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# LETTER TO THE EDITOR

## ETV6 mutations and loss in AML-M0

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*ETV6* (ETS translocation-variant gene 6, located on chromosome 12p), also known as *TEL*, encodes a transcription repressor belonging to the E26 transforming specific (ETS) family of DNA-binding proteins. *ETV6* is known as a proto-oncogene involved in translocation with over 40 partners.[1] In acute myeloid leukemia (AML) only a few rare translocations result in transforming fusion proteins,[1] indicating that the oncogenic role of *ETV6* does not play a major part in AML. However, abnormalities of the short arm of chromosome 12 (12p) are found in about 5% of AML and myelodysplastic syndromes. Most abnormalities consist of total or partial loss of 12p usually affecting *ETV6* and *CDKN1B*, implicating these genes as tumor-suppressor genes.[1–4] Recently, heterozygous mutations of *ETV6*, resulting in loss of repressor activity, were found in AML, adding to the view that *ETV6* might have tumor-suppressor characteristics.[5]

Whereas translocations and deletions involving *ETV6* have been reported in AML-M0, *ETV6* mutations were never investigated specifically in this subtype.[4,6] To better understand the role of *ETV6* in AML-M0, we studied 52 M0 patients using cytogenetic techniques complemented with single-nucleotide polymorphism (SNP) arrays and sequencing of *ETV6*, as described in the Supplementary Information. The cohort was not selected for any parameter rather than being AML-M0, had a median age of 61 years (one patient was a child) and consisted of *de novo*, therapy-related (1 case) and secondary leukemia (4 cases).

Three patients presented deletions ranging from 3.2 to 14.3 Mb in 12p. The minimal overlap region of deletion between these patients included *ETV6* but excluded *CDKN1B*. Two others patients had a t(4;12)(q12;p13). In both patients, we detected an out-of-frame *CHIC2-ETV6* fusion transcript. The reciprocal transcript was not detected, supporting the view that t(4;12) does not result in an oncogenic fusion protein. In addition, we detected *ETV6*-inactivating biallelic mutations in one patient and mutations leading to ETV6 truncated proteins in another two patients (5.7%).

DNA isolated from flow-sorted leukemic cells[7] was compared with T-cell control DNA using GeneChip 10K arrays (Affymetrix, Santa Clara, CA, USA). Deletions in 12p were detected as loss of heterozygosity and copy-number reduction in patients 1, 9 and 45 (Figure 1; Table 1). The deletion in patient 9, confirmed by fluorescent *in situ* hybridization (FISH) analysis (Figure 2a), was accompanied by a t(10;12)(q11;p11) translocation detected by karyotyping (Table 1). Whereas deletions in patients 1 and 45 included both *ETV6* and *CDKN1B*, the proximal deletion break point in patient 9 excluded *CDKN1B* (Figure 1). The minimal deleted overlapping region extended from rs252028 (position 9814 kb) to rs747726 (position 12 561 kb). Together with the studies by Baens *et al.*[2] and La Starza *et al.*,[3] with proximal break point at d12s358 (a marker close to rs747726), these are the most telomeric break points, including *ETV6* ever reported. Both studies set the distal break point at the *PRB3* gene, delimiting a region of 1200 kb (Figure 1c).[2,3] Because inactivation of the second allele of *ETV6*, or another gene, in the deleted region was never found, haplo-insufficiency of *ETV6* has been suggested as an AML mechanism.[4]

