Clinical and molecular correlates of somatic and germline *DDX41* variants in patients and families with myeloid neoplasms

Talha Badar,[1] Ahmad Nanaa,[2,3] James M. Foran,[1] David Viswanatha,[4] Aref Al-Kali,[5] Terra Lasho,[5] Christy Finke,[5] Hassan B. Alkhateeb,[5] Rong He,[4] Naseema Gangat,[5] Mithun Shah,[5] Ayalew Tefferi,[5] Abhishek A. Mangaonkar,[5] Mark R. Litzow,[5] Laura J. Ongie,[6] Timothy Chlon,[7,8] Alejandro Ferrer[4] and Mrinal M. Patnaik[4]

[1]Division of Hematology-Oncology and Bone Marrow Transplant Program, Mayo Clinic, Jacksonville, FL; [2]Division of Hematology, Mayo Clinic, Rochester, MN; [3]John H. Stroger, Jr. Hospital of Cook County, Chicago, IL; [4]Division of Hematopathology, Mayo Clinic, Rochester, MN; [5]Division of Hematology, Mayo Clinic, Rochester, MN; [6]Clinical Genomics, Mayo Clinic, Rochester, MN; [7]Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH and [8]Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, OH, USA

Correspondence: T. Badar
badar.talha@mayo.edu

M. S. Patnaik
Patnaik.Mrinal@mayo.edu

Received: February 1, 2023.
Accepted: May 10, 2023.
Early view: May 18, 2023.

https://doi.org/10.3324/haematol.2023.282867

42 NGS panel at Mayo Clinic

ANKRD26 (NM_014915.2) 5'UTR, exons 1-4, intron c.172, ASXL1 (NM_015338.5) exons 10-13, BCOR (NM_001123385.1) exons 4-15, CALR (NM_004343.3) exon 9, CBL (NM_005188.3) intron 7 last 100bps before start of exon 8, exon 8, intron 8, and exon 9, CEBPA (NM_004364.4) exon 1, CSF3R (NM_000760.3) exons 14 and 17, DDX41 (NM_016222.2) exons 1-17, DNMT3A (NM_022552.4) exons 8-23, ELANE (NM_001972.2) exons 1-5, ETNK1 (NM_018638.4) exons 2-5, ETV6(NM_001987.4) exons 3-8, EZH2 (NM_004456.4) exons 2-20, FLT3 (NM_004119.2) exons 14-20, GATA1 (NM_002049.3) exons 2 and 4, GATA2 (NM_001145661.1) exons 3-7, intron 5, c.1017+1 - 1017+730, IDH1 (NM_005896.3) exon 4, IDH2(NM_002168.3) exon 4, JAK2 (NM_004972.3) exons 12-16, KDM6A (UTX) (NM_021140.3) exons 1-29, KIT (NM_000222.2) exons 8-11 and 17, KRAS(NM_033360.3) exons 2-3, MPL (NM_005373.2) exons 10-12, NPM1(NM_002520.6) exons 9-11, to -30 before exon 11, NRAS (NM_002524.4) exons 2 and 3, PHF6 (NM_001015877.1) exons 2-10, PTPN11 (NM_002834.3) exons 3-4 and 12-13, RAD21 (NM_006265.2) exons 1, 2, 4-7, 9-11, 13, 14, exon 10 flank 15bp, RUNX1 (NM_001001890.2) exons 1-6, intron 4 c.725-13T>A and intron 5 c.886+1-4del, SETBP1 (NM_015559.2) partial exon 4; amino acids 400 - 950, SH2B3 (LNK) (NM_005475.2) exon 2-8, SF3B1 (NM_012433.2) exons 13-16, SRP72 (NM_006947.3) exons 6, 10, SMC3 (NM_005445.3) exons 7, 8, 13, 17, 19, 21, 29, SRSF2 (NM_003016.4) exons 1 and 2, STAG2 (NM_001042750.1) exons 4-34, 12, 17 and 22 flank 15bp, TERT (NM_198253.2) exons 2-16, TET2(NM_001127208.2) exons 3-11, TP53 (NM_000546.4) exons 4-9, U2AF1(NM_001025203.1) exons 2, 6, and 8, WT1 (NM_024426.2) exons 1-10, and ZRSR2 (NM_005089.3) exons 1-11.

