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MYELOID NEOPLASIA

The genetic landscape of germline *DDX41* variants predisposing to myeloid neoplasms

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KEY POINTS

- AML/MDS defined by germline *DDX41* CV represents a unique entity with favorable outcome.
- Germline *DDX41* CVs predisposing patients to MN are often associated with somatic *DDX41* mutations.

Germline *DDX41* variants are the most common mutations predisposing to acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS) in adults, but the causal variant (CV) landscape and clinical spectrum of hematologic malignancies (HMs) remain unexplored. Here, we analyzed the genomic profiles of 176 patients with HM carrying 82 distinct presumably germline *DDX41* variants among a group of 9821 unrelated patients. Using our proposed *DDX41*-specific variant classification, we identified features distinguishing 116 patients with HM with CV from 60 patients with HM with variant of uncertain significance (VUS): an older age (median 69 years), male predominance (74% in CV vs 60% in VUS, $P = .03$), frequent concurrent somatic *DDX41* variants (79% in CV vs 5% in VUS, $P < .0001$), a lower somatic mutation burden (1.4 ± 0.1 in CV vs 2.9 ± 0.04 in VUS, $P = .012$), near exclusion of canonical recurrent genetic abnormalities including mutations in *NPM1*, *CEBPA*, and *FLT3* in AML, and favorable overall survival (OS) in patients with AML/MDS. This superior OS was determined independent of blast count, abnormal karyotypes, and concurrent variants, including *TP53* in patients with AML/MDS, regardless of patient's sex, age, or specific germline CV, suggesting that germline *DDX41* variants define a distinct clinical entity. Furthermore, unrelated patients with myeloproliferative neoplasm and B-cell lymphoma were linked by *DDX41* CV, thus expanding the known disease spectrum. This study outlines the CV landscape, expands the phenotypic spectrum in unrelated *DDX41*-mutated patients, and underscores the urgent need for gene-specific diagnostic and clinical management guidelines.

Introduction

Hereditary hematologic malignancies (HM) typically manifest at earlier ages than de novo disease,[1] usually with substantial familial clustering.[2,3] Inclusion of hereditary HM in the fourth edition of World Health Organization classification of hematopoietic and lymphoid tissues[4] emphasizes the importance of germline evaluation in patients with myeloid neoplasm (MN). The National Comprehensive Cancer Network guidelines on next-generation sequencing (NGS) for patients with MN facilitate comprehensive large-scale screening in the general population for variants of interest, which has revealed the surprisingly high incidence of presumably germline mutations in genes predisposing to HM in children and adults. Approximately 8% of pediatric and adult patients have a pathogenic germline variant, and many patients lack a pertinent family history (FH).[5,6] These recent studies have revealed that familial HM predisposition syndromes, previously thought to be rare diseases, are more common than anticipated.

Recently, our group and others identified *DDX41* as 1 of the most common MN predisposition genes in adults.[7-9] Unlike some other hereditary HMs that present in childhood or adolescence, *DDX41* is associated with late-onset MN, at ages typical of sporadic acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS),[10] years after indolent and mild cytopenia,[7,8,10-12] and these patients often lack FH.[7,8,10-12] The subacute disease course of *DDX41*-associated AML is generally accompanied by bone marrow hypocellularity and a borderline increase in blasts with a near normal immunoprofile, and most patients have a normal karyotype. These features make the initial diagnosis of this inherited AML more challenging than other hereditary HM predisposition syndromes.[8]

Despite increasing integration of NGS assessment in clinical practice, the accurate diagnosis of *DDX41*-associated HM is further complicated by the currently limited ability to distinguish between causal and benign variants. The lack of FH, reduced penetrance and the long disease latency complicate the power of the familial segregation studies to accurately identify causal

variants (CVs) among the increasing pool of novel missense variants identified by NGS. Identification of germline CVs can inform long-term patient management and prevent engraftment failure[13] and donor-derived leukemia[14-18] in some clinical contexts where allogeneic stem cell transplantation (HSCT) is necessary.[7,8] Furthermore, family members also benefit from identification of a germline variant in informing their own risk of developing MN. Unfortunately, no consensus has been reached on guidelines of *DDX41*-specific diagnosis and patient management because of limited awareness of this disease and the inherent challenges in classification of novel missense germline variants. Thus, collaboration on variant curation among expert panels to develop gene-specific diagnostic guidance has become urgent as these novel variants are increasingly detected.

Beyond AML and MDS, germline *DDX41* mutations appear to predispose to other MN, such as chronic myelomonocytic leukemia (CMML), chronic myeloid leukemia (CML), and myelodysplastic/myeloproliferative neoplasm (MDS/MPN), lymphoproliferative disorders (LPD), and potentially nonhematopoietic neoplasms.[7,10,19] Previously reported cases with mixed germline CV and variant of uncertain significance (VUS) provide insufficient support to link germline *DDX41* mutations to LPD and other MN. Moreover, the overall natural course and characteristic pathologic findings of each *DDX41*-associated entity remain unclear, and long-term surveillance for asymptomatic individuals with germline CV and guidelines for early intervention are also needed.

In this study, we identified 176 patients with HM with presumably germline *DDX41* variants in an unselected and unrelated 9821 patient cohort from six institutions. Applying our proposed *DDX41*-specific classification criteria, we analyzed the genomic profiles, demographic characteristics, and clinical outcomes of each specific disease entity. The striking partitioning of these features with variants we deemed CV vs VUS indicates the variant classification criteria are warranted and supports the assertion that AML/MDS with *DDX41* germline CV represent 1 distinct clinical entity with a favorable outcome. Building on the current literature, we further delineated the germline CV landscape, expanded the phenotypic spectrum of HM with germline *DDX41* CV to include MPN and B-cell lymphoma to improve the recognition and refine the management of this HM predisposition syndrome.

Materials and methods

Case selection

Cases with at least 1 *DDX41* variant (n = 195) were identified through retrospective search of the pathology archives from January 2015 to June 2021 at the University of Utah, ARUP Laboratories, Oregon Health and Science University, University of Kansas Medical Center, Emory University, Stanford University, and University of California San Diego in 9821 unrelated and unselected patients with HM (including 3583 AML, 2161 MDS, 1029 MPN, and 3048 other diagnoses) who underwent targeted panel testing by NGS (Figure 1). Nineteen patients (0.2%) exhibited somatic *DDX41* variants without germline variants, whereas 176 patients (1.8%) had at least 1 presumed germline variant. Germline variants were further classified into CV or VUS according to the proposed *DDX41*-specific classification criteria (Figure 1) modified from the American College of Medical Genetics/the Association for Molecular Pathology (ACMG/AMP) guidelines (supplemental Table 3, available on the *Blood* Web site).[20] Demographic data, clinical information, and molecular and cytogenetic profiles were further analyzed to test the proposed classification criteria. Among the 176 patients with HM carrying germline *DDX41* variants, the World Health Organization entities of HM included 84 AML (66 CV and 18 VUS), 40 MDS (28 CV and 12 VUS), 15 MPN (4 CV and 11 VUS), and 37 others. The other category (Figure 2; Tables 1 and 2) included 32 clonal cytopenia of undetermined significance (CCUS, designated as cytopenia), 4 B-cell LPD, and 1 multiple myeloma (MM). Of note, 24 patients with AML with *DDX41* CV have been documented in a previous study.[8] A control cohort of 4307 patients without *DDX41* variants (including 1365 AML, 1109 MDS, 479 MPN, and 1354 other, primarily CCUS) were identified by retrospective search of patients tested at ARUP. All were adult patients (age range, 18-97 years), and their demographic and genetic characteristics were compared with those with germline *DDX41* variants (Figure 1A). Clinical follow-up information was available in 158 patients with AML and 87 patients with MDS and included in overall survival (OS) analysis as the controls (Figure 1A) as they were tested and treated at Huntsman Cancer Institute in the same period as *DDX41* mutant patients. As ethnicity and clinical outcome data were not available for some samples tested at ARUP Laboratories (a national reference laboratory), these patients were excluded from ethnicity-specific and OS analysis. This study was approved by the institutional review boards at the participating institutions.

Targeted NGS

DNA was extracted from fresh bone marrow aspirates and NGS testing was performed using a targeted NGS panel at each institution. The ARUP myeloid malignancy NGS panel included 65 genes (supplemental Table 1), and targeted hybrid-capture sequencing was performed using the SureselectXTHS kit (Agilent Technologies, Santa Clara, CA) following the manufacturer's protocol as described previously.[7,8] The genes listed in NGS panels at each institution and the 53 common genes tested are summarized in supplemental Tables 1 and 2.

DDX41-specific variant classification and interpretation

Variants with a variant allele frequency (VAF) of 40% or above were presumed to be germline variants.[7,8] Germline variants were classified as pathogenic/likely pathogenic variants (PV/LPV), described herein as CV or VUS according to ACMG/AMP guidelines (supplemental Table 3) with the following specific considerations.[20] A pathogenic moderate criterion (PM2) was applied to variants with a Genome Aggregation Database (gnomAD) population frequency less than that of the 2 most frequent known pathogenic *DDX41* variants: p.M1I and p.D140fs (both with gnomAD frequency of 0.008%). Another pathogenic moderate criterion (PM3) was used in a modified manner to account for the known mechanism of *DDX41* second hits in affected individuals. This criterion was applied to the germline variant when a second pathogenic variant (presumed somatic) was also present in an affected patient with this variant in our study or reported in the literature. A pathogenic supporting criterion (PP3) was applied when the REVEL score for the variant was greater than 0.7.

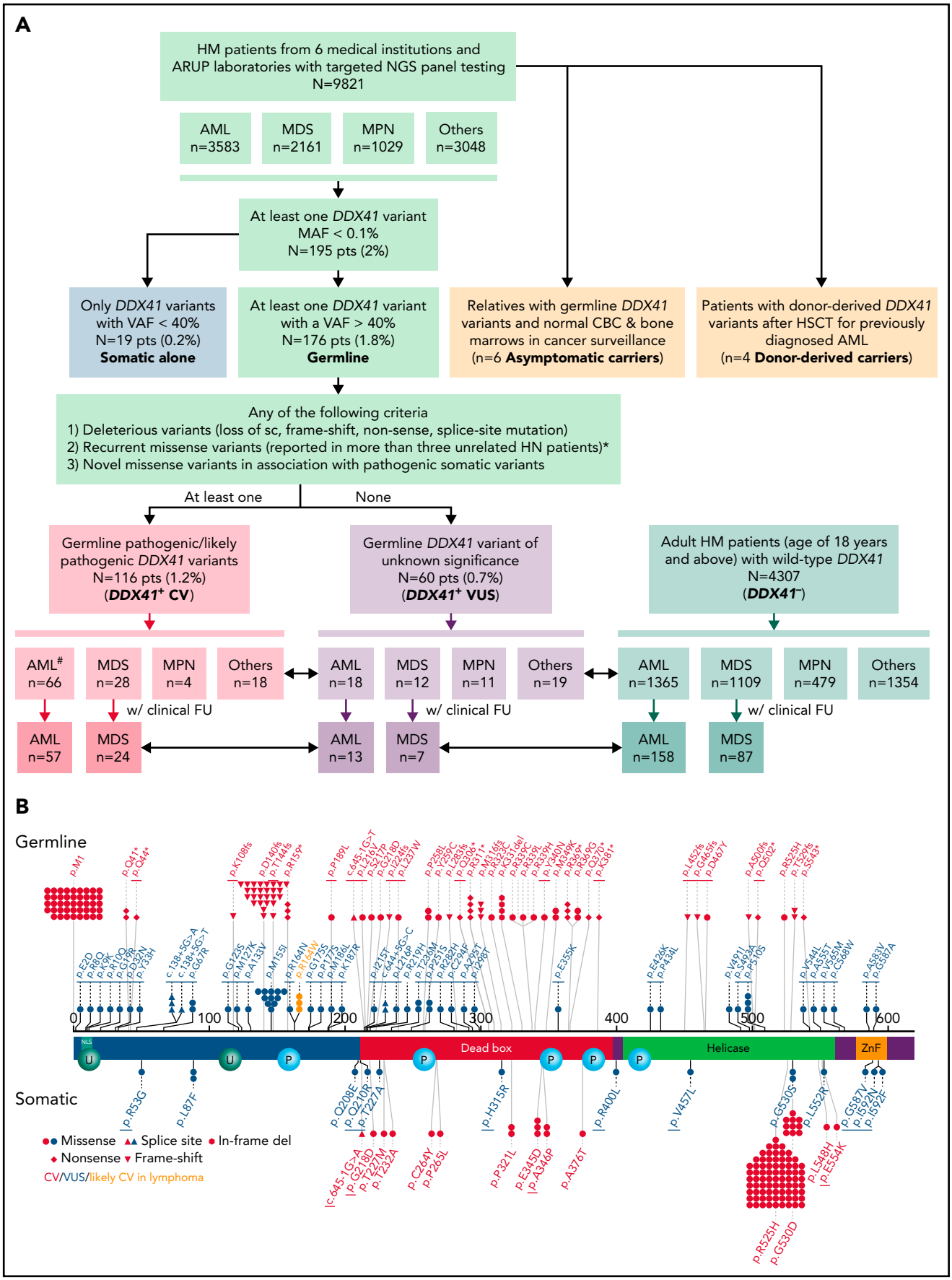


Figure 1.

Germline confirmation

Germline testing was performed prospectively on 12 patients (8 with CV and 4 with VUS) and 6 asymptomatic relatives who were referred to the genetics clinic based on the persistent presence of a *DDX41* variant at near-heterozygous VAF and suspicious FH. Germline confirmation was performed as previously described using skin biopsies.[7] The remaining patients either were not referred for genetic counseling or declined further testing.

Asymptomatic individuals with germline *DDX41* variants under surveillance

Six asymptomatic relatives of the patients with HM with confirmed germline *DDX41* CV underwent cancer surveillance (Tables 1 and 2, patients 196-201, all with CV) with bone marrow biopsies in conjunction with flow cytometric, cytogenetic, and NGS studies to establish a baseline. Bone marrow examination in all cases showed essentially normal trilineage hematopoiesis without evidence of malignancies, as reviewed by P.L. and M.W. independently. Furthermore, 4 patients with donor-derived *DDX41* variants (2 CV and 2 VUS) after HSCT for previously diagnosed AML were included (Tables 1 and 2, patients 202-205), and all had unremarkable complete blood count (CBC) and 100% donor chimerism, confirmed by short tandem repeat testing at post-transplant surveillance.

Statistics

Descriptive statistics were used for patient epidemiologic characteristics and the number of somatic variants per case, and the results are summarized in figures as appropriate. Unpaired *t* test was used for all quantitative data, and Fisher exact test or χ^2 test was used for qualitative data.[8] OS was analyzed as a time-to-event date point using the Kaplan-Meier method.[21-24] Time-to-event data were also analyzed with Cox proportional hazards regression to calculate hazard ratios (HR) by multivariate analysis.[25,26]

Literature review and gnomAD database search

A PubMed search for cases of sporadic and familial HM with germline *DDX41* variant was performed. Individual studies were reviewed, and the variants were reclassified and summarized in Figure 2A. Clinical outcome information from 18 additional AML and high-grade MDS cases with germline *DDX41* variants in association with 186 age-matched patients with *DDX41* wild-type (WT) AML from a previous study[7] was collected. Furthermore, additional data on OS of 3128 age-matched patients with MDS in the literature[27] and 1040 patients with AML in cBioPortal (median age, 68 years; range, 47-99 years; access date, 28 February 2022) were retrieved and reanalyzed to extend OS analysis (supplemental Figure 2A). Where available in publications,[15,28,29] ethnicity was reanalyzed in combination with the current study population and summarized in Figure 2B-E. GnomAD was searched to acquire the minor allele frequencies (MAF) of variants and was incorporated in supplemental Table 3.

