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An Adult Patient with Early Pre-B Acute Lymphoblastic Leukemia with t(12;17)(p13;q21)/ZNF384-TAF15

NIKOLAOS GEORGAKOPOULOS[1], PANAGIOTIS DIAMANTOPOULOS[2], FRANCESCA MICCI[3], NEFELI GIANNAKOPOULOU[2], KONSTANTINOS ZERVAKIS[2], AGLAIA DIMITRAKOPOULOU[4] and NORA-ATHINA VINIOU[2]

[1]*Department of Cytogenetics and Molecular Pathology, Locus Medicus S.A, Athens, Greece;*
[2]*Hematology Unit, First Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece;*
[3]*Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, Oslo University Hospital, Oslo, Norway;*
[4]*Department of Immunology and Histocompatibility, Laikon General Hospital, Athens, Greece*

Abstract. *This is a case report of a 46-year-old man diagnosed with early pre-B acute lymphoblastic leukemia (ALL), bearing the translocation t(12;17)(p13;q21) as the sole chromosomal abnormality. This is a rare chromosomal abnormality that has been reported in approximately 25 cases worldwide. FISH analysis revealed a rearrangement of ZNF384 (12p13) and TAF15 (17q12) genes, which is usually associated with a pre-B ALL phenotype with co-expression of the myeloid markers CD13 and/or CD33. ZNF384 encodes a zinc finger protein, which acts as a transcription factor, regulating the expression of several matrix metalloproteinases and TAF15 belongs to the FET (FUS, EWS, and TAF15) family, consisting of RNA and DNA-binding proteins. Unlike most of the cases where CD10 expression was absent or weak, in our case CD10 was highly expressed. The prognostic significance of ZNF384/TAF15 fusion is not very clear since several reports support a generally good prognosis, while others support a poor clinical outcome. Our patient was treated with the German multicenter ALL (GMALL) protocol for B-ALL, but experienced a fulminant gram-negative sepsis and eventually died during induction therapy.*

Correspondence to: Panagiotis Diamantopoulos, MD, PhD, Hematology Unit, First Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, 11527, Greece. Tel: +30 213 2061985, +30 6976776260, Fax: +30 2132061795, e-mail: pandiamantopoulos@gmail.com

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Translocation t(12;17)(p13;q21) is very rare and has been reported in 25 cases worldwide (1), either as a sole cytogenetic abnormality or coexisting with other chromosomal abnormalities (2-5), mainly in patients with pre-B acute lymphoblastic leukemia (ALL). Only four cases of acute myelogenous leukemia (AML) with t(12;17)(p13;q21) have been reported, one of which was first diagnosed as pre-B ALL, but on the second relapse it was transformed to AML (6). All but one cases (7) were positive for CD13 and/or CD33.

In 11 cases, molecular testing was performed and a fusion of *ZNF384* (mapping on 12p13) and *TAF15* (mapping on 17q21) genes was revealed (Table I). *ZNF384* encodes for a zinc finger protein, which acts as a transcription factor, regulating the expression of several matrix metalloproteinases (*MMP1, MMP3, MMP7* and *COL1A1*) (12). TAF15 belongs to the FET family (*FUS, EWS* and *TAF15*), formerly known as TET family, consisting of RNA and DNA-binding proteins. More specifically, TAF15 is a subunit of transcription factor II D (TFIID), which is part of the polymerase II complex that is formed on the gene promoter site (13). Rearrangements of *ZNF384* have been reported with several other partner genes such as *TCF3* (on 19p13.3), *EP300* (on 22q13.2), *EWSR1* (on 22q12.2) or *ARID1B* (on 6q25.3) (14). Again, all gene alterations were reported in patients with pre-B ALL and expressed CD13 and/or CD33.

Case Presentation

A 46-year-old Greek Caucasian man was admitted due to leukocytosis detected during evaluation of a 2-month history of malaise and a 20-day history of fever without an identifiable site of infection. His past medical history was unremarkable.

