ETV6 mutation in a cohort of 970 patients with hematologic malignancies

The *ETV6* gene (previously known as TEL) belongs to the ETS (E26 transformation specific) family of transcription factors characterized by 2 important domains: the C-terminal Ets domain responsible for specific DNA-binding activities and the N-terminal helix–loop–helix (HLH) oligomerization domain, also known as pointed (PNT) or sterile alpha motif (SAM), that mediates protein–protein interaction with Ets factors.[1-3] The ETV6 protein plays a crucial role in the embryonic development and hematopoietic regulation.[4] *ETV6* is also a versatile element at the center of a network of genes involved in hematologic malignancies through diverse molecular mechanisms, such as fused with other genes and deletions.[3,5-8] *ETV6* was originally identified as a fusion partner of the gene that is fused to *PDGFRB* (platelet derived growth factor receptor beta) gene in chronic myelomonocytic leukemia (CMML) patients with t(5;12)(q33;p13).[8] Subsequently, a growing number of genes have been identified as fusion partners of *ETV6*. At present, 30 partner genes of the *ETV6* gene have been described in a broad spectrum of hematopoietic malignancies.[9] Deregulation of the *ETV6* gene through deletion is also recurrent in leukemia, especially in acute lymphoblastic leukemia patients with t(12;21)(p13;q22).[10]

Recently, point mutations in the *ETV6* gene have been reported in 2.7% cases of myelodysplastic syndromes (MDS),[11] 24-33% of early T-cell precursor ALL (ETP-ALL),[12,13] and a few cases of acute myelogenous leukemia (AML).[14] However, only few data are available on other entities of hematologic malignancies. In order to analyze the frequency of *ETV6* mutations and their clinical impact, we investigated a total of 970 cases. In detail, we analyzed 296 *de novo* AML, 139 B-cell acute lymphoblastic leukemia (B-ALL), 53 T-cell acute lymphoblastic leukemia (T-ALL), 37 mixed-phenotype acute leukemia (MPAL), 169 chronic myeloid leukemia (CML), 101 MDS, 49 chronic lymphocytic leukemia (CLL), 62 myeloproliferative neoplasms (MPN), 28 multiple myeloma (MM), and 36 non-Hodgkin lymphoma (NHL) cases. There were 462 male and 501 female patients in this series; median age was 44 years (range 5-88 years). Main patients' characteristics are summarized in Table 1.

We examined *ETV6* mutation by PCR amplification of the entire coding region followed by direct DNA sequencing. Genomic DNA was extracted from frozen bone marrow mononuclear cells (BMMCs) after Ficoll gradient centrifugation using standard procedures. Point mutations were confirmed in an independent second experiment. Primer sequences and PCR conditions are shown in *Online Supplementary Table S1*. Known single nucleotide polymorphisms are excluded based on the NCBI (Accession number NM_001987, Version NM_001987.4) or the 1000 Genomes databases.

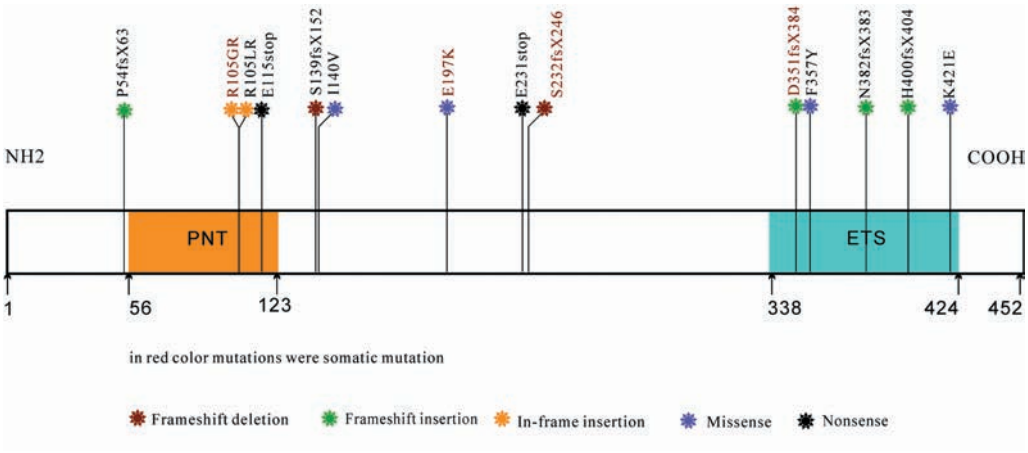
In total, 14 *ETV6* mutations were identified in our study, resulting in an overall frequency of 1.5% (14 of 970). *ETV6* mutations were most frequently detected in CLL (2 of 49, 4.0%), followed by MDS (3 of 101, 2.97%), MPAL (one of 37, 2.7%), B-ALL (3 of 139, 2.2%), AML (4 of 296, 1.35%), and CML (2 of 169, 1.2%). No mutations were found in NHL, MM, T-ALL, or MPN. Among these, frameshift, mis-

