

# Identification of New Translocations Involving *ETV6* in Hematologic Malignancies by Fluorescence In Situ Hybridization and Spectral Karyotyping

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*TEL/ETV6* is the first transcription factor identified that is specifically required for hematopoiesis within the bone marrow. This gene has been found to have multiple fusion partners; 35 different chromosome bands have been involved in *ETV6* translocations, of which 13 have been cloned. To identify additional *ETV6* partner genes and to characterize the chromosomal abnormalities more fully, we studied bone marrow samples from patients known to have rearrangements of 12p, using fluorescence in situ hybridization (FISH) and spectral karyotyping (SKY). FISH analysis was done with 14 probes located on 12p12.1 to 12p13.3. Nine *ETV6* rearrangements were identified using FISH. The aberrations include t(1;12)(p36;p13), t(4;12)(q12;p13) (two patients), t(4;12)(q22;p13), t(6;12)(p21;p13), der(6)t(6;21)(q15;q?)t(12;21)(p13;q22), t(6;12)(q25;p13), inv(12)(p13q24), and t(2;2;5;12;17)(p25;q23;q31;p13;q12). Six new *ETV6* partner bands were identified: 1p36, 4q22, 6p21, 6q25, 12q24, and 17q12. Our present data as well as previous data from us and from other researchers suggest that *ETV6* is involved in 41 translocations. The breakpoints in *ETV6* were upstream from the exons coding for the HLH (helix-loop-helix) domain in six cases. Although cytogenetic analysis identified 12p abnormalities in all cases, FISH and SKY detected new and unexpected chromosomal rearrangements in many of them. Thus, complete characterization of the samples was achieved by using all three techniques in combination. © 2001 Wiley-Liss, Inc.

## INTRODUCTION

*TEL* (translocation–E26 transformation-specific–leukemia), also called *ETV6*, is the first transcription factor identified that is required specifically for hematopoiesis within the bone marrow (Wang et al., 1998). This gene frequently is rearranged in translocations in a wide spectrum of human leukemias (Mitelman, 1998), with the same fusion transcript in both myeloid and lymphoid leukemias (Peeters et al., 1997a). *ETV6* has been found to have many fusion partners. Using fluorescence in situ hybridization (FISH), 35 different chromosome bands have been shown to be involved in *ETV6* translocations, of which 13 have been cloned: *ARNT* (1q21), *ARG* (1q25), *MDS1/EVII* (3q26), *CHIC2/BTL* (4q12), *ASC2* (5q13), *PDGFRB* (5q33), *STL* (6q23), *JAK2* (9p24), *ABL* (9q34), *CDX2* (13q12), *TRKC* (15q25), *CBFA2* (21q22), and *MNI* (22q11) (Golub et al., 1994, 1995; Romana et al., 1995; Buijs et al., 1995; Papadopoulos et al., 1995; Peeters et al., 1997a,b; Suto et al., 1997; Cazzaniga et al., 1999; Chase et al., 1999; Cools et al., 1999; Eguchi et al., 1999; Yagasaki et al., 1999; Iijima et al., 2000; Salomon-Nguyen et al., 2000). The *ETV6* protein contains two critical domains, the 5' helix-loop-helix (HLH) dimerization domain (coded for by exons 3 and 4) and the 3' E26 transformation-

specific (ETS) DNA-binding domain (exons 6 to 8). Little is known about the function of the central region of *ETV6*. The translocation breakpoints are distributed throughout the gene, and *ETV6* contributes to the pathogenesis of leukemia by remarkably diverse molecular mechanisms that are only partially understood.

To identify additional *ETV6* partner genes and to characterize the chromosomal abnormalities more fully, we studied bone marrow samples from patients known to have rearrangements of 12p, using three different techniques: G-banding, FISH, and spectral karyotyping (SKY). Nine patients with *ETV6* rearrangements were identified by FISH. Although cytogenetic analysis pinpointed 12p abnormalities in all cases, FISH and

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SKY detected new chromosomal rearrangements in many of them.

## MATERIALS AND METHODS

### Patients

Fifteen patients with hematologic malignancies and 12p balanced translocations or add(12p) studied at the University of Chicago (U.S.A.) and at the University of Navarra (Spain) were included in the present report. Of these patients, four were studied at diagnosis and 11 at relapse. All samples were obtained with informed consent.