Supplementary Table 1. Demographic, baseline characteristics, genomic profile of patients with *DDX41* variant of unknown significance

VUS Case No.	Diagnosis	Gender	Age at diagnosis (years)	Nucleotide variant (VUS)	Amino acid change (VUS)	Mutation type (VUS)	DDX41 VUS VAF %	Germline status VUS	Pathogenic DDX41 (VAF)	Germline status Pathogenic DDX41	Co mutations	Cytogenetics
76	Anemia	F	63	c.1016G>A	p.Arg339His	Missense	47	Presumed germline	No			
103	Cytopenia	M	72	c.1016G>A, c.1789A>G	p.Arg339His, p.Lys597Glu	Missense, Missense	49, 22	Confirmed germline, Presumed somatic	No			Diploid cytogenetics
84	IPSS Low risk MDS	M	50	c.1018T>A	p.Tyr340Asn	Missense	50	Presumed germline	No			
91	IPSS Intermediate risk MDS	F	39	c.1030G>T	p.Asp344tyr	Missense	49.5	Presumed germline	No			Diploid cytogenetics
85	Thrombocytopenia	M	78	c.1030G>T	p.Asp344tyr	Missense	49	Presumed germline	No		DNMT3A	
29*	IPSS very high risk MDS	F	76	c.1032C>G	p.Asp344Glu	Missense	7	Confirmed somatic	c.434+1G>A, (46%)	Presumed germline	ASXL1	Diploid cytogenetics
38*	CCUS	F	72	c.1032C>G	p.Asp344Glu	Missense	21	Confirmed somatic	c.1A>C, p.M1?, (22%)	Confirmed somatic		Diploid cytogenetics
72	AML	F	41	c.1238A>G	p.Glu413Gly	Missense	49	Presumed germline	No			
59	AML	F	59	c.1283T>C	p.Leu428Pro	Missense	47	Presumed germline	No		CEBPA	
46	Polycythemia Vera	M	72	c.138+5G>T	p.?	Splice site	48	Presumed germline	No			Diploid cytogenetics
56	AML	F	38	c.1436G>A	p.Arg479Gln	Missense	48	Presumed germline	No		CEBPA	
62	AML	F	37	c.1436G>A	p.Arg479Gln	Missense	48	Presumed germline	No			
66	AML	F	83	c.1436G>A	p.Arg479Gln	Missense	46	Presumed germline	No			
2*	AML	F	78	c.1558A>T	p.Ile520Phe	Missense	17	Presumed somatic	c.3G>A, p.M1I, (48%)	Confirmed germline		Diploid cytogenetics
81	Thrombocytopenia	M	46	c.1668G>T	p.Lys556Asn	Missense	49	Presumed germline	No			
45	CHIP	M	40	c.1732+4A>G	p.?	Splice site	49	Presumed germline	No		JAK2	Diploid cytogenetics
99	DDX41 carrier	F	63	c.207A>C	p.Glu69Asp	Missense	69	Presumed germline	No			Inv 12
89	Thrombocytopenia	M	77	c.214G>A	p.Gly72Arg	Missense	50	Presumed germline	No		TET2	
100	Thrombocytopenia	F	67	c.28-3C>T	p.?	Splice site	49	Confirmed germline	No			
49	CCUS	M	74	c.465G>A	p.Met155Ile	Missense	49	Presumed germline	No			Diploid cytogenetics
60	Pancytopenia	M	23	c.465G>A	p.Met155Ile	Missense	48	Presumed germline	No			
64	AML	M	46	c.465G>A	p.Met155Ile	Missense	47	Presumed germline	No			

Supplementary Table 1. Demographic, baseline characteristics, genomic profile of patients with *DDX41* variant of unknown significance

