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Identification of the *TAF15–ZNF384* fusion gene in two new cases of acute lymphoblastic leukemia with a t(12;17)(p13;q12)

Kaja Beate Nyquist [a,b,c,*], Jim Thorsen [a,c], Bernward Zeller [d], Alf Haaland [e], Gunhild Trøen [f], Sverre Heim [a,b,c], Francesca Micci [a,c]

[a] *Section for Cancer Cytogenetics, Institute for Medical Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;* [b] *Faculty of Medicine, University of Oslo, Oslo, Norway;* [c] *Center for Cancer Biomedicine, University of Oslo, Oslo, Norway;* [d] *Department of Pediatrics, Oslo University Hospital, Rikshospitalet, Oslo, Norway;* [e] *Department of Hematology, Oslo University Hospital, Ullevål, Oslo, Norway;* [f] *Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*

We report the clinical, cytogenetic, and molecular data of two patients diagnosed with acute lymphoblastic leukemia characterized by the rare translocation t(12;17)(p13;q12). This translocation has been reported in 25 cases and its putative molecular consequence, the formation of a *TAF15–ZNF384* fusion gene, in only six cases. We used fluorescence in situ hybridization followed by long-range polymerase chain reaction to find the translocation breakpoints. A fusion between *TAF15* and *ZNF384* was identified and confirmed by nucleotide sequencing. Our results confirm that the t(12;17)(p13;q12) leading to a *TAF15–ZNF384* fusion gene characterizes a specific subgroup of acute lymphoblastic leukemia and suggest that two different breakpoints in *TAF15* may be involved. Whether the two variants of the *TAF15–ZNF384* fusion that these correspond to are in any way hematologically or prognostically different, is unknown.

Keywords Acute lymphoblastic leukemia, fusion gene, *TAF15–ZNF384*

Acute lymphoblastic leukemia (ALL) is among the most common malignancies in children, comprising about 20% of all pediatric cancer (1). An abnormal karyotype is found in the majority of ALLs (2,3). Besides numerical and other unbalanced chromosomal changes, whose pathogenetic impact is unknown, ALL may also be brought about by balanced chromosomal translocations leading to the formation of fusion genes with leukemogenic properties.

The t(12;17)(p13;q12) is a relatively rare leukemia-associated translocation that has been reported in 25 cases to date, most of them ALL patients (4). One study found that it leads to fusion between the zinc finger protein 384 (*ZNF384*) gene and the TATA box binding protein (TBP)-associated factor (*TAF15*) gene (5). *ZNF384* maps to chromosomal band 12p13, approximately 5 Mb upstream of *ETV6,* whereas *TAF15* maps to 17q12. The oncogenic properties of the *TAF15–ZNF384* fusion are not fully understood, although cells expressing the fusion gene show transforming properties *in vitro* (5).

We describe two new ALL cases with a t(12;17)(p13;q12), in one case as the only cytogenetic abnormality and in the other as part of a complex karyotype. In both cases, molecular cloning and sequencing of the breakpoint confirmed the presence of a *ZNF384/TAF15* fusion gene.

Case histories

Case 1

A 19-year-old man was admitted to Ullevål University Hospital in 2008 because of fatigue, palpitations, weight loss, sweating, and headache. The physical examination revealed no lymphadenopathy, hepatomegaly, or splenomegaly. Laboratory examinations at the time of presentation revealed severe anemia, leukocytosis, and thrombocytopenia (Table 1). Except for an elevated international normalized ratio of prothrombin time, clotting parameters were normal. A

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* Corresponding author.
E-mail address: k.b.nyquist@medisin.uio.no

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Table 1 Clinical, hematological, and immunophenotypic findings at diagnosis[a]

Characteristic	Case 1	Case 2
Sex/age at diagnosis (y)	M/19	F/3
Follow-up (mo)	27	96[†]
WBC count (/L)	92×10^9	36.5×10^9
Hemoglobin (g/dL)	4.8	2.5
Platelets (/L)	40×10^9	10×10^9
Immunophenotype[b]	($CD10^+$), $CD19^+$, $CD22^+$, $CD34^+$, $CD38^+$, $CD45^+$, $CD58^+$, $CD123^+$, TdT^+, DR^+	($CD10^+$), $CD19^+$, $CD22^+$, ($CD33^+$), $CD79a^+$, TdT^+, DR^+

