Are *DDX41* variants of unknown significance and pathogenic variants created equal?

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A variant of unknown significance (VUS) is a type of genetic mutation whose impact on an individual's health remains undetermined. It falls between benign and pathogenic variants, and we can reclassify it as we gather more evidence. In this issue of *Hematologica*, Bader *et al.* describe a comprehensive study to uncover the clinical impact of VUS for *DDX41* (*DDX41*^{VUS}) and compared it to that of the pathogenic variants of *DDX41* (*DDX41*^{path}) and the associated cytopenia, myelodysplasia syndromes and acute myeloid leukemia.[1]

The *DDX41* gene, also known as DEAD/H-box RNA helicase, is located on chromosome 5q35 and belongs to a class of tumor suppressor genes. It plays a crucial role in hematopoiesis by regulating the processing of small nucleolar RNA, assembling ribosomes, and synthesizing proteins.[2,3] When the function of DDX41 is reduced, it can lead to defects in hematopoietic cells and increase the risk of developing hematologic malignancies, such as certain types of blood cancer. It is important to distinguish precisely between germline and somatic variants of *DDX41*, as well as between *DDX41*^{VUS} and *DDX41*^{path} variants, in order to make accurate prognoses and manage myeloid neoplasms associated with *DDX41* mutations. However, despite the availability of advanced sequencing techniques for diagnosing inherited myeloid neoplasmas,[4] we still face challenges in differentiating between causal variants and those classified as VUS. This difficulty arises from our incomplete understanding of the function of DDX41 protein. As a result, we have yet to fully define the landscape of causal *DDX41* variants.

DDX41^{path}-associated familial myelodysplastic syndrome/acute myeloid leukemia has distinctive features. It typically occurs later in life, at a median age of 65-70 years, and affects males more often than females (ratio of 3:1). There is also variability in the family history of hematologic malignancies.[5-7] Many of these patients (46%) have pre-existing cytopenia and a long latency (5.2 years) before the diagnosis of the myeloid neoplasms.[5] Patients with *DDX41*-mutant myeloid neoplasms typically present with hypocellularity on the bone marrow sample and with normal cytogenetics.[8] Fortunately, patients with *DDX41*^{path}-associated myeloid neoplasms generally respond well to treatment. In fact, a study demonstrated that they had a 100% overall response rate and nearly 90% overall survival at the 2-year mark when treated with intensive chemotherapy or hypomethylating-based agents.[9]

To investigate the potential pathogenicity of *DDX41*^{VUS}, Bader *et al.* screened *DDX41* mutations from 4,524 patients treated at the Mayo Clinic who underwent targeted sequencing for suspected or known myeloid neoplasms. They classified the mutations into *DDX41* causal variants and VUS based on established guidelines from the American College of Medical Genetics and the Association for Molecular Pathology (ACMG/AMP). The researchers carefully categorized the *DDX41* variants as either purely *DDX41*^{VUS} (63 cases) or *DDX41*^{path} (44 cases, with 11 having both pathogenic and VUS variants). The authors described the clinical features and outcomes of this cohort and found comparable features, including molecular profiles, with no differences between patients in variant allele fraction, co-mutation patterns, and cytogenetics. Family history of hematologic malignancies, and the outcomes, including time to initiate treatment, progression-free survival, and overall survival were also comparable between patients with *DDX41*^{vus} and *DDX41*^{path} (Figure 1).

