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Comment on Nishii et al, page 364

Germline ETV6 variants: not ALL created equally

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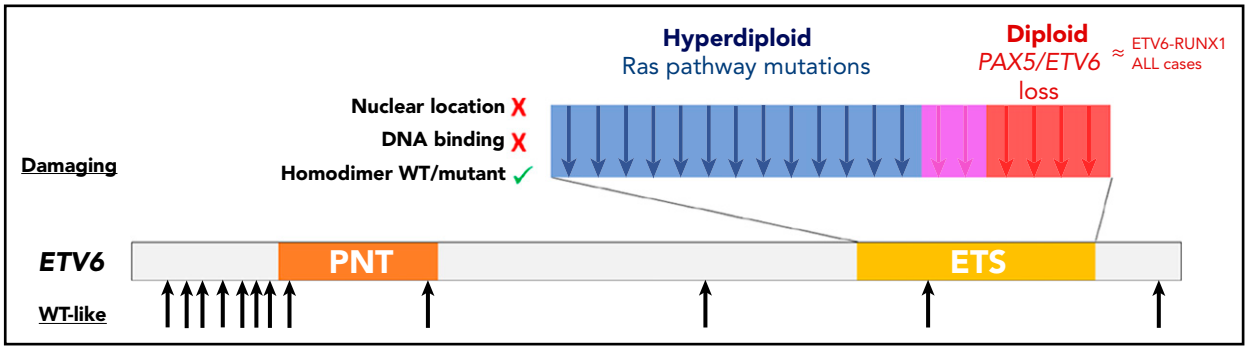
In this issue of *Blood*, Nishii et al complete a comprehensive functional assessment of 34 *ETV6* germline variants identified in a previous screen of 4405 patients with pediatric acute lymphoblastic leukemia (ALL).[1] They show that *ETV6* germline variants are not created equally, with 22 of 34 confirmed as damaging and the remainder considered wild-type (WT) like.

Childhood ALL cases with *ETV6* germline mutations were first reported by 3 groups independently in 2015 and constitute a novel leukemia predisposition syndrome that represents 1% of ALL patients.[2-4] In their manuscript, Nishii et al separate 34 germline variants into 2 groups, damaging and WT-like, based on their effect on ETV6 function. Damaging variants are readily distinguishable from WT-like because they preferentially locate to the ETS functional domain and result in reduced ability to bind DNA and lower transcription repressor activity. Critically, damaging variants retain their dimerization potential and behave in a dominant-negative manner, sequestering the remaining WT ETV6 protein to the cytoplasm and disrupting residual function. In contrast, the 12 designated WT-like variants are dispersed across the entirety of the gene and appear to preserve the function of the WT ETV6 protein (see figure) and, unlike their damaging counterparts, are more frequently detected in a non-ALL control population from the gnomAD dataset.

It is interesting that the subsequent trajectory of the disease does not appear to depend directly on these *ETV6* variants, but instead is affected by the nature of the secondary events acquired before the onset of overt disease. Seventy percent of damaging variants reported by Nishii et al were associated with hyperdiploid ALL, and these possessed a distinctive pattern of somatic mutations affecting the *RAS* pathway and differed from the corresponding diploid cases (30%), which acquire *PAX5* mutations or *ETV6* copy number loss (see figure). On closer inspection of the individual variants, it is notable that patients with identical *ETV6* mutations (eg, p.R359X, p.R433H) subsequently develop either diploid or hyperdiploid forms of ALL. Moreover, analysis of families with segregating germline *ETV6* variants reveal an overall 2:1 ratio of ALL to acute myeloid leukemia occurrence,[5] suggesting that the origin of these acquired mutations will instruct the type of subsequent malignancy.

The contribution of secondary genetic events was further supported by transcriptomic analysis in which a comparison of damaging and WT-like ETV6 with 231



Schematic representation of the distribution and characteristics of the ALL-associated *ETV6* germline variants. Each arrow represents a genetic variant: in black, WT-like germline variants; in blue, damaging germline variants associated with hyperdiploid status; in red, with diploid status; and in purple, common to both.

sporadic cases of pediatric ALL demonstrated that signatures did not reflect the nature of the specific *ETV6* variants, but rather the presence of other genetic features including the ploidy status of the leukemia. Indeed, the authors show that damaging diploid cases cluster with ETV6-RUNX1 ALL cases, which are also enriched in recurrent *PAX5* and *ETV6* copy number losses (see figure). The ability to discriminate damaging from WT-like *ETV6* germline variants was fruitful in defining a transcriptional program activated in the presence of these variants. This signature comprises 94 putative ETV6 target genes, including the Chloride Intracellular Channel 5 (*CLIC5*), which was recently reported as being directly repressed by ETV6.[6] It is difficult to predict with complete confidence, a mechanism contributing to *ETV6*-driven malignancy without further validation, but one possibility worth considering is that the overexpression of *CLIC5* in *ETV6*-mutated cases could impart resistance to oxidative stress, permitting accumulation of DNA damage and acquisition of secondary leukemia driver events.[6]

More broadly, the study by Nishii et al emphasizes the complexity associated with predisposing genes for inherited hematological malignancies, including *CEBPA*, *GATA2*, *SAMD9L*, or *RUNX1*,[7-9] where the type or location of the germline variant and/or the acquisition of an additional mutation in the same gene appears to determine the penetrance and progression of disease. The functional annotation of gene mutations forms an increasingly important component of genetic counseling for inherited disorders, together with an assessment of clinical presentation and family pedigree. The American College of Medical Genetics, in conjunction with the Association for Molecular Pathology and the College of American Pathologists, have developed a consensus framework for the interpretation of sequence variants.[10] Critically, as achieved here by Nishii et al, functional studies showing the deleterious effects of a given variant in a gene previously associated with disease pathogeny, bolster the classification of such a variant to a "likely pathogenic" status, which is particularly valuable for informing medical decisions.

As demonstrated by this study, it is important not to assume that all variants are damaging; for many loci, the absence of a rigorous assessment of the effect of germline variants on gene function necessitates their classification as "unknown significance," thus limiting their diagnostic value. It is certainly reasonable that all variants should be presumed WT-like until proven otherwise, but equally we should remain cognizant that 50% of *ETV6* WT-like variants were not reported in the gnomAD dataset and could conceivably be influencing ETV6 function by as-yet-undetermined means.

Altogether, it is sensible that we encourage more studies like the one described here by Nishii et al, where a rigorous assessment of function is undertaken on the spectrum of variants affecting a given gene. There is a tacet acceptance among researchers that all variants are not created equally, and indeed damaging variants themselves may differ in their penetrance and severity. Although we should be heartened at the increasing awareness of germline predisposition to hematological malignancies, it remains the subject of healthy debate how germline information should be incorporated most effectively into the clinical management of a patient and their family. Confirmation that a variant is deleterious would seem like a good place to start.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Evens et al, page 374

The balancing act in Burkitt lymphoma

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In this issue of *Blood*, Evens et al highlight critical prognostic factors that drive outcomes across the full continuum of adult patients with Burkitt lymphoma (BL) in the United States.[1]

These factors include the age and condition of the patient, the extent of tumor burden as measured by the serum lactate dehydrogenase (LDH) level, and the presence of central nervous system (CNS) disease. Importantly, these prognostic factors do not include infection with human immunodeficiency virus (HIV). Although these factors have been recognized as critical determinants of outcome in both