VUS Case No.	Diagnosis	Gender	Age at diagnosis (years)	Nucleotide variant (VUS)	Amino acid change (VUS)	Mutation type (VUS)	DDX41 VUS VAF %	Germline status VUS	Pathogenic DDX41 (VAF)	Germline status Pathogenic DDX41	Co mutations	Cytogenetics
70	Essential thrombocytosis	F	69	c.465G>A	p.Met155Ile	Missense	48	Presumed germline	No			
69	Pancytopenia	F	39	c.465G>A	p.Met155Ile	Missense	48	Presumed germline	No			
86	Cytopenia	M	30	c.502C>T	p.His168Tyr	Missense	49	Presumed germline	No			
71	AML	F	30	c.505A>G	p.Ile169Val	Missense	49	Presumed germline	No			
104	Leukopenia	M	79	c.511G>C	p.Val171Leu	Missense	48	Confirmed germline	No			Diploid cytogenetics
5*	IPSS High risk MDS	F	81	c.517G>A	p.Gly173Arg	Missense	50	Presumed germline	c.1574G>A, p.Arg525His, (7%)	Presumed somatic		Diploid cytogenetics
30*	CCUS	M	72	c.517G>A	p.Gly173Arg	Missense	46	Presumed germline	c.1574G>A, p.Arg525His, (5%)	Presumed somatic	DNMT3A	Trisomy 8
63	MDS[a]	F	75	c.517G>A	p.Gly173Arg	Missense	47	Presumed germline	No		SMC3	
68	MDS[a]	F	40	c.517G>A	p.Gly173Arg	Missense	48	Presumed germline	No			
82	MDS[a]	M	58	c.517G>A	p.Gly173Arg	Missense	48	Presumed germline	No			
87	Pancytopenia	M	73	c.517G>A	p.Gly173Arg	Missense	51	Presumed germline	No			
75	AML	M	59	c.527T>C	p.Ile176Thr	Missense	91	Presumed germline	No			
61	AML	F	53	c.538A>G	p.Ile180Val	Missense	50	Presumed germline	No			
92	IPSS Intermediate risk (MDS-EB1)	M	65	c.547T>G	p. Phe183Val	Missense	45	Presumed germline	No			Diploid cytogenetics
50	Low risk MDS	M	67	c.566C>T	p.Pro189Leu	Missense	48	Presumed germline	No			Diploid cytogenetics
34*	MDS[a]	F	88	c.571G>A	p.Ala191Thr	Missense	46	Confirmed germline	c.1574G>A, p.Arg525His, (5%)	Presumed somatic		Diploid cytogenetics
47	IPSS High risk MDS (MDS-EB1)	F	61	c.571G>A	p.Ala191Thr	Missense	48	Presumed germline	No			Diploid cytogenetics
28*	MDS[a]	M	73	c.610C>T	p.Pro204Ser	Missense	40	Presumed germline	c.1589G>A, p.Gly530Asp, (46%)	Presumed germline	JAK2	Diploid cytogenetics
52	IPSS Intermediate risk MDS	M	61	c.616C>G	p.Pro206Ala	Missense	14	Presumed somatic	No		DNMT3A, SRSF2, TET2, FLT3	Complex cytogenetics
90	Pancytopenia	M	73	c.616C>G	p.Pro206Ala	Missense	48	Presumed germline	No			
19*	IPSS Very high risk	M	64	c.622C>G	p.Gln208Glu	Missense	11	Confirmed	c.1574G>A,	Confirmed		Diploid cytogenetics

Supplementary Table 1. Demographic, baseline characteristics, genomic profile of patients with *DDX41* variant of unknown significance