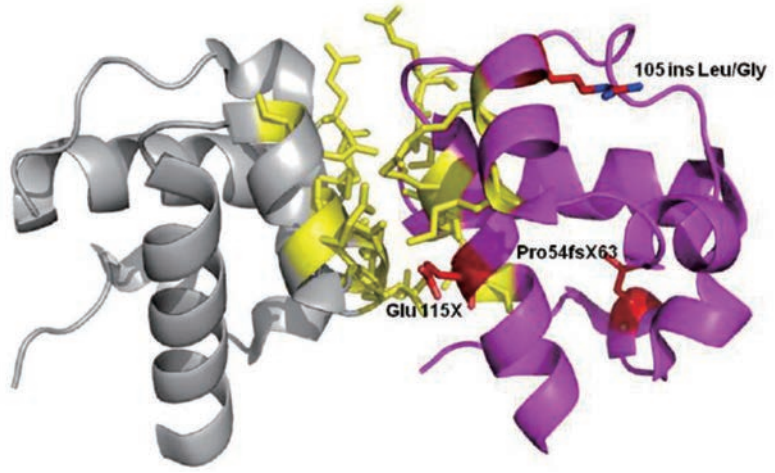
Table 1. The clinical and cytogenetic characteristics of the 970 patients.

Characteristics	Total n. of patients	Median age (range)	N. of *ETV6* mutation	Characteristics	Total n. of patients	Median age (range)	N. of *ETV6* mutation
AML	296	39(13-83)	4	MDS	101	56 (15-88)	3
Male	166			Male	67		
Female	130			Female	34		
M0	3		1	RCUD/RA/RN/RT	16		
M1	55			MDS-RARS	9		
M2	83			MDS-RAEB1	16		
M3	46		1	MDS-RAEB2	18		2
M4	32			MDS-RCMD	22		1
M5	61			MDS-U	20		
M6	5			IPSS subtype			
M7	0			Low	14		
unclassified	11		2	Intermediate I	61		1
B-ALL	139	32(5-75)	2	Intermediate II	17		1
Male	63			High	6		
Female	76			unclassified	3		1
L1	67						
L2	55			CML	169	37 (16-67)	2
L3	1			Male	53		
unclassified	16			Female	116		
T-ALL	53	23 7-56	0	MPN	62	48.5 (5-76)	
Male	15			Male	29		
Female	38			Female	33		
MPAL	37	39 (6-81)	1	MM	28	63 (28-76)	0
Male	14			Male	13		
Female	23			Female	15		
CLL	49	63 37-77	2	NHL	36	43 (15-88)	0
Male	37			Male	9		
Female	12		0	Female	27		

sense, and nonsense mutations respectively accounted for 57.1% (8 of 14), 28.6% (4 of 14) and 14.3% (2 of 14) of all *ETV6* mutations. These mutations are localized in exons 2, 7 and 8 (one case each), and exons 6, 5, 4, and 3 (4, 3, 2, and 2 cases, respectively). None of the 14 mutations have been reported previously. Characteristics of these patients are listed in *Online Supplementary Table S2*. In all available cases (4 of 14), analysis of matched newly diagnosed and remission genomic DNA confirmed the somatic origin of *ETV6* mutations (R105GR, S139fsX152, D351fsX384, and N382fsX383). The *ETV6* mutations detected in the present study are listed in Table 1 and graphically depicted in Figure 1A. We found that, except for the 4 missense mutations (I140V, E197K, F357Y, and K421E), all the mutations were predicted to cause the loss of either the ETS domain or the SAM-PNT domain. This mutation pattern was consistent with previous studies.[12-14]