Abbreviation: WBC, white blood cell.
[a] In case 2, immunophenotyping was performed at the time of first relapse.
[b] Only positive markers are shown. Markers with weak or partial expression are shown in parentheses.
[†] Deceased.

peripheral blood smear showed 80–90% blast cells. A bone marrow biopsy/smear revealed extensive hypercellularity dominated by small, homogeneous blasts with a single inconspicuous nucleolus and abnormal cytoplasm. Initial molecular analysis with reverse transcriptase–polymerase chain reaction (PCR) (6) showed no *BCR–ABL1* fusion transcript or nucleophosmin 1 (*NPM1P1*) mutation, but an internal tandem duplication mutation was found in the fms-related tyrosine kinase 3 (*FLT3*) gene (LeukoStratFLT3 Mutation Assay, InVivoScribe Technologies, San Diego, CA). Analysis of fluid obtained by lumbar puncture revealed no indication of central nervous system involvement. The patient was diagnosed as having B-precursor ALL, and treatment was initiated according to the Hammersmith protocol (7). After completion of the induction therapy, a full repeat examination of the bone marrow was performed; we concluded that complete hematological remission had been achieved, and flow cytometry detected no residual leukemic cells. At the time of writing, 19 months after diagnosis, the patient was still in complete remission.

Case 2

A 3-year-old girl was diagnosed with ALL after a period of malaise, weight loss, and cutaneous and mucosal bleedings, and treatment was initiated with multiple chemotherapeutics in her home country in Eastern Europe. The patient then moved to Norway, where treatment was discontinued 2 years after diagnosis, when no evidence of residual leukemia was observed. No information on possible initial immunophenotyping, cytogenetic, or molecular genetic data is available for the initial diagnosis and treatment given abroad. At 4.5 years after the first diagnosis, the patient experienced renewed symptoms of malaise, and a bone marrow investigation revealed relapse of ALL with 35% lymphoblasts. Immunophenotyping showed a B-precursor ALL (Table 1), and a bone marrow sample was sent for cytogenetic investigation. Treatment was begun according to the NOPHO (Nordic Society for Paediatric Hematology and Oncology) ALL 1992 extra high risk protocol (8) and resulted in a new complete remission. However, immediately before the planned bone marrow transplantation, the patient experienced a second relapse. Despite further therapeutic efforts, she died 2 months later, 8 years after the initial diagnosis.

Materials and methods

The study was approved by the Norwegian Directorate of Health and the Norwegian Biobank Register. Informed consent was obtained from the patients alive during the study period.

G-banding and karyotyping

Bone marrow aspirates were received from the disease debut in case 1 and from the relapse in case 2 and were then short-term cultured according to standard protocols (9). Chromosome preparations were G-banded with trypsin (Difco Laboratories, Detroit, MI) and Leishman staining (BDH, Poole, England). Cytogenetic analysis was performed, and the karyotype was written according to the International System for Human Cytogenetic Nomenclature (ISCN) 2009 guidelines (10).

Fluorescence in situ hybridization analysis

The commercial LSI TEL/AML1 dual-color translocation probe (Abbott, Downers Grove, IL) and bacterial artificial chromosome (BAC) probes were used to identify the position of the breakpoints brought about by the t(12;17) in both cases (see below). The BAC clones were retrieved from the RPCI-11 Human BAC and CalTech Human BAC libraries (P. De Jong Libraries; http://bacpac.chori.org/home.htm). The probes were selected according to their physical and genetic mapping data on chromosomes 12 and 17 as reported by the 2009 assembly of the UCSC Genome Browser database (11). The clone RP11-151M4 maps to 12p13 and overlaps the *ZNF384* gene, whereas the clones CTD-2258F24, RP11-362K1 (accession number AC015849), and CTD-3168J11 map to 17q12 and overlap the *TAF15* gene. All clones were grown in selective media, and DNA was extracted according to standard techniques (12). DNA probes were directly labeled with a combination of fluorescein isothiocyanate (FITC)-12-deoxycytidine triphosphate (dCTP), FITC-12–2-deoxyuridine triphosphate (dUTP), Texas Red-6-dCTP, and Texas Red-dUTP (PerkinElmer, Waltham, MA) by nick translation. Briefly, 1 μg extracted BAC DNA was mixed with 20 μL DNA polymerase I/DNase I (Invitrogen, Carlsbad, CA), 1 μL nucleotide mix (1 mmol/L), and 1.5 μL labeled nucleotides