### Fluorescence In Situ Hybridization

Cytogenetic studies were done on unstimulated short-term bone marrow cultures. G-banded karyotypes were described according to the ISCN (Mitelman, 1995). FISH analysis was done using 11 cosmid and three phage probes located on 12p12.1 to 12p13.3. *TEL/ETV6* was analyzed by 179A6 (exon 1), 50F4 (exon 2), 54D5 (exons 5–8), and 148B6 (exon 8) (all kindly provided by Dr. Peter Marynen, Center for Human Genetics, University of Leuven, Leuven, Belgium) and by a cosmid contig that contained about 60 kb of genomic sequences surrounding exon 3 (TEL 11/18). Two 90-kb P1 phage clones that contained *KIP1/CDKN1B* were used to analyze this gene (Pietenpol et al., 1995) (addresses: 2096 and 2097; Genome Systems, Inc). The order of these probes was telomere-D12S235-D12S237-Phage L24A-D12S229-HTY3049c17-D12S133-179A6-50F4-TEL11/18-54D5-148B6-P12096-P12097-D12S119-D12S20-centromere (Kobayashi et al., 1994; Baens et al., 1996). Three PACs assigned to 4q11-q12 (238H24, 200D9, and 1146G14) (provided by Dr. Peter Marynen, Center for Human Genetics, University of Leuven, Leuven, Belgium) were used to check the involvement of *CHIC2/BTL* in cases with t(4;12).

Additional FISH experiments were performed using whole-chromosome painting probes (WCP) for chromosomes 6, 7, 11, 12, and 13, or centromere-specific probes (CEP) direct labeled with Spectrum Green® or Spectrum Orange®-dUTP (Vysis, Downers Grove, IL). In case 8, we used the LSI *TEL-AML1* probe to determine whether *CBFA2/AML1* was involved (Vysis). The probes were labeled with biotin-11-dUTP or digoxigenin-11-dUTP (Boehringer-Mannheim, Indianapolis, IN) using nick translation and were hybridized to the patients' slides as previously described (Rowley, 1990). The biotin-labeled probes were detected with fluorescein isothiocyanate (FITC)-conju-

gated avidin. The digoxigenin-labeled probes were detected with rhodamine-conjugated sheep anti-digoxigenin antibodies (Boehringer-Mannheim). Chromosomes were identified using counterstaining with 4'6-diamidino-2-phenylindole dihydrochloride (DAPI). The presence or absence of the FISH signals was scored on an average of 12 abnormal metaphase cells (range, 7–20) per probe per patient. Images of the hybridized cells were captured with a liquid-cooled, charge-coupled device camera (Photometrics, Tucson, AZ). Separate gray-scale images for the DAPI and the FITC fluorescence were acquired. The images were merged using Adobe Photoshop (Adobe Systems Inc, San Jose, CA) on a Macintosh computer (Apple Computers, Cupertino, CA).

### Spectral Karyotyping Analysis

The SKY probe mixture and hybridization reagents were obtained from Applied Spectral Imaging (Carlsbad, CA). Slides for spectral karyotyping were hybridized with the probe cocktail as previously described (Rowley et al., 1999) for 2 days at 37°C. For each case, between six and 12 metaphase cells were captured and analyzed, using the SD200 system (Applied Spectral Imaging). Cases with questionable or not obvious chromosome rearrangements were analyzed further using the appropriate painting or centromere-specific probes (Vysis).

## RESULTS AND DISCUSSION

Nine of the 15 patients with 12p balanced translocations (60%) had rearrangements of the *ETV6* gene. These patients had various hematologic malignancies, including four cases of acute myeloid leukemia (AML), three of myelodysplastic syndromes (MDS), one of acute lymphoblastic leukemia (ALL), and one of chronic lymphocytic leukemia. The 12p aberrations were the sole abnormality only in case 2. Eight 12p translocations affecting the *ETV6* gene were identified. The aberrations included t(1;12)(p36;p13), t(4;12)(q12;p13) (two patients), t(4;12)(q22;p13), t(6;12)(p21;p13), der(6)t(6;21)(q15;q?)t(12;21)(p13;q22), t(6;12)(q25;p13), inv(12)(p13q24), and t(2;2;5;12;17)(p25;q23;q31;p13;q12) (Table 1). Six new partner bands were identified: 1p36, 4q22, 6p21, 6q25, 12q24, and 17q12. FISH results are summarized in Table 2. Although some of these abnormalities had been detected previously using G-banding, this is the first study reporting *ETV6* involvement in these rearrangements.

