Fluorescence In Situ Hybridization Characterization of New Translocations Involving TEL (*ETV6*) in a Wide Spectrum of Hematologic Malignancies

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The *ETV6* (also known as *TEL*) gene on chromosome 12p13 is the target of a number of translocations associated with various hematologic malignancies. The contribution of *ETV6* to leukemogenesis occurs through different mechanisms that involve either its helix-loop-helix dimerization domain or its E26 transformation-specific (ETS) DNA-binding domain. Using fluorescence in situ hybridization we characterized seven new *ETV6* rearrangements in chronic myeloid leukemia, acute myeloid leukemia, acute lymphoblastic leukemia, and non-Hodgkin's lymphoma. These aberrations, not always discernible at the cytogenetic level, include a t(5;12)(q31;p13), t(6;12;17)(p21;p13;q25), t(7;12)(p15;p13), t(7;12)(p12;p13), t(7;12)(q36;p13), t(12;13)(p13;q12), and a not completely defined t(12;?)(p13;?). Loss or disruption of the second *ETV6* allele by a del(12)(p12p13) or by an intragenic *ETV6* deletion was detected in two cases. In six cases the 12p13 breakpoint occurred in the 5′ end of *ETV6*, upstream to exons encoding the HLH domain, whereas the remaining case had a breakpoint between the exons coding for the HLH domain and the exons coding for the ETS domain of *ETV6*. These observations provide further evidence for the multiple contributions of *ETV6* in the pathogenesis of a wide range of hematologic malignancies.

RECENT MOLECULAR studies show that the *ETV6* gene (previously known as *TEL*), a member of the E26 transformation-specific (ETS)-family of transcription factors located at 12p13,[1] is involved in different chromosomal translocations associated with human leukemias (Fig 1A). For the t(3;12)(q26;p13), t(5;12)(q33;p13), t(9;12)(q34;p13), t(12;21)(p13;q22), and t(12;22)(p13;q11) the translocation partners were identified.[1-6] These result in the expression of a chimeric transcript consisting of *ETV6* sequences fused to *MDS1/EVI1* (3q26), *PDGFRB* (5q33), *ABL* (9q34), *AML1/CBFA2* (21q22), and *MN1* (22q11), respectively. In *ETV6* translocations involving *PDGFRB*, *ABL*, and *AML1/CBFA2* the helix-loop-helix (HLH) dimerization domain of *ETV6* influences or stimulates the activity of the fusion partner. In leukemias with a t(12;22), the aberrant *MN1-ETV6* protein is believed to have transforming capacity and the DNA-binding domain is thought to be the functional contribution of *ETV6*.[6] In myeloproliferative disorders with a t(3;12)(q26;p13), the chimeric transcript consists of the first two exons of ETV6, which code for no known functional domains, fused to *MDS1/EVI1* sequences suggesting that the oncogenic potential of this translocation could result from the *ETV6* promoter driving the transcription of *MDS1/EVI1*.[2] Molecular analysis of the t(6;12)(q23;p13) recently described by Bohlander et al,[7] in a B-cell acute lymphoblastic leukemia (ALL) cell line identified a novel gene on chromosome 6 named *STL*. The *ETV6* breakpoint was localized in intron 2, upstream to the exon encoding the HLH domain. However, no obvious new chimeric reading frames were found and the hypothesis that the t(6;12)(q23;p13) does not lead to a fusion protein with oncogenic potential but to the elimination of normal ETV6 function was presented.

It is clear that *ETV6* is a versatile element at the center of a network of genes involved in hematologic malignancies through different molecular mechanisms that are only partially understood. Here we report six leukemia cases and one B-cell non-Hodgkin's lymphoma (NHL) case with new chromosomal rearrangements involving *ETV6*. The breakpoints of a t(5;12)(q31;p13), t(6;12;17)(p21;p13;q25), t(7;12)(p15;p13), t(7;12)(p12;p13), t(7;12)(q36;p13), t(12;13)(p13;q12), and t(12;?)(p13;?) were characterized by fluorescence in situ hybridization (FISH) using a panel of DNA probes including an ordered set of cosmids covering the entire *ETV6* gene.

MATERIALS AND METHODS

Patients. Patient material was collected at the Center for Human Genetics in Leuven, Belgium, during the last 3 years. Some clinical and hematologic findings of the reported cases are summarized in Table 1.

Cytogenetics. One-day cultures of bone marrow cells were used for cytogenetic analysis in all cases. Ten to 31 R- and G-banded karyotypes were analyzed and classified according to the International System for Human Cytogenetic Nomenclature.[8]

FISH. FISH was performed as previously described.[9] Chromosome 12p abnormalities were studied with the cosmids 4H9A (*D12S158*, assigned to 12p13.3), 123C12 (*CDKN1B*), and 9 cosmids for *ETV6* ordered from 5′ to 3′ (179A6 - 15A4 - 67C6 - 50F4 - 132B11 - 242E1 - 163E7 - 54D5 - 148B6).[10] The position of the different *ETV6* exons in these cosmids is shown in Fig 1B. Additional FISH experiments were performed using chromosome 5, 6, 9, 12, 13, 17, 19, 20, 21, and 22 painting probes labeled with bio-16-dUTP (Cambio, Cambridge, UK) or Spectrum Orange-dUTP (Vysis, Stuttgart, Germany). Three yeast