It is worth noting that some specific variants of *DDX41*^{VUS}, such as p.P258L, p.G173R, and M155L, were associated with a remarkably high frequency of myeloid neoplasms. For instance, the occurrence of myeloid neoplasms was 86%, 75%, and 40% among patients with these variants, respectively. In addition, 28% (5/18) of patients had cytopenia. Similarly to a previous study led by Li *et al.*,[7] only two patients with *DDX41*^{vus} had concurrent somatic *DDX41*^{path} (R5252H) mutations, which suggests that these *DDX41*^{VUS} alone could be oncogenic. On the other hand, a study by Chlon *et al.* using mouse models demonstrated that a single *DDX41* mutation, known as a monoallelic mutation, is associated with age-dependent hematopoietic

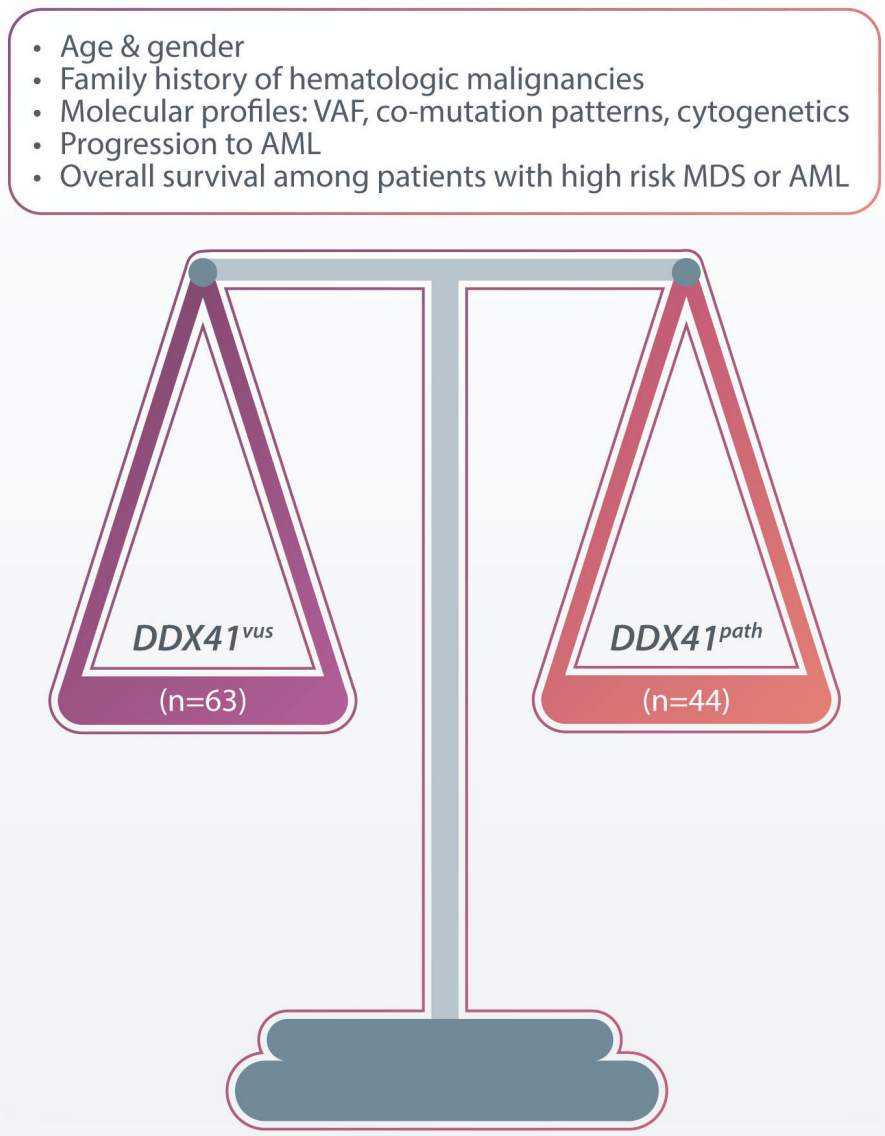

Figure 1. Comparable features between *DDX41*^{vus} and *DDX41*^{path} variants. VAF: variant allele frequency; AML: acute myeloid leukemia; MDS: myelodysplastic syndromes/myeloid neoplasms; *DDX41*^{vus}: variants of *DDX41* of unknown significance; *DDX41*^{path}: pathogenic variants of *DDX41*.

defects.[3] However, the acquisition of a "second-hit" mutation, often R525H, has a disease-modifying effect that accelerates hematopoietic defects and leads to hematologic malignancies.[3] Clinical studies led by Li *et al.*[7] and Duployez *et al.*[8] also demonstrated that 70-80% of patients with *DDX41*^{path} mutations develop acute myeloid leukemia after the acquisition of the “second-hit” *DDX41* somatic mutation. The development of myeloid neoplasms without an additional somatic hit in these variants suggests that these *DDX41*^{vus} might represent *bona fide* risk factors for the progression of myeloid neoplasms rather than trivial or inconsequential findings.