TABLE I. Summary of Clinical Features, Karyotype, and Revised Karyotype Modified by SKY and FISH\*

Case	Sex/age (years)	Diagnosis	Status	Karyotype	Revised karyotype <sup>a</sup>
1	M/5	AML (M2)	R	47,XY,+8[11]/47,idem,t(4;12)(q22;p13)[3]/46,XY[6]	47,XY,+8[4]/47,idem,t(4;12)(q22;p13)[2]/46,XY[3]
2	M/47	AML (M2)	R	46,XY,t(4;12)(q12;p13)[9]/46,XY[2]	46,XY,t(4;12)(q12;p13)[3]/46,XY[3]
3	M/69	AML (M0)	Dx	46,XY,t(4;12)(q12;p13)[24]/46,idem,-7,+11[3]/46,idem,del(7)(q21)[3]	46,XY,t(4;12)(q12;p13)[11]
4	F/34	AML (M2)	R	46,XX,add(12)(p13),del(17)(p12)[15]	46,XX,inv(12)(p13q24),del(17)(p12)[9] 45,XY,der(5)t(2;5)(p25;q13),der(7)t(7;13)(p13;q13)ins(7;13)(q22;q7)[2]/46, XY,t(2;5;12;17)(p25;q23;q31;p13;q12),der(5) t(2;5),der(7)t(1;7)(f;q22),del(13)(q12q32)[6]
5	M/46	MDS (RAEB)	R	46,XX,del(1)(p36),t(9;11)(q34;q13),add(12)(p13)[20]	46,XX,t(1;12)(p36;p13),t(9;11)(q34;q13)[10]
6	F/66	MDS (RAEBT)	R	45,XY,del(6)(q25),-7,der(12)t(12;20)(p13;q12)[11]	45,XY,t(6;12)(q25;p13),der(6)t(6;12),-7, del(20)(q12)[6]
7	M/59	MDS (RAEBT)	R	44,X,-X,del(2)(p15p23),-4,del(6)(q15q23),+del(6)(q15q23),der(12)t(12;7)(p12;q11),del(15)(q15q22),-17[3]/44,idem,add(8)(q24),add(10)(p13),der(21)t(4;21)(p11;p11)[4]/43,X,-X,-4,add(4)(p16),del(6),der(12)t(12;17),del(15),-17,add(18)(q23),der(21)t(4;21)[3]/46,XX[11]	44,X,-X,del(2),-4,der(6)t(6;21)(q15;q22) t(12;21)(p13;q22),+der(6)t(6;21)t(12;21), t(12;21)(p13;q22),der(12)t(12;17)(p12;q11), del(15)(q15),-17[4]/46,XX[2]
8	F/6	ALL (L1)	R	45,XY,del(6)(p21),-9,add(12)(p13),add(16)(q24)[11]/46,XY[2]	46,XY,t(6;12)(p21;p13)[3]/45,idem,-9,der(16)t(9;16)(q21;q24)[4]
9	M/83	LPD (CLL)	Dx		

\*SKY, spectral karyotyping; FISH, fluorescence in situ hybridization; AML, acute myeloid leukemia; MDS, myelodysplastic syndromes; RAEB, refractory anemia with excess blasts; ALL, acute lymphoblastic leukemia; LPD, lymphoproliferative disorder; CLL, chronic lymphocytic leukemia; R, relapse; Dx, newly diagnosed.

<sup>a</sup>Novel translocations or those redefined by the FISH and SKY analysis are highlighted in bold type.

TABLE 2. Results of FISH Analysis with ETV6 Specific Cosmid Probes\*

	Case 1 t(4;12) (q22;p13)	Case 2 t(4;12) (q12;p13)	Case 3 t(4;12) (q12;p13)	Case 4 inv(12) (p13q24)	Case 5 t(2;2; 5;12;17) (p25;q23;q31; p13;q12)	Case 6 t(1;12) (p36;p13)	Case 7 t(6;12) (q25;p13) der(6)t(6;12)	Case 8 der(6)t(6;21) (q15;q22) t(12;21) (p13;q22), t(12;21)	Case 9 t(6;12) (p21;p13)
179A6 (exon 1)	<b>der(4)</b>	<b>der(4)</b>	<b>der(4)</b>	<b>12q</b>	<b>der(17)</b>	<b>der(1)</b>	<b>der(6)x2</b>	<b>der(6)x2,</b> <b>der(21)</b>	<b>der(6)</b>
50F4 (exon 2)	<b>der(4)</b>	<b>der(4)</b>	<b>der(4)</b>	<b>12q</b>	<b>der(17)</b>	<b>der(1)</b>	<b>der(6)x2</b>	<b>der(6)x2,</b> <b>der(21)</b>	der(12)
TEL11/18 (exon 3)	<b>der(4)</b>	der(12)	der(12)	12p	der(12)	der(12)	<b>der(6)x2</b>	<b>der(6)x2</b> <b>der(21)</b> <b>der(6)x2</b> <b>der(21)</b>	der(12)
54D5 (exons 5-8)	der(12)	der(12)	der(12)	12p	der(12)	der(12)	der(12)	der(12)	der(12)
I48B6 (exon 8)	der(12)	der(12)	der(12)	12p	der(12)	der(12)	der(12)	der(12)	der(12)