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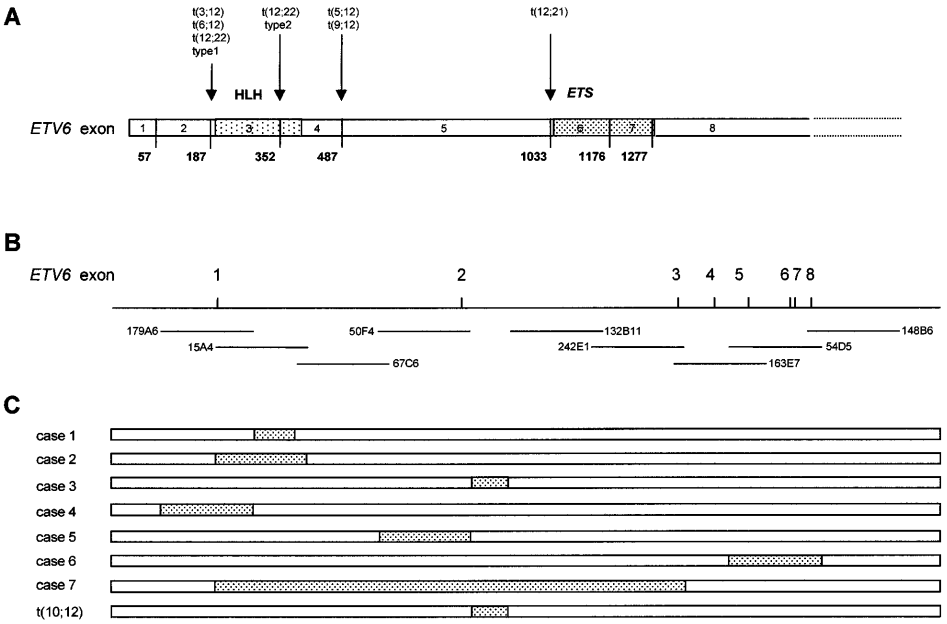

Fig 1. Genomic breakpoints of the *ETV6* gene. (A) Exon structure of the *ETV6* mRNA showing the breakpoints of known translocations involving *ETV6*, (B) genomic structure of the *ETV6* gene showing the positions of the cosmid probes used for FISH, and (C) genomic regions of the *ETV6* breakpoints as determined by FISH are indicated by the shaded areas.

artificial chromosomes (YACs) assigned to 5q31q33 (773B7, 939F12, and 888A7), and 10 YACs assigned to the 7p15p12 region (764B12, 776A4, 207E1, 881B6, 669G6, 16BC5, 959B3, 772B11, 802D6, and 908B12) were selected from the sequence-tagged site (STS)-based map reported by Green et al,[11] Keen et al, [12] and Chumakov et al.[13] The cosB (*PDGFRB*),[14] YAC Y6,[15] and cosmid ICRFCIO2D12118 hybridize to, respectively, 5q33, 14q32, and 21q22.3. Chromosomes 7, 12, and 13/21 were identified by cohybridization with Texas Red-5-dUTP labeled centromere probes (Dupont, Boston, MA) for chromosome 7 (p7t1), 12 (pBR12/D12Z3), and 13/21 (pUC 1.76) in combination with G-banding

Table 1. Clinical Data

Case	Sex/Age	Status/mo After Diagnosis	Diagnosis	Immunophenotype	Organ Involvement*	Treatment/Response	Survival (mo)
1	M/49	P	aCML		BM, PB, spleen, LN	HU; HU + IFN/NR	6†
2	M/13	P	RAEB-t		BM (18%), PB (25%), LN, liver	AEIM/NR	17†
		R/11	AML	CD34$^+$, CD33$^+$, CD13$^+$, CD117$^+$, TdT$^+$, CD14$^-$, CD15$^-$	BM (40%), PB (34%), liver, spleen	AEMAmC/NR	
3	M/4	P	AML-M0	CD34$^+$, CD33$^+$, CD13$^-$, TdT$^+$, CD14$^-$, CD15$^-$	BM (93%), PB (13%)	EORTC-AML 58872/NR	2†
4	M/5	P	ALL-L2	CD34$^+$, CD10$^+$, CD3$^+$, CD20$^+$, TdT$^+$, CD13$^+$, CD33$^+$	BM (96%), PB (37%)	EORTC CLCG-ALL 58881/CR	24
5	M/1	P	AML	CD34$^+$, CD32$^+$, CD13$^+$, CD116$^+$, CD4$^+$, CD56$^+$, CD38$^+$, HLH-DR$^+$	BM (77%), PB (70%) spleen, liver	EORTC CLCG-AML 58921/NR	5
6	F/17	P	ALL-L1	CD10$^+$, CD19$^+$, HLH-DR$^+$, TdT$^+$, slg$^+$, clg:$^+$	BM (98%), PB (79%), LN	ALL BMFT/CR	29†
		R/22 R/28				CVAD, AE, TBI&C, ABMT/CR VIMA, BuM, APBSCT/NR	
7	M/70	P	MZBL	slgM$^+$, CD19$^+$, CD20$^+$, CD22$^+$, FMC7$^+$, HLA-DR$^+$, CD24$^+$, CD5$^-$, CD10$^-$, CD23$^-$, CD15$^-$, CD103$^-$	BM (49%), PB (27%), LN, spleen	Chlorambucil	10

