LETTER TO THE EDITOR

ETV6-related thrombocytopenia: dominant negative effect of mutations as common pathogenic mechanism

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SUPPLEMENTARY DATA

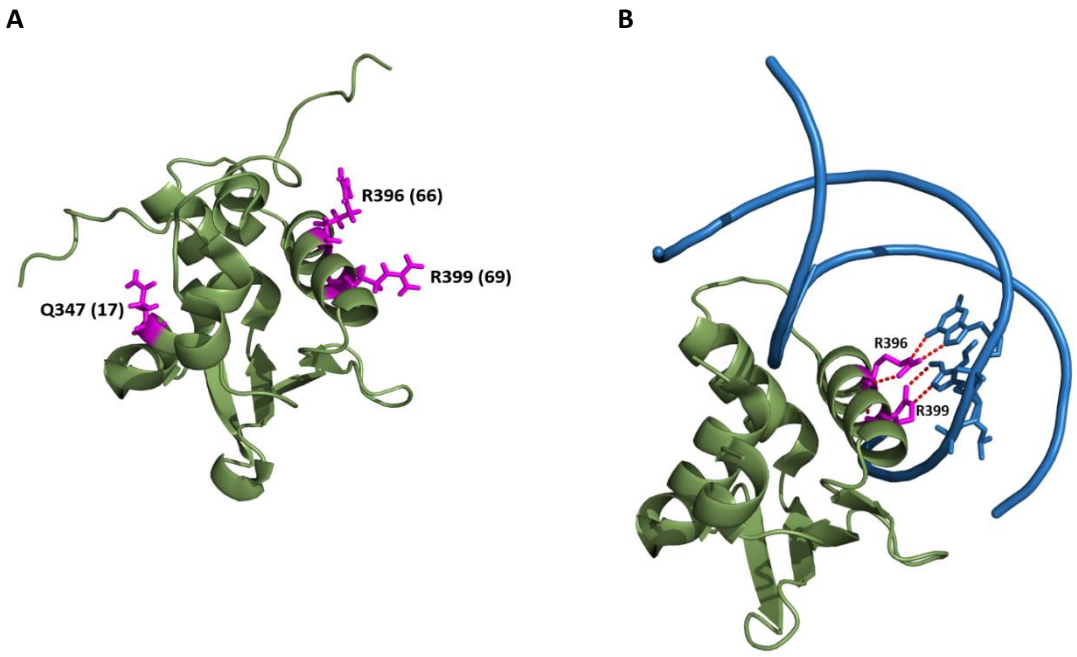
Nucleotide	Protein	Domain	Variant Effect Predictor				MAF (GnomAD)
			PROVEAN (ex SIFT)	Polyphen-2	CADD	ClinVar	
c.65G>A	p.S22N	N-term	T	B	21,4	NR	$7*10^{-6}$
c.672C>G	p.H224Q	CRD	T	B	15,04	NR	$2*10^{-5}$
c.1040A>C	p.Q347P	ETS	D	PD	31	NR	NR
c.1105C>T[1,2]	p.R369W[a]	ETS	D	PD	32	LP	NR
c.1138T>A[1,3]	p.W380R	ETS	D	PD	32	NR	NR
c.1186A>G[4]	p.R396G	ETS	D	PD	26,7	NR	NR
c.1196G>A[2]	p.R399H[a]	ETS	D	PD	32	LP	NR

Supplementary Table 1. In silico pathogenicity prediction of ETV6 variants identified by NGS analysis. The pathogenicity of ETV6 variants was evaluated using the following bioinformatic tools: PROVEAN, PolyPhen-2, CADD and ClinVar provided by Variant Effect Predictor (VEP) (https://grch37.ensembl.org/info/docs/tools/vep/index.html).
Minor allele frequency (MAF) provided by GnomAd (https://gnomad.broadinstitute.org/).