To determine whether these mutations might disrupt the





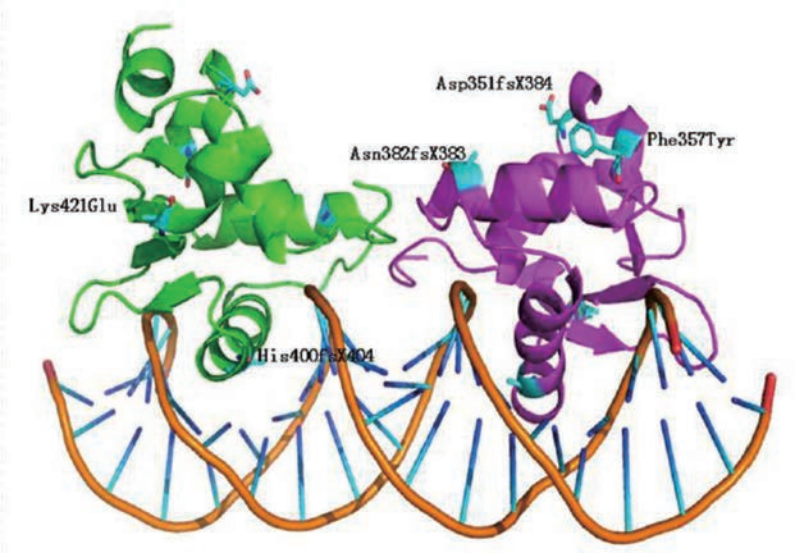


Figure 1 (A). Distribution of somatic sequence mutations in ETV6. Two conserved domains in ETV6 are shown: the PNT domain at its amino terminus which mediates homotypic oligomerization of ETV6 molecules and also heterotypic interaction with other protein and the DNA-binding ETS domain at amino terminus which mediates protein-protein interactions. In red color mutations were somatic mutation and all the mutations have not been reported previously. (B) ETV6 mutations in SAM domain. The structure of a heterodimer with an interface is illustrated by native interface of the SAM domain polymer of TEL. The main residuals from salt-bridges are shown in yellow. The mutation loci discovered in the SAM domain and its relation with the interface of heterodimer are labeled in red. (C). ETV6 mutations in ETS domain. ETS domain (334-437) 3D model of Tel dimmer DNA complex and the mutation residuals also were labeled.

ETS domain or the SAM domain-mediated transcriptional repression activity, structural homology modeling of the etv6 mutant ETS domain DNA binding complex and the SAM domain self-associated oligomerization interface were performed (Figure 1B and C). The structural homology modeling illustrated that the four mutations in the SAM domain (P54fsX63, R105LR, R105GR, and E115X) were near the interface and may disorder Etv6 functions significantly by interfering with the forming of oligomer. While 5 mutations in the ETS domain (D351fsX384, F357Y, N382fsX383, H400fsX404 and K421E) might impede DNA-binding activities of ETV6. Furthermore, 3 out of 5 mutations located in the link region are nonsense (E231X) or out-of-frame mutations (S232fsX246, S139fsX152) that lead to loss of ETS domain.

In cases with translocations involving ETV6, deletion of the non-rearranged ETV6 allele has been identified as an important secondary event that occurs in up to 60% of childhood ALL with *ETV6-RUNX1* fusion gene.[15] In a search for additional genetic abnormalities involving ETV6, we applied genome-wide array-based comparative genomic hybridization (array-CGH) or fluorescence *in situ* hybridization (FISH) technique to patients with and patients without *ETV6* mutations. However, no ETV6 deletion was found in those patients with *ETV6* mutation, while in 73 cases of hemotologic malignancies FISH showed 7 with ETV6 translocation (9.6%) and 12 with haploid deletion (16.4%). Furthermore, 11 patients with deletions were identified in 59 patients by array-CGH. Among these deletions, 2 were in AML (n=7, 28.6%), 6 in ALL (n=25, 24.0 %), 2 in MPAL (n=15,13.3%) and one in CML (n=2).

To evaluate the consequences of mutations on ETV6 expression, quantitative RT-PCR (QRT-PCR) was performed on samples from 12 patients with and 20 patients without *ETV6* mutations. There was no significant difference in the expression of ETV6 between patients with and patients without *ETV6* mutations (P>0.05).

In order to identify genetic defects that might co-operate with *ETV6* mutations in the pathogenesis of leukemia, we sequenced ASXL1, CBL, DNMT3A, EZH2, FLT3, IDH1, IDH2, IKZF1, K-RAS, NPM1, NRAS, P53 RUNX1, TET2, and WT1 in *ETV6*-mutated leukemia samples. We identified the IKZF1 deletion in one B-ALL patient and the *RUNX1* mutation in one patient with CML in blast crisis. No association of *ETV6* mutations with other molecular abnormalities was found in this study.

In summary, the present study describes the frequency and spectrum of the somatic mutations of *ETV6* in a variety of hematologic malignancies. Our results, together with previous reports in the literature, suggest that somatic mutations of *ETV6* are infrequent but recurrent genetic abnormalities in a wider range of myeloid or lymphoid malignancies, including MDS, AML, ETP-ALL, CLL, MPAL, B-ALL, and CML. Structural homology modeling analysis showed that *ETV6* mutations might disrupt the Etv6 structure and impede its function. In addition, it will be necessary to conduct further genetic studies with the novel genomics technologies, such as next generation sequencing, to determine the mutation landscape of *ETV6* in other hematologic malignancies.

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