Results

DDX41 variant landscape, genetic profiles, and ethnic differences of patients with HM

Among the 9821 unrelated and unselected patients with HM, 195 (2%) were found to have at least 1 *DDX41* variant; of those, 176 (1.8%) patients had a putative germline *DDX41* variant and 19 (0.2%) had somatic variants alone (Figure 1A). The 176 HM cases with germline variants (Tables 1 and 2, patients 1-176) included 84 AML, 40 MDS, 15 MPN, 32 cases of cytopenia, 4 B-cell lymphoma, and 1 MM. The 19 cases (Tables 1 and 2, patients 177-195) with only somatic variants included 10 AML, 8 MDS, and 1 cytopenia. Overall, 82 distinct presumed germline variants were identified, among which 39 were classified as CV (red in Figure 1B) and 43 as VUS (blue in Figure 1B; supplemental Table 3) according to the proposed classification criteria. Loss of function variants, recurrent missense variants in association with a low MAF with specific exceptions (eg, p.M155I and p.P510S), and novel missense variants accompanied by pathogenic somatic *DDX41* variants (Figure 1A) were generally considered CV. Here we reported 53 novel germline (15 CV and 38 VUS) and 13 novel somatic variants (4 CV and 9 VUS, underlined in Figure 1B) among which the 5 new missense germline CVs (Table 1, 58, 65, 89, 90, and 93) were all accompanied by previously characterized somatic pathogenic variants (p.R525H, p.G530D, or p.E345D).

The previously reported germline and somatic *DDX41* variants in HM,[7,10,11,28,30-34] together with those in the current study, are summarized in Figure 2A. Most germline CV (63% in this study and 68% by literature review) were loss of function mutations, including start codon loss (p.M1I), nonsense, frameshift, or mutations disrupting splicing sites (Figures 1B and 2A), concentrated upstream to the DEAD box domain. p.M1I and p.D140fs, the

Figure 1. Flowchart of this multi-institutional study and graphical representation of *DDX41* variants found in this study. (A) In this study, 195 (2%) patients with HM with at least 1 *DDX41* variant (MAF < 0.1%) are identified in 9821 unrelated and unselected adult patients from 6 medical centers and at ARUP Laboratories. Among these patients with HM, 3583 are diagnosed with AML, 2160 with MDS, 1030 with MPN, and 3048 with others including cytopenia and other myeloid and lymphoid neoplasms. These *DDX41* variants are further classified into somatic variants alone (variants with a VAF < 40% in isolation) and presumed germline variants (VAFs of 40% or above, with or without concurrent somatic *DDX41* variants). The germline variants are further classified into CV (PV/LPV, n = 116) and VUS (n = 60), according to the proposed gene-specific diagnostic criteria, modified from the ACMG guidelines.[20] Among the 116 patients with germline *DDX41* CV, 66 are diagnosed with AML, 28 with MDS, 4 with MPN, and 18 with cytopenia (others). Similarly, among the 60 patients with germline VUS, 18 are diagnosed with AML, 12 with MDS, 11 with MPN, and 19 with others. Among others, 4 are diagnosed with B-cell LPD, 1 with MM, and 14 with cytopenia. In addition, we select 4307 adult patients with HM (age of 18 years or above) with wild-type *DDX41* (*DDX41*$^-$), confirmed by NGS testing at ARUP laboratories during the same time period. Among these control patients (*DDX41*$^-$), 1365 have a documented AML diagnosis, and the remaining cases include 1109 MDS, 479 MPN, and 1354 others, most of which are cytopenia, similar to those in the cohort of 9821 patients described above. Patients' age, sex, and cytogenetic and molecular profiles are summarized and sorted by each distinct MN entity and correlated with their *DDX41* genotypes (short double-headed arrows indicate the epidemiologic and molecular profile comparisons in between *DDX41*$^+$ CV, VUS, and *DDX41*$^-$ cohorts). Furthermore, we summarize the OS in patients with AML and MDS who were treated at Huntsman Cancer Institute and other medical centers in comparison with the age-matched cohorts (long double-headed arrows indicate the OS comparisons in between *DDX41*$^+$ CV, VUS, and *DDX41*$^-$ cohorts). #Of note, 24 patients with AML with *DDX41* CV have been documented in a previous study.[8] (B) Graphic distribution of variants identified in this study, positioned on the protein sequence (NM_016222.4) with major functional domains (red, DEAD domain; green, helicase domain; orange, Znf, zinc finger domain; teal, NLS, nuclear localization signaling domain) is separated by germline (above-protein sequences) or somatic (below) variants. Each symbol in germline variants represents 1 patient. The underline indicates novel variants reported in this study. Red, *DDX41* CV; blue, *DDX41* VUS; orange, p.R164W, likely CV in lymphoma. *With specific exceptions (eg, p.M155I and p.P510S).

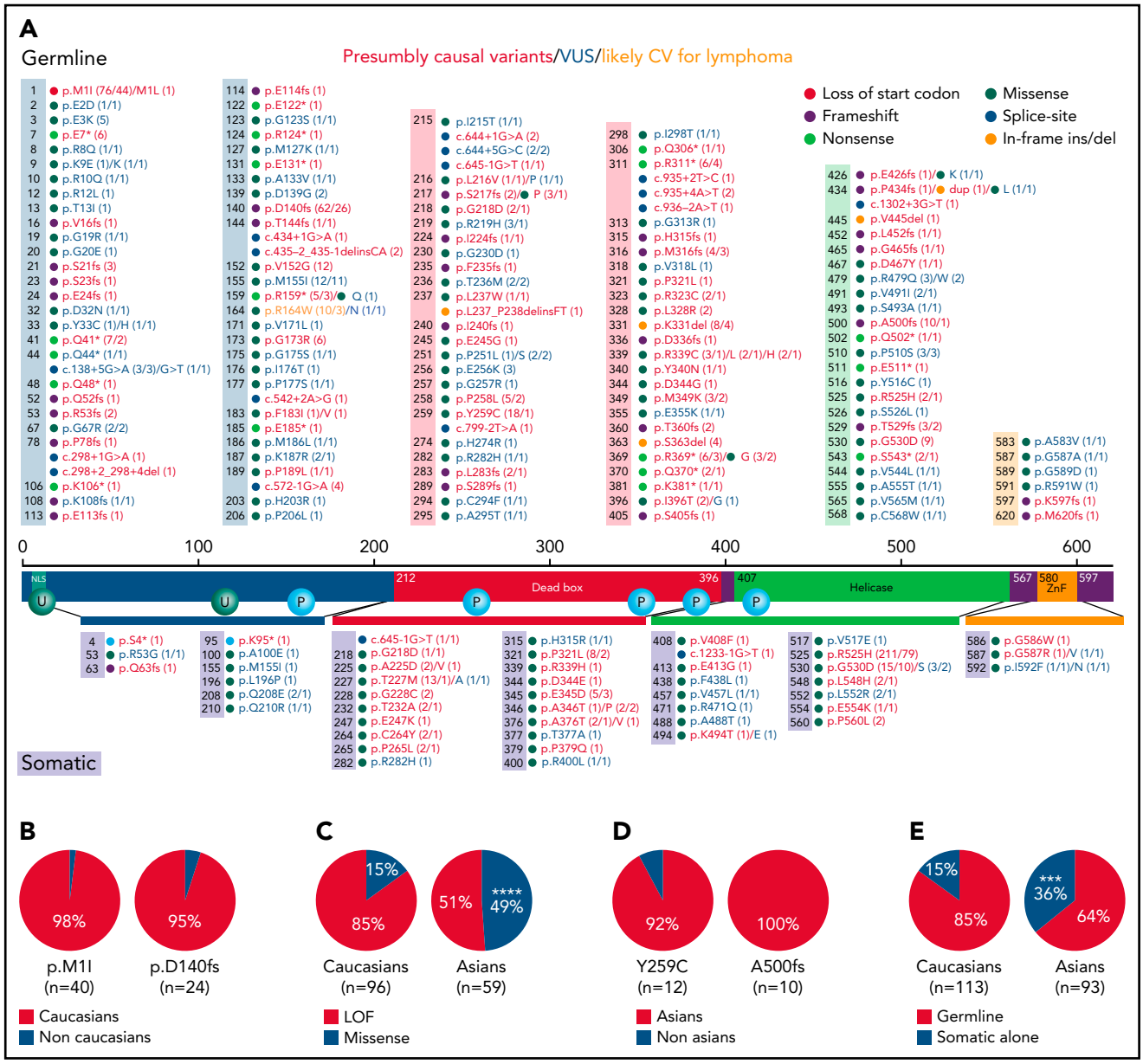


Figure 2. Summary of *DDX41* variants and ethnic difference in germline CV identified in this study and literature. (A) Summary of *DDX41* germline (above the protein sequence) and somatic (below the protein sequence) variants. The colors in the boxes above and the horizontal bars below the protein sequence are designated corresponding to the protein functional domains. Numbers in parentheses alone or before a slash indicate the total times of a certain variant was reported in literature including those reported in this study, whereas numbers after a slash represent variants seen in the current study. Red, CV; blue, VUS; orange, likely CV for lymphoma. (B-E) Ethnic difference in *DDX41* CV as data combined in this study and collected and reanalyzed in literature.[28,29] (B) Germline variants of p.M1I (98%, 39 White and 1 Asian patients) and p.D140fs (95%, 23 White and 1 African American patients) are the leading CVs in White patients. (C) Missense germline variants, although uncommon in Whites (15%), are seen in 49% of Asian patients with HM ($P < .0001$). (D) p.Y592C (92%, 11 Asians and 1 non-Asian) and p.A500fs (100%, 10 all in Asian) appear the most common germline CV in Asian patients. (E) Somatic *DDX41* variants alone, in the absence of associated germline variants, appear more frequently in Asian than White patients (36% in Asian vs 15% in White, $P = .0007$). ***, $P < .001$; ****, $P < .0001$.

most common germline CVs (19% and 15% of all, respectively), were primarily identified in White patients (Figure 2B). Missense germline CV, although less common and most often classified as VUS according to unmodified ACMG guidelines, were reported in this study and literature accompanied by pathogenic somatic variants.[7,10,11,28-34] Interestingly, approximately half of Asian patients with HM carried missense germline CV (Figure 2C), with Y259C being the most common hotspot (Figure 2D), whereas only 1 p.M1I[28] and 0 p.D140fs variants were documented in Asian patients (Figure 2B). Data obtained from the gnomAD database showed similar ethnic differences (not shown). This unique ethnic difference[29] in *DDX41* CV was also highlighted by p.A500fs, seen exclusively in Asian patients (Figure 2D) as the second most common germline CV and more frequent somatic variants alone (Figure 2E).

Concomitant somatic variants were detected by NGS in patients with germline *DDX41* CV (red) and VUS (blue in Figure 3). Beyond somatic *DDX41* variants, *ASXL1* was the second most commonly mutated gene concomitant with germline *DDX41* CV

Table 1. Molecular and cytogenetic profiles of 205 individuals with *DDX41* variants

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
1*	AML	c.3G>A	p.M1I	44	1	c.1574G>A	p.R525H	6	1	ASXL1 c.1900_1922del, p.E635fs	19	1	46,XX[20]
										PFH6 c.811G>A, p.E271K	12	1	
										CUX1 c.607 + 1G>A, p.?	3	1	
										CUX1 c.2786del, p.P929fs	1	1	
2†	AML	c.3G>A	p.M1I	46	1	c.1574G>A	p.R525H	6	1	ASXL1 c.1900_1922del, p.E635fs	6	1	46,XX[20]
										PHF6 c.940A>T, p.I314F	4	2	
										CUX1 c.2485C>T, p.Q829*	5	1	
3	AML	c.3G>A	p.M1I	48	1	c.1574G>A	p.R525H	0	1	ASXL1 c.1627G>T, p.E543*	2	1	NI
										PHF6 c.880A>G, p.I294V	3	2	
4†	AML	c.3G>A	p.M1I	48	1	c.1574G>A	p.R525H	16	1	ASXL1 c.1779dup, p.C594fs	8	1	45,X,-Y[6]/46,XY[14]
										SF3B1 c.2098A>G, p.K700E	4	1	
										JAK2 c.1849G>T, p.V617F	5	1	
5	AML	c.3G>A	p.M1I	48	1	c.1574G>A	p.R525H	3	1	ASXL1 c.2905_2926delinsTACTGTT, p.D969_N971delinsYC*	5	1	NI
										TP53 c.850A>C, p.T284P	5		
										KMT2A c.2830_2847dup, p.D944_T949dup	33	2	
6*,†	AML	c.3G>A	p.M1I	47	1	c.1574G>A	p.R525H	1	1	ASXL1 c.1761_1768del, p.Q588fs	20	1	45,XY,del(5)(q15q35), der(7;16)(q10;q10), add(10)(p11.2),
										BCORL1 c.1339C>T, p.Q447*	1	1	add(10)(q24),+20, -22[13]/45,XY,-4, del(5)(q15q35),
										TP53 c.743G>A, p.R248Q	2	1	der(7)t(7;9)(p11.2;q13), add(12)(p12), -13,+14,
										TP53 c.817C>T, p.R273C	1	1	add(14)(q32), add(18)(q21),-22,+mar[7]
7	AML	c.3G>A	p.M1I	45	1	c.1574G>A	p.R525H	5	1	ASXL1 c.3030_3031delinsTT, p.E1011*	3	1	46,XY[20]
										ASXL1 c.2122del, p.Q708fs	3	1	

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
8	AML	c.3G>A	p.M1I	51	1	c.1574G>A	p.R525H	7	1	ASXL1 c.1960dup, p.A654fs	6	1	46,XY[20]
9†	AML	c.3G>A	p.M1I	58	1	c.1574G>A	p.R525H	3	1	ASXL1 c.3824C>G, p.S1275* ZRSR2 c.202_203del, p.R68fs	11 5	1 1	46,XY[20]
10*,†	AML	c.3G>A	p.M1I	47	1	c.1574G>A	p.R525H	1	1	DNMT3A c.1015-1G>C, p.? SETBP1 c.1977T>A, p.D659G	5 1	1 2	46,XX[20]
11	AML	c.3G>A	p.M1I	51	1	c.1574G>A	p.R525H	4	1	RUNX1 c.385C>G, p.L129V JAK2 c.1849G>T, p.V617F	6 4	2 1	46,XX[20]
12†	AML	c.3G>A	p.M1I	52	1	c.1574G>A	p.R525H	5	1	RUNX1 c.776_777del, p.F259* NF1 c.2033dupC, p.I679fs	5 45	1 1	46,XX[20]
13	AML	c.3G>A	p.M1I	50	1	c.1574G>A	p.R525H	3	1				46,XX[20]
14†	AML	c.3G>A	p.M1I	46	1	c.1574G>A	p.R525H	2	1				trisomy 8
15†	AML	c.3G>A	p.M1I	47	1	c.1574G>A	p.R525H	6	1				46,XX[20]
16†	AML	c.3G>A	p.M1I	50	1	c.1574G>A	p.R525H	7	1				NI
17†	AML	c.3G>A	p.M1I	43	1	c.1574G>A	p.R525H	5	1				46,XY[20]
18†	AML	c.3G>A	p.M1I	49	1	c.1574G>A	p.R525H	7	1				46,XY[19]
19*	AML	c.3G>A	p.M1I	47	1	c.1574G>A	p.R525H	11	1				46,XY[20]
20*,†	AML	c.3G>A	p.M1I	44	1	c.971G>A	p.C264Y	5	1				45,X,-Y[6]/46,XY[14]
21†	AML	c.3G>A	p.M1I	45	1	c.1037C>T	p.A346P	3	1				46,XY[20]
22†	AML	c.3G>A	p.M1I	50	1					ASXL1 c.1934dup, p.G646fs KRAS c.35G>T, p.G12V	13 13	1 1	46, XY[20]
23	AML	c.3G>A	p.M1I	41	1					ASXL1 c.1934dup, p.G646fs	28	1	45, XY, −7[16]/46, XY, [4].