\*FISH, fluorescence in situ hybridization.

The chromosome location of TEL translocation is indicated in bold type.

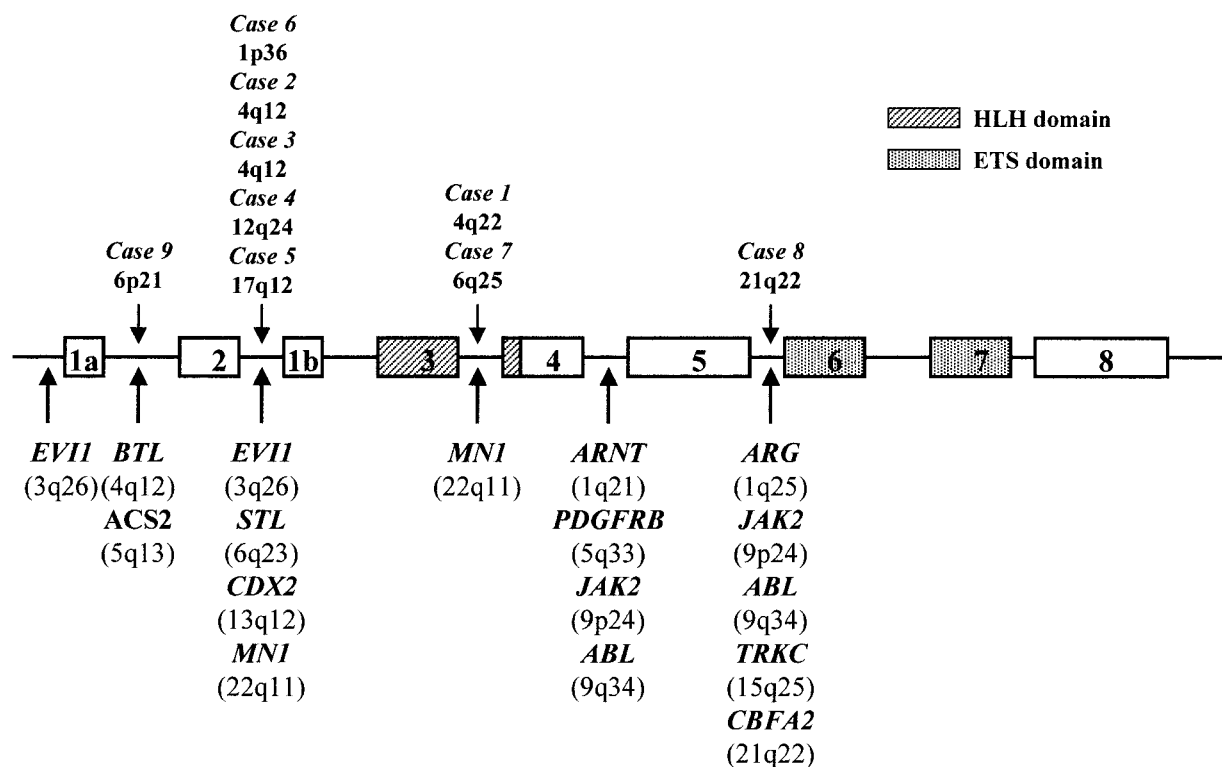


Figure 1. Diagram of the ETV6 gene showing the exons (numbered rectangles) and the protein domains. The location of the breakpoints in patients in this report are above the line; the location of the breakpoints in ETV6 in the cloned translocations already published is shown below the line.

In addition to confirming the FISH results, SKY allowed for the complete characterization of the karyotype of the leukemia samples. In cases 5, 8, and 9, additional FISH experiments were performed using chromosome painting and centromere probes to confirm complex translocations.

