

# Genetic features and clinical outcomes of patients with isolated and comutated *DDX41*-mutated myeloid neoplasms

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## Key Points

- Isolated and comutated *DDX41* myeloid neoplasms have different characteristics.
- *DDX41*-mutated AML has a relatively favorable outcome comparable to core binding factor AML.

*DDX41* mutations (germline and somatic) are associated with late onset myelodysplastic syndromes/acute myeloid leukemia (MDS/AML). Myeloid neoplasms (MN) with germline predisposition was identified as a distinct category in the 2016 WHO classification revision, including MN with germline *DDX41* mutation. We retrospectively analyzed the molecular findings and clinical characteristics of thirty-three *DDX41*-mutated (m*DDX41*) patients at our institution. We identified 14 distinct pathogenic *DDX41* variants in 32 patients and 8 *DDX41* variants of unknown significance (VUS) in 9 patients. Five (16%) patients had a second *DDX41* somatic mutation p.R525H and 13 (40%) had at least one additional oncogenic co-mutation in other genes. The median age at the time of diagnosis was 66 years, with male predominance (72%) and the majority of patients had normal cytogenetics (91%). Two-year overall survival (OS) was 86% and 6 (21%) MDS/AML patients with relatively preserved hematopoietic function were observed without further intervention. In comparison to AML patients with prognostically more favorable subtypes [t(8;21), n=27 and inv(16), n=40], m*DDX41* patients in our cohort showed similarly favorable OS. Our study highlights that m*DDX41*-MN patients often have an indolent course and m*DDX41*-AML has comparable OS to favorable-risk AML.

## Introduction

The DEAD-box helicase 41 (*DDX41*) gene, located on chromosome 5q35, is presumed to be a tumor suppressor gene, encoding a DEAD-box type RNA helicase. *DDX41* is involved in pre-mRNA splicing, rRNA processing and innate immunity.<sup>1-3</sup> Unlike other germline predisposition syndromes, which typically present at an earlier age, *DDX41*-associated germline cases are characterized by late-onset development of myeloid neoplasms (MNs), and may occur as sporadic germline events.<sup>1,2,4-12</sup> *DDX41* mutations account for 0.5% to 5% of adult myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) and typically present as high-risk disease, with male predominance and variable history of preceding cytopenias.<sup>1,2,13-16</sup> Recent studies have reported that *DDX41*-related MN is associated with longer overall survival (OS) and response to lenalidomide.<sup>10,14,17,18</sup> In this study, we describe the clinical and genetic features and survival outcomes of patients with mutated *DDX41*.

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Gene panel sequencing data are available by request to the corresponding author at alkhateeb.hassan@mayo.edu.

The full-text version of this article contains a data supplement.

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## Methods

This is a single-institution study encompassing the Mayo Clinic Cancer Center sites (Rochester, Florida, and Arizona). After institutional review board approval, we retrospectively screened for *mDDX41* cases from 4524 consecutive Mayo Clinic patient samples submitted for a 42-gene MN panel next-generation sequencing clinical assay in the Molecular Hematopathology Laboratory between July 2018 and December 2020. A chart review of *mDDX41* cases between January 2009 and April 2021 was conducted. Of 1404 consecutive patients with the diagnosis of AML, 27 (1.9%) patients with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* and 40 (2.8%) with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* were identified for survival comparison with *mDDX41* AML. All statistical analyses were performed using JMP Pro 14.1.0 Software (supplemental Methods).

## Results and discussion

We identified 33 patients harboring *DDX41* genetic alterations, of which 32 (97%) had at least 1 pathogenic *DDX41* mutation and 1 (3%) had a *DDX41* variant of uncertain significance (VUS) (proven to be a germline variant). Of the 32 patients harboring pathogenic mutations, 5 (16%) had a second *DDX41* mutation (p.R525H), and 9 (27%) harbored a *DDX41* VUS (Figure 1; supplemental Table 1). The germline origin of *DDX41* variants was confirmed in 9 of 10 (90%) tested patients, among them at least 1 variant had variant allele frequency (VAF)  $\geq$  40%.

The median age at diagnosis was 66 years (range, 30-81 years), and 24 (72%) patients were men, consistent with late onset of *mDDX41* MN and male predominance previously reported.<sup>8,14-16,19</sup> All patients with AML were intermediate risk for the European LeukemiaNet (ELN), with a median marrow blast count of 29% (range, 20%-50%). The majority of MDSs were classified as excess blasts-2 (MDS-EB2; N = 13; 68%), similar to that previously reported in the literature.<sup>15,16,19</sup> Eleven (58%) and 4 (21%) patients with MDS were classified as intermediate risk and high risk by the revised International Prognostic Scoring System (IPSS-R), respectively.

