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ETV6 in Hematopoiesis and Leukemia Predisposition

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Abstract

The *ETV6* (also known as *TEL*) gene encodes a transcriptional repressor that plays a critical role in hematopoiesis and in embryonic development. While somatic *ETV6* translocations and missense mutations are frequently observed in human cancers, the role of *ETV6* in malignant transformation was unclear. Recently, autosomal dominant germline *ETV6* mutations were discovered in families with inherited thrombocytopenia and a propensity to develop hematological malignancy, unequivocally demonstrating a role for *ETV6* in leukemogenesis. Studies of germline *ETV6* mutations also uncovered an important function of *ETV6* in megakaryocyte development. Here we discuss our current understanding of the role of *ETV6* in malignancy and in hematopoiesis.

Introduction

ETV6 (also known as *TEL*) was originally discovered in a leukemia-associated chromosomal translocation (1) and has subsequently been identified as a fusion partner in more than 30 chromosomal translocation oncogenes (reviewed in (2)). *ETV6* is a transcriptional repressor that binds DNA via a C-terminal DNA-binding domain (Figure 1), highly conserved among ETS-family transcription factors (reviewed in (3)). The *ETV6* N-terminal Pointed (PNT) domain (Figure 1), also termed helix loop helix (HLH) or sterile alpha motif (SAM) domain, mediates self-association and frequently contributes to fusion proteins as the partner of tyrosine kinases (4). In this setting, the PNT-domain functions as a powerful oligomerization motif that activates leukemogenic kinase activity (5). Such leukemogenic translocations may function entirely independent of the physiological role *ETV6*. However in translocations involving MN1 (6), BTL (7), and PAX5 (8)), the DNA-binding domain of *ETV6* is part of the leukemogenic fusion protein, suggesting that altered expression of normal *ETV6* target genes is involved in the leukemia pathogenesis. Loss of

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ETV6 has firmly been implicated in the pathogenesis of ETV6-RUNX1 (TEL-AML1) - associated childhood leukemia as there is invariably bi-allelic loss of *ETV6* due to deletions of the second (non-translocated) *ETV6* allele (9). Finally more recently, genome wide investigations have uncovered that TEL/ETV6 is subject to heterozygous mutations in hematologic malignancies, including MDS (10, 11), AML (12), early T-cell precursor (ETP) ALL (13, 14), high-risk B-ALL (15), and diffuse large B-cell lymphoma (DLBCL) (16). However, it remained elusive whether and how loss of *ETV6* or modulation of *ETV6* function functionally contributes to leukemogenesis. Now a number of recent studies have uncovered germline *ETV6* mutations in families with a propensity to develop hematological malignancy, unequivocally demonstrating its potential to incite leukemia.

Familial Thrombocytopenia and Predisposition to Hematologic Malignancy

The initial report from Zhang et al identified the link between heterozygous germline *ETV6* mutations to dominantly inherited thrombocytopenia and predisposition to hematologic malignancies in three families (17). Additional studies extended these findings to additional families with unique *ETV6* germline mutations and predisposition to malignancy. (18) (19) (20) (21) (22). With one exception, all of the germline heterozygous *ETV6* mutations identified in family studies cluster within the highly conserved ETS domain shared by all ETS family proteins and responsible for binding to DNA (Figure 1). The P214L mutation was the only mutation identified from family studies to fall outside of the ETS domain. Interestingly, this was reported in 5 different families. The effects of *ETV6* variants on *ETV6* function and platelet production have been investigated as discussed below.

Thrombocytopenia

All families had a highly penetrant autosomal dominant pattern of inherited thrombocytopenia (Table 1). Noetzli et al. (18) screened 24 families with autosomal dominant thrombocytopenia, red cell macrocytosis and identified germline *ETV6* mutations in 3 of the 24 families (12.5%). Melazzini et al. (22) identified germline *ETV6* mutations in 7 out of 130 families (5.4%) with idiopathic familial thrombocytopenia. The severity of thrombocytopenia was highly variable. Many patients had mild thrombocytopenia with platelet counts ranging between $100\text{--}150 \times 10^9/\text{L}$, while others had platelet counts $<50 \times 10^9/\text{L}$. Severe thrombocytopenia $<20 \times 10^9/\text{L}$ was rare in the absence of MDS. Bleeding symptoms were reported in a subset of patients and were generally mild including epistaxis, gum bleeding, easy bruising, and menorrhagia. Platelet size was generally normal, though a subset of patients had large platelets (Table 1).

