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GUIDELINES - CONSENSUS-BASED

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How to manage patients with germline *DDX41* variants: Recommendations from the Nordic working group on germline predisposition for myeloid neoplasms

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Abstract

Increasing recognition of germline *DDX41* variants in patients with hematological malignancies prompted us to provide *DDX41*-specific recommendations for diagnosis, surveillance, and treatment. Causative germline variants in the *DDX41* predispose to the development of myeloid neoplasms (MNs), especially myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Almost 3%–5% of all patients with MDS or AML carry a pathogenic or likely pathogenic germline *DDX41* variant, while half of them acquire a somatic second hit in the other allele. *DDX41*-associated MNs exhibit unique clinical characteristics compared to other hematological malignancies with germline predisposition: MNs occur mostly at advanced age and follow an indolent clinical course. Male carriers are more prone to develop MDS or AML than females. *DDX41*-associated MN is often hypoplastic, and the malignancy may be preceded by cytopenias.

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RECOMMENDATIONS

A summary of the recommendations is provided in Figure 1.

RATIONALE FOR THE ANALYSIS OF *DDX41* IN PATIENTS WITH MYELOID NEOPLASMS

- We recommended that analysis of *DDX41* should be included in the diagnostic work-up of all patients that undergo a genetic investigation for myeloid neoplasms (MNs) such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

This recommendation is based on the following:

- DDX41-associated MN is recognized as a distinct entity in the recent International Consensus and World Health Organization classifications for MNs and the European Leukemia Net diagnostics and therapy recommendations for adults with AML.[1–3]
- Germline *DDX41* variants are present in at least 3%–5% of patients with MNs.[4–6]
- Emerging data suggest a clinical profile with, for example, bone marrow hypocellularity and indolent course in a great proportion of MDS/AML with germline *DDX41* variants.[7–10]
- There are several reports of donor-derived leukemia in patients transplanted with sibling donors who were carriers of a deleterious germline *DDX41* variant. This further highlights the importance of comprehensive germline investigation in patients who undergo allogeneic hematopoietic stem cell transplantation (allo-HSCT).[11,12]
- Emerging data suggest an increased incidence of severe graft-versus-host disease in patients with *DDX41* variants undergoing allo-HSCT.[13]
- Many patients with a causative germline *DDX41* variant have a history of cytopenias, which suggests that surveillance programs might benefit carriers at risk (see surveillance program below).[4]

DDX41 VARIANTS DETECTED DURING SOMATIC PROFILING

Both germline and somatic variants can be found in *DDX41*. Two-thirds of germline *DDX41* variants are truncating, although splice-site variants or missense variants have also been detected.[14] Some recurrent germline variants (p.Met1?, p.Asp140GlyfsTer2) have been reported in Europeans. Additionally, a proportion of private, potentially population-specific variants can be found. Somatic *DDX41* mutations are usually missense variants.[6,14] The most common somatic *DDX41* variant observed in carriers of a germline variant is p.Arg525His (found in two-thirds of the patients). Somatic variants in genes other than *DDX41* may also be present. According to the current literature, a small proportion of cases may only have somatic *DDX41* mutations.[6]

Somatic profiling of the tumor (hematopoietic tissue) can be performed in two ways: (1) tumor-only, where next-generation sequencing (NGS) analyzes patient's bone marrow aspirate or blood sample obtained at diagnosis; or (2) tumor-normal, *i.e.* simultaneous NGS analysis of both tumor and germline tissue samples (11).

- In both the tumor-only and tumor-normal approaches, patients should be informed about the potential for identifying hereditary gene alterations before testing.
- In a tumor-only setting, the detection of a *DDX41* variant with a variant allele frequency suggesting germline origin (near heterozygous) should prompt further investigation.
- To confirm or exclude germline origin, analysis of DNA obtained from cultured fibroblasts is recommended. However, other tissue sources for germline analysis may be used according to institutional policies and experience.