Twenty (60%) patients had an isolated *DDX41* mutation, whereas 13 (40%) had at least 1 additional comutation in other genes. Isolated *mDDX41* cases showed male predominance relative to their comutated counterparts (85% vs 54%;  $P = .05$ ; Table 1). The median number of comutations in the 13 cases was 1 (range, 1-3), and the most common comutations occurred in *DNMT3A* (N = 5, 38%), *ASXL1* (N = 4, 30%), *JAK2* (N = 3, 23%), and *EZH2* (N = 2, 15%; Figure 1; supplemental Tables 1 and 2). The incidence of *TP53* mutation was infrequent (3%), comparable to what was reported by Sébert et al<sup>15</sup> (6%) but lower than that reported by Quesada et al<sup>16</sup> (32%). Similarly, we observed a low incidence of splicing factor comutations (3%), in keeping with the report from Sébert et al.<sup>10,16</sup> Our cohort had fewer comutations (median of 0; range, 0-3) than what reported by recent studies, and interestingly, the comutation median VAF observed here was low 7% (range, 5%-52%).<sup>15,16</sup>

The most common pathogenic mutation type was the initiation codon substitution (start-loss) variant p.M11 (N = 10, 31%; Table 1; supplemental Table 1), previously described as the second most common germline *DDX41* variant in Whites and the most common in Swedish population.<sup>8,14,15</sup> Among isolated cases, 47% had p.M11, whereas only 8% of comutated cases harbored p.M11 ( $P = .02$ ). Twenty-one

(65.6%) *DDX41* mutations clustered in the N-terminal domain (NTD), 4 (12.5%) in the DEAD-box domain, and 7 (22%) in the helicase-C domain (HCD). Mutations located in the HCD were more likely to have a concomitant *DDX41* VUS compared with NTD mutations (N = 6, 86% vs N = 2, 10%;  $P = .0001$ ).

Sixteen patients (52%) had a family history (FH) of solid tumors and 12 (39%) had a FH of hematologic malignancies. Comutated cases were more likely to have FH of solid tumors (77% vs 33%;  $P = .02$ ; Table 1).<sup>20</sup> However, this difference was not significant for hematologic or subgroups of solid malignancies. None of the HCD-mutated cases had FH of solid tumors, in comparison with 70% seen in NTD-mutated cases ( $P = .001$ ), supporting the reported prevalence of germline mutations in the NTD.<sup>8</sup>

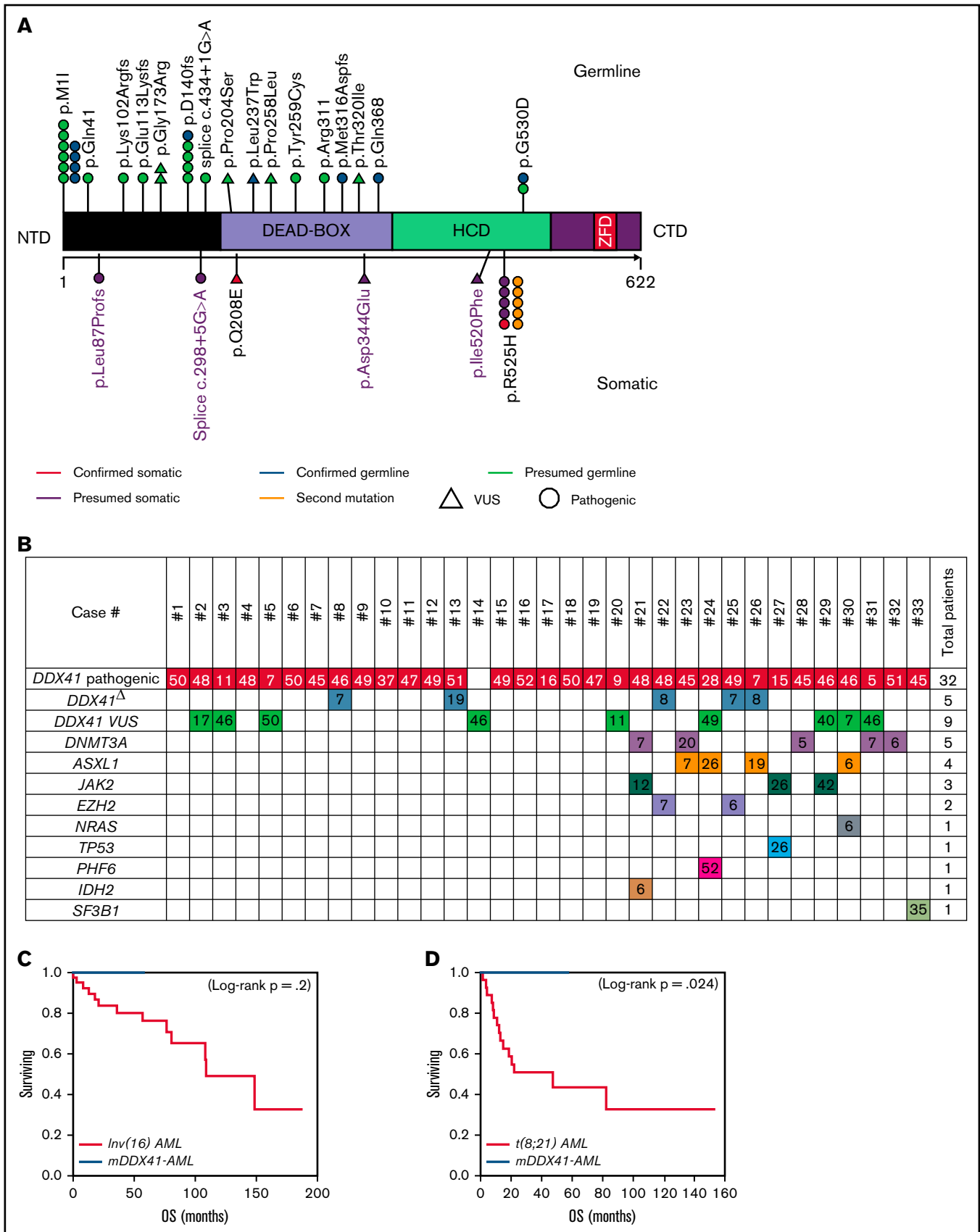
Cytogenetic results were available in 32 cases, and 29 (91%) showed a normal karyotype. Karyotypic abnormalities were thus infrequent (N = 3, 9%), consistent with previous reports.<sup>15,16,19</sup> Interestingly, all 3 cases with karyotypic abnormalities were comutated cases ( $P = .02$ ).