VUS Case No.	Diagnosis	Gender	Age at diagnosis (years)	Nucleotide variant (VUS)	Amino acid change (VUS)	Mutation type (VUS)	DDX41 VUS VAF %	Germline status VUS	Pathogenic DDX41 (VAF)	Germline status Pathogenic DDX41	Co mutations	Cytogenetics
	MDS							somatic	p.Arg525His, (9%)	somatic		
80	MDS[a]	M	81	c.649T>C	p.Ser217Pro	Missense	49	Presumed germline	No		DNMT3A	
83	MDS[a]	M	67	c.653G>A	p.Gly218Asp	Missense	49	Presumed germline	No			
53	IPSS Intermediate risk (MDS-EB2)	M	63	c.653G>A	p.Gly218Asp	Missense	50	Presumed germline	No			Diploid cytogenetics
58	Anemia	M	40	c.656G>A	p.Arg219His	Missense	49	Presumed germline	No		SH2B3	
57	AML	M	54	c.656G>A	p. Arg219His	Missense	49	Presumed germline	No		EZH2	
102	CCUS	F	77	c.680C>T	p.Thr227Met	Missense	48	Presumed germline	No		CBL	Diploid cytogenetics
94	CCUS	F	40	c.6G>T	p.Glu2Asp	Missense	49	Presumed germline	No			Ins (9;?) (p13.8), del20 (q11.2, q13.3)
14*	MDS[a]	M	53	c.710T>G	p.Leu237Trp	Missense	46	Confirmed germline				Diploid cytogenetics
78	Anemia	F	80	c.728G>A	p.Cys243Tyr	Missense	47	Presumed germline	No			
95	IPSS Intermediate risk (MDS-EB1)	M	67	c.740A>G	p.Glu247Gly	Missense	46	Presumed germline	No			Diploid cytogenetics
98	DDX41 carrier	F	45	c.740A>G	p.Glu247Gly	Missense	45	Confirmed germline	No			Diploid cytogenetics
74	MDS[a]	F	38	c.766G>A	p.Glu256Lys	Missense	50	Presumed germline	No			
3*	AML	M	59	c.773C>T	p.Pro258Leu	Missense	46	Confirmed germline	c.1574G>A, p.Arg525His, (11%)	Presumed somatic		Diploid cytogenetics
33*	MDS[a]	M	68	c.773C>T	p.Pro258Leu	Missense	46	Presumed germline	c.1588G>A, p.Gly530Ser, (6%)	Presumed somatic		Diploid cytogenetics
65	AML	M	75	c.773C>T	p.Pro258Leu	Missense	47	Presumed germline	No			
67	MDS[a]	M	61	c.773C>T	p.Pro258Leu	Missense	47	Presumed germline	No			
93	IPSS High risk (MDS-EB2)	M	69	c.773C>T	p.Pro258Leu	Missense	50	Presumed germline	No			Diploid cytogenetics
96	DDX41 carrier	F	36	c.773C>T	p.Pro258Leu	Missense	45	Presumed germline	No			Diploid cytogenetics
97	AML	M	27	c.773C>T	p.Pro258Leu	Missense	45	Confirmed germline	No			
79	Cytopenia	F	71	c.811C>T	p.Arg271Trp	Missense	48	Presumed germline	No			
51	IPSS Low risk MDS	F	62	c.866C>T	p.Ser289Leu	Missense	52	Presumed germline	No			Diploid cytogenetics

Supplementary Table 1. Demographic, baseline characteristics, genomic profile of patients with *DDX41* variant of unknown significance

VUS Case No.	Diagnosis	Gender	Age at diagnosis (years)	Nucleotide variant (VUS)	Amino acid change (VUS)	Mutation type (VUS)	DDX41 VUS VAF %	Germline status VUS	Pathogenic DDX41 (VAF)	Germline status Pathogenic DDX41	Co mutations	Cytogenetics
54	Cytopenia	F	54	c.907G>A	p.Val303Met	Missense	48	Presumed germline	No			Diploid cytogenetics
77	AML	F	76	c.907G>A	p.Val303Met	Missense	47	Presumed germline	No			
23*	AML	M	56	c.959C>T	p.Thr320Ile	Missense	49	Confirmed germline	c.1589G>A, p.Gly530Asp, (28%)	Confirmed somatic	ASXL1	Diploid cytogenetics
106	IPSS Very Low risk MDS	F	39	c.962 C>T, c.1030G>T	p.Pro321Leu, p.Asp344Tyr	Missense, Missense	19, 50	Presumed somatic, Presumed germline	No			Diploid cytogenetics
88	Thrombocytopenia	M	62	c.97T>C	p.Tyr33His	Missense	50	Presumed germline	No			
73	AML	F	70	c.97T>C	p.Tyr33His	Missense	48	Presumed germline	No		CSF3R	
48	IPSS high risk MDS (MDS-EB1)	M	81	c.992_994del	p.Lys331del	Missense	44	Presumed germline	No			-Y
55	Aplastic anemia	M	52	c.992_994del	p.Lys331del	Deletion	43	Presumed germline	No			Diploid cytogenetics
101	Pancytopenia	M	82	c.992_994del	p.Lys331del	Deletion	41	Confirmed germline	No			Diploid cytogenetics

**DDX41* variants previously reported by our group (Alkhateeb, H. B. *et al. Blood adv* [2021])
[a] MDS prognostication data missing.
CCUS; clonal cytopenia of undetermined significance, IPSS; international prognostic scoring system, N/A; not available.