Abbreviations: P, primary diagnosis; aCML, atypical chronic myeloid leukemia; BM, bone marrow; PB, peripheral blood; LN, lymph node; HU, hydroxyurea; IFN, interferon; NR, no response; RAEB-t, refractory anemia with excess of blasts in transformation; AEIM, cytosine-arabinoside, etoposide, idarubicin, mitoxantrone; R, relapse; AML, acute myeloid leukemia; AEMAmC, cytosine-arabinoside, etoposide, mitoxantrone, m-Amsa, cyclosporine; EORTC-AML 58872, cytosine-arabinoside, mitoxantrone, etoposide; ALL, acute lymphoblastic leukemia; EORTC CLCG-ALL 58881, prednisone, vincristine, daunorubicine, L-asparaginase, cyclophosphamide, mercaptopurine, cytosine-arabinoside, methotrexate, dexamethasone, doxorubicine, 6-thioguanine; CR, complete remission; EORTC CLCG-AML 58921, cytosine-arabinoside, mitoxantrone, etoposide; BMFT, daunorubicin, vincristine, prednisone, L-asparaginase, cyclophosphamide, cytosine-arabinoside, 6-mercaptopurine, teniposide; CVAD, cyclophosphamide, vincristine, doxorubicin, dexamethasone; AE, cytosine-arabinoside, etoposide; TBI&C, total body irradiation and cyclophosphamide; ABMT, allogeneic bone marrow transplantation; VIMA, etoposide, ifosfamide, mitoxantrone, cytosine-arabinoside; BuM, busulfane, melphalan; APBSCT, allogeneic peripheral blood stem cell transplantation; MZBCL, marginal zone B-cell lymphoma.

*In brackets % of blasts in case 2-6, or malignant lymphocytes in case 7.

†Died.

Table 2. Karyotypic Findings by Classical Cytogenetics and FISH

Case	Status at Time of Analysis	Cytogenetic Data	Karyotype Update After FISH Studies
1	P	46,XY,t(5;12)(q31;p12)[4]/46,XY[2]	46,XY,t(5;12)(q31;p13)
2	P	46,XY[10]	
	R/11	46,XY,t(6;12)(p21;p13)[1]/46,idem, del(15)(q21q22)[6]/46,XY[3]	46,XY,t(6;12;17)(p21;p13;q25)
3	P	46,XY,t(7;12)(p14;p13)[17]/46,XY[3]	46,XY,t(7;12)(p15;p13)
4	P	45,XY,t(7;12)(p14;p13),−13[30]/46,XY[1]	45,XY,t(7;12)(p12;p13),−13
5	P	46,XY,inv(2)(p11q13),add(7)(q36),del(12)(p12p13), der(16)t(1;16)(q22;p13),add(21)(q22)[19]/46,XY[1]	46,XY,inv(2)(p11q13),t(7;12)(q36;p13),der(16)t(1;16) (q22;p13),add(21)(q22)
6	P	46,XX[10]	
	R/22	46,X,add(X)(q13),del(2)(p23),add(3)(p13),−9, del(12)(p12p13),der(12)t(9;12)(q13;p1?)t(13;20) (q14;p11),del(17)(p11),+mar[14]/46,XX[1]	46,X,add(X)(q13),del(2)(p23),add(3)(p13),der(9)t(9;20) (q13;p12),del(12)(p12p13),der(12)t(12;13;9) (p13;q12q14;p13),der(13)t(12;13)(p13;q12), der(20)t(13;20)(q14;p12),del(17)(p11)
7	P	48,XY,der(1)add(1)(p34)add(1)(q21),der(7)t(1;7) (q21;q31),del(12)(p11),dic(12;17)(p11;p11), add(14)(q32),add(17)(p13),−19,−20,−22, +2-5mar[11]/96,idem[2]/46,XY[5]	48,XY,der(1)t(1;17)(p34;q24)add(1)(q21),der(5) t(5;12;?)(p11;p11p13;?),der(7)t(1;7) (q21;q31),der(12)del(12)(p13p13)t(12;14) (p11;q32),dic(12;17)(p11;p11)del(12)(p13p13), del(14)(q32),der(14)t(12;14)(p11;q32),der(17)t(1;17) (p34;q24),der(17)t(12;17)(p13;q11),−19,−20,−22,+5mar

Abbreviations: P, primary diagnosis; R, relapse/months after diagnosis.

using DAPI counterstaining. Between 5 and 12 abnormal metaphases were studied for each experiment. The FISH data were collected on a Leitz DMRB fluorescence microscope (E. Leitz Inc, Wetzler, Germany) equipped with a cooled black and white CCD camera run by SmartCapture software (Vysis, Stuttgart, Germany).

RESULTS

Seven new chromosome 12p translocations affecting the *ETV6* gene were identified in patients with different malignant hemopathies including chronic myeloid leukemia (CML), acute myeloid leukemia (AML), ALL, and B-NHL. Cytogenetic findings of all seven cases are presented in Table 2. In cases 1, 3, 4, 5, and 7 chromosomal aberrations were identified at diagnosis, whereas in case 2 and 6 the abnormal karyotypes appeared during the course of disease. The 12p aberrations were found to be the sole abnormality in karyotypes of three patients; in one case a t(12)(p13) was associated with a monosomy 13, whereas the karyotypes of the three remaining cases displayed complex multichromosomal changes. The potential involvement of *ETV6* was evaluated by FISH with a panel of cosmid probes covering the complete gene.[10] FISH results are summarized in Table 3.