Table I. *Published cases with TAF15/ZNF384 gene fusion confirmed by the molecular technique.*

Case	S/A	Diagnosis	Karyotype	WBC count ($\times 10^9$/l)	Myeloid markers CD13and /or CD33	CD10 status	Reference
1	M/24	Pro B-ALL	45,X,-Y,t(12;17)(p13;q11)(10)/46,XY(1)	22.9	CD13$^+$, CD33$^-$	CD10$^-$	La Starza *et al.* (8)
2	F/44	Pro B-ALL	46,XX,t(12;17)(p13;q11)(5)/46,idem, del(6)(q16q21)(3)/46,XX(8)	2.9	CD13$^+$, CD33$^+$	CD10$^-$	La Starza *et al.* (8)
3	F/16	Pro B-ALL	46,XX,t(12;17)(p13;q11)(15)	30.4	CD13$^+$, CD33$^-$	CD10$^-$	La Starza *et al.* (8)
4	F/26	Pro B-ALL	46,XX,t(12;17)(p13;q11)(2)/46,XX(7)	4.8	CD13$^-$	CD10$^-$	La Starza *et al.* (8)
5	M/7	Pro B-ALL	46,XY,t(12;17)(p13;q11)(15)/46,XY(3)	7.2	CD33$^+$	CD10$^+$	La Starza *et al.* (8)
6	M/29	AML	46,XY,t(12;17)(p13;q11)(2)/46,idem, i(8)(q10),inc(9)/46,XY(6)	65.6	CD13$^+$, CD33$^+$	NR	La Starza *et al.* (8)
7	F/25	Pro B-ALL	46,XX(10)	3.4	CD33+	CD10$^-$	Grammatico *et al.* (6)
7	FR*	Pro B-ALL	47,XX,t(12;17)(p13;q11),+mar(3)/46,XX(3)	3.1	CD33$^+$	CD10$^-$	Grammatico *et al.* (6)
7	SR**	AML	47,XX,t(12;17)(p13;q11)/+mar(6)/46,XX(6)	1.5	CD13$^+$, CD33$^+$	NR	Grammatico *et al.* (6)
8	F/2.5	Pro B-ALL	46,XX,t(12;17)(p13;q11.2)(8)/46,XX(11)	2.63	CD13$^+$, CD33$^+$	CD10$^-$	Ji-Eun Kim *et al.* (9)
9	M/19	Pro B-ALL	46,XY,t(12;17)(p13;q12)(2)/46,XY(13)	92	NR	CD10^{+weak}	Nyquist *et al.* (10)
10	F/3	Pro B-ALL	42~47,XX,der(6)add(6)(p25)add(6) (q21),add(7)(p11),?add(9)(p22),del(12) (p12), t(12;17)(p13;q12),add(19)(q13),+2, -3mar(cp11)/46,XX(1)	36.5	CD33+weak	CD10^{+weak}	Nyquist *et al.* (10)
11	F/74	MPAL	46,XX,+1,der(1; 18)(q10;q10),t(2; 16) (q13;q13),t(12;17)(p13;q21)(4)/46,XX(16)	2.6	CD13^{+weak}, CD33$^+$	CD10$^-$	Yamamoto *et al.* (11)
This study	M/46	Pro B-ALL	46,XY,t(12;17)(q13;q21)(11)/46,XY(6)	14.5	CD13^{+weak}	CD10$^+$	

S: Sex; A: age; M: male; F: female; FR*: first relapse; SR**: second relapse; NR: not reported (antigen expression is reported as weak when between 20-50).

On clinical examination, he was feverish and had a palpable non-tender spleen about 2 cm below the left costal margin. The initial laboratory evaluation revealed leukocytosis (14.5×10^9/l), normocytic anemia (Hb, 11.3 g/dl) and thrombocytopenia (115×10^9/l). The biochemistry panel revealed high uric acid and LDH levels, while his blood and urine cultures were negative. Examination of a peripheral blood smear revealed 70% blasts that were identified by flow cytometry as CD19$^+$, CD10$^+$, CD79a$^+$, CD34$^+$, CD123$^+$, HLA-DR$^+$, Tdt$^+$ and CD13$^+$ (dim), a picture compatible with B-acute lymphoblastic leukemia (B-ALL). The patient tested negative for a BCR/ABL rearrangement and the cytogenetic analysis revealed t(12;17)(p13;q21) translocation in 11 out of 24 metaphases. A cerebrospinal fluid analysis showed absence of blasts and the patient was treated according to the German multicenter ALL (GMALL) protocol for B-ALL.