The t(4;12)(q12;p13) translocation, although rare, is a recurrent event in AML, particularly in subtype M0.[6,8] Previous studies have mapped the break points of this translocation within *ETV6* and three regions in chromosome 4: *CHIC2*, *HSG2* and an area between these two genes.[6,8] Cools *et al.*[6] reported four cases where the t(4;12)(q12;p13) fused the first three exons in *CHIC2* on 4q12 to exons 2–8 of the *ETV6* gene in 12p13, resulting in the expression of a hybrid *CHIC2-ETV6* transcript in all cases. In our cohort, patients 2 and 43 showed t(4;12)(q12;p13) (Table 1). In patient 2, FISH showed splitting signals of bacterial artificial chromosome (BAC) probes RP11-367N1 and of pooled RP11-96B19 and RP11-418C2 probes corresponding to the positions of *CHIC2* and *ETV6*, respectively (Figure 2b). Unfortunately, metaphases for patient 43 were not available for FISH analysis. We screened both patients for *CHIC2-ETV6* transcripts using the same approach described by Cools *et al.*[6] In both patients, and in contrast with that study, we could only detect an *ETV6-CHIC2* fusion transcript that consisted of the first exon of *ETV6* and exons 2–6 of *CHIC2* (Figure 2c). The fusion transcript, mainly consisting of exon 1 of *ETV6*, encodes a very short out-of-frame protein (Figures 2c and d). We repeatedly failed to detect the reciprocal *CHIC2-ETV6* transcript in both patients, which, if present, would consist of the first exon of *CHIC2* and exons 2–8 of *ETV6*, producing again an out-of-frame fusion transcript. In addition, we did not detect loss of the remaining allele in any of the patients (Figure 1). This finding is in line with the absence of the transforming ability of the CHIC2-ETV6 protein described by Cools *et al.*,[8] and further supports the idea that the fusion protein is not the element of pathogenesis. The heterogeneity of the t(4;12) adds to this view.[1,8] Several AML studies reported different translocations involving *ETV6*, but lacking functional fusion proteins. In some cases, it has been shown that ectopic overexpression of proto-oncogenes at the partner chromosomes might be the malignant event.[1] Still in many other cases, neither a functional fusion protein nor an alternative malignant mechanism was found.[1] This suggests that heterozygous disruption of *ETV6* by the translocation results in *ETV6* haplo-insufficiency and is part of the process resulting in AML.[9] Adding to this, many translocations in AML involving break points outside the *ETV6* locus are accompanied by cryptic deletions that include *ETV6*,[3] suggesting targeting of this gene. This is the case of patient 9 for whom FISH analysis showed that the deletion break points coincided with the translocation break point linking these two events (Figure 2a; Supplementary Figure 1).

We amplified and sequenced all 8 exons of *ETV6* using genomic DNA of the 52 patients. Mutations in *ETV6* have only been reported previously in five AML patients (M1 and M2) and in prostate cancer.[5] We found insertions in patients 6 and 58, and a point mutation in patient 21 (Figure 3; Table 1). Mutations in patient 6 were biallelic, including a frameshift mutation in one allele, resulting in a truncated protein, and an insertion in

