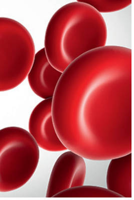

Review Series

GERM LINE PREDISPOSITION TO HEMATOLOGIC MALIGNANCIES

DDX41-associated susceptibility to myeloid neoplasms

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Deleterious germ line *DDX41* variants confer risk for myeloid neoplasms (MNs) and less frequently for lymphoid malignancies, with autosomal dominant inheritance and an estimated prevalence of 3% among MNs. Germ line *DDX41* variants include truncating alleles that comprise about two-thirds of all alleles, missense variants located preferentially within the DEAD-box domain, and deletion variants. The identification of a truncating allele on tumor-based molecular profiling should prompt germ line genetic testing because >95% of such alleles are germ line. Somatic mutation of the wild-type *DDX41* allele occurs in about half of MNs with germ line *DDX41* alleles, typically in exons encoding the helicase domain and most frequently as R525H. Several aspects of deleterious germ line *DDX41* alleles are noteworthy: (1) certain variants are common in particular populations, (2) MNs develop at older ages typical of de novo disease, challenging the paradigm that inherited cancer risk always causes disease in young people, (3) despite equal frequencies of these variants in men and women, men progress to MNs more frequently, suggesting a gender-specific effect on myeloid leukemogenesis, and (4) individuals with deleterious germ line *DDX41* variants develop acute severe graft-versus-host disease after allogeneic hematopoietic cell transplantation with wild-type donors more than others unless they receive posttransplant cyclophosphamide, suggesting a proinflammatory milieu that stimulates donor-derived T cells. Biochemical studies and animal models have identified DDX41's ability to interact with double-stranded DNA and RNA:DNA hybrids with roles in messenger RNA splicing, ribosomal RNAs or small nucleolar RNAs processing, and modulation of innate immunity, disruption of which could promote inflammation and drive tumorigenesis.

Clinical observations

Myeloid neoplasms (MNs) with deleterious germ line *DDX41* variants (Table 1) constitute an autosomal dominant familial myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) disorder as described by the World Health Organization,[21] occurring at an estimated prevalence of 3% among all MNs regardless of the family history.[1] As such, *DDX41* is the most common germ line predisposition gene leading to MNs in adults. Importantly, most cases with this disorder report no family history of hematopoietic malignancy (HM) at diagnosis.[1-6] After the initial descriptions,[7,8] germ line and somatic *DDX41* mutations have garnered the attention of hematologists throughout the world, identifying both deleterious and benign variants and unique clinical features of the MNs that develop in these individuals.[1-6,9,13,14] Although a complete understanding of the disease risk and prognostication is currently lacking, accumulated genetic data afford informed clinical decisions for these patients and their families.

DDX41 variant types

DDX41-mutated MNs define a unique clinical subtype that is distinct from wild-type ones.[1] Among the *DDX41* mutations identified in thousands of MNs, more than 80% are germ line in nature and about 15% are somatic.[1] Deleterious germ line *DDX41* variants include truncating alleles, which comprise about two-thirds of such variants, missense variants encoding changes within the DEAD-box domain, and deletions (Figure 1).[1,4,10-12] Interestingly, very rare congenital cases caused by biallelic deleterious germ line variants have been reported.[22] When molecular profiling panels for MNs include *DDX41*, the identification of a *DDX41* variant should prompt further assessment. Because >95% of truncating alleles are germ line, individuals with such alleles should be prioritized for germ line genetic testing. Increasingly, germ line missense *DDX41* variants are recognized as deleterious,[1] so these should also prompt further testing. Importantly, the c.3G>A, p.M1? start-loss allele, one that to date has only been seen as a germ line allele, often gets detected at a variant allele frequency

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Table 1. Description of HMs arising from deleterious germ line *DDX41* variants in WHO classification vs recent updates

Topics	WHO classification	Updates by recent studies
Prevalence	1.5% of MNs	High in high-risk MDS/sAML (5%-8%), low in MPN (<1%)[1-9]/10 times more enrichment in MNs[1]
Germ line mutations	Half of all the mutations	70%-80%, two-thirds are truncating[4,10-12]
Age at onset	Long latency (mean 62 yo)	Long latency (mean 66 yo)[1-9,11,13-17]
Male predominance	No description	Yes (1.7-3.1 times), compared with WT cases in any disease phenotype[1,2,14]
Hematologic findings	Leukopenia/erythroid dysplasia	Hypocellular bone marrow/higher blast count in MDS[1]
Penetrance	Not fully established	0% of penetrance at 40 yo/50% lifelong penetrance[1]
Karyotype	Frequent normal karyotype	Normal karyotype is 2-4 times more frequent, compared with WT cases[1,7,13,14]
Concomitant mutations	No description	Mutations in *CUX1* and *GNAS* are more frequent compared with WT cases[1] Mutations in *ASXL1*, *TET2*, *DNMT3A*, and *TP53* are frequent but the frequencies are not significantly different compared with those in WT cases[1,2,7,18]
Leukemic evolution	No description	Faster leukemic evolution[1,19]
Prognosis	Poor in cases with somatic deletion	Better in cases with pathogenic variants[1,2,4,14,17,20]
Therapeutics	Lenalidomide may be effective	Hypomethylating agents may be effective[1]

MPN, myeloproliferative neoplasm; WT, wild-type.