Other hematologic parameters

Hemoglobin was normal in most patients. Erythrocyte MCV was generally normal or increased. Neutrophil counts were normal. Examination of the bone marrow revealed frequent immature hypolobated megakaryocytes, mild dyserythropoiesis, and mild nuclear hypolobulation and hypogranulation of myeloid cells. (17) (18) (21) Increased numbers of circulating CD34+ cells were noted in patients harboring the P214L and Y401N mutations compared with healthy controls. (21)

Familial Predisposition to Malignancy

Out of a total of 72 patients with familial *ETV6* mutations reported from family studies, 18 cases of hematologic malignancies (23) were reported (24). An additional 4 cases of hematologic malignancies were noted in thrombocytopenic individuals in affected families, but for whom DNA could not be sequenced. Out of this total of 22 leukemias, 15 had acute lymphoblastic leukemia, typically pre-B cell leukemia with age of presentation ranging from 2-37 years (median 7 years). Six subjects (6/72= 8.3%) had myeloid malignancies including MDS, AML and CMML presenting between the ages of 8-82 years. One patient had pre-B ALL followed by MDS and AML. Additional hematologic malignancies reported included single cases of multiple myeloma, mixed phenotype acute leukemia, and polycythemia vera with a *JAK2* mutation, though the small numbers prevent conclusions regarding the role of *ETV6* in these additional malignancies.

Germline *ETV6* mutations in pediatric ALL

Targeted sequencing of germline *ETV6* in 4405 cases of childhood ALL from the Children's Oncology Group and St. Jude Children's Research Hospital identified 31 *ETV6* exonic variants in 35 cases (0.8%) (20). The source of germline tissue was marrow or blood samples obtained at the time of remission. The mutation types included nonsense, missense, splice site, frameshift, and copy number loss. All thirty one of the variants were absent in non-ALL control populations (MAF <0.01%). 15 of the 31 *ETV6* variants in this study (48%) clustered in the ETS DNA binding domain and were predicted to be deleterious. Hyperdiploid karyotype was over-represented in the germline *ETV6*-mutant cases compared with *ETV6*-wildtype cases. Although somatic *ETV6*-*RUNX1* fusion was common in childhood ALL and found in 455/2007 (23%) of cases with wildtype germline *ETV6*, only one case of somatic *ETV6*-*RUNX1* fusion was found in 14 cases with germline *ETV6* variants. Children with *ETV6* variants were older at diagnosis (10.2 years) than those without *ETV6* variants (4.7 years). There was no association between *ETV6* mutation status and early treatment response (minimal residual disease at end of induction therapy) or risk of relapse. In the absence of functional studies, the role of *ETV6* mutations falling outside of the ETS domain or the P214L site in leukemia development remains unclear.

Secondary somatic mutations

The variable development of leukemias in only a subset of patients and the latency for leukemia development suggest that additional somatic mutations are likely required. Studies of somatic mutations acquired in hematologic malignancies in patients with germline *ETV6* mutations did not reveal mutations in the remaining wild type *ETV6* allele in some cases (17) (19), while two cases of bi-allelic loss of *ETV6* in the leukemic blasts was noted in one study (20). In contrast, loss of the second *ETV6* allele is noted in the context of the *ETV6*-*RUNX1* fusion. (9). Serial assessment of longitudinal samples from study subject III-2 in the report from Zhang et al (17) demonstrated the sequential acquisition of *BCOR* and *RUNX1* mutations at age 17 followed by a *KRAS* mutation at age 21 at the time of presentation with advanced MDS (RAEB-1). Distinct somatic mutations were also acquired upon development of multiple myeloma and colon cancer in study subject II-5 (mother of subject III-2). (17)

The role of additional somatic mutations in malignant transformation remains to be determined.

