX1* Extra Signal (ES), *BCR/ABL* Dual Fusion (DF), and *ETV6* Break Apart (BA) DNA probes (Abbott Vysis, Des Plaines, IL,

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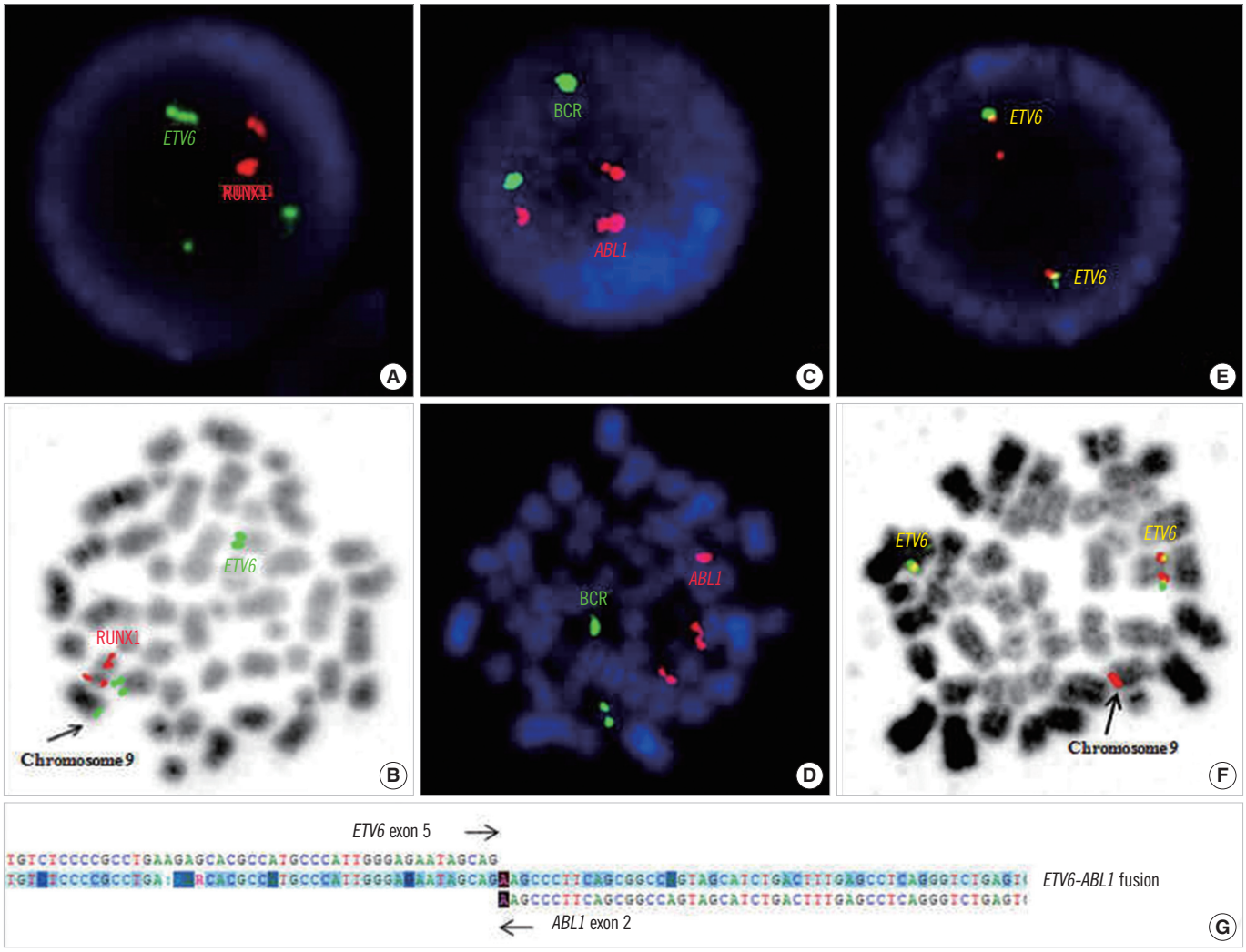

Fig. 1. FISH analysis using locus-specific identifiers for interphase and metaphase cells of bone marrow aspirate. (A) LSI *ETV6/RUNX1* (ES) probe on interphase cells, (B) LSI E*TV6/RUNX1* (ES) probe on metaphase cells, (C) LSI *BCR/ABL1* (DF) probe on interphase cells, (D) LSI *BCR/ABL1* (DF) probe on metaphase cells, (E) LSI *ETV6* (BA) probe on interphase cells, (F) LSI *ETV6* (BA) probe on metaphase cells, and (G) Sequence of fusion transcript products spanning the fusion site, revealing fusion of *ETV6* exon 5 to *ABL1* exon 2.
Abbreviations: LSI, locus-specific identifier; DF, dual fusion; ES, extra signal; BA, break-apart.