(VAF) of about 30% due to a common polymorphism that results in decreased binding of polymerase chain reaction primers made according to the standard DNA sequence. Therefore, the identification of this allele at VAFs in this range should also prompt proper germ line testing.

As with other cancer predisposition genes, *DDX41* variants within MNs are often biallelic, with somatic mutations being detected in about half of the cases with germ line *DDX41* predisposition variants.[1] When they occur, missense somatic mutations are mainly found within the helicase domain.[1-7,9,13,14] Among somatic *DDX41* mutations, R525H is identified in 80% of MNs, G530D/S in 9%, P321L in 7%, and T227A/M in 3%, with less than 5% being truncating alleles.[1] For individuals in whom only somatic *DDX41* mutations are identified within MNs, testing for germ line risk alleles should include the capacity to detect large deletions of multiple exons to avoid missing a deleterious germ line *DDX41* allele.[1]

Population distribution of *DDX41* variants

Deleterious germ line *DDX41* variants have been reported throughout the world, including Europe, the United States, Australia, the United Kingdom, France, Japan, Korea, and Thailand (Figure 2).[2-9,13,15-17,20,23] Certain variants are more frequent within particular populations. For example, the c.3G>A, p.M1? and c.415_418dup, p.D140Gfs*2 alleles are more common in people of Northern European descent, whereas the c.1496dup, p.Ala500Cysfs*9 allele is more frequent in Japanese and Korean individuals, suggesting that these are founder variants. With the availability of large-scale public sequencing

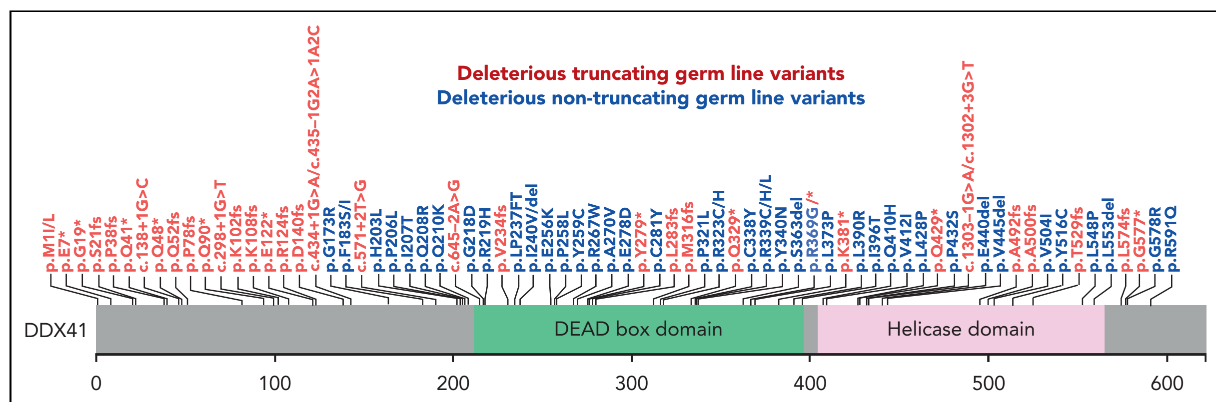

Figure 1. Deleterious DDX41 germ line variants that confer risk to MNs. The DDX41 protein schematic is shown, including the DEAD-box (green) and helicase (pink) domains. Truncating variants are shown in red, and missense variants in blue.

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databases of healthy people, several novel rare truncating *DDX41* variants can be seen, such as in South America, Africa, and India, which likely represent additional germ line variants more common in those populations (Figure 2, olive font). We look forward to germ line testing within those populations for confirmation of those alleles as germ line variants. Most of the germ line variants are not found in both Asian and European populations, but both Asian and European variants are identified in people living in the United Kingdom and the United States, probably because of immigration from Asian areas. For this reason, enrichment of germ line variants should be calculated from patients with MNs vs healthy controls while controlling for the genetic background. Recently, the enrichment of Asian pathogenic variants was assessed by comparing more than 4000 MNs to more than 20 000 healthy controls within Japanese individuals, yielding an enrichment of deleterious germ line *DDX41* variants with an odds ratio (OR) of 10.6 (95% confidence interval: 4.5-22.8).[1]

Age distribution MNs derived from deleterious germ line *DDX41* variants

Deleterious germ line *DDX41* variants challenge the notion that inheritance of a cancer predisposition allele incites tumorigenesis at a younger age. Typically, germ line *DDX41*-mutated MNs develop with long latency at an average age of about 68 years old (yo), which is similar to de novo AML.[2-9,11,13-17] For this reason, the age of onset of MNs should not be a criterion for prioritizing germ line genetic testing.