Solid tumors

A few solid tumors have been reported in patients with *ETV6* mutations; however, the small numbers currently preclude any conclusions regarding the role of germline *ETV6* mutations in solid tumor risk. Eight solid tumors were reported in 7 patients from families with *ETV6* mutations, though *ETV6* was not sequenced in 3 of the 7 patients. DNA was available for 4 study subjects and all 4 harbored *ETV6* mutations. The tumors included Stage III colorectal adenocarcinoma at age 45(17), stage IV colon cancer at age 43(17), breast fibroadenoma (age 35) and meningioma (age 43) in same patient (18) (22), breast carcinoma at age 49 (18) (22), breast fibroadenoma at age 14(22), duodenal adenocarcinoma (19) and renal cell carcinoma (19) (ages not reported). Although somatic variants in *ETV6* have been described in solid tumors such as GI cancers (25), given the small number of cases reported in familial *ETV6* studies, further investigation is needed to understand the role of *ETV6* in promoting the development of solid tumor malignancies.

Additional reported findings

Although a variety of physical anomalies and medical conditions have been reported in a few patients with germline *ETV6* mutations, no characteristic medical findings other than increased bleeding propensity were reported. Additional features described in isolated patients included developmental delay, myopathy, irritable bowel syndrome, arthritis, ankylosing spondylitis, uveitis, secondary amenorrhea, cleft lip/palate, craniofacial/skeletal dysmorphisms, unilateral polydactyly, mitral valve prolapse, renal ectopia, learning disability, and Turner syndrome. (17) (19) (20). (22) Due to the small number of reported cases, it currently remains unclear whether these additional features are associated with the *ETV6* germline mutations.

Molecular structure of ETV6

The human 28-member ETS-family of transcriptional regulators is defined by a highly conserved ~85 amino acid residue ETS-domain that mediates binding of target DNA around a core 5'GGA(A/T)3' ETS-motif (3). Different ETS-factors can often replace each other in the context of over-expression *in vitro* (26), but exhibit functional diversity and individual specificity in DNA-binding beyond the core motif, evident in ChIP-seq studies as well as *in vitro* binding site selections assays (3, 27). *ETV6* has the capacity to form polymers with to head-to-tail binding of two different protein surfaces within its PNT-domain (5). *ETV6*, rendered monomeric by mutating the PNT-domain polymerization surfaces, binds DNA with very low affinity due to an autoinhibitory domain located C-terminal of the ETS-domain (28). The intact protein likely binds as a multimer to several ETS-motifs with high affinity and considerable tolerance to their orientation and spacing, owing to the size and flexibility of the ~200 residue Linker domain (Fig. 1) (3). The PNT-domain has also been reported to bind to the transcription factors FLI1 and *ETV7* (29, 30), potentially modulating the DNA-binding pattern of *ETV6*. In addition to their impact on DNA-binding, the major domains of

ETV6 also appear to bind a plethora of co-repressors, which have been mapped to interact with the PNT-domain (L3MBTL1, KAP1, SIN3A (31-33)), Linker Domain (HDAC3, N-COR (31, 34)), and ETS-domain (SIN3A, N-COR, SMRT, TIP60 (22, 35)). It has been proposed that ETV6 polymers may facilitate repression by compacting DNA over long distances; such compacted structures may include co-repressors, similar to Polycomb complexes (5). However, direct evidence for such a model is lacking.