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
24†	AML	c.3G>A	p.M1I	49	1					RUNX1 c.743dupA, p.N248fs	21	1	46,XY, del(5)(q13q33)[2]/47, sl,+21[2]/
										TP53 c.827C>A, p.A276D	7	1	46~48, sdl1,t(1;2) (p36.3;q31), t(1;6)(^{32}P;q27)t(1;2), +mar[cp16]
25	AML	c.323del	p.K108fs	51	1	c.1574G>A	p.R525H	9	1	TET2 c.3965T>A, p.L1322Q	9	1	46,XY[20]
										SRSF2 c.284C>T, p.P95L	13	1	
										TP53 c.743G>A, p.R248Q	5	1	
26	AML	c.415_418dup	p.D140fs	49	1	c.1574G>A	p.R525H	22	1	ASXL1 c.1924_1928del, p.G644fs	4	1	46,XX[20]
										TET2 c.2456dup, p.Y819*	4	1	
										TET2 c.2459G>A, p.S820N	4	2	
										SH2B3 c.1200dup, p.Y401fs	36	1	
27	AML	c.415_418dup	p.D140fs	43	1	c.1574G>A	p.R525H	3	1	ASXL1 c.1900_1922del, p.E635fs	2	1	46,XY[20]
										DNMT3A c.976C>T, p.R326C	3	12	
										CSF3R c.1640G>A, p.W547*	48		
28†	AML	c.415_418dup	p.D140fs	43	1	c.1574G>A	p.R525H	5	1	ASXL1 c.1919_1929del, p.A640fs	7	1	46,XY[20]
										PHF6 c.834G>T, p.M278I	13	2	
29	AML	c.415_418dup	p.D140fs	50	1	c.1574G>A	p.R525H	7	1	ASXL1 c.2275_2284del, p.Gln760fs	3	1	46,XY, inv(11)(q21q23)[20]
										TP53 c.830G>A, p.C277Y	6	1	
30*,†	AML	c.415_418dup	p.D140fs	45	1	c.1574G>A	p.R525H	16	1	TET2 c.1847del, p.P616fs	1	1	46,XY[20]
										TET2 c.782_786del, p.S261*	1	1	
31	AML	c.415_418dup	p.D140fs	45	1	c.1574G>A	p.R525H	1	1	TET2 c.5577_5578del, p.I1859fs	2	1	NI

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
32†	AML	c.415_418dup	p.D140fs	46	1	c.1574G>A	p.R525H	2	1	TET2 c.2340dup, p.V781fs DDX41 c.138 + 5G>T, p.?	3 47	1 2	NI
33	AML	c.415_418dup	p.D140fs	46	1	c.1574G>A	p.R525H	1	1	CUX1 c.2459G>A, p.W820*	1	1	NI
34†	AML	c.415_418dup	p.D140fs	46	1	c.1574G>A	p.R525H	2	1				46,XX[20]
35	AML	c.415_418dup	p.D140fs	42	1	c.1574G>A	p.R525H	6	1				46,XY[20]
36	AML	c.415_418dup	p.D140fs	43	1	c.1589G>A	p.G530D	35	1	ASXL1 c.3001dup, p.T1001fs EZH2 c.349C>T, p.Q117* SETBP1 c.2608G>A, p.G870S	34 31 4	1 1 1	NI
37	AML	c.415_418dup	p.D140fs	46	1								NI
38†	AML	c.415_418dup	p.D140fs	49	1								46,X,-X,der1, (X;1)(p11.3;p36.3), inv9(p12q13)c, +14[4]/46, XX, inv9c[16]
39†	AML	c.415_418dup	p.D140fs	47	1								46,XY[20]
40†	AML	c.415_418dup	p.D140fs	46	1								46,XY[19]
41	AML	c.415_418dup	p.D140fs	44	1								NI
42	AML	c.415_418dup	p.D140fs	46	1								46,XX[20]
43	AML	c.415_418dup	p.D140fs	44	1								46,XY[20]
44	AML	c.668dup	p.I224fs	45	1	c.1574G>A	p.R525H	16	1	ASXL1 c.2541del, p.T848fs PHF6 c.138 + 1G>A, p.? PHF6 c.255C>G, p.C85W	17 5 9	1 1 2	NI
45†	AML	c.847del	p.L283fs	43	1	c.1574G>A	p.R525H	5	1				NI
46	AML	c.946_947del	p.M316fs	52	1	c.1574G>A	p.R525H	3	1	CUX1 c.3855del, p.S1286fs	1	1	46,XY[20]
47	AML	c.1394del	p.G465fs	46	1	c.1574G>A	p.R525H	1	1	DNMT3A c.2026C>T, p.R676W ASXL1 c.2423_2427del, p.P808fs ASXL1 c.2060_2061del, p.C687fs	6 5 3	1 1 1	46,XY[20]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
48	AML	c.121C>T	p.Q41*	48	1	c.1574G>A	p.R525H	1	1	ASXL1 c.1900_1922del, p.E635fs	10	1	N/A
										DNMT3A c.2256_2263del, p.W753*	15	1	
										PHF c.820C>T, p.R274*	22	1	
49	AML	c.121C>T	p.Q41*	45	1					TET2 c.4133G>A, p.C1378Y	44	1	NI
										GATA2 c.599dup, p.S201*	2	1	
										KDM6A c.3704 + 1G>C, p.?	67	1	
										ZRSR2 c.505C>T, p.R169*	93	1	
										NPM1 c.860_863dup, p.W288fs	35	1	
50	AML	c.475C>T	p.R159*	51	1	c.1589G>A	p.G530D	2	1	U2AF1 c.101C>A, p.S34Y	3	1	46,XY[20]
51	AML	c.931C>T	p.R311*	49	1	c.1589G>A	p.G530D	5	1	PHF6 c.730-1G>A, p.?	3	1	NI
										DNMT3A c.2255_2257del, p.F752del	1	1	
52	AML	c.1105C>T	p.R369*	46	1	c.1574G>A	p.R525H	18	1	ASXL1 c.1900_1922del, p.E635fs	13	1	46,XY[20]
										SETBP1 c.2602G>A, p.D868N	5	1	
										SETBP1 c.2608G>A, p.G870S	9	1	
										SETBP1 c.2612T>C, p.I871T	5	1	
53	AML	c.1105C>T	p.R369*	47	1	c.1574G>A	p.R525H	1	1	TET2 c.3632G>A, p.C1211Y	4	1	NI
										SH2B3 c.794G>A, p.R265Q	48	2	
54	AML	c.1108C>T	p.Q370*	48	1	c.1588G>A	p.G530S	25	1	JAK2 c.1849G>T, p.V617F	22	1	46,XY[20]
55	AML	c.1504C>T	p.Q502*	49	1	c.1035G>C	p.E345D	10	1	ASXL1 c.2693G>A, p.W898*	9	1	45,X,-Y[14]/46,XY[6]
56†	AML	c.645-1G>T	p.?	45	1	c.1574G>A	p.R525H	1	1	ASXL1 c.3824C>G, p.S1275*	1	1	46,XY[20]
										DNMT3A c.1572T>A, p.C524*	1	1	

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
57	AML	c.992_994del	p.K331del	48	1	c.1035G>C	p.E345D	14	1	ASXL1 p.E635fs	5	1	46,XY, der(7)add(7)(p13) add(7)(q11.2)[10]/ 45,XY, -der(7)[5]/46,XY[13]
										EZH2 p.N546K	7	2	
58*,†	AML	c.646C>G	p.L216V	51	1	c.1035G>C	p.E345D	30	1	DNMT3A c.2656C>T, p.Q886*	31	1	NI
59	AML	c.653G>A	p.G218D	50	1	c.1589G>A	p.G530D	4	1	TP53 c.488A>G, p.Y163C	6	1	NI
										RUNX1 c.288_291delinsAAA, p.N96fs	3	1	
										DNMT3A c.1627G>T, p.G543C	5	1	
										DNMT3A c.1578C>G, p.Y526*	5	1	
60	AML; breast cancer	c.773C>T	p.P258L	56	1	c.1574G>A	p.R525H	15	1				46,XY[20]
61	AML	c.967C>T	p.R323C	45	1	c.1574G>A	p.R525H	3	1	TET2 c.3585_3588delnsAG, p.A1196fs	5	1	NI
										SRSF2 c.284C>A, p.P95H	4	1	
62	AML	c.1046T>A	p.M349K	45	1								46,XY[20]
63	AML	c.1046T>A	p.M349K	50	1								46,XY[20]
64	AML	c.1105C>G	p.R369G	48	1	c.1574G>A	p.R525H	5	1				46,XX[20]
65	AML	c.1399G>T	p.D467Y	45	1	c.1589G>A	p.G530D	13	1	ASXL1 c.1934dup, p.G646fs	13	1	NI
										ASXL1 c.1900_1922del, p.E635fs	2	1	
										EZH2 c.2022G>C, p.L674F	3	1	
										SETBP1 c.2608G>A, p.G870S	4	1	
										EZH2 c.2197T>A, p.Y733N	10	1	
										SETBP1 c.2612T>C, p.I871T	8	1	
66	AML	c.1574G>A	p.R525H	54	1								NI
67	MDS	c.3G>A	p.M1I	45	1	c.1574G>A	p.R525H	2	1	DNMT3A c.1010C>G, p.S337*	1	1	NI

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
68	MDS	c.3G>A	p.M1I	49	1	c.1574G>A	p.R525H	6	1				46,XY[20]
69	MDS	c.3G>A	p.M1I	43	1	c.1574G>A	p.R525H	4	1				46,XX[20]
70	MDS	c.3G>A	p.M1I	53	1	c.1574G>A	p.R525H	2	1				46,XY[20]
71	MDS	c.3G>A	p.M1I	50	1	c.694A>G	p.T232A	3	1				46,XY[20]
72	MDS	c.3G>A	p.M1I	49	1	c.962C>T	p.P321L	14	1	DNMT3A c.1792C>T, p.598*	14	1	46,XY[20]
73	MDS	c.3G>A	p.M1I	49	1	c.962C>T	p.P321L	14	1				46,XY[20]
74	MDS	c.3G>A	p.M1I	48	1	c.1037C>T	p.A346P	3	1				46,XY[20]
75	MDS	c.3G>A	p.M1I	46	1	c.1643T>A	p.L548H	4	1				46,XX[20]
76	MDS; MM; MBL	c.3G>A	p.M1I	50	1					DNMT3A c.2645G>A, p.R882H	15	1	46,XX[20]
77	MDS	c.3G>A	p.M1I	46	1					PTPN11 c.215C>T, p.A72V RUNX1 c.1036dup, p.R346fs	42 32	1 1	47,XY,del(5)(q13q33), +21[2]/48,sl,t(9;21)(q10;q10), +21[12]/
78	MDS	c.3G>A	p.M1I	49	1					JAK2 c.1849G>T, p.V617F	1	1	46,XY[20]
79	MDS	c.3G>A	p.M1I	49	1								45,X,-Y[6]/46,XY[14]
80	MDS	c.415_418dup	p.D140fs	44	1	c.1574G>A	p.R525H	10	1	ASXL1 c.4127dup, p.P1377fs	12	1	NI
81	MDS	c.415_418dup	p.D140fs	45	1	c.794C>T	p.P265L	27	1	DNMT3A c.1579C>T, p.Q527*	9	1	46,XY[20]
82	MDS	c.1496dup	p.A500fs	47	1	c.1660G>A	p.E554K	2	1				46,XY[20]
83	MDS	c.130C>T	p.Q44*	60	1	c.1126C>T	p.A376T	2	1	DNMT3A c.2207G>A, p.R736H	7	1	46,XX[20]
84	MDS	c.475C>T	p.R159*	47	1	c.1589G>A c.1574G>A c.1588G>A	p.G530D p.R525H p.G530S	6 3 1	1 1 2				NI
85	MDS	c.931C>T	p.R311*	51	1	c.1574G>A	p.R525H	6	1	SRSF2 c.284C>T, p.P95L	3	1	NI
86	MDS	c.931C>T	p.R311*	50	1	c.1574G>A	p.R525H	7	1				NI

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
87	MDS	c.992_994del	p.K331del	43	1	c.1574G>A	p.R525H	14	1	TET2 c.3866G>T, p.C1289F	3	1	46,XY[20]
88	MDS	c.992_994del	p.K331del	48	1								46,XY[20]
89	MDS	c.566C>T	p.P189L	49	1	c.1574G>A	p.R525H	4	1	EZH2 c.434T>C, p.F145S	3	1	NI
90	MDS	c.710T>G	p.L237W	49	1	c.1589G>A	p.G530D	13	1				46,XY[20]
91	MDS	c.1016G>A	p.R339H	51	1					IDH1 c.605del, p.S202fs	31	2	45,X,-Y/46,XY[15]
92	MDS	c.1015C>T	p.R339C	49	1	c.680C>T	p.T227M	13	1	TET2 c.1793del, p.N598fs	2	1	46,XY[20]
93	MDS	c.1018T>A	p.Y340N	47	1	c.1574G>A	p.R525H	7	1	CUX1, c.2389del, p.Q797fs EZH2 c.371A>G, p.D124G	13 15	1 2	46,XY[20]
94*	MDS	c.1105C>G	p.R369G	47	1	c.645-1G>A	p.?	1	1	TET2 c.1263del; p.G422fs TET2 c.3860_3869del, p.F1287fs	31 1	1 1	NI
95	Pancytopenia	c.3G>A	p.M1I	47	1	c.1574G>A	p.R525H	14	1	JAK2 c.1849G>T, p.V617F TET2 c.1648C>T, p.R550*	15 7	1 1	NI
96	Pancytopenia	c.3G>A	p.M1I	47	1	c.1574G>A	p.R525H	2	1				NI
97	Pancytopenia	c.415_418dup	p.D140fs	46	1	c.1574G>A	p.R525H	9	1				46,XY, del(20)(q11.2q13.1)[1]/46,XY[20]
98	Pancytopenia	c.430del	p.T144fs	45	1	c.1574G>A	p.R525H	4	1	ASXL1c.4002del, p.S1335fs TP53 c.586C>T, p.R196* TP53 c.916C>T, p.R306* IDH2 c.419G>A, p.R140Q	21 4 3 2	1 1 1 1	NI
99	Pancytopenia	c.946_947del	p.M316fs	47	1	c.1574G>A	p.R525H	5	1	ASXL1 c.2644C>T, p.Q882* PHF6 c.941T>C, p.I314T	6 11	1 2	46,XY[20]
100	Pancytopenia	c.1354del	p.L452fs	48	1	c.1574G>A	p.R525H	8	1	DNMT3A c.929T>C, p.I310T	5	1	46,XY[20]
101	Pancytopenia	c.475C>T	p.R159*	48	1	c.1574G>A	p.R525H	12	1	SRSF2 c.284C>A, p.P95H STAG2 c.1243C>T, p.H415Y	10 16	1 2	NI