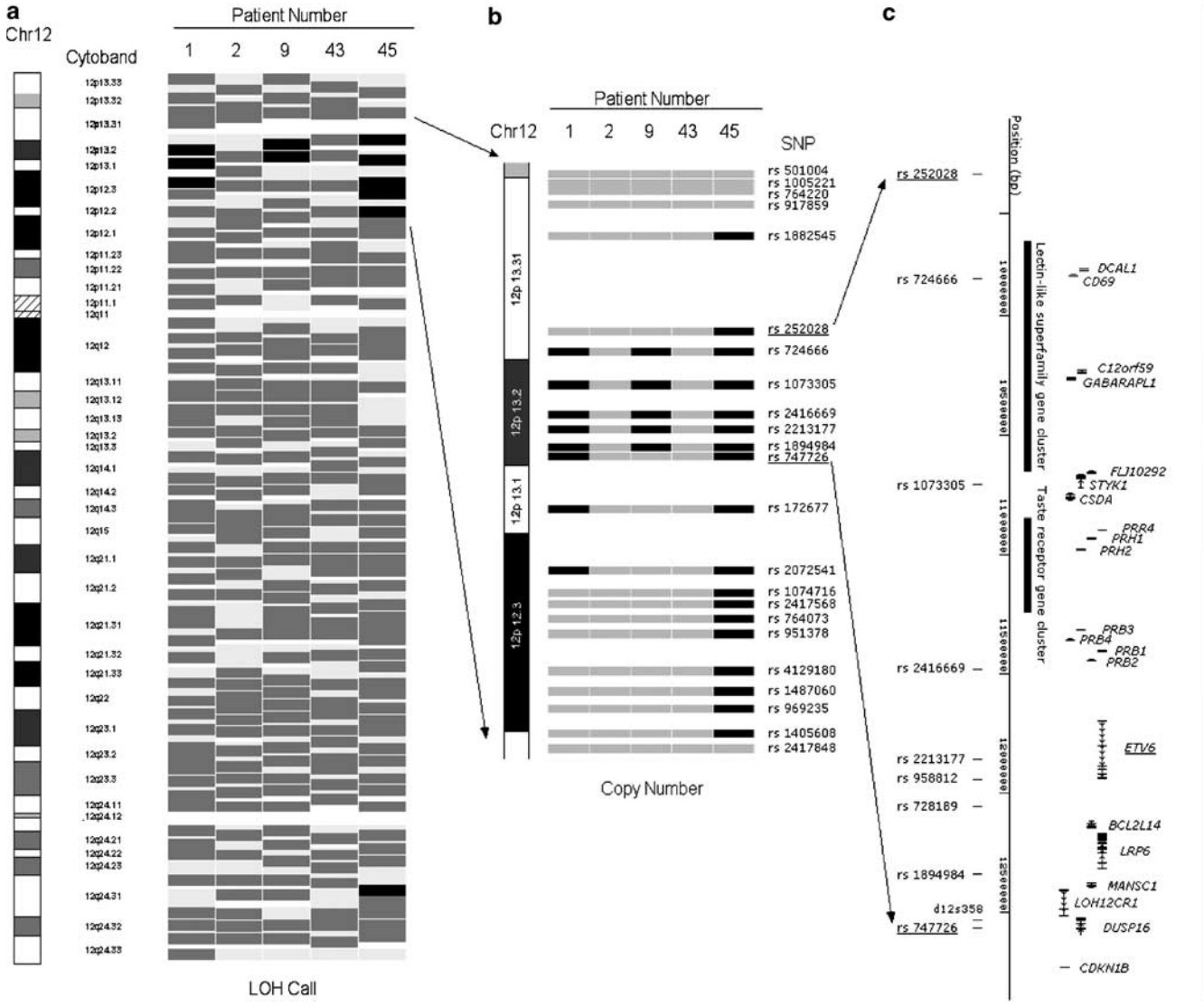


**Figure 1** Single-nucleotide polymorphism analysis of patients with chromosome 12 abnormalities. (**a**) Loss-of-heterozygosity values based on haplotype, using the paired normal. Each box represents the combined call between tumor sample and respective control (T cells). Black boxes represent loss of heterozygosity, dark gray boxes no loss and light gray boxes non-informative markers. Boxes are displayed proportionally to the position of the SNP that they represent in relation to the cytogenetic band to the left of the panel. (**b**) Chromosome copy number calculated for the common region showing loss of heterozygosity in panel a (amplified). Copy number for the tumor samples was inferred using the paired normal as reference and a median smoothing. Gray boxes represent two copies and black boxes one copy (deletion) for each chromosome locus. (**c**) Schematic representation of the genes present in the minimal deleted overlapping region defined in (**b**) based on the UCSC Genome Browser (http://genome.ucsc.edu/). Solid black boxes represent clusters of related genes. SNP, single-nucleotide polymorphism.

**Table 1** Clinical, hematological, cytogenetic features and mutational status of *ETV6*

| *Patient* | *Age* | *Diagnoses* | *Karyotype* | ETV6 | |
|---|---|---|---|---|---|
| | | | | *Allele 1* | *Allele 2* |
| 1[a] | 65 | AML-M0 | 46,XX,del(16)(q22?),i(17)(q10),del(20)(q?) | Deleted | WT |
| 2[a] | 67 | sAML-M0 | 47,XX,t(4;12)(q12;p13),−21,+der21del(q?)x2 | Translocated | WT |
| 6[a] | 37 | AML-M0 | 46,XY | S107DfsX21 | V345_Y346insR |
| 9[a] | 47 | sAML-M0 | 52,XX,t(1;4)(p13;p12),+6,+8,t(10;12)(q11;p11),+18,+19,+20,+21 | Deleted | WT |
| 21 | 59 | AML-M0 | 46,XY | R360X | WT |
| 43 | 64 | AML-M0 | 46,XY,t(4;12)(q12;p13) | Translocated | WT |
| 45 | — | AML-M0 | ND | Deleted | WT |
| 58 | 50 | AML-M0 | 46,XY,idic(21)(p11.2) | F103LfsX11 | WT |