SUMMARY OF RECOMMENDATIONS

- *DDX41* should be included in the myeloid genetic panels used in the diagnostic work-up of patients with suspected AML and MDS.
- Patients with a verified germline *DDX41* variant should be referred for genetic counseling and to a hematology clinic with expertise in the management of patients with germline predisposition.
- IPSS-M should be used with caution in patients with MDS and germline *DDX41* variants
- Adult first-degree relatives of a patient with hematological disease and a verified germline *DDX41* variant should be offered testing at the age of 50 or 10 years before the earliest onset of MN occurring in the family.
- Carriers of germline *DDX41* variants should be excluded as hematopoietic stem cell donors.
- Active surveillance of asymptomatic carriers aims at early detection of potentially actionable hematological disorders and is indicated from the age of 50 or 10 years before the earliest onset of MN occurring in the family.
- Baseline hematological investigation should be offered at the time of identification of a *DDX41* germline variant.

FIGURE 1 Summary of recommendations. AML, acute myeloid leukemia; IPSS-M, Molecular International Prognostic Scoring System; MDS, myelodysplastic syndrome; MN, myeloid neoplasm.

- In a tumor-normal setting, we suggest a re-analysis of the germline sample for germline *DDX41* variants when a somatic *DDX41* variant is detected.

INTERPRETATION OF CONFIRMED GERMLINE *DDX41* VARIANTS AND GENETIC COUNSELING

- Upon confirmation of germline origin, the *DDX41* variant should be classified according to the American College of Medical Genetics criteria.[15] Variants classified as likely pathogenic/pathogenic (LP/P) can be used for predictive testing in relatives (see below).
- For now, since official international recommendations for the classification of *DDX41* variants are lacking, caution is advised when interpreting the presence of a pathogenic somatic *DDX41* variant as a criterion to upgrade a germline variant of unknown significance (VUS) to likely pathogenic, as proposed for other disorders.[16] Nonetheless, recent data suggest that such somatic findings could be used to modify the strength of current ACMG criteria PP4 to a very strong level.[17] Importantly, we strongly encourage discussion of such variants within an expert group and re-evaluation after 2 years from the initial investigation.
- The index patient should be referred for genetic counseling and to a hematology clinic with expertise in the management of patients with germline predisposition.
- We recommend collecting a three-generation family history and verifying cancer and cytopenia diagnoses in the family.
- Carriers of germline *DDX41* variants should receive counseling indicating dominant inheritance for a moderate-risk gene associated with cytopenias and/or MNs with late onset.[18,19] In a large international study, the penetrance for myeloid malignancies was estimated to be up to 10% by age 60 and about 50% by age 90,[6] with higher penetrance observed in males.[6] The typical age of onset for DDX41-associated MNs is often at the end of the seventh decade.[6]
- Emerging data suggest that truncating germline variants may be associated with a higher risk for AML transformation and increased penetrance.[6,7,14] However, for now, we recommend providing similar genetic counseling regardless of the variant's nature (e.g., missense vs. truncating).
- Adherence to national legislation and/or institutional policies regarding counseling for prenatal diagnostics and preimplantation genetic testing is advised. Relatives of childbearing age who are at risk for carrying the *DDX41* variant should be offered genetic counseling.
- We encourage participation in registries and/or academic studies investigating further the role of *DDX41* in hematological diseases.
- To exclude other causative predisposition variants, we recommend a comprehensive germline investigation according to the Nordic guidelines[20] for index patients with a germline *DDX41* variant and a diagnosis of MDS before the age of 50.

PREDICTIVE TESTING IN RELATIVES

- Adult first-degree relatives of a patient with hematological disease and a verified germline *DDX41* variant should be offered testing at least at the age of 50 years or 10 years before the earliest occurrence of MNs in the family, if surveillance is warranted (see below). This proposed age threshold can be adjusted based on the family history, individual psychosocial state, and preferences. Testing minors (below 18 years of age) for carrier status is not recommended.
- Caution should be exercised for testing second-degree relatives, especially in families lacking multiple cases of hematological malignancies. In such cases, we encourage discussion with an expert group.

SURVEILLANCE OF HEALTHY CARRIERS OF LP/P *DDX41* VARIANTS

- Asymptomatic carriers of germline LP/P *DDX41* variants should be offered a surveillance program.
- Active surveillance for asymptomatic carriers aims at early detection of a potentially actionable hematological disorder (e.g., MDS) and is indicated from the age of 50 years or 10 years before the earliest MN occurring in the family.
- A baseline investigation should be offered at the time of identification of the germline variant (Table 1).