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
102	Pancytopenia	c.1628C>G	p.S543*	50	1	c.1574G>A	p.R525H	4	1	ASXL1 c.1934dup, p.G646fs	3	1	NI
										RUNX1 c.540del, p.F180fs	3	1	
										PHF6 c.941T>C, p.I314T	7	1	
										KDM6A c.2665del, p.T889fs	1	1	
103	Pancytopenia	c.649T>C	p.S217P	48	1	c.1589G>A	p.G530D	9	1	ASXL1 c.2467_2468insA, p.L823fs	7	1	NI
										KRAS c.437C>T, p.A146V	3	1	
										SRSF2 c.47T>A, p.L16H	7	2	
104	Pancytopenia	c.773C>T	p.P258L	47	1	c.1574G>A	p.R525H	4	1	KDM6A c.3107delT, p.F1036fs	2	1	NI
										SRSF2 c.284C>A, p.P95H	7	1	
105	Pancytopenia	c.776A>G	p.Y259C	48	1	c.1574G>A	p.R525H	6	1	FBXW7 c.62G>A, p.G21D	19	2	46,XY[20]
106	Pancytopenia	c.1016G>T	p.R339L	50	1	c.1574G>A	p.R525H	1	1				NI
107	Thrombocytopenia	c.415_418dup	p.D140fs	45	1	c.1574G>A	p.R525H	10	1	ASXL1 c.1900_1922del, p.E635fs	2	1	NI
										CUX1 c.2161C>T, p.Q721*	8	1	
										SMC1A c.2132G>A, p.R711Q	4	2	
108	Thrombocytopenia	c.1586_ 1587delCA	p.T529fs	52	1					TP53 c.1024C>T, p.R342*	40	1	46,XX[20]
109	Thrombocytopenia	c.3G>A	p.M1I	45	1	c.962C>T	p.P321L	23	1	RUNX1 c.281G>A, p.S94N	30	1	NI
										TP53 c.742C>T, p.R248W	35	1	
										SRSF2 c.284C>G, p.P95R	26	1	
110	Neutropenia	c.3G>A	p.M1I	48	1	c.1574G>A	p.R525H	10	1				NI
111	Neutropenia	c.3G>A	p.M1I	47	1	c.1574G>A	p.R525H	1	1				NI
112	Anemia	c.1105C>T	p.R369*	45	1	c.1574G>A	p.R525H	5	1	ASXL1 c.1934dup, p.G646fs	7	1	46,XX,+1, der(1;7)(q10;p10) [16]/46,XX[4]
										EZH2 c.786dup, p.N263fs	4	1	
										SETBP1 c.2613_2614delinsGC, p.I871_G872delinsMR	4	2	
113	MPN	c.415_418dup	p.D140fs	49	1								46,XX[20]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
114	MPN	c.946_947del	p.M316fs	48	1								NI
115	MPN	c.916C>T	p.Q306*	48	1								46,XX[20]
116	MPN	c.1141A>T	p.K381*	44	1	c.1574G>A	p.R525H	7	1	CUX1 c.988C>T, p.Q330*	7	1	46,XY[20]
117	AML	c.6G>T	p.E2D	48	2					FLT3 c.1805_1806ins42, p.K602_W603ins14	n/a	1	46,XX[20]
										NPM1 c.863_864insCCTG, p.W288fs	35	1	
										WT1 c.1141_1144dup, p.A382fs	8	1	
118*	AML	c.55G>A	p.G19R	48	2					FLT3 ITD c.1837 + 11_1837 + 12ins114, p.?	n/a	1	NI
										NPM1 c.863_864insCCTG, p.W288fs	43	1	
										IDH1 c.395G>A, p.R132H	41	1	
										DNMT3A c.1627G>T, p.G543C	42	1	
119*	AML	c.97T>C	p.Y33H	45	2					FLT3 c.1770_1811dup42, p.W603_E604ins14	n/a	1	NI
										NPM1 c.860_863dup, p.W288fs	18	1	
										DNMT3A c.860_863dup, p.W288fs	27	1	
										TET2 c.2490dup, p.Q831fs	23	1	
120	AML	c.465G>A	p.M155I	47	2					NPM1 c.860_863dup, p.W288fs	7	1	46,XY[20]
										SRSF2 c.284C>G, p.P95R	28	1	
										TET2 c.2244dup, p.Q749fs	44	1	
121	AML	c.465G>A	p.M155I	47	2					NPM1 c.863_864insCTTG, p.W288Cfs	33	1	46,XY[20]
										GATA2 c.599dup, p.S201*	2	1	
122*	AML	c.491G>A	p.R164N	48	2	c.1774A>T	p.I592F	35	2	NPM1 c.863_864insCTTG, p.W288Cfs	33	1	46,XX[20]
123	AML	c.1528C>T	p.P510S	48	2					NPM1 c.860_863dup, p.W288fs	34	1	46,XY[20]
										SRSF2 c.284C>T, p.P95L	42	1	
										KRAS c.35G>A, p.G12D	11	1	

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
124	AML	c.380T>A	p.M127K	49	2					FLT3 c.2505T>G, p.D835E	3	1	46,XY,+12, der(17)t(17;18) (p10;q10),-18[8]
										TP53 c.400T>A, p.F134I	46	1	
										TP53 c.458_462del, p.P153fs	3	1	
										U2AF1 c.101C>A, p.S34Y	6	1	
										KRAS c.351A>T, p.K117N	27	1	
125	AML	c.465G>A	p.M155I	47	2					FLT3 p.N841K	7	1	46,XY[20]
										KRAS c.35G>T, p.G12V	3	1	
126	AML	c.199G>C	p.G67R	50	2					ASXL1 c.2959G>T, p.G987*	47	1	46,XY[20]
										CEBPA c.985_986insCC, E329fs	42	1	
										CEBPA c.68del, p.P23fs	46	1	
										IDH2 c.418C>T, p.R140W	48	1	
										JAK2 c.1849G>T, p.V617F	6	1	
127	AML	c.199G>C	p.G67R	48	2					ASXL1 c.2959G>T, p.G987*	22	1	46,XY[20]
										IDH2 c.418C>T, p.R140W	25	1	
										SRSF2 c.284C>A, p.P95H	28	1	
										STAG2 c.1196+1G>A, p.?	4	1	
										STAG2 c.1999del, p.R667fs	15	1	
128*	AML	c.465G>A	p.M155I	51	2								48,XY,+8,+22[20]
129	AML	c.883G>A	p.A295T	53	2					TP53 c.743G>A, p.R248Q	35	1	44,XY,-3,add(5) (q11.2),+8, add(8)(q22), der(12;17)(q10;q10),
										TP53 c.818G>A, p.R273H	15	1	−14,i(14)(q10), i(21)(q10)[12]/ 43-45, sl,-add(5)(q11.2), i(5)(q10)[cp4]/88 <4n>,slx2[1]
130	AML	c.893T>C	p.I298T	49	2					DNMT3A c.989G>A, p.W330*	2	1	NI
										TP53 c.844C>T, p.R282W	61	1	
										TP53 c.535C>T, p.H179Y	4	1	
										PTPN11 c.1504T>C, p.S502P	2	1	

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
131	AML	c.1063G>A	p.E355K	47	2					PHF6 c.418 + 2T>C, p.? KMT2A c.10462C>T, p.Gln3488*	21 25	1 2	46,XX[7]
132	AML	c.465G>A	p.M155I	48	2					NRAS c.181C>A, p.Q61K	15	1	46,XY,inv(16)(p13.1q22)[20]
133	AML	c.465G>A	p.M155I	47	2					CSF3R c.1843A>G, p.T615A CSF3R c.1853C>T, p.T618I	22 4	1 1	46,XX, der(8)t(8;21)(q22;q22), der(8)?(8pter->8p22::?::8p11.2->8q?13::8q22->8qter), der(21)(21pter->21q22::8?q13->8q?22::8q22->8qter)[7]/45,sl,-X[8]/46,XX[5]
134	AML	c.138 + 5G>A	p.?	50	2					NRAS c.181C>A, p.Q61Lys	39	1	47,XY, +8,inv(16)(p13.1q22)[20]
135	MDS	c.367G>A	p.G123S	48	2					ASXL1 c.2239_2244delinsCC, p.S747fs ASXL1 c.1934dup, p.G646fs TET2 c.5543C>G, p.S1848* EZH2 c.1119dup, p.T374fs IDH2 c.419G>A, p.R140Q SF3B1 c.2347G>A, p.E783K JAK2 c.1849G>T, p.V617F	42 2 1 2 31 2 1	1 1 1 1 1 2 1	46,XX[20]
136	MDS	c.465G>A	p.M155I	43	2					JAK2 c.1849G>T, p.V617F DNMT1 c.4663G>A, p.V1555M SH2B3 c.127C>T, p.R43C	27 32 35	1 2 2	NI
137	MDS	c.644 + 5G>C	p.?	43	2					SH2B3 c.947_953del, p.E316fs JAK2 c.1849G>T, p.V617F	21 40	1 1	47,XY, +9,del(20)(q11.2q13.1)[19]/46,XY[1]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
138	MDS	c.523G>A	p.G175S	48	2					NRAS c.34G>A, p.G12S	44	1	NI
										TET2 c.2290dup, p.Q764fs	50	1	
										GATA2 c.599del, p.G200fs	1	1	
										SRSF2 c.284C>A, p.P95H	47	1	
139	MDS	c.529C>T	p.P177S	45	2					SMC1A c.197A>G, p.H66R	9	2	NI
										BCOR c.441dup, p.Ile148fs	50	1	
										U2AF1 c.101C>T, p.S34F	37	1	
										RUNX1 c.601del, p.R201fs	10	1	
140	MDS	c.1301C>T	p.P434L	49	2					GATA2 c.599dup; p.S201*	31	1	46,XY[20]
										NPM1 c.867_868insAGGA, p.W290fs	48	1	
141	MDS	c.1528C>T	p.P510S	48	2					ASXL1 c.1934dup, p.G646fs	35	1	NI
										CBL c.800G>A, p.G267D	2	2	
142	MDS	c.1704C>G	p.C568W	48	2					KIT c.2446_2447delinsAT, p.D816I	10	1	NI
										KIT c.2447A>T, p.D816V	1	1	
										PTPN11 c.154A>G, p.T52A	5	1	
										PTPN11 c.1508G>A, p.G503E	3	2	
143	MDS/MPN	c.1760G>C	p.G587A	47	2					CSF3R c.1853C>T, p.T618I	3	1	47,XY,+8[20]
										KRAS c.436G>C, p.A146P	37	1	
										ASXL1 c.1900_1922del, p.E635fs	44	1	
										STAG2 c.1191dup, p.Q399fs	92	1	
144	MDS	c.1276G>A	p.E426K	49	2					SRSF2 c.284C>A, p.P95H	46	1	NI
										IDH2 c.419G>A, p.R140Q	45	1	
145	MDS	c.1528C>T	p.P510S	48	2					TP53 c.734G>A, p.G45D	20	1	NI
146	MDS	c.1663G>A	p.A555T	43	2					TP53 c.706dup, p.Y236fs	39	1	44-47,XY, del(5)(q22q35), -7,der(11)t(11;13) (p15;q14),
										TP53 c.713G>A, p.C238Y	37	1	
147	Pancytopenia	c.27G>A	p.K9K	43	2								NI

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
148	Pancytopenia	c.465G>A	p.M155I	50	2					SF3B1 c.1996A>G, p.K666E	19	1	45,X,-Y[14]/46,XY[6]
										U2AF1 c.101C>T, p.S34F	5	1	
149	Pancytopenia	c.556A>T	p.M186L	47	2					ETNK1 c.731A>G, p.N244S	24	1	NI
	Small cell carcinoma									KRAS c.468C>G, p.F156L	18	2	
150	Pancytopenia	c.656G>A	p.R219H	49	2	c.679A>G	p.T227A	30	2				46,XY[20]
151	Pancytopenia	c.845G>A	p.R282H	40	2					TP53 c.377A>G, p.Y126C	67	1	NI
										BRAF c.1391G>T, p.G464V	38	1	
152	Pancytopenia	c.881G>T	p.C294F	48	2					CBL c.1211G>A, p.C404Y	2	1	NI
153	Pancytopenia	c.1477T>G	p.S493A	47	2					ASXL1 c.1900_1922del, p.E635fs	15	1	NI
										ASXL1 c.1934dup, p.G646fs	3	1	
										ASXL1 c.2295del, p.S766fs	1	1	
										SH2B3 c.703C>G, p.R235G	16	1	
154	Thrombocytopenia	c.465G>A	p.M155I	50	2								46,XY[20]
155	Thrombocytopenia	c.707C>T	p.T236M	47	2								NI
156	Thrombocytopenia	c.138 + 5G>A	p.?	54	2								NI
157	Thrombocytopenia	c.644 + 5G>C	p.?	47	2					RAD21 c.507_508del, p.E169fs	38	1	NI
										NRAS c.35G>A, p.G12D	20	1	
										TP53 c.818G>A, p.R273H	31	1	
										TP53 c.559 + 1G>A, p.?	42	1	
158	Neutropenia	c.138 + 5G>A	p.?	50	2								NI
159	Anemia	c.751C>T	p.P251S	46	2								46,XX[20]
160	Anemia	c.1748C>T	p.A583V	47	2					PTPN11 c.227A>T, p.E76V	45	1	NI
161	MPN, PV	c.23G>A	p.R8Q	47	2					JAK2 c.1849G>T, p.V617F	45	1	46,XY[20]
162	MPN, PV	c.94G>A	p.D32N	48	2					JAK2 c.1849G>T, p.V617F	85	1	46,XY[20]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
163	MPN, ET	c.398C>T	p.A133V	44	2					CALR c.1122_1125del, p.K374fs U2AF1 c.470A>G, p.Q157R ZRSR2 c.236_237del, p.E79Afs	44 45 87	1 1 1	NI
164	MPN	c.644T>C	p.I215T	48	2					JAK2 c.1849G>T, p.V617F ASXL1 c.1934dup, p.G646fs	26 1	1 1	NI
165	MPN	c.647T>C	p.L216P	48	2	c.1655T>G	p.L552R	14	2	JAK2 c.1849G>T, p.V617F ASXL1 c.1900_1922del, p.E635fs	12 8	1 1	NI
166	MPN, ET	c.707C>T	p.T236M	50	2					JAK2 c.1849G>T, p.V617F SF3B1 c.2110A>T, p.I704F	18 15	1 1	46,XY[20]
167	MPN	c.1471G>A	p.V491I	52	2					JAK2 c.1849G>T, p.V617F TP53 c.743G>A, p.R248Q TP53 c.742C>T, p.R248W NF1 c.2035del, p.I679fs	47 6 5 15	1 1 1 1	46,XY[20]
168	MPN, ET	c.1630G>T	p.V544L	49	2					JAK2 c.1849G>T, p.V617F SF3B1 c.1997A>C, p.K666T	26 16	1 1	46,XY[20]
169	MPN, ET	c.138 + 5G>T	p.?	49	2								NI
170	MPN, ET	c.465G>A	p.M155T	49	2								46,XX[20]
171	MPN	c.560A>G	p.K187R	46	2								NI
172	LPL with pancytopenia	c.490C>T	p.R164W	50	2					SF3B1 c.2098A>G, p.K700E	41	1	46,XY[20]
173	γ heavy chain disease MYD88 negative LPL	c.490C>T	p.R164W	49	2								46,XY[20]
174	CLL; breast cancer	c.490C>T	p.R164W	47	2								46,XY[20]
175	CLL	c.751C>T	p.P251S	50	2								46,XY, del(13)(q12;q22), add(18)(p11.20[4]/ 46,XY[16]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
176	MM	c.29G>A	p.R10Q	46	2					TP53 c.376T>G, p.Y126D	24	1	44∼46,X, add(X)(p10), del(6)(q21q23), i(8)(q10),
										DNMT3A c.2339T>C, p.I780T	18	2	t(11;14)(q13;q32), del(13)(q12q22),-14,-17,+1∼2mar[cp4]/46,XX[16]
										KDM6A c.1354_1355del, p.G452fs	26	1	
177	AML					c.1574G>A	p.R525H	12	1	ASXL1 c.1774C>T, p.Q592*	4	1	46,XY,t(1;4)?(q21;q31)[2]/46,XY[6]
										PHF6 c.58del, p.C20fs	17	1	
178	AML					c.1574G>A	p.R525H	25	1	ASXL1 c.2077C>T, p.R693*	2	1	46,XY[21]
179	AML					c.1574G>A	p.R525H	1	1	CBL c.1211G>A, p.C404Y	15	1	46,XX[20]
										U2AF2 c.766G>A, p.D256G	16	2	
180	AML					c.1574G>A	p.R525H	4	1	SH2B3 c.519_523del, p.R175fs	3	1	45,X,-Y[5]/46,XY[15]
										PHF6 c.635G>T, p.C212F	8	2	
181	AML					c.1574G>A	p.R525H	16	1	SRSF2 c.284C>A, p.P95H	17	1	46,X,del(X)?(q22q26)[2]/46,XX[18]
182	AML					c.1574G>A	p.R525H	9	1	SETBP1 c.2602G>C, p.D868H	2	1	NI
						c.944A>G	p.H315R	8	2	CUX1 c.439C>T, p.R147*	7	1	
										STAG2 1693G>T, p.E565*	5	1	
										EZH2 c.1967C>T, p.A656V	4	1	
										ASXL1 c.2156del, p.E719fs	8	1	
183	AML					c.1589G>A	p.G530D	15	1	ASXL1 c.2985del, p.H995fs	5	1	46,XY[20]
						c.629A>G	p.Q210R	15	2	ASXL1 c.2083C>T, p.R695*	3	1	
										ASXL1 c.2077C>T, p.H963*	5	1	
										EZH2 c.2213del, p.A738fs	15	1	
										NF1 c.3774G>C, p.W1258C	12	1	
184	AML; DLBCL					c.1589G>A	p.G530D	6	1				46,XY[20]
185	MDS					c.1574G>A	p.R525H	4	1	DNMT3A c.2645G>A, p.R882H	3	1	46,XX[11]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
186	MDS					c.1574G>A	p.R525H	9	1				45,X,-Y[4]/46,XY[16]
187	MDS					c.1574G>A	p.R525H	10	1				46,XX[20]
188	MDS					c.1574G>A	p.R525H	1	1				46,XY[20]
189	MDS					c.1574G>A	p.R525H	4	1				46,XY[20]
190	AML					c.157C>G	p.R53G	5	2	SF3B1 c.2098A>G, p.K700E	32	1	NI
										PTPN11 c.214G>A, p.A72T	37	1	
191	AML					c.1760_1761TT	p.G587V	4	2	DNMT3A c.2645G>A, p.R882H	7	1	46,XY[20]
										DNMT3A c.2095G>A, p.G699R	3	2	
192	MDS					c.622C>G	p.Q208E	14	2	SETBP1 c.2608G>A, p.G870S	6	1	46,XY,del(7)(q22)[6]/46,XY[14]
										SETBP1 c.2602G>A, p.D868N	2	1	
										DNMT3A c.2645G>A, p.R882H12	12	1	
										EVT6 c.313C>G, p.R105G	2	2	
										TET2 c.4079T>C, p.L1360P	1	2	
193	MDS					c.1199G>T	p.R400L	3	2	ASXL1 c.2324T>G, p.L775*	20	1	46,XY, del(5)(q31q33)[13]/46,XX[7]
194	MDS					c.1369G>C	p.V457L	2	2	TP53 c.818G>A, p.R273H	30	1	46,XY, del(5)(q31q33)[14]/46,XX[6]
										TP53 c.578A>C, p.H193P	2	2	
195	Neutropenia					c.1775T>A	p.I592N	4	2	SRSF2 c.284C>T, p.P95L	4	1	NI
										RUNX1 c.606dup, p.P203fs	2	1	
										TET2 c.330G>C, p.Lys110N	3	2	
196*	Normal	c.3G>A	p.M1I	53	1								46,XY[20]
197*	Normal	c.3G>A	p.M1I	50	1								46,XX[20]
198*	Normal	c.415_418dup	p.D140fs	46	1								Not done
199*	Normal	c.415_418dup	p.D140fs	45	1								NI
200*	Normal	c.415_418dup	p.D140fs	56	1								46,XY[20]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.