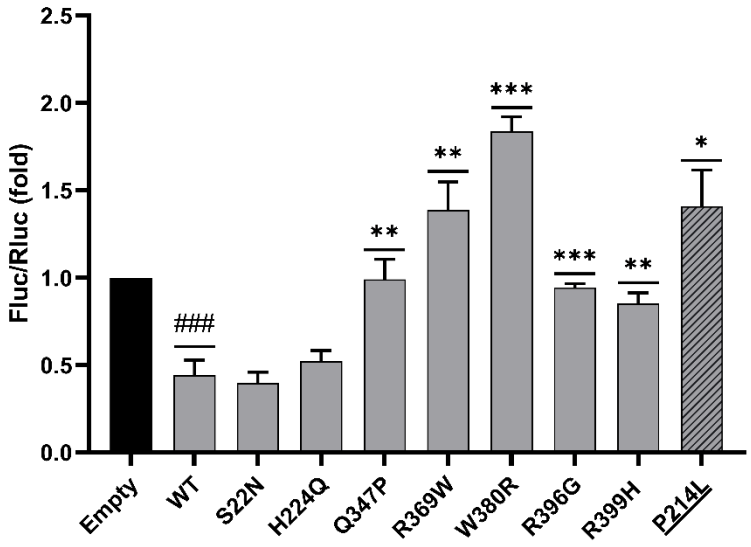
1. Melazzini F, Palombo F, Balduini A, et al. Clinical and pathogenic features of ETV6-related thrombocytopenia with predisposition to acute lymphoblastic leukemia. Haematologica 2016;101(11):1333–1342.
2. Nishii R, Baskin-Doerfler R, Yang W, et al. Molecular basis of ETV6-mediated predisposition to childhood acute lymphoblastic leukemia. Blood 2021;137(3):364–373.
3. Kozubik KS, Radova L, Reblova K, et al. Functional analysis of germline ETV6 W380R mutation causing inherited thrombocytopenia and secondary acute lymphoblastic leukemia or essential thrombocythemia. Platelets 2021;32(6):838–841.
4. Poggi M, Canault M, Favier M, et al. Germline variants in ETV6 underlie reduced platelet formation, platelet dysfunction and increased levels of circulating CD34+ progenitors. Haematologica 2017;102(2):282–294.

[a] Same amino acids involved in other substitutions described in Zhang et al., 2015 and Nishii et al., 2021.

PROVEAN legend: D: deleterious, T: tolerated. Polyphen-2 legend: B: benign, PD: probably damaging. CRD: central regulatory domain; ETS: C-terminal DNA-binding. ClinVar legend: NR: not reported, LP: likely pathogenic.



Supplementary Figure 1. Mutations exert a different effect on protein structure (A) Analysis of the structure of ETS domain of ETV6 (PDB ID 2DAO) reveals that mutations Q347P and R396G can destabilize the fold of the α-helixes to which they belong, affecting the entire protein stability and folding. Indeed, proline residues cannot form the canonical backbone α-helix hydrogen bonds having no amide hydrogen. Furthermore, their ring structure sterically inhibits the formation of α-helix turns resulting in bending the helix' axis. On the other hand, glycine residues are too flexible and do not suit with the well constrained α-helix structure. (B) The substitution R399H might have effects on the DNA binding function of the protein. Indeed, structural analysis of ETS bound to a specific DNA sequence (PDB ID 4MHG) revealed that in the wild type protein, R399 is involved in electrostatic interactions with its DNA cognate. The substitution with a histidine residue might destroy these interactions and affect the protein function. Similarly, this might also happen for the substitution R396G being R396 also involved in electrostatic interactions with the DNA binding partner. The protein structures were displayed and analysed by the graphic program Pymol (The PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC).



Supplementary Figure 2. ETV6 mutations impair protein repression ability. Dual luciferase assay of HEK293 cells 48 hours after transfection with wild type or mutated ETV6, firefly luciferase cloned downstream MMP3 promoter and *Renilla* luciferase under the control of CMV promoter as normalizer. Histogram shows the firefly/renilla light emission ratio. The ratio is normalized on empty vector (black bar) levels. P214L (striped column) was used as control mutation. Error bars represent standard deviation of three independent experiments. ### = p<0.001, versus empty vector. * = p<0.05, ** = p<0.01, *** = p<0.001, versus ETV6 WT overexpression, Student's T test.