A t(5;12)(q31;p13) was found in a patient with atypical CML associated with marked eosinophilia. FISH with *ETV6* cosmids showed that 179A6 (exon 1 of *ETV6*) hybridized to the der(5), whereas cosmid 67C6 (intron 1), 50F4 (exon 2), and 148B6 (exon 8) hybridized to the der(12) (Fig 2A). The chromosome 5 breakpoint was analyzed with a *PDGFRB* (5q33) probe (cosB)

Table 3. Results of FISH Analysis With 12p Specific Cosmid Probes

Case	Chromosome	Probes:	4H9A	179A6	15A4	67C6	50F4	132B11	242E1	163E7	54D5	148B6	123C11
		Loci:	*D12S158*					*ETV6*					*CDKN1B*
1	der(5)t(5;12)(q31;p13)			+		−	−					−	
	der(12)t(5;12)(q31;p13)			−		+	+					+	
2	der(6)t(6;12;17)(p21;p13;q25)			−	−	−	−			−		−	
	der(12)t(6;12;17)(p21;p13;q25)			−	+	+	+			+		+	
	der(17)t(6;12;17)(p21;p13;q25)			+	+	−	−			−		−	
3	der (7)t(7;12)(p15;p13)			+			+	−	−	−	−	−	
	der(12)t(7;12)(p15;p13)			−				+	+	+	+	+	+
4	der(7)t(7;12)(p12;p13)			+		−	−					−	
	der(12)t(7;12)(p12;p13)			+		+	+					+	
5	der(7)(t(7;12)(q36;p13)			+			+					−	
	der(12)t(7;12)(q36;p13)			−			+					+	
6	der(12)t(12;13;9)(p13;q12q14;q13)		−	−			−	−	−	−	+	+	+
	der(13)t(12;13)(p13;q12)		+	+			+	+	+	+	+	−	−
	del(12)(p12p13)		+	−			−	−	−	−	−	−	−
7	der(5)t(5;12;?)(p11;p11p13,?)		−	−						+		+	+
	dic(12;17)(p11;p11)		−	−						−		−	−
	der(12)t(12;14)(p11;q32)		−	−						−		−	−
	der(14)t(12;14)(p11;q32)		+	+						−		−	−
	der(17)t(12;17)(p13;q11)		+	−						−		−	−