*Germline variants confirmed by skin biopsies.

†Patients reported in a prior study.[9]

Table 1. (continued)

Patient	Diagnosis	gl DDX41	gl DDX41	VAF	Tier	s DDX41	s DDX41	VAF	Tiers	Concomitant variants	VAF (%)	Tier	Cytogenetics
201*	Normal	c.992_994del	p.K331del	47	1								Not done
202	Normal∼(donor)	c.931C>T	p.311*	50	1								47,XX,+8[19]
203	Normal∼(donor)	c.465G>A	p.M155I	46	2								46,XY[20]
204	Normal∼(donor)	c.1693G>A	p.V565M	50	2								46,XY[20]
205	Normal∼(donor)	c.1585dup	p.T529fs	46	1								44,XY,inc[1]/46,XX[16]

AF, variant allele frequency by %; ET, essential thrombocythemia; gl, germline; NI, no information; PMF, primary myelofibrosis; s, somatic; Tier 1, CV; Tier 2, VUS.
*Germline variants confirmed by skin biopsies.
†Patients reported in a prior study.[9]

28%), followed by *DNMT3A* (13%) and *TET2* (11%), similar to those in HM without germline *DDX41* variants.[35] In stark contrast, the most frequent concomitant variant in patients with HM with *DDX41* VUS was the *JAK2* p.V617F mutation (18%; Figure 3), and most (82%, 9 of 11) exhibited a leading VAF (Table 1), suggestive of a disease driver mutation. The genetic profiles in 19 patients with somatic *DDX41* CV alone appeared similar to those with somatic *DDX41* VUS (Figure 3; supplemental Figure 1). Interestingly, the median age of patients with HM with CV (68 years; Table 3) was greater than patients with VUS (63 years; $P = .01$). Similar to previous reports,[7,8,10,32] there was a striking male predominance of patients with CV (74%, Table 3) compared with WT control cohorts (50%, $P < .0001$), which was markedly diminished in patients with VUS (62%).

AML/MDS with germline *DDX41* CV is a distinct and the most common HM

By the proposed variant classification framework, patients with AML/MDS with *DDX41*-presumed germline CV and VUS were readily distinguished by differing genetic characteristics, epidemiologic features, and OS. Seventy-nine percent of patients with CV developed later-onset AML/MDS, whereas approximately 52% of those carrying VUS manifested with AML/MDS including a subset of early-onset AML (Tables 2 and 3). Specifically, the median age at the time of AML diagnosis was 69 years in patients carrying CV in contrast to those with VUS, in which some were children or young adults (median, 62 years; $P = .02$), and sporadic AML in adults (median, 64 years; $P = .002$; Table 3). Interestingly, the median age at MDS diagnosis (72 years) was similar to that in patients with AML with germline CV (Table 3; $P > .05$), whereas the median ages of patients with MDS carrying either VUS or WT *DDX41* was older than those in patients with AML with the same genotype (Table 3; 69 in MDS vs 62 in AML with VUS, $P = .08$; 74 in MDS vs 64 in AML with WT, $P < .0001$), as MDS is primarily a disease of the elderly.

More frequent somatic *DDX41* variants (Figure 4A) and a lower somatic mutation burden (Figure 4B) were observed in patients with AML and MDS with germline *DDX41* CV compared with those with VUS or without germline *DDX41* variants (Figure 4B). Mutated *NPM1*, rarely seen in patients with AML with CV (1.5%), was the most common concomitant variant, some associated with FLT3-ITD, in AML with VUS (37%; $P < .0001$; Figures 3 and 4C-D). In addition, t(8;21), inv(16), and biallelic *CEBPA* mutations were identified in 4 patients with AML with VUS, whereas none were seen in cases with CV (Figures 3 and 4E; $P < .0001$). Furthermore, mutations involved in tyrosine kinase and RAS/MAPK pathways were significantly more frequent in patients with AML/MDS with VUS than those with CV (Figures 3 and 4C-E; $P < .0001$). Germline CV-related AML/MDS cases shared similar mutational profiles; however, a higher somatic mutation burden was observed in patients with AML (Figure 4B, red bars; 1.6 in AML vs 0.7 in MDS; $P = .0016$). Mutations in other splicing factors, although previously reported to be mutually exclusive to *DDX41* variants,[36] were seen in patients with CV and enriched in those with VUS (Figures 3 and 4E). *TP53* mutations were infrequent, seen in 7% of AML/MDS with CV, 12.5% with VUS, and 9% with WT *DDX41* (Figure 4D-E; $P > .05$). In patients with AML/MDS with CV with cytogenetic results, 80% (53 of 66) were associated with a normal karyotype,

Table 2. The demographic features, family history, and overall survival of 205 individuals with *DDX41* variants

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
1*	AML	62	M	NI	p.M1I	NI	NI	NI	NI	NI
2†	AML	78	F	Caucasian	p.M1I	365	Y	No	No	No
3	AML	77	M	Caucasian	p.M1I	122	Y	NI	NI	NI
4†	AML	64	M	Caucasian	p.M1I	519	Y	No	No	Breast cancer (mother)
5	AML	80	M	Caucasian	p.M1I	336	Y	NI	NI	NI
6*,†	AML	77	M	Caucasian	p.M1I	2161	Y	No	No	No
7	AML	62	M	Caucasian	p.M1I	151	Y	Leukemia (father)	No	No
8	AML	76	M	Caucasian	p.M1I	1214	Y	No	No	No
9†	AML	68	M	Caucasian	p.M1I	822	Y	No	No	No
10*,†	AML	76	F	Caucasian	p.M1I	2161	Y	No	No	Breast cancer (mother and sister); pituitary tumor (brother); rhabdomyosarcoma (son)
11	AML	88	F	Caucasian	p.M1I	98	Y	No	No	No
12†	AML	48	F	Caucasian	p.M1I	700	Y	No	No	No
13	AML	63	F	Caucasian	p.M1I	NI	NI	NI	NI	NI
14†	AML	78	M	Caucasian	p.M1I	1034	Y	No	No	No
15†	AML	65	M	Caucasian	p.M1I	NI	NI	NI	NI	NI
16†	AML	73	M	Caucasian	p.M1I	91	Y	No	No	Cancer of unknown origin (mother)
17†	AML	72	M	Caucasian	p.M1I	822	N	No	No	No
18†	AML	74	M	Caucasian	p.M1I	396	Y	No	No	Cancer of unknown origin (brother)
19*	AML	75	M	Caucasian	p.M1I	608	Y	NI	NI	NI

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
20*#	AML	60	M	Caucasian	p.M1I	639	Y	No	No	No
21#	AML	65	M	Caucasian	p.M1I	365	Y	No	No	Cancer of unknown origin (sister)
22#	AML	64	M	Caucasian	p.M1I	304	Y	No	No	Lung cancer (father); cervical cancer (daughter)
23	AML	68	M	Caucasian	p.M1I	400	Y	No	No	Lung cancer (mother)
24†	AML	69	M	Caucasian	p.M1I	1216	Y	NI	NI	NI
25	AML	71	F	Caucasian	p.K108fs	NI	NI	NI	NI	NI
26	AML	71	F	Caucasian	p.D140fs	NI	NI	NI	NI	NI
27	AML	57	M	Caucasian	p.D140fs	561	Y	NI	NI	NI
28†	AML	57	M	Caucasian	p.D140fs	403	N	No	No	No
29	AML	57	M	Caucasian	p.D140fs	1080	Y	No	Lymphoma (father)	Prostatic cancer (paternal uncle)
30*,†	AML	59	M	Caucasian	p.D140fs	639	Y	No	No	Pancreatic cancer (brother)
31	AML	78	M	Caucasian	p.D140fs	NI	NI	NI	NI	NI
32	AML	81	M	Caucasian	p.D140fs	260	Y	NI	NI	NI
33	AML	63	M	Caucasian	p.D140fs	61	Y	NI	NI	NI
34†	AML	90	F	Caucasian	p.D140fs	176	N	No	No	No
35	AML	66	M	Caucasian	p.D140fs	913	Y	MDS (father and paternal uncle)	No	No
36	AML	67	M	NI	p.D140fs	580	Y	NI	NI	NI
37	AML	50	M	Caucasian	p.D140fs	245	Y	NI	NI	NI
38†	AML	70	F	AA	p.D140fs	183	Y	No	No	Brain cancer (sister)
39†	AML	53	M	Caucasian	p.D140fs	945	Y	No	No	Lung cancer (mother); colon cancer (father)

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
40†	AML	70	M	Caucasian	p.D140fs	488	Y	No	No	Cancer of mouth and throat (brother)
41	AML	57	M	NI	p.D140fs	675	Y	NI	NI	NI
42	AML	54	F	Caucasian	p.D140fs	300	Y	No	No	No
43	AML	61	M	Caucasian	p.D140fs	145	Y	No	MM (father)	No
44	AML	73	M	Asian	p.I224fs	731	Y	NI	NI	NI
45†	AML	54	F	Caucasian	p.L283fs	580	Y	No	No	No
46	AML	58	F	Caucasian	p.M316fs	1071	Y	No	No	No
47	AML	76	F	NI	p.G465fs	640	Y	NI	NI	NI
48	AML	63	M	Caucasian	p.Q41*	212	Y	No	No	No
49	AML	70	M	Caucasian	p.Q41*	NI	NI	NI	NI	NI
50	AML	71	M	Caucasian	p.R159*	31	Y	NI	NI	No
51	AML	61	M	Caucasian	p.R311*	NI	NI	NI	NI	NI
52	AML	68	M	Caucasian	p.R369*	548	N	No	No	No
53	AML	78	M	NI	p.R369*	120	Y	NI	NI	NI
54	AML	70	M	Caucasian	p.Q370*	408	Y	Leukemia (mother)	No	No
55	AML	82	M	Caucasian	p.Q502*	NI	NI	NI	NI	NI
56†	AML	68	F	Caucasian	p.?	905	Y	Leukemia (maternal aunt)	No	No
57	AML	74	M	Caucasian	p.K331del	232	N	No	No	Thyroid and colon cancer (mother)
58†	AML	65	F	Caucasian	p.L216V	275	Y	MDS (brother)	No	No
59	AML	66	M	Caucasian	p.G218D	31	Y	NI	NI	NI
60	AML; breast cancer	47	F	Caucasian	p.P258L	653	Y	No	No	No

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
61	AML	60	M	NI	p.R323C	956	Y	NI	NI	NI
62	AML	69	M	Caucasian	p.M349K	365	Y	NI	NI	NI
63	AML	69	M	Caucasian	p.M349K	1134	Y	No	No	Prostatic cancer (father)
64	AML	81	F	Caucasian	p.R369G	151	Y	AML (paternal cousin)	No	No
65	AML	85	M	Asian	p.D467Y	458	Y	NI	NI	NI
66	AML	61	M	Asian	p.R525H	243	N	MDS (father); Leukemia (paternal grandma) Hematologic cancer (paternal uncle)	No	No
67	MDS	72	M	Caucasian	p.M1I	NI	NI	NI	NI	NI
68	MDS	73	M	Caucasian	p.M1I	539	Y	No	No	No
69	MDS	81	F	Caucasian	p.M1I	670	Y	No	No	No
70	MDS	76	M	Caucasian	p.M1I	362	Y	No	No	No
71	MDS	79	M	Caucasian	p.M1I	763	Y	No	No	Prostatic cancer (brother)
72	MDS	61	F	Caucasian	p.M1I	192	Y	No	No	Breast cancer (sister)
73	MDS	60	M	Caucasian	p.M1I	1795	Y	No	No	Throat cancer (father); melanoma (sister)
74	MDS	65	M	Caucasian	p.M1I	153	Y	NI	NI	No
75	MDS	63	M	NI	p.M1I	NI	NI	NI	NI	NI
76	MDS; MM; MBL	67	F	Caucasian	p.M1I	98	Y	No	No	No
77	MDS	69	M	Caucasian	p.M1I	92	Y	No	No	No
78	MDS	72	M	Caucasian	p.M1I	2722	Y	No	No	No