Supplementary Table 2: In silico prediction of pathogenicity of *DDX41* variants of unknown significance

Genomic change (Chr38)	cDNA change (NM_016222.4)	Predicted protein impact	CADD score	REVEL score	gnomAD (% All population)	# Patients	ClinVar entry	ClinVar classification	ACMG criteria	Current ACMG classification	Previous references	Novel variant
Chr5:177513767C>T	c.1016G>A	p.Arg339His	28.8	0.395	0.0032%	2	434921	Conflicting - Likely Pathogenic, VUS	PM2_Supporting, PM5	VUS	PMID: 31713024, 32307695	No
Chr5:177512609C>T	c.1436G>A	p.Arg479Gln	24.3	0.416	0.0067%	3	978208	VUS	PM2_Supporting	VUS	N/A	No
Chr5:177516838G>A	c.28-3C>T	p.?	16.75	0	Not described	1	Not described	N/A	PM2_Supporting, BP4_Supporting	VUS	N/A	Yes
Chr5:177515791C>T	c.465G>A	p.Met155Ile	22.6	0.669	0.023%	5	1254618	VUS	PS4_Moderate, PM2_Supporting, PP3_Supporting	VUS	PMID: 25920683, 31911633	No
Chr5:177515745C>G	c.511G>C	p.Val171Leu	23.9	0.153	0.002%	1	1336347	VUS	PM2_Supporting, BP4_Moderate	VUS	PMID: 33332384	No
Chr5:177515058C>T	c.656G>A	p.Arg219His	31	0.488	0.0032%	2	Not described	N/A	PM2_Supporting	VUS	N/A	No
Chr5:177514974T>C	c.740A>G	p.Glu247Gly	29.3	0.352	0.0004%	2	1333084	Likely Pathogenic	PM2_Supporting, PM5	VUS	N/A	No
Chr5:177514941G>A	c.773C>T	p.Pro258Leu	25.8	0.641	0.0004%	6	1338483	Likely Pathogenic	PS4_Moderate, PM2_Supporting, PP3_Supporting	VUS	PMID: 33850299, 32307695, 31256854, 35987913	No
Chr5:177513789_177513791del	c.992_994del	p.Lys331del	N/A	N/A	0.0014%	3	Not described	N/A	PS4_Moderate, PM2_Supporting, PM4	VUS	PMID: 35671390	No
Chr5:177515739C>T	c.517G>A	p.Gly173Arg	29.2	0.454	0.0032%	6	978206	Likely Pathogenic	PS4_Moderate, PM2_Supporting	VUS	PMID: 35987913	No
Chr5:177513765A>T	c.1018T>A	p.Tyr340Asn	23.6	0.648	Not described	2	Not described	N/A	PM2_Supporting, PP3_Supporting	VUS	N/A	Yes
Chr5:177513753C>A	c.1030G>T	p.Asp344Tyr	29.9	0.967	Not described	3	Not described	N/A	PM2_Supporting, PP3_Strong	VUS	N/A	Yes
Chr5:177513751G>C	c.1032C>G	p.Asp344Glu	18.15	0.903	Not described	2	Not described	N/A	PM2_Supporting, PS3_moderate	VUS	N/A	Yes
Chr5:177513075T>C	c.1238A>G	p.Glu413Gly	30	0.901	0.002%	1	Not described	N/A	PM2_Supporting, PP3_Supporting	VUS	PMID: 30963592	No
Chr5:177513030A>G	c.1283T>C	p.Leu428Pro	32	0.961	Not described	1	1336572	VUS	PM2_Supporting, PP3_Strong	VUS	N/A	No
Chr5:177512385T>A	c.1558A>T	p.Ile520Phe	26.8	0.590	Not described	1	Not described	N/A	PM2_Supporting	VUS	N/A	Yes
Chr5:177512160C>A	c.1668G>T	p.Lys556Asn	21.9	0.3	0.008%	1	977491	VUS	PM2_Supporting	VUS	N/A	No
Chr5:177512092T>C	c.1732+4A>G	p.?	8.666	N/A	0.0008%	1	Not described	N/A	PM2_Supporting	VUS	N/A	No
Chr5:177516379T>G	c.207A>C	p.Glu69Asp	10.86	0.082	Not described	1	Not described	N/A	PM2_Supporting, BP4_Moderate	VUS	N/A	Yes
Chr5:177516372C>T	c.214G>A	p.Gly72Arg	36	0	Not described	1	Not described	N/A	PM2_Supporting, BP4_Very Strong	VUS	N/A	Yes
Chr5:177515754G>A	c.502C>T	p.His168Tyr	22.9	0.116	0.0004%	1	Not described	N/A	PM2_Supporting, BP4_Moderate	VUS	N/A	No
Chr5:177515751T>C	c.505A>G	p.Ile169Val	25.1	0.245	Not described	1	Not described	N/A	PM2_Supporting, BP4_Supporting	VUS	N/A	Yes
Chr5:177515745C>G	c.511G>C	p.Val171Leu	23.9	0.153	0.002%	1	1336347	VUS	PM2_Supporting, BP4_Moderate	VUS	PMID:33929502	No
Chr5:177515729A>G	c.527T>C	p.Ile176Thr	24.4	0.214	0.0004%	1	Not described	N/A	PM2_Supporting, BP4_Supporting	VUS	N/A	No
Chr5:177515718T>C	c.538A>G	p.Ile180Val	23.3	0.063	0.0065%	1	Not described	N/A	PM2_Supporting, BP4_Moderate	VUS	N/A	No
Chr5:177515709A>C	c.547T>G	p. Phe183Val	29	0.675	Not described	1	Not described	N/A	PM2_Supporting, PP3_Supporting	VUS	PMID: 3358199	No