ETV6 as a transcriptional repressor

The repressive function of ETV6 has primarily been studied utilizing reporter plasmids with short promoters containing ETS-motifs, such as the promoter of stromelysin, an ETV6-target in RAS-transformed fibroblasts (36). Such reporter assays have been useful to characterize the structure (36) and interactions of ETV6 (32, 34) as well as *ETV6* germline mutants discovered in families (Table 3) and in sporadic mutants in leukemias (12, 13). As PNT-domain mediated multimerization is required for high affinity DNA-binding, it is, like the ETS-domain, essential for transcriptional repression (36). Importantly, truncated ETV6 proteins resulting from frameshift mutations in AML and retaining either the PNT-domain or the ETS-domain were shown to exhibit dominant-negative activity on repression by wildtype ETV6, as did an AML-associated point mutation in the ETS domain (12). Such dominant negative activity was also demonstrated for 9/9 familial germline mutations (Table 3) (17-19, 21). This suggests that the pathogenic activity of *ETV6* mutants not only includes loss of function but also interference with the non-mutated wildtype allele. As mutants affecting different regions of the protein share dominant negative activity, the mechanisms vary for individual mutants, and likely involve sequestration of the wildtype protein to the cytoplasm, competition for co-repressors, or reducing the affinity of repressive complexes to DNA by changing or deleting the DNA-binding activity of the ETS-domain. The ETS-domain is required and sufficient for the nuclear localization of ETV6 although it does not harbor a known nuclear localization motif (37). Accordingly, 5/5 familial point mutations affecting the ETS-domain resulted in more prominent cytoplasmic and reduced nuclear localization (Table 3) (17-19, 21). The only familial mutation of *ETV6* outside of the ETS-domain, P214L, also leads to increased cytoplasmic localization, albeit by an unknown mechanism (17, 18, 21). Increased cytoplasmic retention of mutant ETV6 protein represents a potential mechanism for dominant negative activity because the mutant proteins may sequester wild type ETV6 protein in the cytoplasm as a result of interaction of the PNT domains.

Functional impact of *ETV6* mutations

While simple reporter assays may underestimate the complexity of the abnormalities resulting from individual mutations, studying the functional impact of *ETV6* mutations is important, especially for variants that have not been implicated to confer disease risk in the context of family studies. *ETV6* variants have been observed in ~0.16% of a large population not selected for a cancer history (67209 individuals from the NHLBI GO ESP and the ExAC cohorts) (20). The mutations affected 24 different residues, 17 in single cases (20). Remarkably, the PNT-domain and the ETS-domain were almost spared from variations (only one variant in each), and no truncating nonsense or frame shift mutations were identified in these control cohorts (20). In the same study, 31 variant germline alleles, were

discovered in childhood B-ALL cohort that did not occur in the control cohorts (26 single cases). Based on previous functional studies and absence in the control cohorts, it appears highly likely that the ALL-associated germline mutations in the ETS-domain (~50%) as well as truncating non-sense or frameshift mutations contribute to the disease pathogenesis, but this is less certain for other individual variants. Conversely, with the challenge of so many diverse singular variants that may have different biology, it is difficult to exonerate all variants in the control cohort from a suspicion of pathogenic potential. Further study on the genetics and biology of the novel ALL-associated germline mutations is warranted to shed light on these issues.

ETV6 in embryonic development

Homozygous germline disruption of *Etv6* in mice results in embryonic lethality associated with failure of yolk sac angiogenesis (38). An important role for *ETV6* in vascular biology is also supported by studies utilizing *Xenopus* (39), zebrafish, and human vascular cell lines (40, 41). Moreover, *ETV6* is strictly required for maintaining the function of hematopoietic stem cells (HSCs) in the bone marrow (42, 43). Thus it is unlikely that bi-allelic germline loss of *ETV6* could be survived and, consistently, bi-allelic germline deletions or mutations have not been described. Notably, heterozygous disruption of *Etv6* in mice is not associated with obvious phenotypes, providing a potential explanation for the prevalence of disease associated germline mutations with dominant negative activity: complete loss of *ETV6* is lethal but the development of abnormalities requires more than heterozygous loss. In this context it is noteworthy that somatic truncating mutations predominate in high risk B-ALL (15) and early precursor T-ALL (13), but much less frequently occur in the germline. Likewise, a considerable number of somatic mutations in the pointed domain have been reported (17) but these seem to be rare in the germline (20) (Table 1). It is tempting to speculate that such mutations exhibit more potent dominant-negative activity, reducing viability.