MN penetrance

Importantly however, the penetrance of MNs derived from deleterious germ line *DDX41* variants has not been established.[24] Because such information is necessary for appropriate genetic counseling, researchers have used the kin (first-degree relatives) cohort method recently to estimate the penetrance of *DDX41*-mutated MNs, as was performed for *BRCA*^{mut}-associated breast cancer. This calculation of disease incidence considers competing risks associated with other causes of death using the Fine-Gray model, which theoretically attributes more precise risks typically in older populations. For deleterious *DDX41* germ line variants, the risk of any MN is almost negligible before 40 yo, but rapidly increases to 49% by the age of 90.[1]

Gender distribution of MNs derived from deleterious germ line *DDX41* variants

Several groups have demonstrated that men with deleterious *DDX41* variants are more likely to develop MNs than women with such alleles,[2,14] despite equal frequencies of pathogenic germ line *DDX41* variants in men vs women, suggesting a gender-specific effect on myeloid leukemogenesis. The male gender is more frequent in *DDX41*-mutated cases than in wild-type ones, with an OR ranging from 1.7 to 3.1. Accordingly, the enrichment of *DDX41* pathogenic variants is more remarkable in men (OR, 20.7) than in women (OR, 5.0), although both OR are significant compared with healthy individuals. The penetrance is also (~4 times) higher in men than in women.[1]

Disease phenotypes and co-occurring secondary mutations

Within specific types of MNs, deleterious germ line *DDX41* variants are found in 3% to 5% in MDS, 5% to 9% in secondary AML, 1% to 2% in MDS/myeloproliferative neoplasms, and <0.5% in myeloproliferative neoplasms. Compared with

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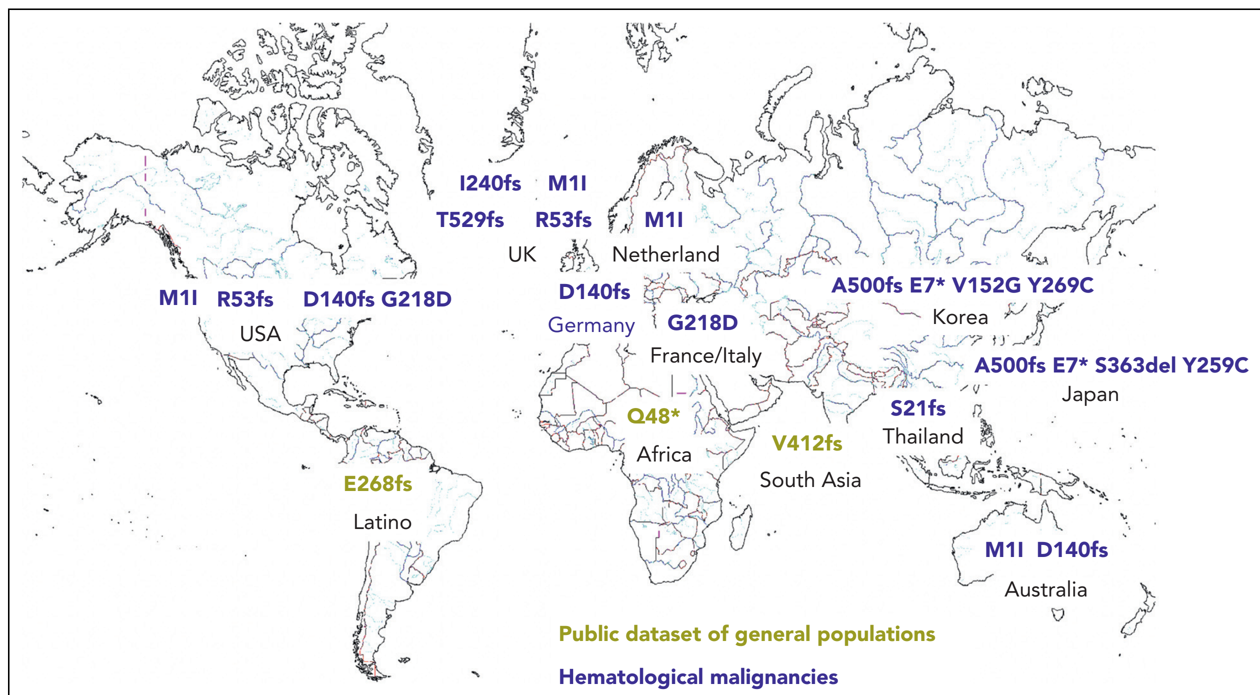

Figure 2. Ethnic diversity of deleterious germ line DDX41 alleles. A world map is displayed with common deleterious germ line DDX41 alleles present within particular geographic areas. Standard variant nomenclature is used. Deleterious germ line variants identified within HMs are shown in blue type, and those identified from public databases are given in olive type.