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
79	MDS	60	M	Caucasian	p.M1I	395	Y	NI	NI	No
80	MDS	88	F	Caucasian	p.D140fs	476	Y	NI	NI	NI
81	MDS	69	M	Caucasian	p.D140fs	1078	Y	NI	NI	NI
82	MDS	63	M	Asian	p.A500fs	761	Y	NI	NI	No
83	MDS	76	F	Asian	p.Q44*	701	Y	No	No	No
84	MDS	77	M	Caucasian	p.R159*	876	N	NI	NI	NI
85	MDS	85	F	Caucasian	p.R311*	NI	NI	NI	NI	NI
86	MDS	84	M	Caucasian	p.R311*	NI	NI	NI	NI	NI
87	MDS	77	M	Caucasian	p.K331del	1793	Y	Leukemia (father)	No	No
88	MDS	62	M	Caucasian	p.K331del	900	Y	No	No	Prostatic cancer, melanoma and stomach cancer (paternal uncles); Pancreatic cancer (maternal aunt); lung cancer (maternal cousins); Bile duct cancer (maternal cousin); breast cancer (maternal great aunt)
89	MDS	84	F	Caucasian	p.P189L	2015	Y	No	No	No
90	MDS	73	M	Caucasian	p.L237W	134	Y	MDS (father)	No	No
91	MDS	74	M	Caucasian	p.R339H	2023	Y	No	No	No
92	MDS	76	M	Caucasian	p.R339C	305	Y	NI	NI	NI
93	MDS	63	M	Caucasian	p.Y340N	90	Y	NI	NI	NI
94*	MDS	76	M	Caucasian	p.R369G	864	Y	NI	NI	NI
95	Pancytopenia	85	M	NI	p.M1I	NI	NI	NI	NI	NI

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
96	Pancytopenia	56	M	NI	p.M1I	NI	NI	NI	NI	NI
97	Pancytopenia	82	M	Caucasian	p.D140fs	NI	NI	NI	NI	NI
98	Pancytopenia	80	M	Caucasian	p.T144fs	NI	NI	NI	NI	NI
99	Pancytopenia	79	M	NI	p.M316fs	NI	NI	NI	NI	NI
100	Pancytopenia	83	M	Caucasian	p.L452fs	NI	NI	NI	NI	NI
101	Pancytopenia	65	M	Caucasian	p.R159*	61	Y	NI	NI	NI
102	Pancytopenia	56	M	NI	p.S543*	NI	NI	NI	NI	NI
103	Pancytopenia	55	M	NI	p.S217P	NI	NI	NI	NI	NI
104	Pancytopenia	67	M	NI	p.P258L	NI	NI	NI	NI	NI
105	Pancytopenia	77	M	Asian	p.Y259C	NI	NI	NI	NI	NI
106	Pancytopenia	58	M	Asian	p.R339L	NI	NI	NI	NI	NI
107	Thrombocytopenia	81	M	NI	p.D140fs	NI	NI	NI	NI	NI
108	Thrombocytopenia	37	F	Caucasian	p.T529fs	423	Y	AML (father)	No	No
109	Thrombocytopenia	68	M	Caucasian	p.M1I	NI	NI	NI	NI	NI
110	Neutropenia	64	M	NI	p.M1I	NI	NI	NI	NI	NI
111	Neutropenia	78	F	Caucasian	p.M1I	NI	NI	NI	NI	NI
112	Anemia	70	F	NI	p.R369*	NI	NI	NI	NI	NI
113	MPN	40	F	Caucasian	p.D140fs	278	Y	Leukemia (family members, not specified)	No	Breast and uterine cancers (mother)
114	MPN	76	F	Caucasian	p.M316fs	NI	NI	NI	NI	NI
115	MPN, ET	41	F	NI	p.Q306*	NI	NI	NI	NI	NI
116	MPN	85	M	NI	p.K381*	NI	NI	NI	NI	NI
117	AML	48	F	Caucasian	p.E2D	96	NI	NI	NI	NI

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
118*	AML	54	F	NI	p.G19R	61	Y	NI	NI	NI
119*	AML	62	F	NI	p.Y33H	516	Y	NI	NI	NI
120	AML	76	M	Caucasian	p.M155I	NI	NI	NI	NI	NI
121	AML	56	F	NI	p.M155I	613	N	No	No	No
122*	AML	63	F	Caucasian	p.R164N	365	Y	No	No	No
123	AML	84	M	Caucasian	p.P510S	216	Y	NI	NI	NI
124	AML	81	M	Caucasian	p.M127K	15	N	No	No	No
125	AML	47	F	Caucasian	p.M155I	NI	NI	NI	NI	NI
126	AML	72	M	Caucasian	p.G67R	180	Y	No	No	No
127	AML	72	M	Caucasian	p.G67R	150	Y	No	No	No
128*	AML	46	M	Caucasian	p.M155I	146	Y	No	No	Cancer of unknown origin (mother)
129	AML	79	M	Caucasian	p.A295T	241	Y	No	No	Cancer of unknown origin (mother and father)
130	AML	55	M	NI	p.I298T	NI	NI	NI	NI	NI
131	AML	47	F	Caucasian	p.E355K	19	N	No	No	No
132	AML	67	M	Caucasian	p.M155I	1056	Y	No	No	No
133	AML	7	F	NI	p.M155I	NI	NI	NI	NI	NI
134	AML	64	M	NI	p.?	28	Y	NI	NI	NI
135	MDS	79	F	Caucasian	p.G123S	NI	NI	NI	NI	NI
136	MDS	63	M	NI	p.M155I	NI	NI	NI	NI	NI
137	MDS	73	M	Caucasian	p.?	420	N	NI	NI	NI
138	MDS	50	M	Caucasian	p.G175S	NI	NI	NI	NI	NI

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
139	MDS	84	M	Caucasian	p.P177S	NI	NI	NI	NI	NI
140	MDS	60	M	Caucasian	p.P434L	303	Y	NI	NI	NI
141	MDS	83	F	Caucasian	p.P510S	NI	NI	NI	NI	NI
142	MDS	78	M	Caucasian	p.C568W	516	N	No	No	No
143	MDS/MPN	56	M	Caucasian	p.G587A	183	Y	NI	NI	No
144	MDS	92	M	Caucasian	p.E426K	NI	NI	NI	NI	NI
145	MDS	65	F	NI	p.P510S	456	Y	NI	NI	NI
146	MDS	62	M	Caucasian	p.A555T	255	Y	NI	NI	NI
147	Pancytopenia	63	M	NI	p.K9K	NI	NI	NI	NI	NI
148	Pancytopenia	81	M	Caucasian	p.M155I	NI	NI	NI	NI	NI
149	Pancytopenia; SCC	77	M	NI	p.M186L	NI	NI	NI	NI	NI
150	Pancytopenia	85	M	NI	p.R219H	NI	NI	NI	NI	NI
151	Pancytopenia	84	F	NI	p.R282H	NI	NI	NI	NI	NI
152	Pancytopenia	85	F	NI	p.C294F	550	Y	NI	NI	NI
153	Pancytopenia	70	M	NI	p.S493A	NI	NI	NI	NI	NI
154	Thrombocytopenia	88	M	Caucasian	p.M155I	37	Y	No	No	No
155	Thrombocytopenia	78	F	NI	p.T236M	NI	NI	NI	NI	NI
156	Thrombocytopenia	39	M	Caucasian	p.?	NI	NI	NI	NI	NI
157	Thrombocytopenia	73	M	Caucasian	p.?	31	Y	NI	NI	NI
158	Neutropenia	43	F	Caucasian	p.?	NI	NI	NI	NI	NI
159	Anemia	69	F	Caucasian	p.P251S	1777	Y	No	No	No
160	Anemia	78	M	Caucasian	p.A583V	NI	NI	NI	NI	NI
161	MPN, PV	53	F	Caucasian	p.R8Q	87	Y	No	No	Cancer of unknown origin (brother)

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
162	MPN, PV	61	M	Caucasian	p.D32N	395	Y	NI	NI	NI
163	MPN, ET	72	M	NI	p.A133V	NI	NI	NI	NI	NI
164	MPN	49	F	NI	p.I215T	943	Y	NI	NI	NI
165	MPN	85	F	NI	p.L216P	NI	NI	NI	NI	NI
166	MPN, ET	69	M	Caucasian	p.T236M	918	Y	No	No	No
167	MPN	66	M	Caucasian	p.V491I	3839	Y	No	No	No
168	MPN, ET	54	M	Caucasian	p.V544L	664	Y	NI	NI	NI
169	MPN, ET	39	F	NI	p.?	NI	NI	NI	NI	NI
170	MPN, ET	7	F	Asian	p.M155T	NI	NI	NI	NI	NI
171	MPN	46	F	Caucasian	p.K187R	NI	NI	NI	NI	NI
172	Pancytopenia; LPL	77	M	AA	p.R164W	10	Y	No	No	No
173	Y heavy chain disease; MYD88 negative LPL	52	M	NI	p.R164W	7955	Y	Myelofibrosis (mother)	No	Lung cancer (father)
174	CLL; breast cancer	51	F	NI	p.R164W	1186	Y	No	FL (mother)	Bladder & prostatic cancer (father)
175	CLL	70	M	Caucasian	p.P251S	1004	Y	No	No	No
176	MM	82	F	Caucasian	p.R10Q	471	N	No	No	No
177	AML	64	M	Asian		1703	Y	Leukemia (maternal uncle)		Gastric cancer (paternal grandfather)
178	AML	74	M	Caucasian		578	Y	No	No	Sarcoma (father)
179	AML	69	F	Caucasian		974	Y	NI	NI	No
180	AML	54	M	Caucasian		42282	N	No	No	Colon cancer (father)

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
181	AML	70	F	Caucasian		455	Y	No	No	Breast cancer (paternal aunt)
182	AML	77	M	Caucasian		NI	NI	NI	NI	NI
183	AML	70	M	Caucasian		81	Y	No	No	No
184	AML; DLBCL	74	M	Caucasian		NI	Y	No	No	Breast cancer (sisters × 2)
185	MDS	75	M	Caucasian		1127	Y	No	No	No
186	MDS	71	M	Caucasian		2053	Y	No	No	Cancer of unknown origin (mother and sister)
187	MDS	65	F	Caucasian		2466	Y	No	No	Lung cancer (father); breast cancer (father's sister)
188	MDS	54	M	Caucasian		151	Y	No	No	No
189	MDS	69	M	Caucasian		689	N	No	No	Prostatic cancer (father)
190	AML	30	M	NI		304	Y	NI	NI	NI
191	AML	63	M	Caucasian		21	N	No	No	No
192	MDS	81	M	Caucasian		NI	NI	NI	NI	NI
193	MDS	62	M	Caucasian		212	Y	No	No	Yes
194	MDS	80	F	Caucasian		NI	NI	NI	NI	NI
195	Neutropenia	86	M	Caucasian		NI	NI	NI	NI	NI
196*	Normal	43	M	Caucasian	p.M1I	65	Y	MDS (father)	No	No
197*	Normal	69	F	Caucasian	p.M1I	496	Y	Myeloid neoplasm (mother)	No	No
198*	Normal	28	M	Caucasian	p.D140fs	1044	Y	AML (father)	No	No

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

Table 2. (continued)

Patient	Diagnosis	Age	Sex	Ethnicity	gl DDX41	FU (d)	Survival (Y/N)	FH_MN	FH_LN	FH_Other
199*	Normal	59	F	Caucasian	p.D140fs	NI	Y	Leukemia (father)	NI	NI
200*	Normal	66	M	Caucasian	p.D140fs	NI	Y	MDS (brother); AML (brothers); ALL (daughter)	No	Lung cancer (father)
201*	Normal	33		Caucasian	p.K331del	614	Y	MDS (father)	No	Prostatic cancer (paternal uncle); pancreatic cancer (maternal aunt); lung cancer (maternal cousins); bile duct cancer (maternal cousin); melanoma and stomach cancer (paternal uncle); breast cancer (maternal great aunt)
202	Normal~(donor)	71	F	Caucasian	p.311*	367/PHSCT	Y	No	No	
203	Normal~(donor)	59	M	Caucasian	p.M155I	2061/PHSCT	Y	No	No	No
204	Normal~(donor)	60	M	Caucasian	p.V565M	725/PHSCT	Y	NI	NI	NI
205	Normal~(donor)	71	M	Caucasian	p.T529fs	459/PHSCT	Y	No	No	No

AA, African American; F, female; M, male; N, deceased; NI, no information; PHSCT, post-HSCT; Y, survived.

*Germline variants confirmed by skin biopsies.

†Reported in a prior study.[9]

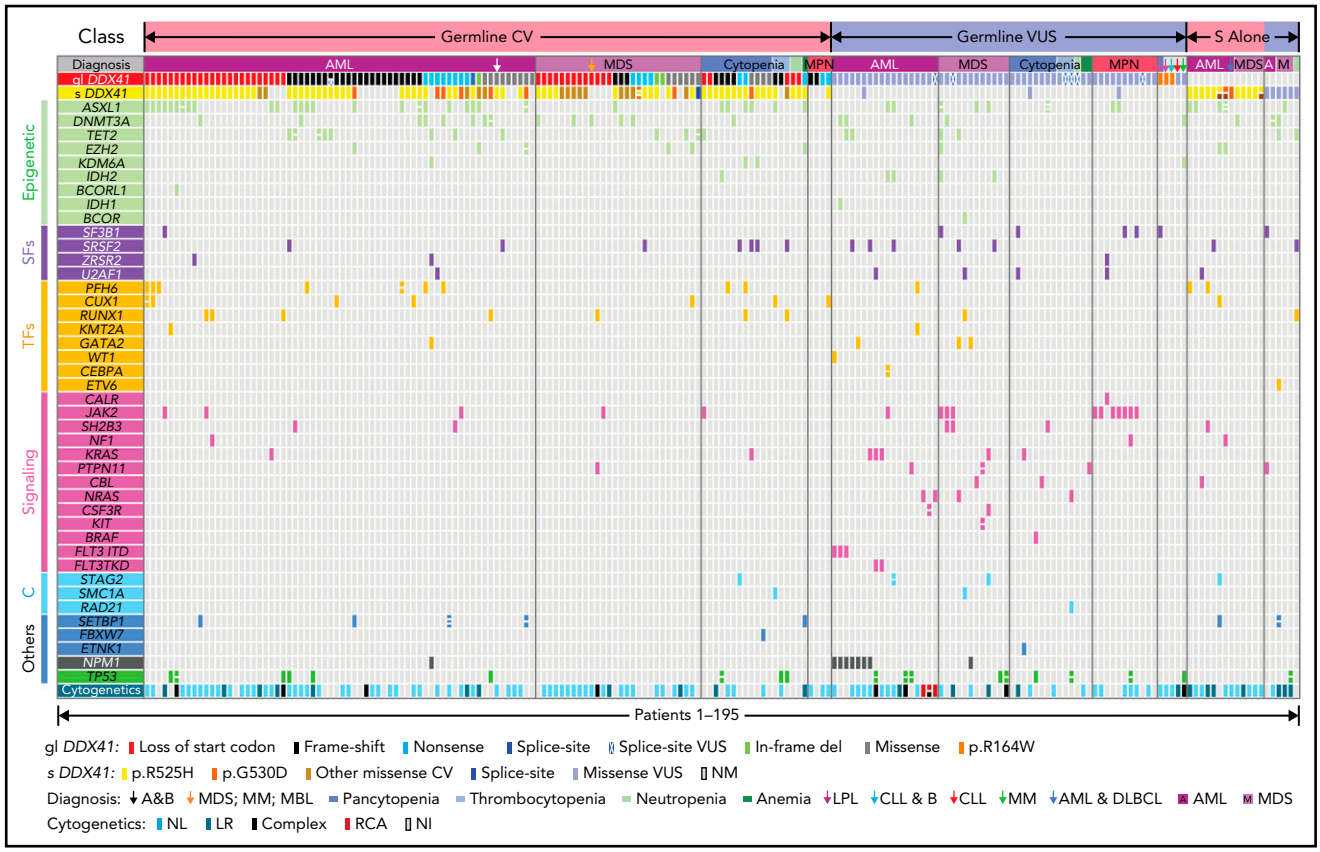


Figure 3. Integrated genetic profiles of the 195 HM patients with epidemiologic characteristics grouped by different HM diagnoses. A total of 176 patients with presumed germline (gl *DDX41*) 116 CV and 60 VUS are grouped (CV in red and VUS in blue, respectively), along with the associated somatic *DDX41* (s *DDX41*), concomitant somatic variants, and cytogenetics. In addition, 19 patients with HM with somatic *DDX41* variants in the absence of germline variants are appended to the right of the variant table, 13 CV in red and 6 VUS in blue. Each column represents 1 patient. The concomitant variants are grouped into 6 categories based on gene function: epigenetic, epigenetic regulators, genes involving DNA methylation or histone acetylation, and deacetylation (light green); SFs, RNA splicing factors (purple); TFs, transcription factors (orange); signaling, molecules in tyrosine kinase pathway or RAS/MAPK pathways (pink); C, cohesins (light blue); and others (dark blue), genes with function beyond the above categories. Each bar represents 1 variant, and split bars indicate 2 or more variants in the same gene. A&B, AML and breast cancer; CLL & B, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and breast cancer; complex, complex karyotype; DLBCL, diffuse large B-cell lymphoma; gl DDX41, germline DDX41 variants; LPL, lymphoplasmacytic lymphoma (MYD88 negative); LR, low risk; MBL, monoclonal B-cell lymphocytosis; MM, multiple myeloma; NI, no information; NL, normal; NM, no mutation; RCA, recurrent cytogenetic abnormalities in AML; s DDX41, somatic DDX41 variants.