ETV6 in megakaryocytic development

The high or complete penetrance of thrombocytopenia in families with germline *ETV6* mutations is consistent with the results from lineage-selective gene disruption in mice (42). Over-expression of *ETV6* was reported to suppress (29, 44, 45) or enhance (46) the megakaryocyte program in different systems, the latter more easy to reconcile with the recent clinical findings. Disruption of *ETV6* after the HSC commitment to the megakaryocyte/erythroid lineage utilizing Gata1-Cre resulted in consistent, marked reduction of platelet counts (42). The mice exhibited increased megakaryocyte colony-formation from the bone marrow, as was recently reported after *in vitro* differentiation of CD34⁺-cells from patients with *ETV6* mutations (21). Proplatelet formation after *in vitro* differentiation was defective in three independent studies, re-enforcing the notion that *ETV6* has critical intrinsic role in the megakaryocyte lineage (18, 21, 22). *ETV6* interacts with FLI1 in cell lines (29, 47), which, like *ETV6*, is implicated in vascular and megakaryocyte development as well as inherited thrombocytopenia (Jacobsen or Paris-Trousseau Syndrome)(48). However, MYH10 expression was found markedly increased in platelets from patients with FLI1 mutations but not *ETV6* mutations (22). Likewise, CDC42 and RHOA expression were

decreased in platelets from patient with *ETV6* mutations but normal in platelets from FLI1-mutant bearing patients, suggesting that the molecular pathology of the conditions is distinct (22). The abnormalities in CDC42 and RHOA expression and the documented defects platelet spreading *ex vivo* (21, 22) provide explanations for the increased risk of bleeding seen in these patients (49), which is not likely to be due to the moderate reduction in their platelet numbers alone. Future studies are needed to understand the variability in bleeding risk associated with germline *ETV6* mutations. Regardless of the remaining and new questions, *ETV6* is now firmly established as a major intrinsic regulator of megakaryocytes and platelets.

***ETV6* in erythroid development**

The erythroid lineage is not affected by *ETV6* loss in an obvious way, comparable to the megakaryocytic lineage. Anemia is infrequent in patients with germline *ETV6* mutations (17-19, 21, 22) and absent in mice after lineage-selective *ETV6* disruption (42). However, increases in mean erythrocyte volume were reported in a fraction of patients in two studies, raising the possibility that *ETV6* plays a non-essential role in erythropoiesis (18, 21). Supporting this notion, over-expression of *ETV6* driven by the *Gata1* promoter in transgenic mice resulted in subtle increases in hemoglobin (46), and this was consistent with the results of over-expression of *ETV6* in cell lines (44, 50). However, as the increases in MCV only occur in a fraction of patients with *ETV6* mutations and over-expression of *ETV6* may be subject to artifacts from binding non-physiological ETS-sites. Thus, while intriguing, an intrinsic role for *ETV6* in the erythroid lineage remains somewhat uncertain.

Mechanism of tumor initiation by *ETV6* mutants

The malignancy predisposition conferred by germline *ETV6* mutations remains poorly understood. No animal or cell line models of hematologic malignancy resulting from mutant or absent *ETV6* have been reported. This may be in part related the strict requirement for continuous *Etv6* expression in HSCs precludes stable disruption in all bone marrow cells (42). It has been demonstrated that over-expressed *ETV6* has the potential to repress phenotypes associated with RAS-transformation of fibroblasts (31, 50-52). Given the association of *ETV6* mutations with diverse hematologic malignancies and possibly solid tumors, a role for *ETV6* in suppressing a common pathway to transformation is attractive but mechanistic data are still lacking. The highest risk for patients with germline *ETV6* mutations is for B-ALL, which often presents in childhood but in some cases as late as age 37 (Table 2). Among cases with childhood B-ALL, germline *ETV6* mutations were associated with older age (20). This late onset suggests that secondary hits are required for the pathogenesis. Consistently, lineage-specific disruption of *ETV6* in B-cells in mice was not associated with transformation or major abnormalities in B-cell development (42). Likewise, despite up to 33% somatic mutations in *ETV6* in early T-cell precursor ALL cases (13, 14), after lineage-specific disruption of *ETV6* in T-cells did not perturb T-cell development no transformation was observed (42). Likely, the identification of second hits in leukemia with germline *ETV6* mutations will inform the development of models for these malignancies that will be useful to gain much needed insights into the role of *ETV6* malignancies.