individuals without deleterious germ line *DDX41* variants, patients with MDS and AML are 10 times more enriched for such alleles.[1] The most common preceding diagnosis in those who developed secondary AML was MDS.[1] Patients with such *DDX41* alleles who develop MNs usually present with leukopenia and hypocellular bone marrow with prominent erythroid dysplasia. An initial sequencing study of erythroleukemia revealed that the category of *DDX41* mutant cases is a subset with distinct genetic profile,[25] but such variants were less frequent in another study of erythroleukemia.[26] Among MDS subtypes, refractory anemia with excess blasts is the most frequent. In accordance with the phenotypic features, deleterious germ line *DDX41* variants are significant risk factors of leukemic evolution,[19] which is confined to truncating alleles, but very rarely observed in the cases with missense variants.[1] Despite higher risk phenotypes, the most frequent cytogenetic pattern in the MNs that develop is a normal karyotype, confirmed in phenotype-matched subgroup analysis.[1,7,13,14] Most frequent co-occurring somatic mutations within the MNs occur in *ASXL1*, *CUX1*, *TP53*, *DNMT3A*, and *TET2*,[2,7,9,18] rather than mutations in genes typically associated with secondary AML, such as *NRAS*, *FLT3*, and *NPM1*. Among these, only *CUX1* mutations are preferentially observed in *DDX41*-mutated cases compared to wild-type ones.[1] Of note, the clone sizes of co-occurring somatic mutations are smaller than somatic *DDX41* mutations, suggesting that their acquisition occurs after the somatic *DDX41* mutation.[1] Although MNs are the overwhelming majority of HMs diagnosed in these individuals, lymphoid malignancies have been observed, also in the adult age range.[2,3,8,16,27] Solid tumors have also been observed in individuals with deleterious germ line *DDX41* variants, although the contributions of these variants to tumorigenesis is unclear.

Cellular functions of DDX41

DDX41 is a member of the DEAD-box RNA helicase family, although unlike the name implies, it interacts with double-stranded DNA (dsDNA) and RNA:DNA hybrids.[28] Expressed widely across many tissues,[29] DDX41 is found in both the cytoplasm and nucleus with distinct and pleiotropic functions in each cellular compartment (Figure 3).[30] Our understanding of how DDX41 insufficiency contributes to the development of HMs is still in its infancy.

DDX41 as a splicing regulator

DDX41 is a metazoan-specific spliceosomal component that is part of the catalytically active complex C spliceosome (Figure 3).[7] In line with this connection, spliceosomal components are among the top DDX41-interacting proteins identified in both *Caenorhabditis elegans* and human cells (Table 2).[7,34] Several groups have identified significant alternative splicing changes in *DDX41* mutant or knockdown hematopoietic (hematopoietic stem and progenitor cells [HSPCs], erythroid progenitors, and leukemic cells) and nonhematopoietic cell types across multiple organisms (*C elegans*, zebrafish, mice, and humans) indicating high conservation for DDX41 in splicing regulation.[7,31,32,34,37] The functional significance of the specific splicing alterations identified in these studies remains untested. However, 1 study showed patients with deleterious *DDX41* variants rarely have somatic mutations in spliceosomal components.[7] This negative selection for mutational co-occurrence supports the model that the function of DDX41 in splicing is critical for hematopoiesis. Consistent with the mutual exclusivity observed in patients, genetic interaction studies in *C elegans sacy-1* (*DDX41* homolog) mutants revealed synthetic lethality with splicing factor mutants.[34] Combined, these findings indicate that although the precise function of DDX41 in premessenger RNA (mRNA) processing is unknown, it is essential for cellular survival.

DDX41 in ribosomal RNA (rRNA) and small nucleolar RNA (snoRNA) processing

In addition to a role in pre-mRNA splicing, DDX41 affects the processing of RNAs critical for ribosome assembly and function (Figure 3).[30,33] Human cells overexpressing a common somatic hypomorphic mutant form of DDX41 (R525H) displayed defects in pre-rRNA processing, which correlated with diminished cellular proliferation.[30] Pre-rRNAs are transcribed and processed in the nucleolus, where they are then assembled into ribosomes. Part of their processing and maturation is mediated by snoRNA. Defects in snoRNAs will alter pre-rRNA cleavage and pseudouridylation, which are required for proper ribosome functioning.[39] As an evolutionarily conserved coupling mechanism, most snoRNAs are located within the introns of ribosomal protein genes.[40] Thus, the first step in snoRNA processing requires proper splicing. A recent study demonstrated that DDX41 could be a previously unrecognized player involved in snoRNA processing.[33] *Ddx41*-deficient murine HSPCs displayed a marked increase in snoRNA levels. Moreover, the identification of DDX41-interacting RNAs revealed a strong enrichment for snoRNAs, suggesting that the effect could be direct, potentially via the removal of snoRNA-containing introns via splicing regulation. Consistent with this function being important for hematopoiesis, knockdown of several snoRNAs in murine HSPCs diminished cell viability and differentiation.[33] Currently, it remains unclear if the effect of DDX41 on ribosome biogenesis is via direct regulation to both snoRNAs and pre-rRNAs or due to snoRNA defects that then alter pre-rRNA processing. In addition, the relative contribution of splicing defects in protein coding vs noncoding genes on *DDX41*-mutant hematopoietic pathology is an unanswered question in the field.