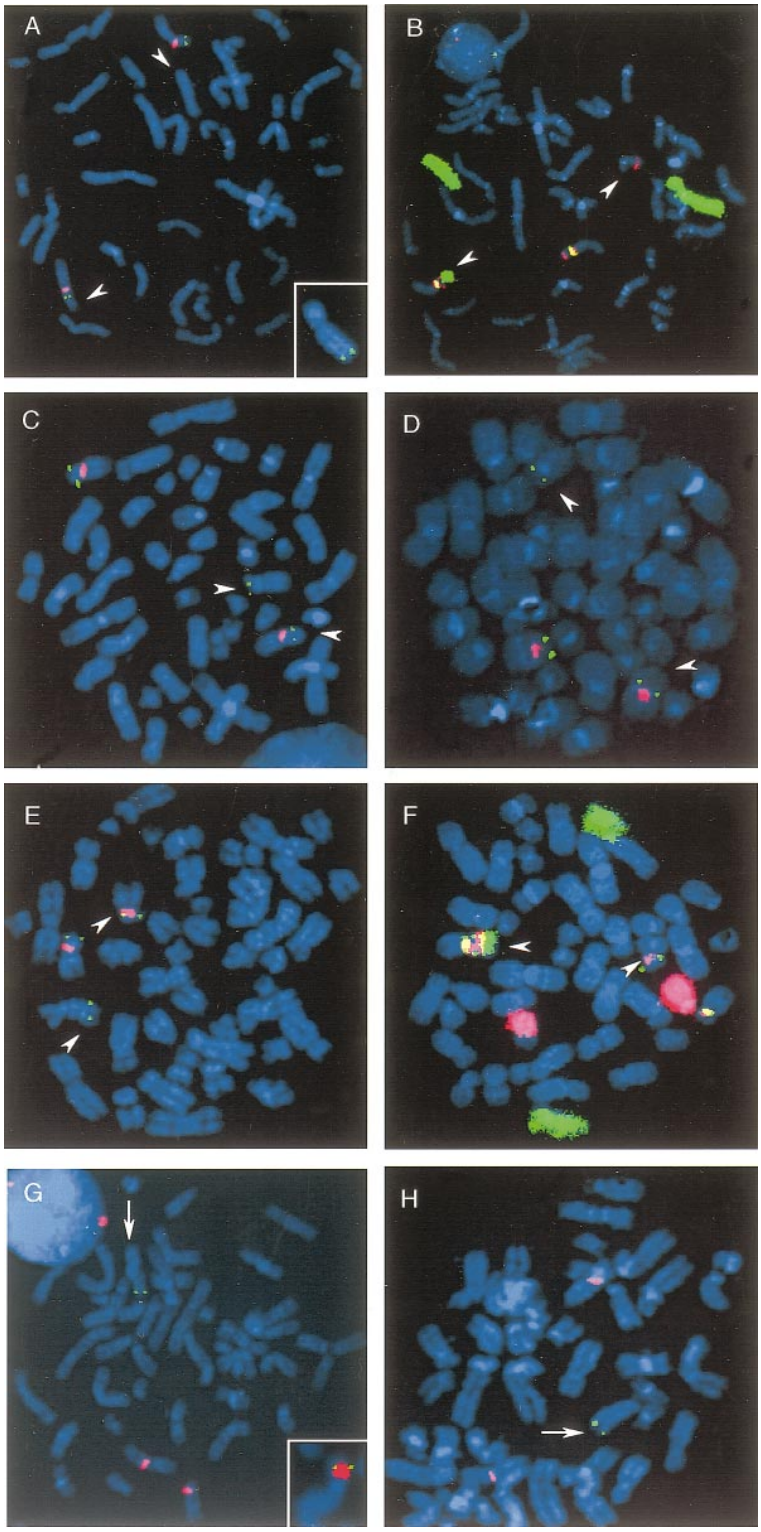

Fig 2. FISH analysis of *ETV6* rearrangements. (A) Case 1 with a t(5;12)(q31;p13); (B) case 2 with a t(6;12;17)(p21;p13;q25), (C) case 3 with a t(7;12)(p15;p13); (D) case 4 with a t(7;12)(p12;p13); (E) case 5 with a t(7;12)(q36;p13); (F) case 6 with a t(12;13)(p13;q12); and (G and H) case 7 with a der(12)t(5;12;?)(p11;p11p13;?). (A through F) arrowheads indicate derivatives of particular t(12)(p13); (G and H) arrows indicate der(5) and der(14) chromosomes, respectively. All green signals result from probes labeled with a bio-16-dUTP, red signals are generated by probes labeled with Texas Red-5-dUTP, yellow signals result from a mixture of bio-16-dUTP–labeled and Texas Red-5-dUTP–labeled probes in ratio 2;1. The probes used for FISH include (A) cosmid 67C6 (green) and a chromosome 12 centromeric probe pBR12 (red); (A inset) cosmid 179A6 (green); (B) cosmid 15A4 (red), library 6 (green) and pBR12 (yellow); (C) mixture of two cosmids 50F4 and 132B11 (green) and pBR12 (red); (D) cosmid 179A6 (green) and pBR12 (red); (E) cosmid 50F4 (green) and pBR12 (red); (F) cosmid 54D5 and library 9 (green), library 13 (red) and a chromosome 12 centromeric probe pBR12 (yellow); (G) cosmid 148B6 (green) and pBR12 (red); (G inset) cosmid 163E7 (green) and library 12 (red); and (H) cosmid 179A6 (green) and pBR12 (red). Note the hybridization of 179A6 and 67C6 to a der(5) and der(12), respectively, in case 1; hybridization of 15A4 with a der(12) and der(17) in case 2; separation of 50F4 and 132B11 cosmids on both derivative chromosomes in case 3; split signal from 179A6 and 50F4 in cases 4 and 5, respectively; split signal from 54D5 on der(12) and der(13), absence of a second 54D5 signal on a del(12)(p12p13), and presence of a chromosome 13 and 9 material on a der(12) in case 6; appearance of only one hybridization signal from cosmids 148B6 (G) and 179A6 (H) on a der(5) and a der(14), respectively, and the presence of additional material on a der(5)t(5;12;?)(p11;p11p13;?) upstream of the *ETV6*-specific cosmid 163E7 (G inset) in case 7.

and three YACs (773B7, 939F12, 888A7) assigned to 5q31q33. All these probes hybridized to the der(12) indicating that the breakpoint occurred proximal to 773B7, the most centromeric probe examined (Fig 3A).

A second 12p13 translocation resulting in an *ETV6* rearrangement was found in a pediatric patient with AML and a previous history of myelodysplastic syndrome (MDS). Cytogenetically the translocation was described as a t(6;12)(p21;p13). FISH with the cosmid 148B6 showed a hybridization signal on a der(12), whereas the cosmid 179A6 hybridized to 17q25. This implied a three-way translocation t(6;12;17)(p21;p13;q25), which was confirmed by FISH using chromosome 6 and 17

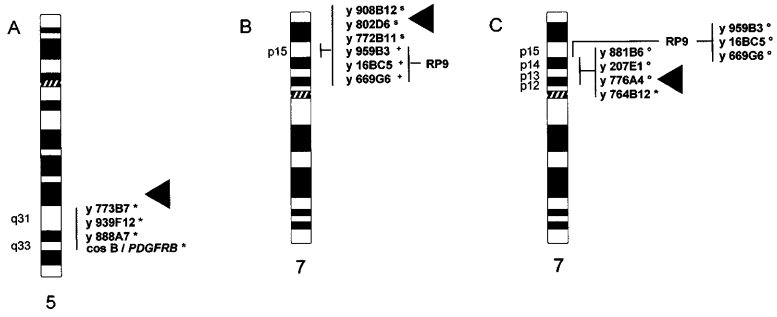

Fig 3. Localization of probes from chromosome 5q or 7p used for FISH detection of the breakpoints in case 1 with a t(5;12)(q31;p13) (A), case 3 with a t(7;12)(p15;p13) (B), and case 4 with a t(7;12)(p12; p13) (C). Abbreviations: (A) Asterisk (*) indicates hybridization with a der(12)t(5;12); (B) plus sign (+) indicates hybridization with a der(7)t(7;12) and s indicates split signal on der(7) and der(12); (C) ° indicates hybridization with a der(12)t(7;12), and asterisk (*) indicates hybridization with a der(7)t(7; 12); ◄ indicates position of breakpoint.

painting probes. Further FISH analysis showed that cosmid 15A4 (intron 1) hybridized to both the der(12) and der(17) (Fig 2B), which locates the breakpoint on chromosome 12 in the first intron of *ETV6* (Fig 1C).