ETV6 as a regulator of HSCs

The most critical physiological role for *ETV6* in hematopoiesis is its capacity to maintain HSCs. After disruption of *ETV6* in adult mice, HSCs are rapidly cleared from the bone marrow whereas short-lived progenitor cells are not affected and transiently sustain blood formation (42). The molecular basis of the requirement for *ETV6* expression in HSCs is unresolved. However, ETS factors are often effectors of signaling pathways (3). Similar to Yan, its closest relative in *Drosophila*, (53), *ETV6* is subject to phosphorylation, sumoylation, and ubiquitination *in vivo* (31, 50, 54-57). In cell lines, *ETV6* is phosphorylated by p38 and ERK2, representatives of two of the four major MAPKs pathways (50, 58). Phosphorylation of *ETV6* negatively regulates its activity either by inhibition of DNA binding, or by contributing to export from the nucleus, perhaps in combination with sumoylation (31, 50). As Tel/*Etv6* is ostensibly not required for the emergence and expansion of HSCs in embryonic development (38, 43), we hypothesize that it mediates a continuous survival signal for HSCs, specifically within the hematopoietic niche of the bone marrow. Since *ETV6* mutations affect malignancies of many different hematopoietic lineages, it is likely that a better definition of *ETV6*'s molecular role in HSCs will also have bearing for understanding how it predisposes to malignancy.

Conclusions

The recent discovery of familial germline mutations in *ETV6* has established its clinical importance as a cause of thrombocytopenia and bleeding propensity, as well as a major cancer predisposition gene that is associated with close to 1% of childhood B-ALL, as well as myeloid malignancies. Despite very rapid progress, our understanding of the clinical impact of *ETV6* mutations and physiological role of *ETV6* remains incomplete. Most individual germline *ETV6* mutations have been identified in single families or patients and it is unclear whether different mutations result in different propensities to develop specific types malignancy or are associated with different risks of bleeding. Germline and somatic *ETV6* mutations are frequently observed in cancers, but more work is needed to help definitively distinguish disease-associated alleles from harmless polymorphisms or passenger mutations. The complete spectrum of malignancies and conditions that individual with specific *ETV6* mutations are at risk for remains to be defined. Importantly, a better understanding for the physiological role of *ETV6* and further insights into the molecular pathology of its mutants are urgently needed. This may inform risk stratification and medical management of affected individuals and may lead to novel targeted interventions for treatment of malignancies.

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Three Key points

1. Germline heterozygous *ETV6* mutations cause autosomal dominant thrombocytopenia with normal platelet volume and a variable propensity for increased bleeding.
2. A variety of hematologic malignancies are associated with germline *ETV6* mutations, most commonly childhood pre-B ALL but also other hematologic malignancies such as MDS and AML.
3. Mutant *ETV6* proteins may act as dominant inhibitors of wildtype proteins in transcriptional repression.

Figure 1. ETV6 structure and location of familial ETV6 mutations

The ETV6 structural domains and locations of familial mutations are shown. The N-terminal pointed domain (PNT) mediates oligomerization with itself as well as with other factors. The C-terminal DNA-binding domain (ETS) is conserved among ETS-family transcription factors. The majority of the mutations identified in family studies cluster within the ETS domain. The P214L mutation, which resides in the linker region, has been recurrently identified in different families.