The revised karyotypes summarizing the G-banding, FISH, and SKY results are shown in Table 1. FISH and SKY confirmed the G-banding results in cases 1, 2, and 3. The t(4;12) found in cases 2 (AML-M2) and 3 (AML-M0) seems to be the same translocation reported by Cools et al. (1999) that

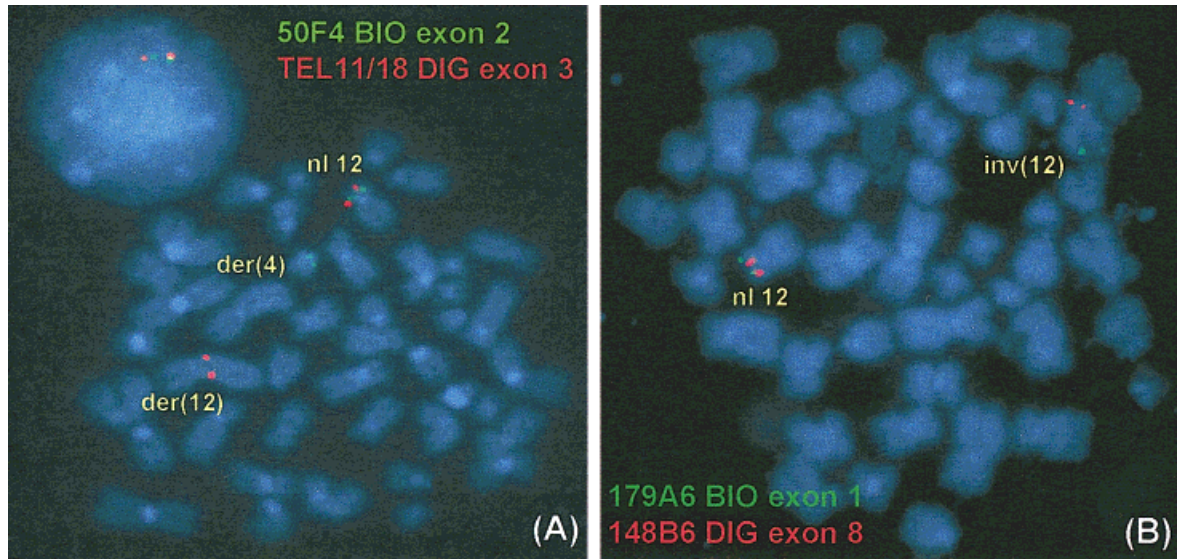


Figure 2. Examples of fluorescence in situ hybridization analysis of *ETV6* rearrangements. **A:** Patient 2 with a  $t(4;12)(q12;p13)$ . The red and green signals both label the normal 12p, whereas the green signal representing the telomeric probe is translocated to the der(4) and the red centromeric probe labels the der(12) chromosome. **B:** Patient 4 with  $inv(12)(p13q24)$ . The red and green signals are together on the normal 12p, and they are separated on the  $inv(12)$ , with exon 8 remaining on 12p and exon 1 labeling 12q.

fuses *ETV6* and *CHIC2/BTL*. In both cases the breakpoint was in intron 2 of *ETV6* (Fig. 2), upstream from the exons coding for the HLH domain, and in intron 3 of *CHIC2/BTL*. Only four other cases with this rearrangement have been reported: three were classified as AML-M0 and one as myeloid/natural killer (NK) cell leukemia (Cools et al., 1999). Although more FISH studies are required to confirm the involvement of these genes in cases with  $t(4;12)$ , the *CHIC2-BTL-ETV6* fusion gene could be specific for an early hematopoietic cell phenotype in AML.

Our case 2 seems to be similar to case 4 described by Cools et al. (1999). The breakpoint in *ETV6* is between exon 2 and 3 in both cases, and we were unable to detect a *CHIC2-BTL-ETV6* fusion transcript in the RNA of the patient (data not shown). In case 1, the breakpoint in chromosome 4 was on 4q22, and FISH confirmed that it was telomeric to *CHIC2/BTL*. In cases 4 and 6, the translocation of 12p13 with an unknown chromosome resulted in an  $inv(12)(p13q24)$  (Fig. 2) and a  $t(1;12)(p36;p13)$ . The breakpoint was between exons 2 and 3 in both cases. Identification of the new fusion partner genes is in progress. There are several candidate genes on 1p36. Among the candidate genes on 12q24 are *PRKAB1* (protein kinase, AMP-activated, beta 1 non-catalytic subunit) and *CIT*.