USA). LSIs were used in both interphase and metaphase cells of bone marrow aspirate (Fig. 1A-F). The *BCR/ABL* DF probe revealed interphase cells with 2 green signals for *BCR* and 3 orange signals for *ABL1*, suggesting a rearrangement of *ABL1*. The *ETV6/RUNX1* ES probe revealed 2 green and 2 orange signals for *ETV6* and *RUNX1*, respectively, with a third, albeit significantly smaller, *ETV6* signal. The *ETV6* BA probe revealed an additional orange signal for the 5' end of *ETV6* with 2 normal fusion signals. These results were different from what we had expected, since *ETV6/ABL1* rearrangement with a breakpoint at intron 5 of *ETV6* may not affect the *ETV6* signal, encompassing exons 1-5 of *ETV6* on the *ETV6/RUNX1* ES probe, and also may not produce split *ETV6* signals on the BA probe. Metaphase cell analysis revealed abnormal signals for *BCR* and *ETV6* on chromosomes 12 and 9, respectively.

Collectively, these results indicate a fusion between *ETV6* and *ABL1* with inverse chromosomal orientation, which could occur via 2 events (Fig. 2): 1) a balanced t(9;12)(q34;p13) results in the juxtaposition of part of *ABL1* (exons 2-10) in the vicinity upstream of *ETV6*, exon 1 at 12p13; followed by 2) an inversion within the 12p13 segment of der(12)t(9;12) after breakage at intron 5 of *ETV6*, and the boundary of the *ABL1* segment bordered by upstream of *ETV6*. The International System for Human Cytogenetic Nomenclature (ISCN) for the abnormal clone demon-

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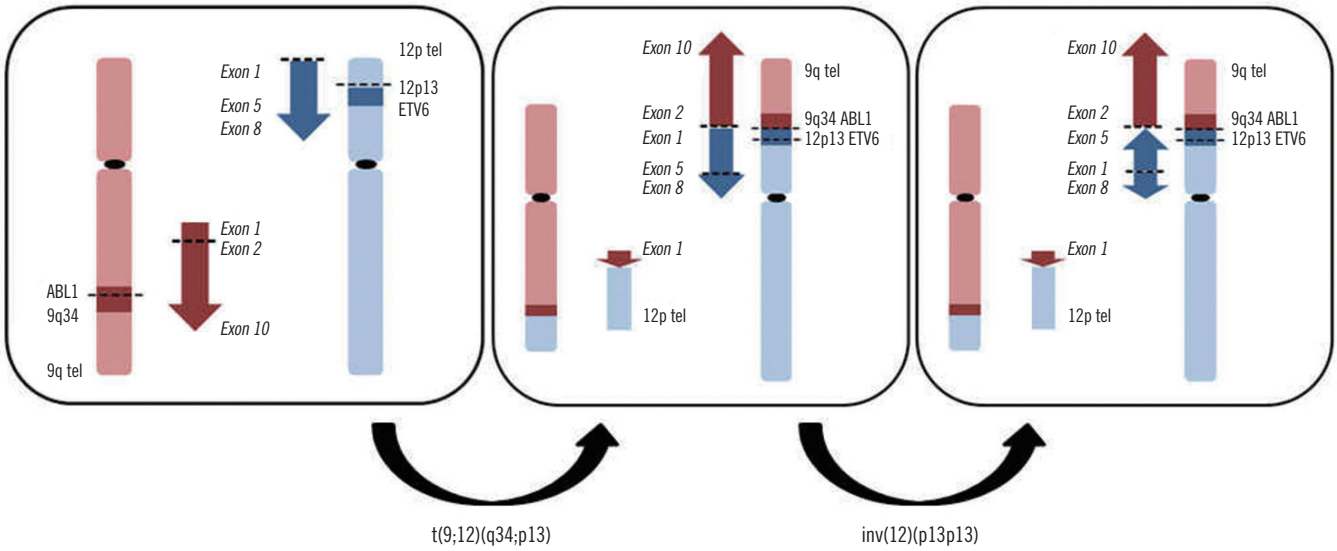

Fig. 2. Schematic representation of the suspected chromosomal mechanism leading to *ETV6/ABL1* rearrangement in this case.

strated by FISH is: 47,XX,+5. ish der(9)t(9;12)(q34;p13),der(12)t(9;12)(q34;p13)inv(12)(p13p13). The patient received induction chemotherapy and, after consolidation treatment, underwent allogenic peripheral blood stem cell transplantation; however, she experienced relapse of B-ALL 5 months after transplantation.

There are various descriptions in the literature of in-frame fusion products involving *ETV6* and *ABL1* with varying orientations, but *ETV6/ABL1* rearrangement is often not detected using conventional chromosome analysis because of the cryptic nature of this rearrangement [4]. These cryptic rearrangements were also due to similar G banding patterns of the long arm of chromosome 9 and short arm of chromosome 12. RT-PCR screening for leukemia-related fusion transcripts (using methods such as HemaVision) is a useful tool to detect these cryptic rearrangements; however, this RT-PCR screening has been mainly performed in acute leukemia and not in other hematologic malignancies, such as MPN. Therefore, the frequency of *ETV6/ABL1* rearrangement among total hematologic malignancies is likely underestimated.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest to this article were reported.

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