and 20% (13 of 66) had an abnormal karyotype (9 low-risk and 4 complex karyotypes; Figures 3 and 5A; Table 1).

Similar to that reported previously,[8] indolent courses of cytopenia (Figure 5A) were seen prior to an overt MN, and there was a borderline increase in blasts in patients with AML with *DDX41* CV (31% in AML vs 8% in MDS; $P < .0001$). Similar genetic features and *DDX41* CV types were seen in both normo/hypocellular AML/MDS with CV (Figure 5A). Fifty-seven patients with AML with CV had a favorable OS (Figure 5B-D; supplemental Figure 2A; median OS not reached) compared with 13 with VUS (613 days; $P = .02$) or 158 WT patients; a similar trend was seen in patients with MDS (Figure 5B-D). This superior OS, similar to a previous study[7] (supplemental Figure 2A), appeared independent of blasts (Figure 5B-E; $P = .30$), patient age (Figure 5E; supplemental Figure 2B; $P = .69$), sex ($P = .61$), somatic variant burden, presence of somatic *DDX41* variants (Figure 5E; supplemental Figure 2C; $P = .95$), and other concomitant variants ($P = .50$) including *TP53* (supplemental Figure 2D; $P = .39$), regardless of cytogenetic abnormalities ($P = .91$) or type of germline *DDX41* CV (Figure 5E).

Among the 50 patients with AML/MDS with CV and available FH, only 18% (9 of 50) had FH of MN, and 2% (1 of 50) had FH of lymphoma, whereas nonhematologic tumors were rather common (32%, 16 of 50; Table 2). In contrast, none of the patients with VUS had FH of myeloid or lymphoid neoplasms (Table 2). In this study, 2 patients with MN with CV had concomitant lymphoid or solid tumors, similar to previous reports (Table 1, patients 60 and 76).[7,19]

Germline *DDX41* CV predisposing to MPN and lymphoma

We further focused on 15 patients (4 CV and 11 VUS) with MPN. Male predominance was not observed here in contrast to patients with AML/MDS (Table 3). A similar tendency for more frequent somatic *DDX41* mutation (Figure 6A; $P < .0001$) and lower somatic mutation burden (Figure 6B; $P = .05$) was seen in patients with CV compared with those with WT *DDX41*. Interestingly, *JAK2* V617F and *CALR* mutations, absent in all patients with CV, were identified in 72% of patients with VUS (Figures 3 and 6C), most (7 of 8) being a leading clone (Table 1; VAFs at 45%, 85%, 44%, 26%, 18%, 47%, and 26%, respectively), whose

Table 3. Summary of ages and sexes of patients with HM with *DDX41* CV, and VUS and controls

Disease	Germline *DDX41*	*DDX41*$^{+}$ CV (reference)	*DDX41*$^{+}$ VUS	*P* (a)	*DDX41*$^{-}$ WT	*P* (b)
HM	n	111	60		4307	
	Median age (y, range)	68 (37-90)	63 (7-92)	.01*	67 (18-100)	.35
	Sex male/female (M%)	82/29 (74%)	37/23(62%)	.10	2154/2153 (50%)	<.0001****
AML	n	66	18		1365	
	Median age (y, range)	69 (47-90)	62 (7-84)	.02*	64 (18-90)	.002**
	Sex male/female (M%)	49/17 (71%)	10/8 (56%)	.12	737/628 (54%)	.001**
MDS	n	28	12		1109	
	Median age (y, range)	72 (60-88)	69 (50-92)	.49	74 (36-98)	0.64
	Sex male/female (M%)	21/7 (75%)	9/3 (75%)	>.99	555/554 (50%)	.009**
MPN	n	4	11		470	
	Median age (y, range)	59 (40-85)	54 (7-85)	.64	66 (18-95)	.74
	Sex male/female (M%)	1/3 (25%)	5/6 (45%)	.33	192/287 (40%)	.36

P value (a) applies to *DDX41*$^{+}$ CV vs *DDX41*$^{+}$ VUS, and *P* value (b) applies to *DDX41*$^{+}$ CV vs *DDX41*$^{-}$ WT.
*$P < .05$; **$P < .01$; ****$P < .0001$.

frequency was almost identical to that in patients with *DDX41* WT (Figure 6C-E). Karyotypes in patients with MPN with CV were normal, and *TP53* mutations were not identified (Figures 3 and 6D).

Beyond MN, 3 unrelated patients with B-cell lymphomas were linked by an identical presumably germline *DDX41* variant, p.R164W (Tables 1 and 2, patients 172-174). Two patients (patients 173 and 174) carrying this variant developed earlier-onset lymphoma at age 51 and 52 years, respectively. Both patients had affected family members diagnosed with either myelofibrosis or follicular lymphoma (Table 2), adding further support to this likely CV predisposing to lymphoma. A third patient with this variant (pt 172) developed *MYD88*-negative lymphoplasmacytic lymphoma (LPL) and pancytopenia at age 77 years without significant FH. A somatic *SF3B1* variant was also identified in this patient, which might potentially contribute to the patient's reported pancytopenia. Importantly, p.R164W was previously reported in a family with LPD, in which all 5 affected individuals developed lymphoma (4) and multiple myeloma (1), whereas all 3 unaffected individuals of similar age did not.[19]

The prevalence of disease entities in patients with HM with germline *DDX41* CV can be summarized as follows: AML/MDS (Figure 6F, 79%), as a distinct clinical entity, is the most common disease, followed by cytopenia (15%), MPN (3%), and lymphoma (3%). Per the data collected at ARUP Laboratories, approximately 3.0% (41 of 1406, 29 CV and 12 VUS) of patients with AML, 1.4% (16 of 1125, 9 CV and 7 VUS) of patients with MDS, and 2.0% (10 of 489, 3 CV and 7 VUS) of patients with MPN carried a presumed germline *DDX41* variant. The prevalence of *DDX41*-related lymphoma remains uncertain, as this disease is not fully acknowledged, and NGS testing for patients with LPD is not yet a standard of care.

Asymptomatic carriers with germline CV

Six asymptomatic individuals with normal CBC and germline *DDX41* CV who were related to patients with HM in this study underwent tumor surveillance (Tables 1 and 2, patients 196-201); their median age (51 years; range, 28-69 years) was significantly lower compared with patients with overt diseases (supplemental Figure 3A; $P < .0001$). No somatic *DDX41* or other mutations were identified by NGS testing (supplemental Figure 3B), and all 5 patients who underwent cytogenetic testing showed a normal karyotype (Table 1). Furthermore, 4 patients with HSCT for previously diagnosed AML were found to have donor-derived *DDX41* variants (2 CV and 2 VUS) during surveillance (Table 1, patients 202-205). All 4 had unremarkable CBC and complete engraftment confirmed by 100% donor chimerism with a median follow-up of 30 months in surveillance (Table 2) without biopsy proving recurrent/residual AML.

Discussion

In this study, we analyzed the genetic, epidemiologic and hematologic features, and clinical outcomes of 116 patients with HM with germline *DDX41* CV and 60 with VUS identified by NGS. Using the proposed *DDX41*-specific variant classification framework, we identified a phenotype encompassing primarily AML/MDS and rarely MPN and lymphoma associated with germline CV. A complete germline CV landscape is critical to direct appropriate clinical management, preventive care, and family screening.

In this largest cohort to date of 176 patients with HM with *DDX41* germline variants, we proposed that the acquisition of a pathogenic somatic *DDX41* variant is a compelling criterion for causality in germline variant interpretation. The marked segregation in genetic profiles, epidemiologic features, and clinical behavior separating patients with AML/MDS with germline CV from those with VUS provided validation for this modified variant

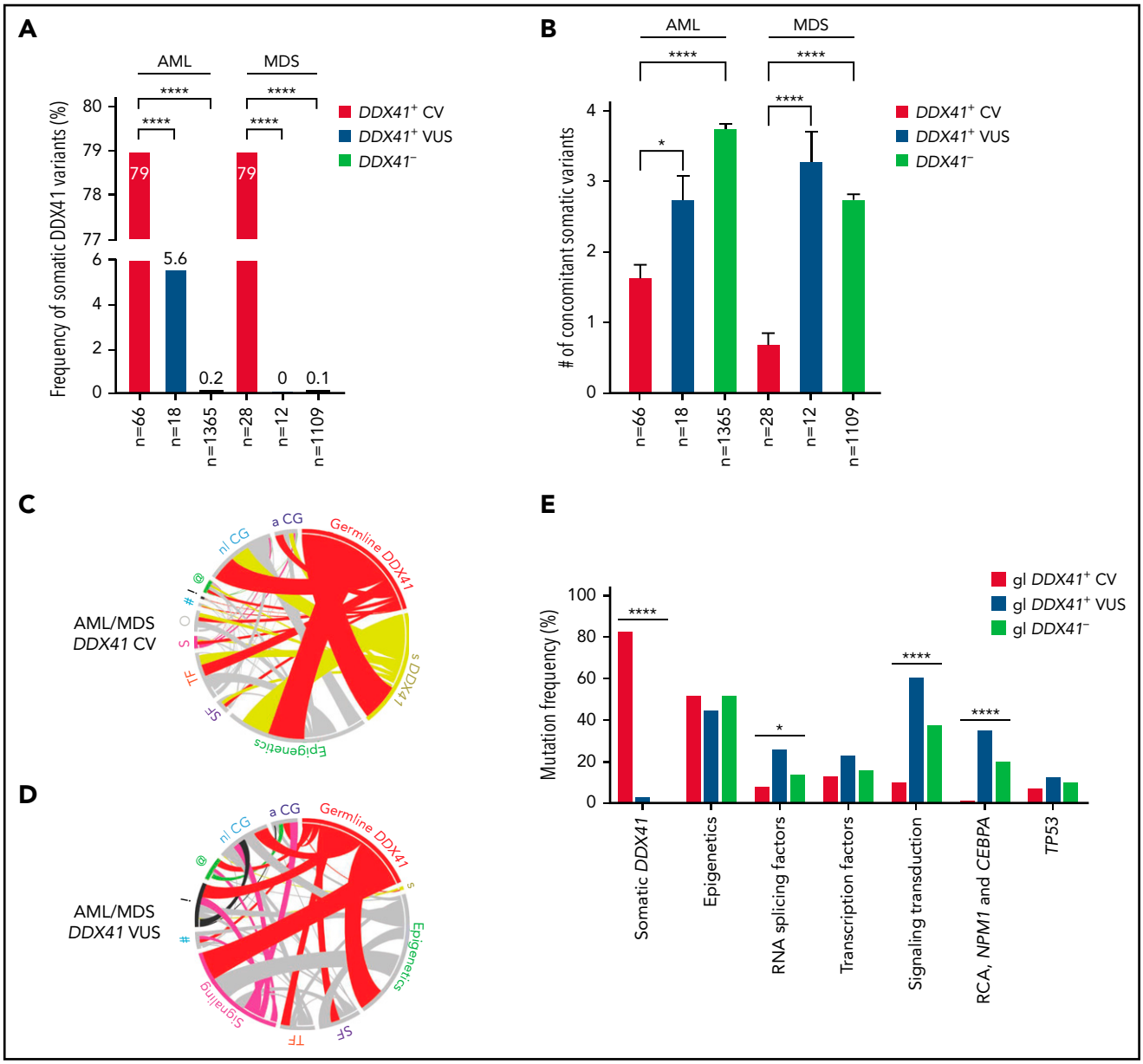


Figure 4. Genetic characteristics of patients with AML/MDS with germline variants in *DDX41*. (A) The occurrence of somatic *DDX41* variants in patients with AML is closely linked to the presence of germline *DDX41* CV (79%; 52 of 66), in comparison with patients with VUS (5.6%, 1 of 18, $P < .0001$) or patients not carrying germline *DDX41* variants (*DDX41*$^{-}$, 0.2%; 3 of 1365, $P < .0001$). A similar trend is seen in patients with MDS (79% in CV, 0% in VUS, and 0.1% in *DDX41*$^{-}$, $P < .0001$). (B) A lower somatic mutation burden, calculated by the number of total concomitant somatic variants (excluding somatic *DDX41* variants) per case, is seen in patients with AML with CV (mean ± standard error of the mean: 1.6 ± 0.2) compared with patients with AML with wild-type *DDX41* (*DDX41*$^{-}$, 3.7 ± 0.07, $P < .0001$) and those with VUS (2.6 ± 0.4, $P = .03$). Similarly, in MDS, a lower somatic mutation burden is seen in patients with CV (0.7 ± 0.1) in contrast to those with wild-type *DDX41* (*DDX41*$^{-}$, 2.7 ± 0.06, $P < .0001$) or VUS (3.2 ± 0.5, $P < .0001$). (C-D) Circos plot diagrams illustrate the pairwise co-occurrence of somatic variants and cytogenetic abnormalities in 94 patients with AML/MDS with germline CV (C) and 30 with VUS (D). Genetic variants and cytogenetic events listed in Figure 3 appear in descending order clockwise, starting at 12 o'clock. Each link (ribbon) indicates pairwise co-occurrence of mutational events, and the width of the ribbons indicates the frequency of the co-occurrent events. The occurrence of germline and somatic *DDX41* variants is indicated in red and yellow ribbons, respectively. Variants in signaling and RAS/MAPK pathways are labeled in pink; *NPM1* and *TP53* variants are labeled in black and green, respectively; the remaining variants are labeled in gray. s (yellow) in D, somatic *DDX41*; SF (purple), RNA splicing factors; TF (orange), transcription factors; S (pink) in panel C, signaling; O (gray), others; # (blue), cohesin; ! (black), *NPM1*; @ (green), *TP53*; nl CG, normal cytogenetics; a CG, abnormal cytogenetics. (E) Frequencies of somatic *DDX41* and other concurrent variants in AML/MDS patients. For each gene or genetic category, the percentage of mutations is displayed, associated with either germline CV (red bars, gl *DDX41*$^{+}$ CV), VUS (blue bars, gl *DDX41*$^{+}$ VUS), or wild-type *DDX41* (green bars, gl *DDX41*$^{-}$). s *DDX41*, somatic *DDX41* variants; epigenetics, genes involving DNA methylation or histone acetylation and deacetylation; signaling transduction, molecules in tyrosine kinase pathway or RAS/MAPK pathways; RCA, recurrent cytogenetic abnormalities in AML. *$P < .05$; ****$P < .0001$.