DDX41 in innate immunity

DEAD-box helicases also play critical roles in immunity.[41] This is best characterized in myeloid effector cells such as macrophages and dendritic cells that activate type I interferon (IFN) signaling in response to either infection or exposure to DNA-type pathogen-associated molecular patterns.[41] DDX41 is one of the pattern recognition receptors that is stimulated by these pathogen-associated molecular patterns, specifically bacterial cyclic dinucleotides as well as viral dsDNA and RNA:DNA hybrids.[28,42,43] It is thought that cytoplasmically localized DDX41 initiates the type I IFN signaling cascade, starting with the stimulator of IFN genes (STING, also known as TMEM173, MITA, MPYS, or ERIS), which then activates TANK-binding kinase 1, IFN response factor 3, and nuclear factor κB (Figure 3).[42,43]

HMs, especially MNs, have a strong connection to inflammatory dysfunction.[44] Because of the connection between DDX41 and type I IFN, this pathway has been examined in DDX41-mutant hematopoiesis.[31,32,35] Functionally hypomorphic zebrafish *ddx41* mutants display aberrant HSPC expansion.[31] Gene expression analysis of the *ddx41* mutant compared with normal HSPCs revealed deregulation of type I IFN pathway

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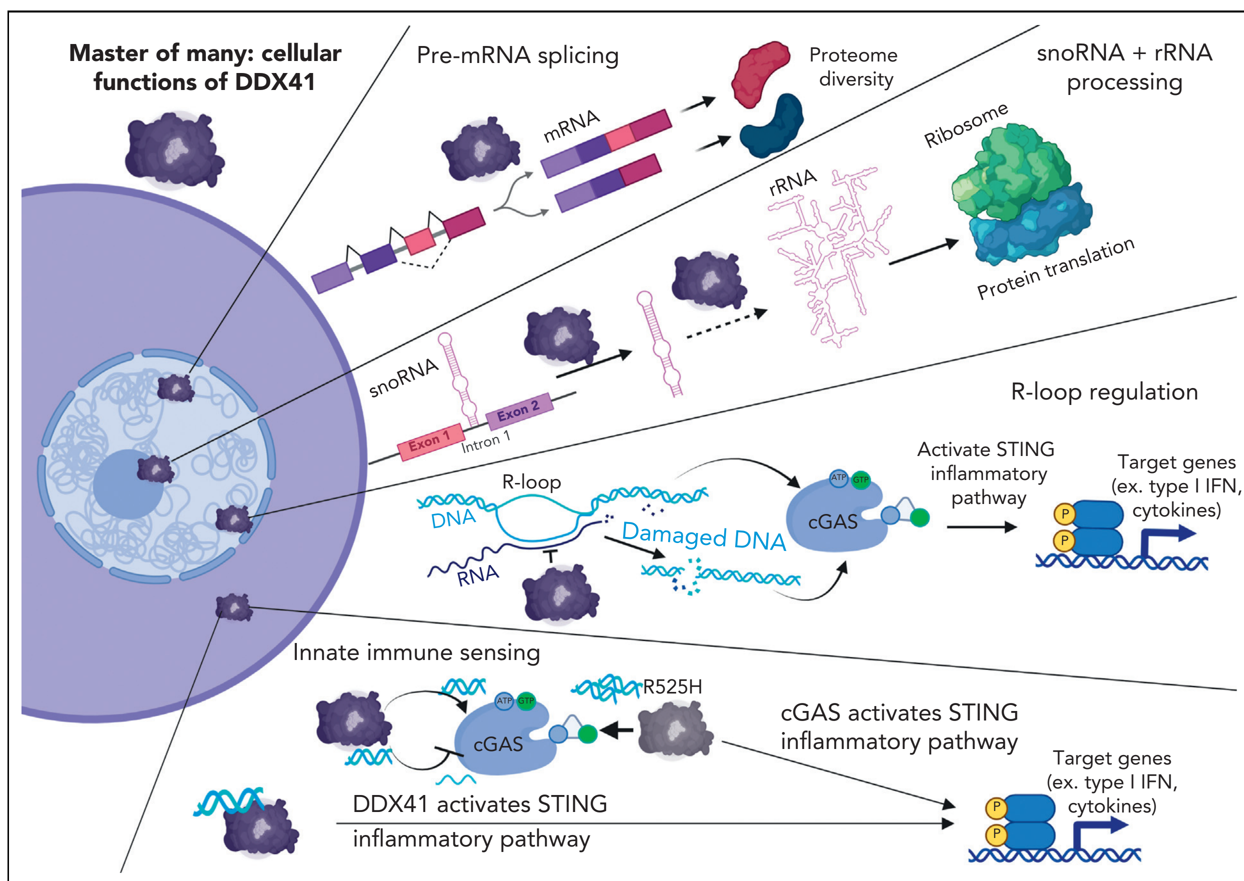