Supplementary Table 2: In silico prediction of pathogenicity of *DDX41* variants of unknown significance

Genomic change (Chr38)	cDNA change (NM_016222.4)	Predicted protein impact	CADD score	REVEL score	gnomAD (% All population)	# Patients	ClinVar entry	ClinVar classification	ACMG criteria	Current ACMG classification	Previous references	Novel variant
Chr5:177515690G>A	c.566C>T	p.Pro189Leu	27.3	0.285	0.00071	1	1336295	VUS	PM2_Supporting, BP4_Supporting	VUS	PMID: 35671390	No
Chr5:177515685C>T	c.571G>A	p.Ala191Thr	34	0.069	Not described	2	1330726	Conflicting – Likely Pathogenic, VUS	PM2_Supporting, BP4_Moderate	VUS	N/A	No
Chr5:177515220G>A	c.610C>T	p.Pro204Ser	27.1	0.673	Not described	1	Not described	N/A	PM2_Supporting, PP3_Supporting	VUS	PMID: 35987913	No
Chr5:177515214G>C	c.616C>G	p.Pro206Ala	25.7	0.276	0.0048%	2	Not described	N/A	PM2_Supporting, BP4_Supporting	VUS	N/A	No
Chr5:177515208G>C	c.622C>G	p.Gln208Glu	26	0.918	0.0004%	1	Not described	N/A	PM2_Supporting, PP3_Moderate	VUS	PMID: 35987913	No
Chr5:177515065A>G	c.649T>C	p.Ser217Pro	24.4	0.235	0.0008%	1	1188500	Likely Pathogenic	PM2_Supporting, BP4_Supporting	VUS	PMID: 30963592	No
Chr5:177515061C>T	c.653G>A	p.Gly218Asp	27	0.531	0.0018%	2	1217660	Conflicting – Likely Pathogenic, VUS	PM2_Supporting	VUS	PMID: 31911633	No
Chr5:177515034G>A	c.680C>T	p.Thr227Met	29.4	0.835	Not described	1	Not described	N/A	PM2_Supporting, PP3_Moderate	VUS	PMID: 33626862, 32307695	No
Chr5:177516940C>A	c.6G>T	p.Glu2Asp	22.9	0.076	0.0079%	1	1275746	VUS	PM2_Supporting, BP4_Moderate	VUS	PMID: 32890400	No
Chr5:177515004A>C	c.710T>G	p.Leu237Trp	27.7	0.486	Not described	1	Not described	N/A	PM2_Supporting	VUS	N/A	Yes
Chr5:177514986C>T	c.728G>A	p.Cys243Tyr	25.9	0.261	Not described	1	Not described	N/A	PM2_Supporting, BP4_Supporting	VUS	N/A	Yes
Chr5:177514948C>T	c.766G>A	p.Glu256Lys	24.8	0.415	0.0016%	1	1338053	VUS	PM2_Supporting	VUS	N/A	No
Chr5:177514825G>A	c.811C>T	p.Arg271Trp	31	0.299	0.0008%	1	Not described	N/A	PM2_Supporting	VUS	N/A	No
Chr5:177514770G>A	c.866C>T	p.Ser289Leu	23.1	0.08	Not described	1	Not described	N/A	PM2_Supporting, BP4_Strong	VUS	N/A	Yes
Chr5:177514729C>T	c.907G>A	p.Val303Met	20.6	0.184	0.0093%	2	Not described	N/A	PM2_Supporting, BP4_Supporting	VUS	N/A	No
Chr5:177513824G>A	c.959C>T	p.Thr320Ile	27.9	0.772	Not described	1	1338592	VUS	PM2_Supporting, PP3_Supporting	VUS	N/A	No
Chr5:177513821G>A	c.962 C>T	P.Pro321Leu	29.8	0.682	Not described	1	Not described	N/A	PM2_Supporting, PP3_Supporting	VUS	PMID: 30144193, 33332384, 30963592, 34918362, 25920683, 32307695, 32508881	No
Chr5:177516766A>G	c.97T>C	p.Tyr33His	25.1	0.144	0.0068%	2	1338019	VUS	PM2_Supporting, BP4_Moderate	VUS	N/A	No