In three cases, additional studies using FISH and SKY resulted in a deletion 6 identified by G-banding, which is more accurately described as a

translocation with chromosome 12:  $t(6;12)$  (cases 7 and 9) and  $der(6)t(6;21)t(12;21)$  (case 8). The breakpoints in chromosome 6 were different, and we could not identify a recurring rearrangement. Duplication of the abnormal 6 was found in cases 7 and 8 (Table 1). Case 8 represents a 6-year-old girl with ALL. Both chromosomes 12 were abnormal. G-banding detected a  $der(12)t(12;17)$ , and FISH and SKY completed the analysis, showing that the patient had the classic  $t(12;21)$ , with an *ETV6-CBFA2* fusion confirmed by FISH. Surprisingly, in addition to the normal chromosome 6, two der(6) with a complex  $der(6)t(6;21)t(12;21)$  were detected (Fig. 3). *ETV6* was deleted in the  $der(12)t(12;17)$ .

Figure 3. Examples of the analysis of cells from two patients. **A:** Patient 8. Fluorescence in situ hybridization and spectral karyotyping analysis showing the classic *ETV6-CBFA2* fusion on the der(21) and two  $der(6)t(6;21)t(12;21)$ . In the upper left panel, the AML1 probe (red) from chromosome 21 labels the der(21), both der(6) chromosomes, and the der(12). There is no normal chromosome 21. The *ETV6* probe (green) labels the der(21) and both der(6) chromosomes; it is deleted from the der(12). There is no normal chromosome 12. The upper right panel shows with the CEP6 probe (red) that there is one normal 6 and two der(6) chromosomes. The painting probe for chromosome 12 (green) labels both der(6), one der(21), the  $der(12)t(12;17)$ , and the  $der(12)t(12;21)$  chromosomes. Bottom SKY classified image shows involvement of chromosomes 6, 12, and 21 in a complex rearrangement. **B:** The lower set of images represent SKY analysis of patient 5. Reverse 4'-diamidino-2-phenylindole dihydrochloride (DAPI), spectral, and classified images are shown. The reverse DAPI image is at bottom left, the spectral image is in the upper left, and the classified image is in the upper right. The abnormal chromosomes are identified with arrows. The karyotype using the classified image is shown in the lower right.