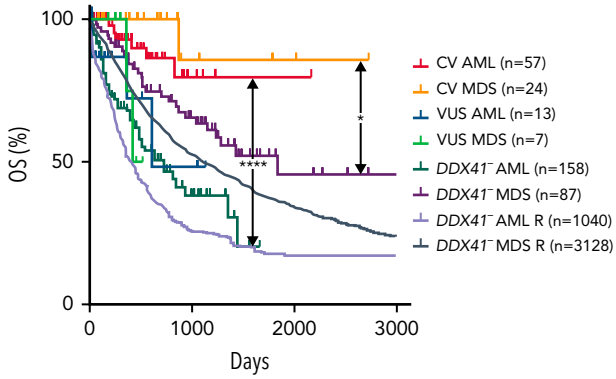
classification strategy.[7,20,29] Patients with HM with germline *DDX41* VUS behaved similarly to patients who were WT, in which canonical somatic mutations or recurrent genetic alterations in other genes were common as drivers of tumorigenesis.

Patients with AML and MDS, the most common entities associated with germline CV, present during their late 60s or early 70s with indolent cytopenia years before overt myeloid neoplasia, with a male predominance.[7,8,10,29,32,37] Furthermore, both were

A

DDX41 CV factors	AML (n=57)	MDS (n=24)	P values
Pre-existing cytopenia (months)	16.9 ± 2.9	50.4 ± 19.7	0.02*
WBC (μL)	2.1 ± 0.2	2.5 ± 0.3	NS
Hgb (g/dL)	10.5 ± 0.3	10.4 ± 0.5	NS
PLT ($\times 10^3$/μL)	81.6 ± 8.8	95.3 ± 13.8	NS
Hypo/normal cellularity, %	86	75	NS
BM blasts (%)	30.7 ± 2.0	7.7 ± 1.1	<0.0001****
Normal cytogenetics, %	79	85	NS
gl *DDX41* LOF/missense, %	84/26	75/25	NS
gl *DDX41* p.M1I/p.D140fs/others	26/12/19	23/2/9	NS
s *DDX41* presence, %	74	67	NS
Other CSV presence, %	54	46	NS
CSV number/patient	1.3 ± 0.2	0.6 ± 0.2	0.03*

B



C

Genotype	AML, N	Median OS, days	MDS, N	Median OS, days	P value
DDX41$^+$ CV	57	Undefined	24	Undefined	0.30
DDX41$^+$ VUS	13	613	7	468	0.86
DDX41$^-$	158	630	87	1425	0.0009***
DDX41$^-$ R	1040	433	3128	1116	<0.0001****

D

P value	CV MDS	VUS AML	VUS MDS	WT AML	WT MDS	WT AML R	WT MDS R
CV AML	NS	0.02*	NS	****	NS	****	0.003**
CV MDS		0.01*	0.003**	0.0002***	0.03*	****	0.003**
VUS AML			NS	NS	NS	NS	NS
VUS MDS				NS	NS	NS	NS
WT AML					0.0009***	NS	****
WT MDS						****	NS
WT AML R							****
WT MDS R							

E

DDX41 CV factors	HR	(95% CI)
Age	1.03	(0.85–1.30)
Cytopenia duration	0.98	(0.92–1.00)
WBC	0.99	(0.41–1.91)
HGB	0.79	(0.48–1.24)
PLT	0.98	(0.95–1.01)
Cellularity	0.99	(0.93–10.2)
BM blasts	1.04	(0.99–1.14)
Abnormal cytogenetics	0.82	(0.04–5.66)
Missense g *DDX41*	0.61	(0.07–5.32)
Non p.M1I/p.D140fs	7.44	(0.7–162.02)
Absence of s *DDX41*	1.71	(0.22–10.49)
Absence of CSV	1.74	(0.28–13.27)
CSV number	2.39	(0.68–15.21)

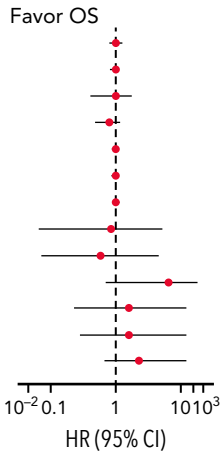


Figure 5. Common clinical, hematologic, pathologic, and genetic features and superior OS in AML/MDS patients with germline *DDX41* CV. (A) Both patients with AML and MDS with germline *DDX41* CV present a similarly indolent and chronic course of cytopenia years before the diagnosis of an overt myeloid neoplasm. Furthermore, the bone marrow examination shows predominantly normal to hypocellular marrow in AML (86%) and MDS (75%, $P > .05$), and a borderline increase in blasts is seen in patients with AML (31% in AML vs 8% in MDS, $P < .0001$). Most patients with AML (79%) and MDS (85%, $P > .05$) carry normal karyotypes with similar germline *DDX41* variant subtypes and somatic mutation profiles. (B) The median OS of 57 patients with AML with CV (red line, CV AML, not reached) is significantly longer than that of 13 patients with AML with VUS (blue line, VUS AML, 613 days, $P = .02$) or 158 patients with *DDX41* wild-type AML (dark green line, *DDX41*$^-$ or WT AML, 630 days, $P < .0001$) in the current study and 1040 patients documented in cBioPortal (lavender line, *DDX41*$^-$ or WT AML R 433 days, $P < .0001$). Similarly, the median OS of 24 patients with MDS with CV (orange line, CV MDS, not reached) is significantly longer than that of 7 patients with MDS with *DDX41* VUS (green line, VUS MDS, 468 days, $P = .003$) or 87 patients with *DDX41* WT MDS (purple line, *DDX41*$^-$ or WT MDS 1425 days, $P = .03$) in this study and 3128 patients reported recently (navy blue line, *DDX41*$^-$ or WT MDS R, 1116 days, $P = .003$).[27] (C-D) Statistical characteristics of the median OS in each genotype and disease group (C) and *P* values in pairwise comparisons (D) are listed in the tables. (E) The results of univariate analysis for different factors predicting OS in patients with AML/MDS with *DDX41* CV show that the superior OS is not impacted by patient's age, duration or severity of cytopenia, blast count, presence of abnormal cytogenetics, somatic DDX41 or other concomitant variants, somatic mutation burden, or different types of germline *DDX41* CV. Each circle represents the mean HR calculated by Cox proportional hazards regression, and the horizontal lines represent the 95% confidence interval (CI) for the subgroup's HR. Right of the dashed vertical line (HR = 1), unfavorable OS; left of the dashed line, favorable OS. WBC, white blood cells; Hgb, hemoglobin; PLT, platelet count; BM, bone marrow; gl *DDX41*, germline *DDX41* CV, s *DDX41*, somatic *DDX41* variants. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$; NS, not significant, $P > .05$.

characterized by frequent somatic *DDX41* variants, infrequent other somatic mutations, largely normal karyotype, normo/hypocellular marrow, and a favorable OS.[7,8,10,29,32,37] This superior OS was independent of blast counts or additional genetic abnormalities, regardless of the patients' age, sex, or specific germline CV. This unique disease was also characterized by near mutual exclusion of recurrent cytogenetic abnormalities and canonical mutations in *FLT3*, *NPM1*, and *CEPBA* in sporadic AML. Thus, AML/MDS caused by *DDX41* CV appear to be a spectrum of the same disease, unlike sporadic de novo AML and MDS, caused by completely different pathogenic mechanisms. Beyond AML/MDS, unrelated patients with MPN and B-cell lymphoma were linked to germline *DDX41* CV. Further studies identifying more germline CV are warranted to provide more insights into disease prevalence, characteristic pathologic features, the underlying mechanisms, and genotype-phenotype correlation in MPN and lymphoma[19] to further refine clinical management.

Unique ethnic differences were highlighted by different recurrent CVs seen nearly exclusively in White or Asian patients and more common missense CV in Asian patients.[7,10,11,28-34] There is also an urgent need for gene-specific classification guidelines by expert panels, without which a large number of missense CV are classified as VUS, and for international collaboration to fully characterize the CV landscape. Further studies are needed to address the currently uncertain significance of rare missense germline variants, especially those accompanied by rare noncanonical somatic *DDX41* variants (patient 150, Table 1). Germline confirmation of *DDX41* variants was limited in this study, partially because of the setting of a national reference laboratory.

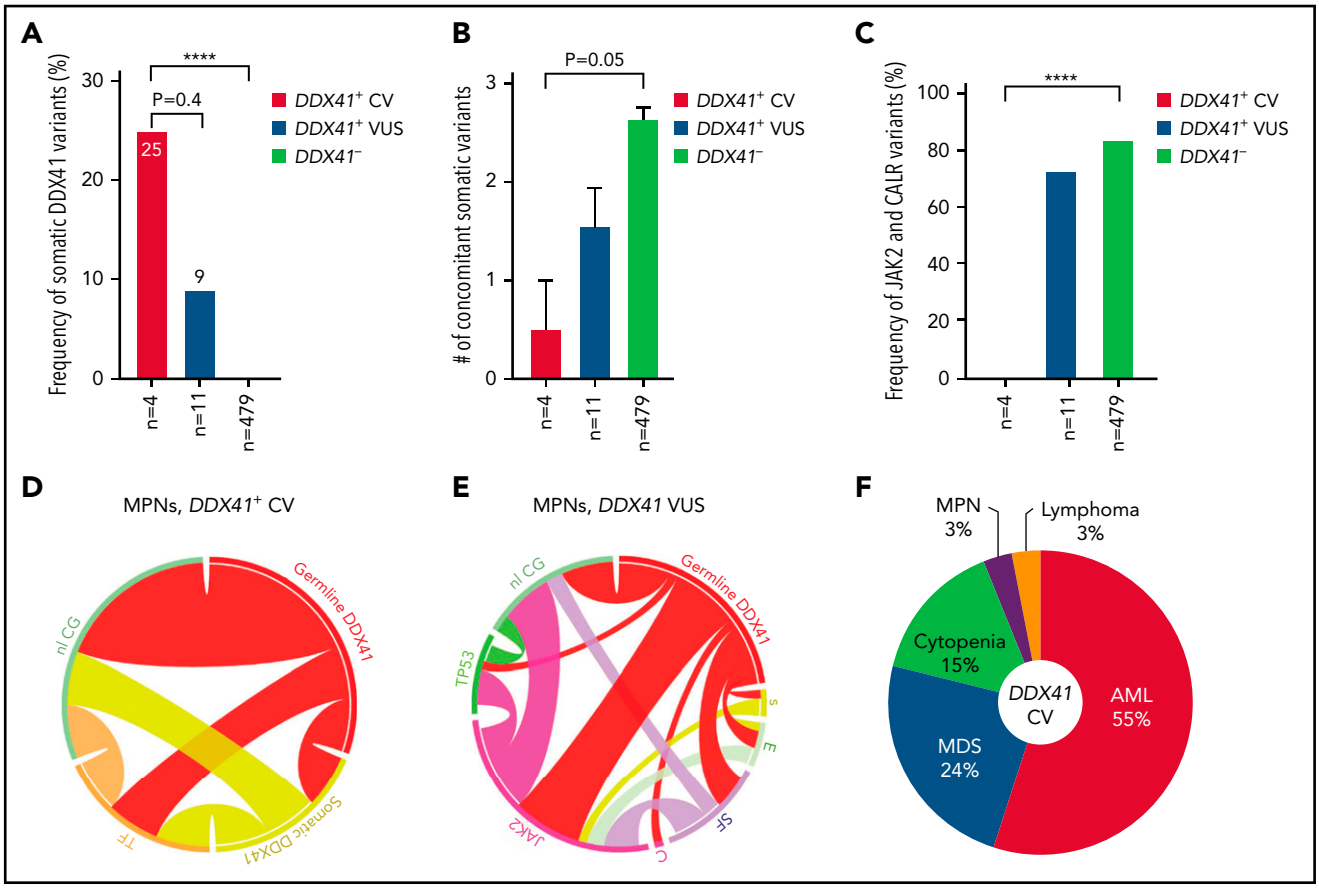


Figure 6. MPN and lymphoma predisposed by germline *DDX41* CV. (A) The occurrence of somatic *DDX41* variants in MPN patients is more frequent in patients with germline CV (25%) compared with patients with VUS (9%; 1/11, $P = .4$) or patients not carrying germline *DDX41* variants (*DDX41*$^{-}$, 0%, $P < .0001$). (B) There appears to be a lower concomitant somatic mutation burden in patients with CV (mean ± standard error of the mean: 0.5 ± 0.5), compared with those with WT *DDX41* (*DDX41*$^{-}$, 2.6 ± 0.1, $P = .05$) and VUS (1.5 ± 0.4, $P > .05$). (C) No mutations in *JAK2* or *CALR* were seen in MPN with CV, whereas these canonical variants are seen in 73% (8 of 11) of MPN patients with VUS and 82% in the WT cohort ($P < .0001$). (D-E) Circos plot diagrams illustrate the pairwise co-occurrence of variants and cytogenetic events in MPN patients with germline CV (D) and VUS (E). Genetic variants and cytogenetic events listed in Figure 2 appear in descending order clockwise starting at 12 o'clock. Each link (ribbon) indicates the pairwise co-occurrence of mutational events, and the width of the ribbons indicates the frequency of the co-occurrent events. TF (orange), transcription factors; nl CG (green), normal cytogenetics; s (yellow), somatic *DDX41;* E (light green), epigenetic modulators; SF (purple), RNA splicing factors; C (pink), CALR. (F) HM predisposed by germline *DDX41* CV. AML (55%) and MDS (24%) are the most common entities predisposed by *DDX41* CV, followed by cytopenia (16%), MPN (3%), and lymphoma (3%). ****$P < .0001$.

Creating gene-specific diagnostic and management guidelines could raise awareness of this disease and provide necessary guidance for germline confirmation.

This is the first study to expand the link of germline *DDX41* CV to MPN and lymphoma beyond AML/MDS by outlining the CV landscape in unselected and unrelated patients. AML/MDS caused by germline CV is 1 distinct clinical entity with relative indolent course and favorable outcomes, as shown by this and other studies. Our study presents the first and most complete characterization of germline CV profiles to date and highlights the need for guidelines addressing variant classification, patient management, carrier surveillance, and stem cell donor selection.

Authorship

Contribution: P.L. designed the study and drafted the manuscript; T.W., S.B., M.W., W.X., W.C., D.P., H.-Y.W., L.L., and C.A.K. collected patients' clinical and family history and cytogenetic and molecular data; J.V. and T.K. examined patients and performed *DDX41* germline testing; P.L., T.W., W.X., W.C., D.P., H.-Y.W., S.S.M., L.L., C.A.K., and S.B. interpreted and classified all variants by NGS testing; P.L. and M.W. examined the bone marrow biopsies for healthy individuals in cancer surveillance; and all authors reviewed and approved the final manuscript.

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Footnotes

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For original and additional data, please contact Peng Li via peng.li@aruplab.com or peng.li@hsc.utah.edu.

The online version of this article contains a data supplement.

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