CADD: combined annotation dependent depletion, REVEL: rare exome variant ensemble learner, VUS: variant of unknown significance, #; number

Supplementary Table 3. Clinical characteristics and outcome of patients with concurrent somatic mutations (N=31)			
Variables	**DDX41 pathogenic (N= 15)**	**DDX41 VUS (N=16)**	**P value**
Age years, median(range)- at diagnosis	68 (50-76)	68 (38-81)	>0.99
Gender (male), n (%)	9 (60)	9 (56)	>0.99
Hemoglobin G/dL, median (range)	9.6 (7-22.1)	12.8 (9.6-17.6)	0.36
WBC x 10^9/L, median (range)	2.5 (1.6-21)	3.6 (2.3-18.8)	0.36
Platelet x 10^9/L, median (range)	91 (26-440)	231 (72-571)	0.06
BM blasts %, median (range)	12 (0-30)	1 (0-8.0)	0.14
Myeloid disease classification			
MDS	5 (33)	10 (66)	0.06
AML	3 (20)	3 (19)	>0.99
MPN	1 (7)	1 (7)	>0.99
CCUS	1 (7)	2 (12.5)	>0.99
***DDX41* VAF (%), median (range)**	46 (5-51)	48 (15-52)	0.27
Abnormal cytogenetics	6 (40)	2 (12.5)	>0.99
Progression to AML	2 (14)	2 (12.5)	0.31
Requiring treatment	13 (87)	3 (19)	0.08
Time on observation, median in months	28.7 (11.2-33.9)	19.1 (17.1-27.6)	>0.90
Time to treatment, median in months	0.60 (0.30-83.6)	2.17 (1.67-12.13)	0.20
Overall survival, median in months	61.6	55.73	0.69
N; number, WBC; white blood cell count, BM; bone marrow, MDS; myelodysplastic syndrome, AML; acute myeloid leukemia, MPN; myeloproliferative neoplasm, CCUS; clonal cytopenia of undetermined significance, VAF; variant allele frequency			

References

1. Polprasert, C., et al., *Novel DDX41 variants in Thai patients with myeloid neoplasms.* Int J Hematol, 2020. **111**(2): p. 241-246.
2. Qu, S., et al., *Molecular and clinical features of myeloid neoplasms with somatic DDX41 mutations.* Br J Haematol, 2021. **192**(6): p. 1006-1010.
3. Polprasert, C., et al., *Inherited and Somatic Defects in DDX41 in Myeloid Neoplasms.* Cancer Cell, 2015. **27**(5): p. 658-70.
4. Li, S.T., et al., *Rare germline variant contributions to myeloid malignancy susceptibility.* Leukemia, 2020. **34**(6): p. 1675-1678.
5. Byrjalsen, A., et al., *Nationwide germline whole genome sequencing of 198 consecutive pediatric cancer patients reveals a high incidence of cancer prone syndromes.* PLoS Genet, 2020. **16**(12): p. e1009231.
6. Singhal, D., et al., *Targeted gene panels identify a high frequency of pathogenic germline variants in patients diagnosed with a hematological malignancy and at least one other independent cancer.* Leukemia, 2021. **35**(11): p. 3245-3256.
7. Mangaonkar, A.A., et al., *Clinical Applications and Utility of a Precision Medicine Approach for Patients With Unexplained Cytopenias.* Mayo Clin Proc, 2019. **94**(9): p. 1753-1768.
8. Baranwal, A., et al., *Outcomes of allogeneic transplant in patients with DDX41 mutated myelodysplastic syndrome and acute myeloid leukemia.* Bone Marrow Transplant, 2022: p. 1-3.