Two other translocations, both cytogenetically defined as a t(7;12)(p14;p13), were found in pediatric patients with AML-M0 or ALL-L2. FISH analysis showed that in the AML case the translocation breakpoint was flanked by 50F4 and 132B11 (Fig 2C), and thus occurred near exon 2 of *ETV6* (Fig 1C). In the second case, cosmid 179A6 (exon 1) spanned the breakpoint (Fig 2D), and three cosmids 3′ to 179A6 hybridized with the der(12). To determine the breakpoint on the translocation partner, FISH analysis with chromosome 7p YACs was performed (Fig 3B and C and Fig 4). All three YACs (959B3, 16BC5, and 669G6) from a contig covering the Retinitis Pigmentosa 9 locus at 7p15[12] hybridized to the der(7) in the AML case or the der(12) in the ALL case, indicating a different 7p breakpoint for them. FISH analysis was then performed with YACs centromeric (881B6, 207E1, 776A4, and 764B12) or telomeric (772B11, 802D6, and 908B12) to *RP9*.[11,13] The breakpoint of the AML t(7;12) was found to be spanned by two overlapping probes, 802D6 (D7S516, D7S1808, and D7S2416/ 1790 kb) and 908B12 (D7S1808, D7S2416, and D7S2564/1300 kb). A third overlapping YAC, 772B11 (D7S516 and D7S1808/ 1500 kb), hybridized only to the der(7) chromosome. These results suggested the localization of the breakpoint near D7S2416 at 7p15. The breakpoint of the ALL t(7;12) was narrowed down to the area flanked by the YACs 776A4 and 764B12 (7p12).

In a karyotype of the other pediatric patient with AML, complex chromosomal abnormalities, including add(7)(q36) and del(12)(p12p13), were observed. FISH with 179A6 (exon 1) and 148B6 (exon 8) showed hybridization signals on add(7) and del(12) chromosomes, respectively, and implied a t(7; 12)(q36;p12). The *ETV6* breakpoint of this translocation was localized in a region covered by 50F4 that was split between both derivative chromosomes (Fig 2E). Because exon 2 is located at the 3′ end of 50F4, the breakpoint could be mapped to intron 1 of *ETV6* (Fig 1C).

The sixth *ETV6* translocation identified by FISH as a t(12;13)(p13;q12) was masked by complex chromosomal rearrangements not detected during cytogenetic analysis. FISH with *ETV6* cosmids 179A6 (exon 1) and 148B6 (exon 8) yielded hybridization signals on a small, unidentifiable chromosome and the der(12), respectively, whereas cosmid 54D5 (exons 5, 6, 7, and 8) spanned the breakpoint. All cosmids 5′ to 54D5 hybridized with the same small marker chromosome. However, none of them, nor a cosmid for *CDKN1B* (123C12), showed hybridization signals on the second chromosome 12, which was cytogenetically defined as a del(12)(p12p13). FISH analysis with the 12p13.3 cosmid 4H9A (*D12S158*) yielded hybridization signals on this del(12)(p) chromosome, confirming that the deletion is interstitial. Hybridization of the *ETV6* probes to the small marker suggested a cryptic t(12;21)(p13;q22), masked by the cytogenetically recognized t(9;12)(q13;p13), but FISH with a chromosome 21 paint and a 21qter cosmid (ICRFC102D12118) showed no rearrangement of chromosome 21. The use of painting probes for chromosome 9, 12, 13, and 20 allowed the identification of this small chromosome as a der(13) from a reciprocal t(12;13)(p13;q12) masked by an additional and

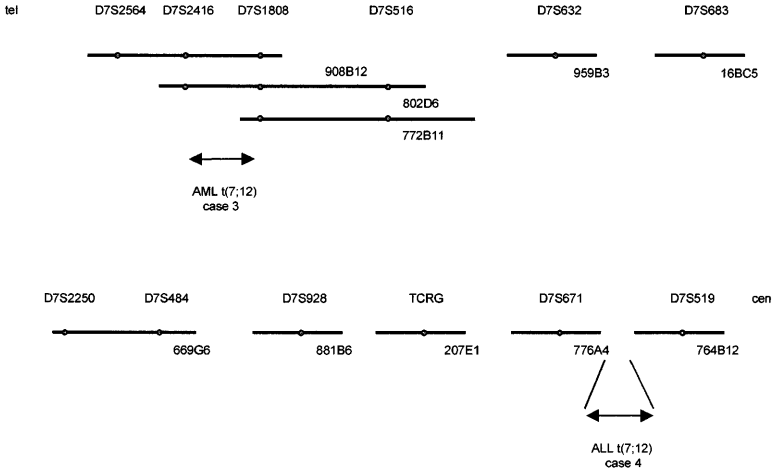

Fig 4. Scheme of the loci on 7p (ordered from telomere to centromere). The YACs used for this study, their STS content, and their breakpoint regions of two t(7;12) are shown.

probably secondary t(9;13;20)(p13;q14;p12) involving the der(12)t(12;13)(p13;q12) (Fig 2F).