Overall, 2 (7%) patients died after a median follow-up of 20 months. Six (20%) patients (5 MDS and 1 AML) were observed because of stable blood indices with a median follow-up of 6.6 months (range, 1.5-32.6 months). Twenty-three (80%) patients received treatment with median time from diagnosis to treatment initiation of 0.7 months (range, 0-92 months). Overall response rate in patients with MDS/AML was 77%, and median time to response was 3.2 months (range, 0.9-20.5 months; supplemental Table 3). Fifteen (68%) patients achieved complete remission (CR), 2 (9%) patients had hematologic improvement, and 3 (23%) patients did not respond. Patients with AML had 100% CR when treated with induction chemotherapy or hypomethylating agents (HMA) plus venetoclax regimen, and median time to CR was 1 month (range, 0.86-4.1 months). Of the 9 (39%) patients who received second-line therapy, 6 (75%) achieved CR. Four (21%) of 19 patients with MDS progressed into AML with a median time to progression of 16 months (range, 1.3-27.6 months), and the 2-year progression-free survival rate for patients with MDS was 62%.

In *mDDX41* MDS/AML, the median OS was not reached, and the 2-year OS was 86% (95% confidence interval: 57%-97%). There was no statistically significant difference in OS between 2-year OS for isolated and comutated ( $P = .99$ ) responders and nonresponders (90% vs 50%;  $P = .38$ ) and treatment compared with the 2-year OS for the observation group (83% vs 100%;  $P = .52$ ; supplemental Figure 1). Twelve (41%) patients with MDS/AML received hematopoietic stem cell transplantation (HSCT), and there was no difference in 2-year OS between patients who received HSCT vs no HSCT (87% vs 86%;  $P = 1.0$ ; supplemental Figure 1).

All patients with *mDDX41* AML were alive at the end of follow-up without reaching median OS. Comparing the OS of *mDDX41* AML to the prognostically favorable group of core-binding factor AML, a statistically significant superior outcome was observed in *mDDX41*-AML compared with AML with t(8;21) (2-year OS, 100% vs 51%;  $P = .024$ ; Figure 1D). In comparison with AML with inv(16), *mDDX41* AML showed a trend to better OS; however, statistical significance was not achieved (2-year OS, 100% vs 84%;  $P = .2$ ), supporting at least noninferior clinical outcome (Figure 1C).