On day 22, the patient had an episode of neutropenic fever treated with meropenem and vancomycin. Unfortunately, despite timely treatment initiation, he developed severe septic shock, rhabdomyolysis and acute renal injury and eventually died from fulminant gram-negative sepsis.

Materials and Methods

G-Banding preparation. Bone marrow aspirates were obtained at diagnosis and short-term cultures were set up according to standard protocols (15). Cells were cultured in RPMI (Roswell Park Memorial Institute) medium 1640 (1×) complete with Glutamax supplemented with 20% Fetal bovine serum (Gibco, Grand Island, NY, USA) and 2% penicillin/streptomycin. Colcemide (Invitrogen, Carlsbad, CA, USA) was added to the cultures 15 minutes before harvest. Cells were harvested after treatment with 0.075 M KCl hypotonic solution and Carnoy's fixative solution: 3:1 (v/v) methanol/glacial acetic acid. G-Banding analysis was prepared by treatment with 2.5% trypsin (10×) (Gibco) and Leishman's eosin blue solution in methylane staining (VWR Chemicals, Leuven, Belgium). Karyotype analysis was undertaken using Olympus microscope BX51 and CytoVision™ v3.6 Applied Imaging software.

FISH preparation. Three FISH probes were used: LSI Vysis *ETV6* Break Apart FISH Probe Kit (Vysis, Des Plaines, IL USA) and two different break-apart FISH probes for *ZNF384* and *TAF15* genes. Both probes were designed from BAC clones RP11-151M4 and RP11-362K1 overlapping the *ZNF384* and *TAF15* gene, respectively (10).

Slides with fixed cells were co-denatured with *ETV6* Break Apart FISH probe on Thermo Brite machine (Abbott, Chicago, IL, USA) for 5 min at 75˚C and then incubated in a humified chamber for 16 h at 37˚C. The slides were then washed twice in 50% formamide and 2xSSC (saline sodium citrate) for 10 min each time, to remove random non-specific hybridizations. Finally, the slides were washed with 0.15% NP40 (Vysis) and counterstained with DAPI (4,6-diamidino-2-phenylindole) (Vysis). Slides were analyzed under the Fluorescent Microscope Olympus BX51 using the CytoVision™ v3.6 software system of Applied Imaging. Detailed FISH procedures were previously reported (10).

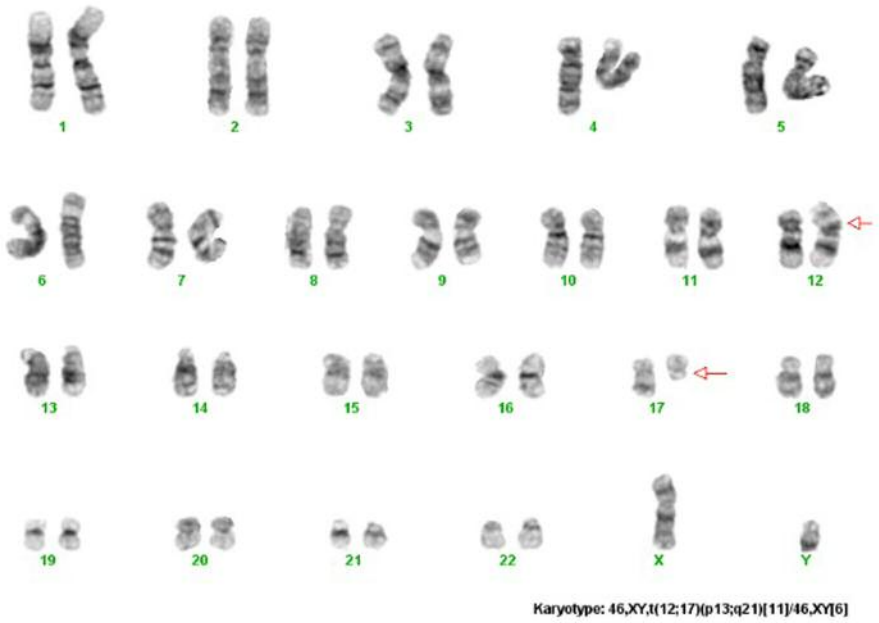

Figure 1. *Cytogenetic analysis of G-Banded chromosomes of the patient. Red arrows indicate aberrant chromosomes 12 and 17, that are involved in t(12;17)(p13;q21).*