Abbreviations: AML, acute myeloid leukemia; *ETV6*, ETS translocation-variant gene 6; ND, not done; s, secondary; WT, wild type.
[a]Patients in the study by Silva *et al*.[7]

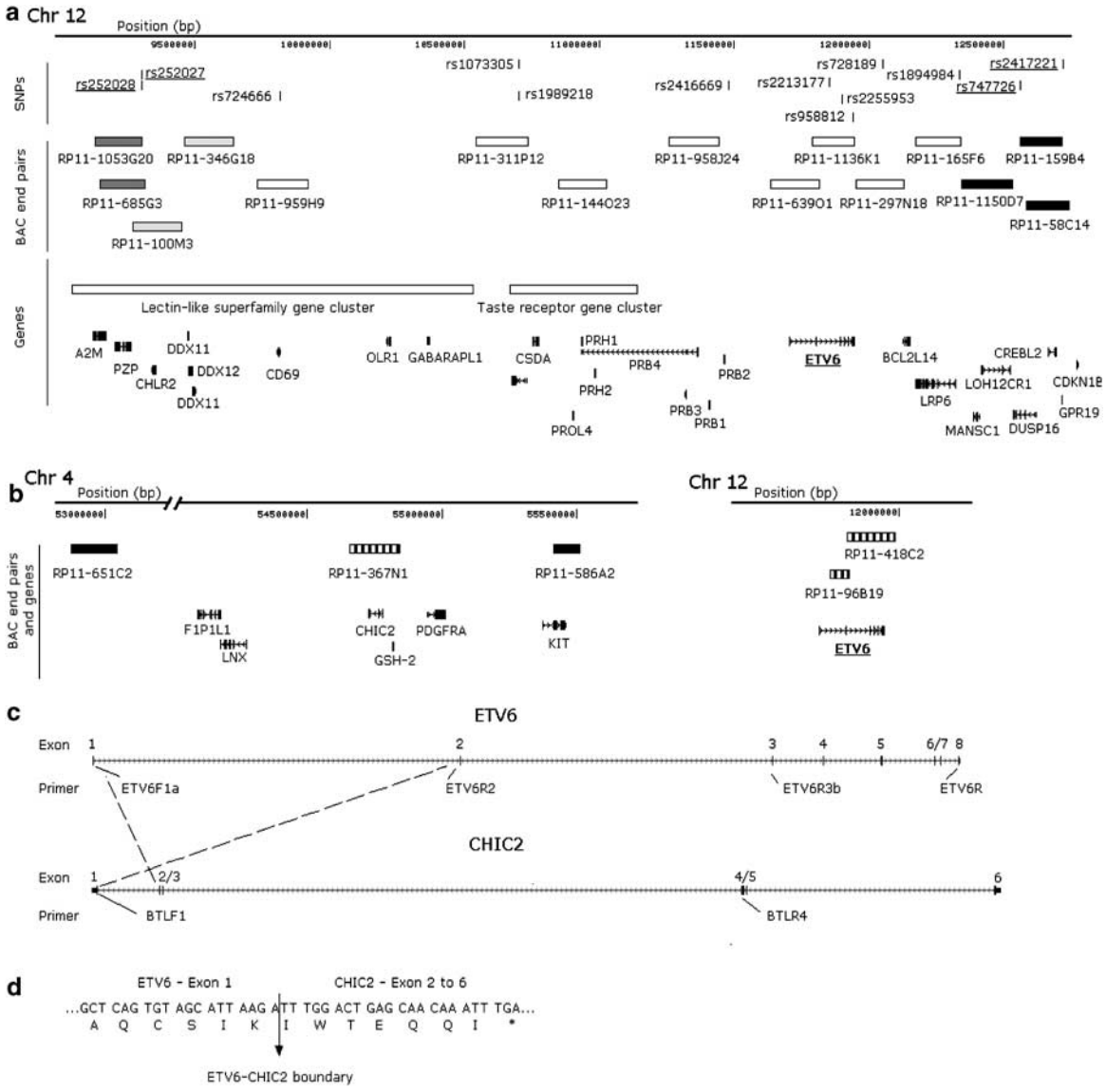


**Figure 2** Schematic representation of FISH analysis of the breakpoints in t(10;12) in patient 9 and t(4;12) in patient 2, and fusion transcripts in patients 2 and 43. (**a**) Determination of the deletion and translocation break points in patient 9. The deleted region in chromosome 12 is localized between SNP rs252027 to SNP rs747726 (SNPs present in a single copy are not underlined) as determined using the GeneChip 10K array (Figure 1b). By FISH analysis, we determined that the distal translocation break point occurred between BACs RP11-685G3 and RP11-959H9, whereas the proximal break point occurred between BACs RP11-165F6 and RP11-1150D7. BACs represented by white boxes are hemizygously deleted, by black boxes are retained in the original chromosome, by dark gray boxes are translocated to the partner chromosome, whereas light gray boxes show an intrachromosomal cross-hybridization signal on der(12) and a split signal with der(10.) Only known genes are represented and white boxes represent clusters of related genes. (**b**) BAC clones on chromosome 4 and 12 used to determine the t(4;12) break point in patient 2. The gap in chromosome 4 is approximately of 0.75 Mb. Color code for the BAC probes as in panel (a), with the exception of striped boxes, which represent BACs showing a split signal. (**c**) Genomic structure of *CHIC2* (4q12) and *ETV6* (12p13) according to UCSC Genome Browser and positions of primers used for PCR and sequencing (genes are not represented in the same scale). Dotted crossed lines represent the probable area where the t(4;12) occurred in patients 2 and 43. (**d**) *ETV6-CHIC2* transcripts detected in both patients 2 and 43. Sequence of *ETV6-CHIC2* cDNA showed an out-of-frame fusion between exon 1 of *ETV6* and exon 2 of *CHIC2*. The arrow indicates the boundary between the *ETV6* and *CHIC2* exons. BACs in all panels were chosen according to the latest version of the UCSC Genome Browser. BAC, bacterial artificial chromosome; *ETV*, ETS translocation-variant gene; FISH, fluorescent *in situ* hybridization.