Figure 3. Master of many: cellular functions of DDX41. DDX41 localizes to both cytoplasmic and nuclear compartments and plays distinct roles in each location. In the nucleus, DDX41 functions in at least 3 processes. Via its association with the catalytically active spliceosome, DDX41 regulates pre-mRNA splicing. Here, it also modulates snoRNA processing, potentially via promoting excision of snoRNA-containing introns found in ribosomal protein genes. DDX41 can also modify pre-rRNA processing, either indirectly via its control of snoRNA processing or perhaps directly via an unknown mechanism. In the nucleus, DDX41 also interacts with R-loops, which comprise RNA:DNA hybrids and ssDNAs. DDX41 insufficiency leads to R-loop accumulation and subsequent increased dsDNA breaks that trigger a cGAS-STING–mediated type I IFN response. Cytoplasmically localized DDX41 can bind to infection-derived or damaged endogenous dsDNA and then promote STING activation and type I IFN response. In addition, DDX41 can modulate cGAS activity via its dsDNA unwinding capabilities by altering the relative amounts of dsDNA-to-ssDNA. For example, the R525H DDX41 mutant, which has intact dsDNA binding activity but diminished unwinding activity, can overstimulate cGAS leading to a heightened type I IFN response. Created with BioRender.com. DDX41, DEAD-box helicase 41; ssDNA, single-stranded DNA.

components and targets. Similar results were observed in human hematopoietic cells upon *DDX41* knockdown.[35] Surprisingly, type I IFN signaling appeared upregulated, not downregulated in these studies. This result is seemingly contradictory with a function of DDX41 as an activator of this pathway, thus another role of DDX41 might be driving this inflammation.

Within the nucleus, DDX41 interacts with R-loops, which are structural DNA variants comprising single-stranded DNAs and RNA:DNA hybrids (Figure 3).[35,36] DDX41 is a helicase that is stimulated by RNA:DNA hybrid binding, suggesting it could unwind and destroy these molecules.[28] In line with this model, both zebrafish and human cells with insufficient DDX41 levels accumulate RNA:DNA hybrids, which result in elevated dsDNA breaks.[31,35] Since the discovery of DDX41 as a DNA pattern recognition receptor, new data indicate the main DNA sensor in most cells is cyclic guanosine monophosphate- adenosine 5′-monophosphate (GMP-AMP) synthase (cGAS).[45] cGAS is activated by many infectious and damage-associated stimuli, including dsDNA, RNA:DNA hybrids, micronuclei, and damaged mitochondria, among others.[45] Ligand binding to cGAS stimulates its enzymatic activity, leading to the production of the second messenger cyclic GMP-AMP, which can bind to several proteins, including STING, leading to an inflammatory signaling cascade that results ultimately in activation of the type I IFN pathway.[45] In the zebrafish *ddx41*-mutant model, R-loops and/or the ensuing damaged DNA orchestrated a cGAS-STING–mediated inflammatory response that promoted HSPC expansion.[31] These data imply that DDX41 regulation of R-loop levels is important in hematopoietic homeostasis.

Other connections between DDX41 and cGAS are emerging. DDX41 binds the bacterial second messengers cyclic-di-guanosine monophosphate and cyclic-di-adenosine monophosphate, which are structurally similar to cGAS-produced cyclic GMP-AMP, suggesting cGAS could modulate DDX41 activity.[42] In addition, a recent study uncovered a mechanism for DDX41 modulation of cGAS activity.[38] Loss of DDX41 in monocytic cells lowered dsDNA-triggered activation of the cGAS-STING pathway. In contrast, expression of the R525H hypomorphic mutant DDX41, which displays dampened

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Table 2. Contrast of phenotypes in animal vs human studies

	Animal studies	Human observations
Effect on HSPCs	Hypomorphic zebrafish *ddx41* mutants display aberrant HSPC expansion, with deregulation of type I IFN pathway components and targets[31] Mouse *Ddx41* KO HSPC show diminished survival and transplantation capacity[32,33] Mouse HSPCs with *Ddx41* heterozygous KO show competitive advantage[33] Mice with *Ddx41* heterozygous KO show age-dependent hematopoietic defects including anemia, thrombocytopenia, and BM hypocellularity[33] Animals with heterozygous KO of *Ddx41* function do not develop hematopoietic malignancies[31-33]	Patients present with leukopenia, hypocellular bone marrow, and erythroid dysplasia[1] Patients with deleterious germ line *DDX41* variants develop hematopoietic malignancies, mostly of myeloid lineage, with late onset[1-17,20,22-24]
DDX41-interacting proteins	Spliceosomal components (*C elegans*)[34]	Spliceosomal components[7] R-loop–interacting proteins[35,36]
Alternative splicing changes in *DDX41*-mutant or knockdown cells	Increased alternative exon usage, intron retention, alternative 5′ and 3′ splice site usage (*C elegans*, zebrafish, mice)[31,34,37]	Increased exon inclusion and intron retention[7]
Mutual exclusivity with splicing mutations?	Yes. *C elegans* sacy-1 mutants show synthetic lethality with germ line splicing factor mutants[34]	Yes. Co-occurrence of somatic mutations in genes encoding splicing factors is rare, suggesting that DDX41's splicing function is critical[7]
Role in ribosome biogenesis (snoRNA and pre-rRNA processing)	*Ddx41*-deficient HSPCs display increased snoRNA levels[33]	Cells overexpressing DDX41 R525H display defects in pre-rRNA processing and had diminished cellular proliferation[30] DDX41-interacting RNAs are enriched for snoRNAs, suggesting that DDX41 could remove snoRNA-containing introns via splicing regulation[33]
R-loop interactions	Increased RNA:DNA hybrids result from insufficient Ddx41 levels, which induces a cGAS-STING-mediated inflammatory response that directs HSPC expansion (zebrafish)[31]	Increased RNA:DNA hybrids and consequently elevated dsDNA breaks result from insufficient DDX41 levels[35]
cGAS-STING interactions	Murine fetal liver Ddx41-null HSPCs display diminished inflammatory gene expression[32]	Loss of DDX41 in monocytic cells lowered dsDNA-triggered activation of the cGAS-STING pathway. However, expression of DDX41 R525H, with intact dsDNA binding but dampened helicase activity, activates the STING pathway[38] Human hematopoietic cells show paradoxical increased type I IFN signaling upon DDX41 knockdown[35]