Our study reaffirms some previous observations and demonstrates several novel findings in patients with MN and *mDDX41*. Isolated



**Figure 1. Characteristics of patients with *DDX41* mutation.** (A) Representation of *DDX41* variants detected, positioned on the *DDX41* protein and its functional domains. (B) Patterns of the mutations identified in the cohort of 33 patients with *DDX41* mutation. The number reported in the box represents the VAF of each mutation. (C-D) Kaplan-Meier survival curves in 10 patients with *mDDX41* AML compared with (C) 40 patients with *inv 16* AML and (D) 27 patient with *t(8;21)* AML. CTD, C-terminal domain; ZFD, zinc finger domain;  $\Delta$ , second mutation.

**Table 1. Characteristics and hematologic features of patients with isolated and comutated *DDX41* mutation**

Variable	Isolated	Comutated	P
No. of patients, (%)	20 (60)	13 (40)	
Age, y, median (range), at diagnosis	65 (30-81)	66 (50-76)	.767
Sex (male), n (%)	17 (85)	7 (54)	.0496*
Hemoglobin, g/dL, median (range)	11.2 (7.5-15.6)	10.05 (6.6-14)	.1988
Leukocytes, 10 <sup>9</sup> /L, median (range)	2.15 (1-4.4)	2.4 (1.6-8.5)	.1239
Thrombocytes, 10 <sup>9</sup> /L, median (range)	87 (28-241)	94 (63-571)	.1443
ANC, median (range)	0.925 (0.16-3.73)	1.005 (0.65-4.78)	.2058
MCV median (range)	104.3 (85.2-114.8)	105.6 (90-115)	.7151
RDW, median (range)	14.2 (12.4-23.4)	15.05 (12.5-21.3)	.2824
BM blasts, median (range)	13 (1-45)	12 (0-50)	.6056
BM blasts (AML only), median (range)	34 (20-45)	25.5 (21-50)	.91
Number of comutations, median (range)	0	1 (1-3)	
<i>DDX41</i> VAF %	48 (7-52)	45 (5-51)	.1656
<i>DDX41</i> mutations > 1	2 (10)	3 (23)	.306
<b>Pathogenic mutation type</b>			
Missense	5 (26)	3 (23)	.8354
Nonsense	1 (5)	2 (15)	.3347
Frameshift	4 (21)	5 (38)	.2820
Splice site mutation	0	2 (15)	.0774
Start-loss variant	9 (47)	1 (7)	.0174*
<b>Diagnosis</b>			
MDS	13 (65)	6 (46)	.2845
AML	6 (30)	4 (30)	.96
MPN	0	2 (15)	
Carrier	1 (5)	0	
CCUS	0	1 (7)	
Abnormal cytogenetics	0	3 (25)	.0188*
<b>Family history</b>			
Solid or hematologic malignancies	11 (61)	12 (92)	.0501
Solid tumors	6 (33)	10 (77)	.0166*
Hematologic malignancies	8 (44)	4 (30)	.4405
Gastrointestinal malignancies	3 (15)	3 (23)	.6040
Genitourinary malignancies	2 (10)	4 (30)	.1496
Lung cancer	1 (5)	3 (23)	.1345
Breast cancer	2 (10)	2 (15)	.6832
<b>Any personal history</b>			
Hematologic malignancies	0	1 (7)	.2078
Solid tumors	1 (5)	3 (23)	.1200
<b><i>DDX41</i> VUS</b>			
Yes	5 (25)	4 (30)	.7161
No	15 (75)	9 (70)	

ANC, absolute neutrophil count; BM, bone marrow; CCUS, clonal cytopenia of undetermined significance; MCV, mean corpuscular volume; MPN, myeloproliferative neoplasms; RDW, red cell distribution width.

\*Statistically significant.

*DDX41* mutations were associated with male predominance (85%), the start-loss p.M1I mutation (47%), normal cytogenetics (100%), and less frequent association with a FH of solid tumors (33%) compared with their comutated counterpart. Patients with *mDDX41* MN

have a low incidence of *TP53* and splicing factor gene comutations. Despite the categorization of all patients with *mDDX41* AML intermediate risk for ELN, we found they fit favorable-risk AML in our study cohort.<sup>21-23</sup> Finally, some *mDDX41* MN cases can be

observed for a long time if they have preserved hematopoiesis, and therapeutic intervention could be delayed. We acknowledge that our study is limited by the retrospective nature and small sample size.

## Authorship

Contribution: A.N., A.A.-K., H.B.A., and D.V. designed the study, interpreted the data, and wrote the manuscript; A.N. collected the data and conducted the statistical analysis; M.V.S., J.M.F., T.B., A.T., M.R.L., M.M.P., N.G., A.A.M., L.S., H.B.A., and A.A.-K., cared for the patients and provided patient information; R.H., P.N., D.J., and D.V. performed the next-generation sequencing; P.G. performed

cytogenetic analysis; and all authors critically reviewed and approved the manuscript.

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