Results

Cytogenetic analysis. Cytogenetic analysis of G-Banded chromosomes revealed that t(12;17)(p13;q21) translocation was the only identified chromosomal abnormality and was observed in 11 out of 17 metaphases: 46,XY,t(12;17)(p13;q21)(11)/46,XY(6) (Figure 1).

FISH analysis. FISH analysis using *ETV6* break-apart probe detected a normal hybridization pattern in interphase nuclei, as well as in metaphase nuclei, excluding the possibility of *ETV6* gene rearrangement (Figure 2A). In a metaphase stained with DAPI antifade, it was shown that both fluorescent probes for *ETV6* remained on chromosome 12, indicating that the breakpoint position was more distal on 12p (Figure 2B, C).

FISH analysis using break-apart probes for *ZNF384* and *TAF15* detected rearrangements of both genes, confirming the *ZNF384-TAF15* fusion (Figure 2D, E).

Discussion

There is an unequivocal relation between t(12;17)(p13;q21) rearrangement and specific antigen(s) expression. Regardless of the partner gene (*TCF3, EP300, EWSR1, ARID1B* or *TAF15*), with which *ZNF384* was rearranged, CD13 and/or CD33 antigen expression was consistently observed in all reported cases. In the great majority of cases, CD10 expression was weak or absent, but it was highly expressed in our case (2, 8). CD10 expression has been associated with poor clinical outcome due to decreased tendency of cells to undergo apoptosis (2, 4). Interestingly, from a cohort of 260 pediatric patients with precursor B cell acute lymphoblastic leukemia, 26% of patients that expressed CD13 and/or CD33 but did not express CD10, were positive for *ZNF384* rearrangements. Prognosis also seemed to be favorable regarding pediatric patients with several other genetic aberrations and adult patients with the same gene fusion (14).

In reported cases with t(12;17)(p13;q12), several breakpoints had been assigned to derivative chromosome 17, ranging from q11 to q21, but it's highly probable that most, if not all, involved *TAF15* gene rearrangements take place on 17q12 (10).

Although several reports support a generally good prognosis for patients with the ZNF384/TAF15 rearrangement (16), other reports support a poor clinical outcome. This is probably because, despite CD13 and/or CD33 expression, these cases show a high degree of heterogeneity concerning different breakpoints within *TAF15* ranging from exon 4 to exon 10, broader gene mutation status, additional chromosomal abnormalities, diversity in CD10 expression, age of onset and clinical features (6, 10, 17).

Fusions between genes coding FET proteins and transcription factors have been described in several solid