Previous studies showed that the enrichment of somatic mutations in *ASXL1*, *EZH2*, and *SRSF2* is associated with acute myeloid leukemia progression, and patients with *DDX41* variants that result in truncation of the protein experience a rapid progression to acute myeloid leukemia compared to those with non-truncating variants.[10] However, within the current study, the authors did not find significant differences in clinical features or outcomes when comparing patients with isolated *DDX41* variants and those with co-mutations nor between patients with protein-truncating variants *versus* non-protein-truncating variants. It is important to note that this lack of significance may be due to the small number of cases included in the analysis.

Collectively, the high frequency of myeloid neoplasms observed in patients with *DDX41*^{VUS}, along with the comparable clinical features and outcomes between those with *DDX41*^{VUS} and *DDX41*^{path}, suggest that these VUS might actually be oncogenic. This study underscores the importance of accurately classifying variants as VUS or pathogenic and understanding the causal landscape of *DDX41* variants. It is crucial to establish a standardized classification specifically for *DDX41* variants. For hematologists, the identification of *DDX41*^{VUS} should prompt increased vigilance in terms of genetic counseling referrals, monitoring blood counts, and tailoring management accordingly. Future studies should focus on performing functional analyses of *DDX41*^{VUS} to overcome the challenges associated with interpreting these variants of uncertain relevance.

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Contributions

ZX wrote the initial draft; both authors reviewed, provided edits to subsequent manuscript versions, and approved the final manuscript for submission.

References

1. Badar T, Nanaa A, Foran JM, et al. Clinical and molecular correlates of somatic and germline DDX41 variants in patients and families with myeloid neoplasms. Haematologica. 2023;108(11):3033-3043.
2. Polprasert C, Schulze I, Sekeres MA, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. Cancer Cell. 2015;27(5):658-670.
3. Chlon TM, Stepanchick E, Hershberger CE, et al. Germline DDX41 mutations cause ineffective hematopoiesis and myelodysplasia. Cell Stem Cell. 2021;28(11):1966-1981.e6.
4. Bannon SA, Routbort MJ, Montalban-Bravo G, et al. Next-generation sequencing of DDX41 in myeloid neoplasms leads to increased detection of germline alterations. Front Oncol. 2021;10:1.
5. Sébert M, Passet M, Raimbault A, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. Blood. 2019;134(17):1441-1444.
6. Quesada AE, Routbort MJ, DiNardo CD, et al. DDX41 mutations in myeloid neoplasms are associated with male gender, TP53 mutations and high-risk disease. Am J Hematol. 2019;94(7):757-766.
7. Li P, Brown S, Williams M, et al. The genetic landscape of germline DDX41 variants predisposing to myeloid neoplasms. Blood. 2022;140(7):716-755.
8. Duployez N, Largeaud L, Duchmann M, et al. Prognostic impact of DDX41 germline mutations in intensively treated acute myeloid leukemia patients: an ALFA-FILO study. Blood. 2022;140(7):756-768.
9. Alkhateeb HB, Nanaa A, Viswanatha D, et al. Genetic features and clinical outcomes of patients with isolated and comutated DDX41-mutated myeloid neoplasms. Blood Adv. 2022;6(2):528.
10. Makishima H, Saiki R, Nannya Y, et al. Germ line DDX41 mutations define a unique subtype of myeloid neoplasms. Blood. 2023;141(5):534-549.