The karyotype of a seventh patient with a B-NHL showed rearrangements of both 12p chromosomes, cytogenetically defined as a del(12)(p11) and dic(12;17)(p11;p11). FISH analysis with cosmid 179A6 (exon 1) yielded surprisingly only a single hybridization signal on the terminal end of the add(14)(q32) suggesting a t(12;14)(p11;q32). This reciprocal translocation was confirmed by FISH with a cosmid 4H9A (*D12S158*) showing a signal on the add(14)(q32) and with a 14q32 YAC (Y6), which hybridized to a chromosome defined cytogenetically as a del(12p). Only one Y6 signal was observed in all abnormal cells analyzed, indicating a cryptic del(14)(q32). Cosmid 148B6 (exon 8) did not hybridize with the add(14)(q32), showing an intragenic deletion of part of *ETV6*, which was then shown to extend as far as *CDKN1B* (123C12) (Table 3). However, cosmids 148B6 and 123C12 yielded hybridization signals with a chromosome band 5p suggesting a 5;12 translocation. As cosmid 179A6 did not hybridize with this der(5), the second *ETV6* allele also appeared to be inactivated by an intragenic deletion. Moreover, the 12p13.3 cosmid 4H9A (*D12S158*) was found to be translocated to another chromosome identified as a der(17)t(12;17)(p13;q11) using the chromosome 17 painting probe. Chromosome 17 material was also detected on a dic(12;17)(p11;p11), on the add(1)(p34) and a partially painted chromosome 17 suggesting a t(1;17)(p34;q24), and on a long arm of one of five marker chromosomes. All these data indicated the involvement of three copies of chromosome 17 in different chromosomal aberrations. Translocation of the telomeric 12p13.3 sequences (4H9A) to 17q11, deletion of the 5′ end of *ETV6*, and localization of hybridization signals from 163E7 and 148B6 cosmids on a distal but not terminal end of a der(5) chromosome (Fig 2G, inset) suggested a masked translocation of unknown material to the retained 3′ region of *ETV6* on a der(5). Despite using chromosome 5, 12, 19, 20, and 22 libraries, the origin of this material could not be identified by FISH because of its small size, complex aberrations, and insufficient material resources. Summarizing the FISH and G-banding results, the chromosome 12 abnormalities found in this patient can be described as follows: a der(5)t(5;12;?)(p11;p11p13;?), der(12)del(12)(p13p13)t(12;14)(p11;q32), dic(12;17)(p11;p11) del(12)(p13p13), der(14)t(12;14)(p11;q32), and der(17)t(12;17)(p13;q11).

The breakpoint of a previously reported MDS case with a t(10;12)(q24;p13)[16] was also further refined using the *ETV6* cosmids. The *ETV6* breakpoint occurred in intron 2 and was flanked by cosmids 50F4 and 132B11 (Fig 1C).

DISCUSSION

Seven new chromosomal aberrations involving the *ETV6* gene were detected in patients with CML, AML, ALL, and B-NHL and were further characterized by FISH. Although cytogenetic analysis already indicated 12p abnormalities in all these cases, FISH studies for most of them showed new and unexpected chromosomal rearrangements.

The (5;12)(q31;p13) translocation found in atypical CML showed a breakpoint gene in the first intron of *ETV6* and a chromosome 5 breakpoint centromeric to 773B7, mapped proximally to *PDGFRB*. These findings clearly indicate that this translocation is different from the t(5;12)(q33;p13) resulting in the *ETV6-PDGFRB* fusion cloned by Golub et al.[1] Insufficient material did not allow to identify the target gene at 5q31 by FISH; however, the breakpoint is localized in a region where many growth factors and hormone receptor genes have been mapped including members of the interleukin (IL) gene family (*IL3, IL4, IL5, IL9, IL12B, IL13*), interferon regulatory factor-1 gene (*IRF1*), early growth response-1 gene (*EGR1*), colony stimulating factor-2 gene (*CSF2*), CD14 antigen and cell division cycle 25C gene (CDC25C). It is interesting to note that the t(5;12)(q31;p13) presented here, as well as the translocation 5;12 resulting in the *ETV6-PDGFRB* fusion protein, occurred in patients with marked eosinophilia.

In the second case a three-way t(6;12;17)(p21;p13;q25) disrupting the *ETV6* gene in intron 1 was identified. The 5′ end of *ETV6*, including exon 1, was translocated to 17q25, whereas the remaining part of the gene with the HLH and ETS DNA-binding domains was retained on the der(12) and became juxtaposed to unknown 6p21 sequences. Among the candidate genes on 6p21 are *PBX2* (pre–B-cell leukemia transcription factor 2), *TNFA* (tumor necrosis factor-α), *PIM1*, *CDKN1A/WAF1* (cyclin-dependent kinase inhibitor 1A), *CBFA1/AML3* (α subunit of a core binding factor), and *CCND3* (cyclin D3).

Two apparently similar translocations, both cytogenetically defined as t(7;12)(p14;p13), appeared to be molecularly different after FISH analysis. Although both translocations have a breakpoint in the 5′ end of *ETV6*, they affected different regions on chromosome 7p (see Results). The breakpoint of the AML t(7;12) was near the D7S2416 locus at 7p15. The breakpoint is contained in the Centre d'études du Polymorphisme Humain YACs 802D6 and 908B2. The *HOX-A* gene cluster was mapped to 7p15 and can be considered candidate genes. However, polymerase chain reaction (PCR) analysis with expressed sequence tours developed for *HOXA1* and *HOXA4* did not detect the presence of these genes on either YAC excluding these candidates (results not shown). Of interest in a perspective of our finding is another published case of a t(7;12)(p15;p13) found in a 4-year-old boy with a minimally differentiated AML (French-American-British classification AML-M0).[17] This could be a relevant association because the AML-M0 is poorly characterized at the cytogenetic level. The largest published series of AML-M0 comprises 21 adult patients with clonal abnormalities,[18] and although 12p chromosomal aberrations were found in three patients, no t(7;12) was present among these cases. Moreover, involvement of this region in myeloid leukemia with a typical t(7;11)(p15;p15) or with a del(7p) has been already reported.[19,20]

Another 7;12 translocation involving the long arm of chromosome 7 (q36) and *ETV6* was found in a 1-year-old boy with AML. The *ETV6* breakpoint of this translocation was localized in intron 1 of *ETV6* upstream to exons encoding the HLH domain. The partner gene at 7q36 remains unknown, among the candidate genes is a cyclin-dependent kinase 5 (*CDK5*) involved in a regulation of cell cycle.