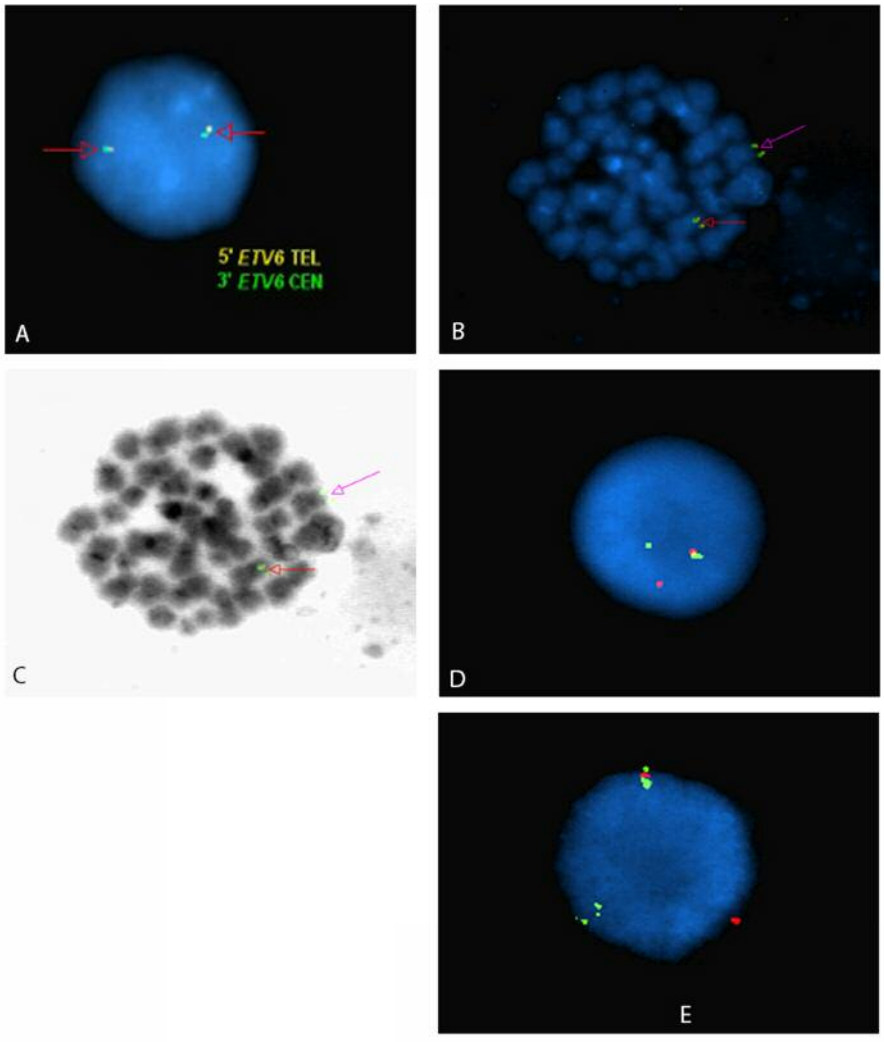

Figure 2. *A: FISH using ETV6 break-apart probe showing two fusion signals (green and orange signals represent 5' and 3' ETV6 gene positions); a hybridization pattern showing absence of ETV6 gene rearrangement. B, C: FISH using ETV6 break-apart probe on a metaphase nucleus stained with DAPI (B) and inverted DAPI. Red and purple arrows show ETV6 fusion signal on derivative chromosome 12 and normal chromosome 12, respectively. In Figure 2C, shows that ETV6 gene is not rearranged and remains on chromosome 12, suggesting that chromosomal breakage has occurred downstream of ETV6 with a direction towards the 12p telomere. D: FISH on a nucleus using TAF15 break-apart probe showing one fusion and one split signal, indicative of TAF15 gene rearrangement. E: FISH on a nucleus using ZNF384 break-apart probe showing one fusion and one split signal, indicative of ZNF384 gene rearrangement.*

tumors of mesenchymal origin and acute leukemia (17). It is thought that transcriptional deregulation is the main oncogenic event. In acute leukemia, the ZNF384 transcription factor is recurrently fused to two FET proteins, EWSR1 and TAF15. On both cases the amino-terminal SYGQ-rich region, is fused to the entire ZNF384 protein sequence (13). The transactivating properties of both fusion proteins were investigated on HEK293T cell extracts. Surprisingly, neither enhanced nor decreased expression of metalloproteinases was observed (17). Although EWSR1/ZNF384 and TAF15/ZNF384 fusion proteins do not cause tumorigenesis by elevated expression of metalloproteinases, their oncogenic potential in acute leukemias is beyond any doubt. Alternative oncogenic pathways, such as their effect on pre-mRNA splicing, are under investigation.

The incidence of this rare gene rearrangement, followed by a severe infection makes this case interesting. Metalloproteinases, among others, are involved in regulating activation and release of cytokines, chemokines, growth

factors and antibiotic peptides, contributing actively to innate and adaptive immunity (18). Although *ZNF384* acts as a transcription factor on several metalloproteinases, its fusion to TAF15 protein does not alter their transcription level in HEK293T cells (17), but this may happen in other cell types. The fact as well, that none of the reported cases, correlated this gene rearrangement with increased infection risk, cannot support a biological connection between the *TAF15/ZNF384* fusion and severe sepsis.

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