BM, bone marrow; DDX41, DEAD-box helicase 41; KO, knockout.

helicase activity with intact dsDNA binding, increased pathway activation (Figure 3). The findings indicate a complex interplay among the nucleic-acid sensing pathways that could have a significant impact on hematopoiesis and immunity.

Interplay of DDX41 functions in hematopoiesis

The examination of how the pleiotropic cellular functions of DDX41 affect hematopoiesis and how they cross talk is in its infancy. The differences among the studies in various hematopoietic and nonhematopoietic cells could be explained by cell-type selective functions and dependencies. In addition, DDX41 isoforms may localize to distinct cellular compartments.[8,30] Full-length DDX41 appears largely nuclear, whereas a shorter isoform with a truncated N-terminus is found predominantly in the cytoplasm.[30] The relative levels of these 2 isoforms could alter how a particular cell deploys DDX41. Expression of the corresponding transcripts across hematopoietic cell types and during HSPC development is unknown.

Management implications

MN pathogenesis

Deleterious germ line *DDX41* variants challenge the accepted paradigm that germ line predisposition variants cause cancer at ages younger than expected because MNs rarely develop in people younger than 40 years and typically in the late

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60s.[2-7,9,13,14] This inverted age distribution of MNs suggests a unique pathogenesis. Cascade testing in families has identified many relatives who share the deleterious familial *DDX41* allele. These individuals are being closely followed decades before MNs develop, which will allow the natural history and clonal progression of these malignancies to be described. Because we postulate that MNs arise as the sequential acquisition of somatic mutations, beginning with the phenomenon of clonal hematopoiesis (CH), an open question is whether CH precedes the development of MNs in those with deleterious germ line *DDX41* alleles. If so, it is possible that the presence of CH represents a molecular clock that marks the initiation of MN development. Future studies will demonstrate whether CH-associated somatic mutations occur within expected genes (eg, *DNMT3A*, *TET2*, *ASXL1*, and others) or within a gene set unique to germ line *DDX41*-associated MNs. Because MNs occur within the expected age range, the role of the pathogenic germ line *DDX41* variant throughout most of an individual's lifetime and its impact on any CH that might develop remain a mystery. If these questions could be answered by future studies, we might be able to design specific interventions that could be instituted decades before MN development to delay or prevent these cancers.

Multiple recent studies have shown that deleterious germ line *DDX41* variants are associated with a better prognosis than de novo MNs.[2,4,14,17,20] Initially, however, MNs arising in those with deleterious germ line *DDX41* variants were described as having a poor prognosis, likely because cases with somatic deletion of the *DDX41* locus, typically caused by the deletion of the telomeric region of chromosome 5q with complex karyotypes and biallelic *TP53* mutations, were included in the mutated cohort. Table 1 contrasts initial descriptions of MNs arising in those with deleterious germ line *DDX41* variants[46] with a more updated understanding of the features of these malignancies.[2-6,9,13,14] A recent detailed study showed that the bone marrow blast percent is higher in *DDX41*-mutated MDS compared with *DDX41*–wild-type MDS. In contrast, the bone marrow blast percent is lower in *DDX41*-mutated secondary AML compared with such cases with wild-type *DDX41*, suggesting that with deleterious germ line *DDX41* variants, bone marrow blasts increase until leukemic evolution (at ~20%) but tend not to progress rapidly after that point.[1]

Therefore, the discrepancy between frequent leukemic evolution and better prognosis should be addressed. Another possible explanation of this discrepancy between frequent leukemic evolution and better overall survival is that *DDX41*-mutated cases respond to specific therapy. Early data suggest that patients may respond to lenalidomide,[7] and the most recent study showed that hypomethylating agents might be effective and improve overall survival in *DDX41*-mutant MN cases (hazard ratios: 0.25-0.60) compared with wild-type *DDX41* ones.[1] This retrospective study also showed that treatment with hypomethylating agents before hematopoietic stem cell transplantation (HSCT) dramatically improved overall survival in *DDX41*-mutated cases compared with wild-type cases, which should be validated by prospective clinical trials.

Implications for allogeneic HSCT

For most individuals with germ line predisposition variants, the risk of developing a HM is relieved by allogeneic HSCT using a donor who lacks the deleterious familial allele. However, given the common nature of some germ line *DDX41* variants within particular populations, several instances have been described in which allogeneic donors, either related or unrelated, had a deleterious germ line *DDX41* variant that was transferred to the recipient during allogeneic HSCT.[47-50] For this reason, we advocate the following: (1) the addition of *DDX41* to all molecular profiling panels being used to prognosticate for HMs, because the identification of a truncating *DDX41* allele at VAF >30% is likely to be germ line; however, the use of such panels should not replace proper germ line testing, because these panels are generally unable to detect all types of germ line alleles;[51,52] and (2) testing of all allogeneic stem cell donors for germ line predisposition variants, including *DDX41*.