FISH analysis of the sixth case diagnosed as ALL-L2 showed disruption of *ETV6* by a cryptic t(12;13)(p13;q12) masked by additional multichromosomal changes not discernible by cytogenetic analysis. The *ETV6* breakpoint of t(12;13) is covered by cosmid 54D5, containing exons 5 to 8 of *ETV6*, and the

translocation is associated with loss of the second *ETV6* allele as a consequence of del(12)(p12p13). The 13q12 region involved in the t(12;13) is known to carry the *FGF9* (fibroblast growth factor 9), *FLT1* and *FLT3* (fms-related tyrosine kinase 1 and 3), *IPF1* (insulin promoter factor 1), and *BRCA2* (breast cancer 2) genes. Some other ALL cases with an analogous t(12;13) were previously reported.[21-23] Although in some cases the breakpoints were interpreted differently, there is a possibility that all of them involve *ETV6* and the same unknown sequences at 13q12.

The most complex karyotypic changes affecting both 12p chromosomes and *ETV6* were observed in a B-NHL case. FISH analysis showed that one chromosome 12 was rearranged by a t(12;14)(p11;q32) with a breakpoint typical for B-NHL lymphoma at 14q32 corresponding to the Ig heavy chain gene locus. The second chromosome 12p was involved in three different translocations, a t(5;12;?)(p11;p11p13;?), a t(12;17)(p13;q11), and a dic(12;17)(p11;p11). The 12p13 breakpoint in the t(5;12;?) that might have resulted in an *ETV6* fusion transcript was localized in the 5′ end of the gene upstream to cosmid 163E7 and upstream to the HLH coding exons. The partner chromosome involved in this translocation could not be identified. FISH analysis resulted in the detection of two different cryptic microdeletions of 12p13 affecting both *ETV6* alleles. One of them, independent from a t(12;14)(p11;q32), covered the 3′ end of *ETV6* (163E7 and 148B6) and *CDKN1B* (123C12), whereas the second intragenic microdeletion was associated with a t(5;12;?)(p11;p11p13;?) and involved the 5′ end of *ETV6* (179A6). This latter finding indicated that the putative reciprocal chimeric transcript of a t(5;12;?)(p11;p11p13;?) containing the 5′ end ETV6 domain was absent in the malignant cells and did not play a significant role in the development and/or progression of this lymphoma. It is noteworthy that deletion of the second *ETV6* allele is a commonly observed secondary phenomenon in pre–B-ALL cases with a t(12;21)(p13;q22).[24,25] In two cases the deletion was shown to be intragenic in *ETV6*,[25,26] suggesting that the gene was probably the actual target of the deletion. It was already hypothesized that wild type *ETV6* might reduce the oncogenic and growth-stimulating properties of the fusion proteins and that, therefore, its loss would provide an additional proliferative advantage to the malignant cells. The characterization of two new cases with biallelic *ETV6* rearrangements [together with at least one more leukemia case characterized by a t(9;12;14)(q34;p13;q22)/*ETV6-ABL* and del(12)(p11;p13)[27]; B-cell ALL cell line with a t(6;12)(q23;p13)/*ETV6-STL* and a microdeletion of the other *ETV6* allele[7]; and a myeloid leukemia case with two translocations involving *ETV6*, namely, t(3;12)(q26,p13) and t(9;12)(p24;q15;p13)[2]] indicates that biallelic aberrations of *ETV6* are not exclusively associated with a subtype of ALL with a t(12;21).

In summary, seven new chromosomal abnormalities affecting *ETV6* have been identified in patients with atypical CML associated with eosinophilia in MDS, in young patients with acute leukemias and, for the first time, in a case of B-NHL. In two leukemia cases the *ETV6* aberrations appeared during the course of the disease suggesting that the disruption of this gene can not only initiate the development but also be involved in the progression of the malignant disorder. The latter can be supported by the karyotypic findings in a case of B-NHL where the *ETV6* affected translocation was coexisting with a typical 14q32/IgH translocation regarded as a primary rearrangement in lymphomagenesis. The frequency of these new *ETV6* translocations in hemopathies is difficult to evaluate because of multichromosomal aberrations impeding cytogenetic detection.

FISH analysis of seven *ETV6* rearrangements showed that in six of them the breakpoint occurred in the 5′ end of *ETV6* upstream of exons coding for the HLH domain. Together with the previously reported t(10;12)(q24;p13),[16] they might generate chimeric proteins with features of transcriptional activators analogous to the t(12;22)(p13;q11) fusion protein, but it is also conceivable that the oncogenic properties of some of these translocations result from the *ETV6* promoter driving the partner gene as suggested for a t(3;12)(q26;p13) or from the disruption of as yet unidentified domain(s) encoded by the first two exons. In the remaining case with a t(12;13)(p13;q12), the breakpoint was localized in the 3′ end of *ETV6*, probably upstream to exons coding for its ETS DNA-binding domain as was previously reported for the t(5;12)(q33;p13), t(9;12)(q34;p13), and t(12;21)(p13;q22), which might lead to the expression of chimeric transcripts containing either the HLH domain or the ETS domain of *ETV6*. On the other hand, regarding that *ETV6* is a candidate tumor suppressor gene, it is possible that some of the *ETV6*-related translocations, especially those associated with a complete or partial deletion of the second *ETV6* allele, might inactivate the *ETV6* gene as hypothesized for a t(6;12)(q23;p13).

The data presented here support the hypothesis of the multiple contributions of *ETV6* in the pathogenesis of hematologic disorders and show the occurrence of *ETV6* aberrations in hemopathies as diverse as CML, AML, ALL, and B-NHL. The cryptic deletions of the nontranslocated *ETV6* allele found in two cases further emphasize the significance of the inactivation of the wild type *ETV6* protein for the development and/or the progression of some hematologic malignant disorders.

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