the remaining allele (Figure 3; Table 1). ETV6-mutant proteins with truncation and insertions comparable to ours (Table 1; Figure 3) were shown to have impaired transcriptional repression activity.[5] Interestingly, all our mutants resulted in loss of the ETS domain or its binding activity, but not of the pointed (PNT) domain (Figure 3). Similar mutants reported by Barjesteh van Waalwijk van Doorn-Khosrovani *et al.*[5] showed a dominant-negative effect when *ETV6* wild-type constructs were co-transfected with higher amounts of mutant construct. However, it is possible that the dominant-negative effect is due to the overexpression of mutant ETV6 protein. In fact, expression of mutated ETV6 protein was not detected in the patients in the Barjesteh van Waalwijk van Doorn-Khosrovani *et al.*[5] study, arguing against a dominant-negative effect.

In conclusion, the number and variety of *ETV6* translocations not resulting in a fusion protein, together with loss of *ETV6* by

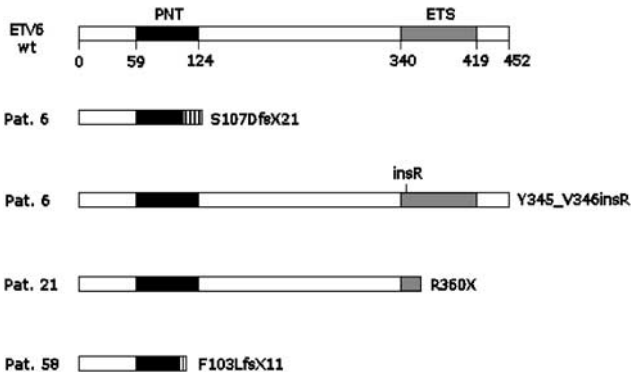


**Figure 3** Schematic representation of the predicted ETV6-mutant products. Amino-acid positions are shown under the wild-type ETV6 protein. The pointed (PNT) and ETS DNA-binding domain are represented by a black and a gray box, respectively. New open reading frames resulting from frame shifts are drawn as stripped boxes. Patient 6 has two predicted proteins corresponding to each one of the mutated alleles. ETV6, ETS translocation-variant gene 6.

deletion and heterozygous or homozygous mutations, makes a mutually supportive and compelling case for loss and haploinsufficiency of *ETV6* as a leukemogenic step in AML-M0.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)