Table 1

Hematologic characteristics from ETV6 family studies

Reference	Family	ETV6 mutation	Family members with ETV6 mutation	Mean corpuscular volume	Platelet count	Mean platelet volume
Zhang et al	A	R399C	4	76.1-94.2	34-136	7.1-9.5
Zhang et al	B	R369Q	5 *	91-107.1	42-138	6.3-10.2
Zhang et al	C	P214L	1	90	42	NR
Noetzli et al	1	P214L	5	92.5-101.2	67-132	9-11.3
Noetzli et al/Melazzini et al	2/B	P214L	3	88-97	44-115	10-10.4
Noetzli et al/Melazzini et al	3/G	R418G+N385Vfs ^{*7}	2	97	101	7.6-7.8
Bottomka et al	1	L349P	9	NR	NR	NR
Bottomka et al	2	N385fs	2	NR	NR	NR
Moriyama et al		R359X	4	89.3-94.7	122-217	7.4-8.2
Poggi et al	F1	P214L	9	83-101	38-210	7.2-12.4
Poggi et al	F2	A377T	3	81-102	60-111	7.9-9
Poggi et al	F3	Y401N	3	86-88	80-125	9.4-10
Poggi et al	F4	I358M	4	91-98	29-134	9.8-11.7
Poggi et al	F5	R396G	3	ND	58-76	9.9-11.2
Poggi et al	F6	Y401H	1	100	77	ND
Melazzini et al	A	P214L	3	68-99	59-115	8.2-8.8
Melazzini et al	C	R369W	2	86-103	87-112	NR
Melazzini et al	D	R369W	2	79-97	109-110	8.4-9.2
Melazzini et al	E	W380R	5	86-101	55-105	7.4-9.1
Melazzini et al	F	N385Vfs ^{*7}	3	97-107	57-105	8.6-8.9

* one obligate carrier not tested

Table 2

Hematologic malignancies in familial ETV6 studies

Reference	Family	Gender	ETV6 mutation	MDS/Leukemia	Other Hematologic Malignancy
Zhang et al	A	F	R399C		Multiple myeloma, age 51
Zhang et al	A	F	R399C	Pre-B ALL, age 7.5	
Zhang et al	A	F	R399C	RAEB-1, age 22	
Zhang et al	B	M	ND	CMML-2, age 82	
Zhang et al	C	F	P214L		Mixed phenotype acute leukemia, age 50
Noetzli et al	I	F	P214L	Pre-B ALL, age 37	
Noetzli et al	I	F	P214L	Pre-B ALL, age 3	
Noetzli et al/Melazzini et al	2/B	F	P214L	Pre-B ALL, age 14	
Bottomka et al	I	NR	ND	ALL	
Bottomka et al	I	NR	L349P	pre-B ALL	
Bottomka et al	I	NR	L349P	pre-B ALL	
Bottomka et al	I	NR	ND	pre-B ALL	
Bottomka et al	2	NR	N385fs	pre-B ALL, MDS, AML	
Moriyama et al	*	F	R359X	pre-B ALL, age 9	
Moriyama et al	*	F	R359X	pre-B ALL, age 3	
Moriyama et al	*	F	R359X	pre-B ALL, age 2	
Poggi et al	F1	M	P214L	MDS, RAEB-2, age 70	
Poggi et al	F2	F	ND	MDS, RAEB	
Poggi et al	F4	M	I358M	AML, M0, age 8	
Melazzini et al	A	F	P214L	ALL age 7	
Melazzini et al	E	M	W380R	ALL age 7	
Melazzini et al	F	F	N385Vfs* 7	ALL age 3	
Melazzini et al	E	M	W380R		JAK2 V617F Polycythemia vera, age 37

NR: not reported ND: Not done

Table 3

Functional studies of familial ETV6 mutations

ETV6 mutant	Transcriptional repression	DNA binding	Nuclear localization	Megakaryocyte development	Proplatelet formation	Interaction with N-CoR, SMRT, mSin3 A	References
P214L	Decreased		Decreased	abnormal	Decreased	Yes	Zhang/Noetzli/Poggi et al
L349P	Decreased		Absent				Bottomka et al
R369Q	Decreased	Impaired	Decreased	abnormal			Zhang et al
A377T	Decreased					No	Poggi et al
N385fs	Decreased		Absent				Bottomka et al
385_418 del	Decreased						Noetzli et al
R399C	Decreased	Impaired	Decreased	abnormal			Zhang et al
Y401N	Decreased				Decreased	No	Poggi et al
R418G	Decreased		Decreased	abnormal	Decreased		Noetzli et al