We advocate transplantation for individuals diagnosed with MNs in clinical remission when they have deleterious germ line *DDX41* variants (ie, likely pathogenic or pathogenic variants). Like in other germ line cancer predisposition disorders, only deleterious germ line variants are clinically actionable. Germ line variants deemed to be of uncertain significance, likely benign, or benign do not prompt further studies or cascade testing in families unless the variant gets upgraded into likely pathogenic or pathogenic classification with additional functional or segregation data.

Observational studies have shown that after allogeneic HSCT using wild-type donors, individuals with deleterious germ line *DDX41* variants suffer more graft-versus-host disease (GVHD) than others.[53,54] One study showed that patients with deleterious germ line *DDX41* variants had more stage 3 to 4 acute GVHD (38%) than those with deleterious *CHEK2* variants (0%), other hereditary HM variants (12%), or patients without such germ line variants (9%) unless they had received posttransplant cyclophosphamide as GVHD prophylaxis.[53] We postulate that individuals with deleterious germ line *DDX41* variants have a proinflammatory milieu generated from the germ line allele that remains in all of their nontransplanted organs, which stimulates donor-derived T cells and causes severe GVHD. Based on these observations, we advocate consideration of lower intensity transplantation regimens, optimizing donor selection, and use of posttransplant cyclophosphamide as GVHD prophylaxis when individuals with deleterious germ line *DDX41* variants undergo allogeneic HSCT, even when using wild-type donors.

Population screening and monitoring

Because truncating and deleterious germ line *DDX41* variants can be identified in screening sequencing databases from populations across the world (Figure 2), we recognize that it is possible to do population-based screening for deleterious germ line *DDX41* variants. Currently, we advocate that once an individual is identified as having a deleterious germ line *DDX41* variant, baseline blood work, including a complete blood cell count and differential, and a bone marrow biopsy be performed to test for the presence of cytopenias and/or bone marrow dysplasia, respectively. It is important to establish the degree of bone marrow dysplasia before MN progression so that an accurate diagnosis of MDS can be made over time for patients. We advise including molecular profiling that includes *DDX41* and cytogenetic analysis at the time of bone marrow biopsy because the presence of the DDX41 R525H somatic mutation

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and/or del(5q) could signal impending or overt MN. The age at which baseline studies are performed and when surveillance monitoring begins should be decided ideally in collaboration with genetic counselors experienced in hereditary hematopoietic malignancies and discussions that consider the family history and the personal preferences of the affected individuals. Once the baseline studies have been performed, we advise repeating a complete blood cell count and differential 3 to 4 times per year with consideration of an annual bone marrow examination. Evidence for progression toward MN includes the identification of somatic mutations and/or karyotypic abnormalities, and some advocate for preemptive allogeneic HSCT if such changes are demonstrated above baseline studies even in the absence of increasing blast burden in the bone marrow. The hope is that future studies will define the molecular progression to MNs for individuals with germ line *DDX41* predisposition alleles, allowing us to intercede and slow or prevent MN development without the need for allogeneic HSCT.

Conclusion

Identified in about 3% of MNs, deleterious germ line *DDX41* variants constitute the most common germ line predisposition disorder causing myeloid, and less often lymphoid, malignancies in adults. Several unique features of these alleles are important for optimal patient management, as follows: (1) the vast majority of truncating *DDX41* alleles are germ line, and others, such as the c.3G>A, p.M1? start-loss allele, have only been seen as germ line alleles; therefore, when such alleles are detected on tumor-based molecular profiling platforms, they should prioritize that individual for proper germ line testing; (2) MNs that develop in people with deleterious germ line *DDX41* alleles occur at ages typical of de novo disease, challenging the paradigm that inherited cancer risk always causes disease at young ages; (3) men with these alleles progress to MNs more frequently, suggesting a gender-specific effect on myeloid leukemogenesis; (4) because certain variants are quite common in particular populations, unrelated allogeneic HSCT donors with these alleles have inadvertently been used, resulting in the transfer of a deleterious germ line *DDX41* variant into recipients; The presence of these common *DDX41* variants may allow donor-based and/or population-based screening in the future; (5) when wild-type donors are used in allogeneic HSCT for those with deleterious germ line *DDX41* variants, posttransplant cyclophosphamide appears to be an effective means of posttransplant GVHD prophylaxis that prevents the otherwise severe GVHD that occurs in these individuals. The exact mechanism(s) by which these variants confer risk to MNs is unclear, but biochemical studies and animal models demonstrate that DDX41 interacts with dsDNA and RNA:DNA hybrids with roles in mRNA splicing, rRNAs/snoRNAs processing, and modulation of innate immunity, which could lead to an inflammatory milieu that drives tumorigenesis.

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Authorship

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Footnote

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