The surveillance program facilitates proactive planning of clinical management, avoidance of unnecessary medication, and establishment of a channel of communication between the carrier and the health system. In addition, the surveillance program may address psychological issues related to cancer predisposition.

- The surveillance program should be performed by or in collaboration with a hematology clinic with expertise in the management of patients with germline predisposition to hematological neoplasms.
- When performed by the primary health care and in the presence of significant cytopenia, bone marrow dysplasia, or clonal evolution, the carrier should be referred to a specialized clinic.

ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION

- Carriers of germline *DDX41* variants should be excluded as donors in an allo-HSCT setting.
- The risk classification of MN and the decision to perform an allo-HSCT in patients with a germline *DDX41* variant should be based on the diagnosis-specific guidelines. According to the current knowledge, the presence of an LP/P germline *DDX41* variant is not considered an indication for allo-HSCT.
- Likely due to *DDX41*-specific features and biology, a higher Molecular International Prognostic Scoring System (IPSS-M) score tends to be assigned to patients with *DDX41* variants, and a recent large retrospective study suggested that the IPSS-M score did not enable significant prognostic discrimination in *DDX41*-mutated patients.[21] Hence, the IPSS-M should be used with caution in patients with MDS and germline *DDX41* variants.

Recent retrospective studies in cohorts of *DDX41*-mutated AML or MDS showed no significant survival benefit in patients who underwent allo-HSCT.[10,22] Prospective studies are warranted to confirm these findings. There are emerging data supporting a high risk for GVHD after allo-HSCT in patients with a germline *DDX41* variant.[13] These data still need to be confirmed in further studies.

DDX41 AS AN INCIDENTAL FINDING

- We advise that clinical management of patients with incidentally identified germline LP/P *DDX41* variants should be performed according to the institutional policy and national legislation of the

TABLE 1 Proposed baseline evaluation and surveillance program for carriers of an LP/P germline *DDX41* variant.

	At the identification of an LP/P germline *DDX41* variant	Follow-up from the age of 50 years or earlier if indicated (cytopenia and/or clonal abnormalities)
CBC and clinical assessment[a]	Yes	Every 12 months if previously normal CBC
Bone marrow aspirate/biopsy and cytogenetics	Only in the presence of aberrant CBC or previously identified somatic variants (clonal hematopoiesis)	If progression is suspected from CBC/clinical assessment/NGS[b] results
NGS panel of recurrently mutated genes in MN (including *DDX41*)	Yes (bone marrow or blood samples in case of aberrant or normal CBC, respectively)	Every second year (blood) if no signs of progression are detected[c]

Abbreviations: AML, acute myeloid leukemia; CBC, complete blood count; LP/P, likely pathogenic/pathogenic; MDS, myelodysplastic syndrome; MN, myeloid neoplasm; NGS, next-generation sequencing.

[a]Clinical assessment should include investigation for signs and symptoms of MDS/AML/other malignancies.

[b]Somatic myeloid panel according to the institutional policy.

[c]The emergence of a clone should warrant the exclusion of disease evolution, especially in the case of a somatic *DDX41* second hit. Please note that the interval between the NGS analysis may vary on individual basis.

respective country. For now, *DDX41* is not included in the ACMG list for reportable incidental findings.[23]

METHODOLOGY

These guidelines have been written by a multidisciplinary working group of active members of the Nordic MDS Study Group, a scientific and educational organization founded in 1984. The aim of this group is to improve management of patients with MDS and to facilitate MDS-related research. The working group mainly consisted of clinical geneticists and hematologists from the Nordic countries (Denmark, Finland, Norway, and Sweden) with clinical experience in diagnosing and managing patients with germline predisposition to myeloid malignancies.[20] The development of the recommendations was based on the best available evidence from the literature to November 2023 and expert consensus. The working group collectively formulated the recommendations, which were reviewed, further discussed, and revised at multiple meetings during 2022–2023 and approved in December 2023.

AUTHOR CONTRIBUTIONS

All authors took part in the discussions and meetings that led to the formulation of the guidelines. Panagiotis Baliakas, Bianca Tesi, Jörg Cammenga, and Ulla Wartiovaara-Kautto drafted the manuscript. All authors reviewed and approved the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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