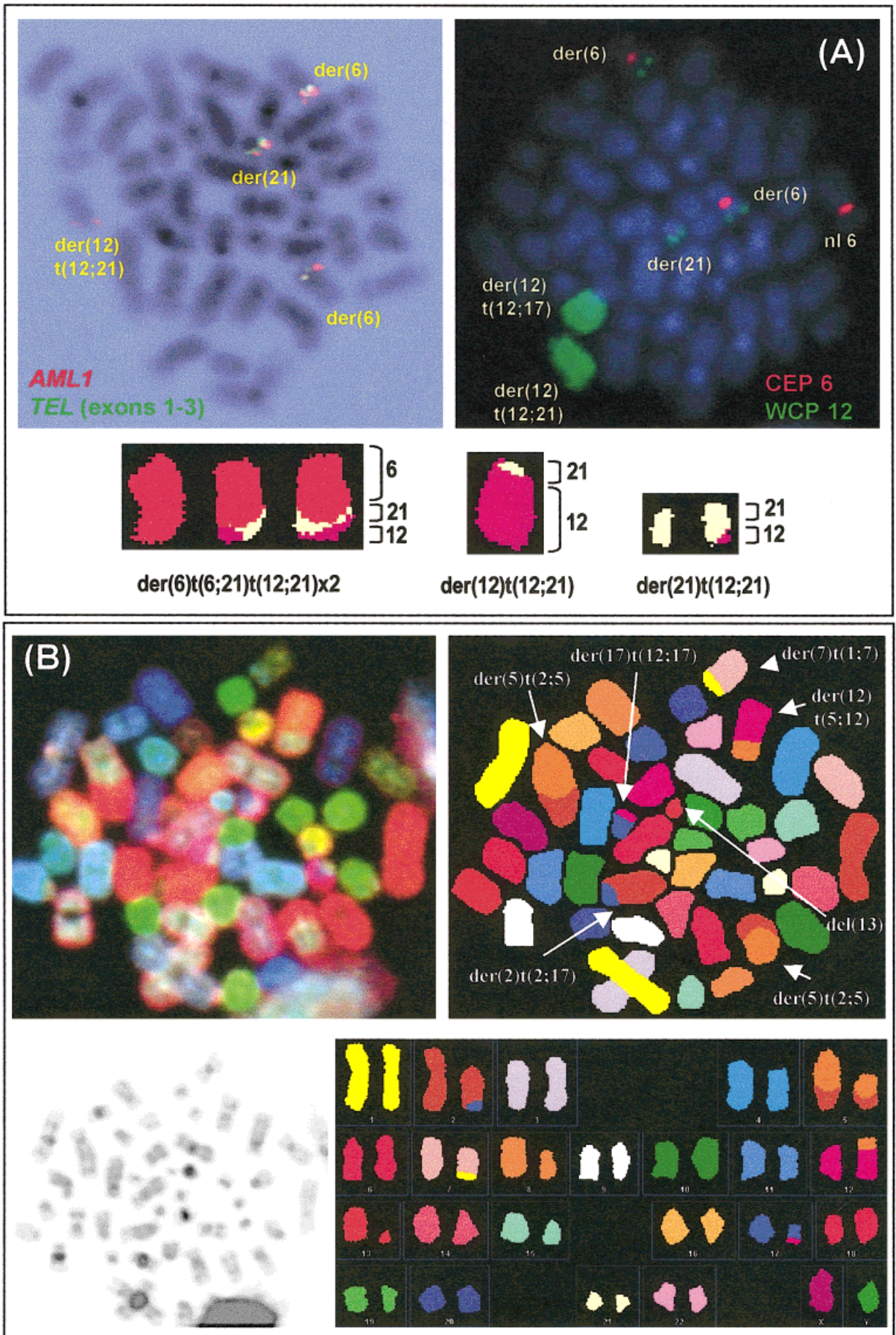


Figure 3.

TABLE 3. Review of the Literature of the Cases Reported With Reciprocal Translocations Involving *ETV6*\*

TEL band partner	Diagnosis	Reference <sup>a</sup>
1p36	MDS	Odero et al. (2001)
<b>1q21 (ARNT)</b>	AML	Berger et al. (1997), <b>Salomon-Nguyen et al. (2000)</b>
<b>1q25 (ARG)</b>	AML	Sato et al. (1997), <b>Cazzaniga et al. (1999)</b>
2q31	NHL-B	Kobayashi et al. (1994)
<b>3q26 (EVII)</b>	AML, MDS	Raynaud et al. (1996), <b>Peeters et al. (1997b)</b> , Streubel et al. (1998)
<b>4q12 (CHIC2/BTL)</b>	AML	Andreasson et al. (1998), <b>Cools et al. (1999)</b>
4q22	AML	Odero et al. (2001)
5q13	AML, ALL	Kobayashi et al. (1994)
5q31 ( <b>ACS2</b> )	CML	Wlodarska et al. (1997), <b>Yagasaki et al. (1999)</b>
<b>5q33 (PDGFRβ)</b>	AML, MDS	<b>Golub et al. (1994)</b> , Höglund et al. (1996), Sato et al. (1997)
6p11-p12	MDS	Streubel et al. (1998)
6p21	CLL	Odero et al. (2001)
6p2?5	ALL	Kobayashi et al. (1994)
6q21	ALL	Raimondi et al. (1997)
<b>6q23 (STL)</b>	ALL	<b>Suto et al. (1997)</b>
6q25	MDS	Odero et al. (2001)
7p12	ALL	Wlodarska et al. (1998)
7p15	AML	Wlodarska et al. (1998)
7q22	MDS	Tosi et al. (1998)
7q32	AML	Satake et al. (1999)
7q36	AML	Tosi et al. (1998), Wlodarska et al. (1998), Slater et al. (1999), Tosi et al. (2000)
8p21	ALL	Raimondi et al. (1997)
8q11	MDS	Kobayashi et al. (1994)
<b>9p24 (JAK2)</b>	ALL, CML	Berger et al. (1997), <b>Peeters et al. (1997a)</b>
9q11	ALL	Tosi et al. (1998)
<b>9q34 (ABL)</b>	ALL, CML, AUL	Kobayashi et al. (1994), <b>Papadopoulos et al. (1995)</b> , Andreasson et al. (1997)
11q2?	AML	Andreasson et al. (1998)
12q24	AML	Odero et al. (2001)
<b>13q12 (CDX2)</b>	AML, ALL	Streubel et al. (1998), Tosi et al. (1998), Wlodarska et al. (1998), <b>Chase et al. (1999)</b>
14q22	AUL	Kobayashi et al. (1994)
<b>15q25 (TRKC)</b>	AML	<b>Eguchi et al. (1999)</b>
16q24	MDS	Sato et al. (1997)
17p13	AML, MDS	Kobayashi et al. (1994)
17q12	MDS	Odero et al. (2001)
17q25	AML	Wlodarska et al. (1998)
19q13	CML	Kobayashi et al. (1994)
<b>21q22 (AML1)</b>	AML	<b>Golub et al. (1995)</b> , <b>Romana et al. (1995)</b>
<b>22q11 (MNI)</b>	AML	<b>Buijs et al. (1995)</b>
22q12	AML	Höglund et al. (1996)
22q13.1	AML	Kobayashi et al. (1994)
Xq28	MDS	Berger et al. (1997)

\*MDS, myelodysplastic syndromes; AML, acute myeloid leukemia; NHL-B, non-Hodgkin lymphoma, B cell; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; AUL, acute undifferentiated leukemia.

<sup>a</sup>Cloned *ETV6* band partners are highlighted in bold type.

Another complex translocation t(2;2;5;12;17) involving *ETV6* was detected in a patient diagnosed as having MDS refractory anemia with excess blasts (RAEB) (case 5). FISH with *ETV6* cosmid showed that this gene was involved, with the breakpoint between exons 2 and 3 (Table 2). The partner bands were 5q31 and 17q12. The *ETV6* partner gene on 5q31 could be *ACS2* (Yagasaki et al., 1999). This is the first time that band 17q12 has been reported to be involved in rearrangements with *ETV6*. SKY confirmed the translocation and identified the add(5) as a der(5)t(2;5) (Fig. 3). The

partial loss of chromosome 5 material seen in this patient is a recurrent event in cases of myeloid disorders. The fact that *ETV6* rearrangements occurred as subclones in two cases (cases 1 and 5) suggests that these *ETV6* translocations could be secondary abnormalities in myeloid malignancies. FISH analysis with the other probes located on 12p12.1 to 12p13.3 showed no 12p deletions in these cases.

The breakpoints in *ETV6* in our cases were between exons 1 and 2 (one case) and exons 2 and 3 (five cases), upstream from the exons coding for the

HLH domain, and between exons 3 and 5 (two cases) and exons 5 and 8 (one case) (Table 2 and Fig. 1). In three patients (cases 2, 3, and 6), the location of the breakpoint was confirmed by sequencing (data not shown). It is difficult to predict the molecular consequences of these rearrangements, owing to the variety of mechanisms described in the 13 fusion genes cloned (Golub et al., 1994, 1995; Romana et al., 1995; Buijs et al., 1995; Papadopoulos et al., 1995; Peeters et al., 1997a,b; Suto et al., 1997; Cazzaniga et al., 1999; Chase et al., 1999; Cools et al., 1999; Eguchi et al., 1999; Yagasaki et al., 1999; Iijima et al., 2000; Salomon-Nguyen et al., 2000). In the six cases in which the 12p13 breakpoint occurred upstream from the exons coding for the HLH domain, in intron 1 or 2, the oncogenic potential of some of these translocations could result from the *ETV6* promoter's driving the transcription of the partner gene, as has been suggested for the *ETV6-MDS1/EVI1* fusion (Peeters et al., 1997b). The importance of the *ETV6* gene in hematopoiesis makes these results interesting for future studies. Identification of some of the new fusion partner genes is in progress.

Although the same fusion transcript has been found in the translocations involving *ETV6* in both myeloid and lymphoid leukemias (Peeters et al., 1997a), all the cases that have been cloned with breakpoints between exons 2 and 4 are myeloid neoplasias (Buijs et al., 1995; Peeters et al., 1997a,b; Chase et al., 1999). In our series, the breakpoints of the cases of myeloid malignancies were between exons 2 and 5, whereas they were outside this region in the two lymphoid cases (Table 2). *ETV6* and *MLL* are unique in having more than 40 partner genes involved in translocations leading to fusion genes. Our present data, along with other of our data and the data of other researchers, suggest that *ETV6* is involved in 41 translocations (Table 3).

By combining G-banding, FISH, and SKY analysis, we redefined the aberrations described by G-banding. FISH allowed the identification of six new *ETV6* translocations. In all instances, the breakpoints of chromosomal rearrangements were identified by comparing the banding and painting patterns, but SKY confirmed the FISH findings and clarified the abnormalities in two patients with complex karyotypes. This approach allowed the identification of hidden translocations in six cases. Thus, complete characterization of the samples was achieved by using all three techniques in combination.

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