Next-generation sequencing reveals the presence of DDX41 mutations in acute lymphoblastic leukemia and aplastic anemia

Yang Zhang1 | Fang Wang1 | Xue Chen1 | Hong Liu1 | Xiaoliang Wang1 | Jiaqi Chen1 | Panxiang Cao1 | Xiaoli Ma1 | Hongxing Liu1,2,3

1 | Division of Laboratory Medicine, Hebei Yanda Lu Daopei Hospital, Langfang, China
2 | Division of Laboratory Medicine, Beijing Lu Daopei Hospital, Beijing, China
3 | Beijing Lu Daopei Institute of Hematology, Beijing, China

Correspondence
Hongxing Liu, Beijing Lu Daopei Institute of Hematology, 22 South Tongji Road, Beijing, 100176, China.
Email: starliu@pku.edu.cn

Abstract
Limited studies have been described DEAD-box helicase 41 (DDX41) mutations in hematological diseases other than myeloid neoplasms. In this study, DDX41 mutations were identified in 0.8% of myeloid neoplasms, 0.9% of acute lymphoblastic leukemia (ALL), and 1.0% of aplastic anemia (AA). A total of 15 causal DDX41 variants in 14 patients were detected; seven of which have not been reported previously. In myeloid neoplasms, the median age of patients with germline missense was lower than that of germline nonsense mutations. In ALL, the characteristics of DDX41 mutation were distinct. This study first reported DDX41 mutations in ALL and AA, expanding its mutation and phenotypic spectrum.

KEYWORDS
acute lymphoblastic leukemia, aplastic anemia, DDX41 mutations, genetic predisposition

1 | INTRODUCTION

Myeloid neoplasms with germline DEAD-box helicase 41 (DDX41) mutation have been included in the 2016 revised World Health Organization (WHO) myeloid neoplasms classification as a new diagnostic category [1]. DDX41 mutation has been reported mainly in about 2%–5% of myeloid neoplasms [2–6], a few in chronic myeloid leukemia, lymphomas, multiple myeloma, sarcoidosis, and blastic plasmacytoid dendritic cell neoplasm [2,7]. However, limited studies are describing the mutation profile of DDX41 other than myeloid neoplasms in hematological disease. We analyzed the prevalence and characteristics of DDX41 mutations in an unselected cohort of patients with hematological disorders to advance our understanding of this gene.

2 | METHODS

2.1 | Patients

From February 2017 to May 2020, bone marrow and peripheral blood samples from 1753 patients who were admitted at Hebei Yanda Lu Daopei Hospital were collected, including 720 subjects with acute myeloid leukemia (AML), 91 with myelodysplastic syndromes (MDS), 41 with myeloproliferative neoplasms (MPN), 16 with MDS/MPN, 585 with B-cell acute lymphoblastic leukemia (B-ALL), 164 with T-cell ALL (T-ALL), 42 with mixed phenotype acute leukemia, and 94 with aplastic anemia (AA). The diagnosis criteria were referred to the 2016 World Health Organization classification of myeloid neoplasms and acute leukemia [1].

2.2 | Gene sequencing and mutation analysis

Mutational hotspots or whole coding regions of 86 genes (Table S1) known to be frequently mutated in hematologic malignancies (HMs) were sequenced using a targeted amplicon-based high-throughput sequencing protocol, as we previously reported [8]. DDX41 genes were tested for the whole coding region in this multigene panel. DDX41 germline variants were classified for pathogenicity according to the American College of Medical Genetics and Genomics guidelines [9], and acquisition of a somatic DDX41 mutation was also considered as a
firm criterion for causality. The fingernail specimens or blood samples in complete remission (CR) of acute leukemia were taken as a control to verify the possible germline origin.

2.3 Follow-up

The end of the follow-up period was December 30, 2020. CR was defined as morphologic CR. Overall survival (OS) is measured from the date of diagnosis to the date of death or the date of the last follow-up; relapse-free survival (RFS) is measured from the date of achievement of CR until the date of relapse, death, or to the date of the last follow-up.

2.4 Statistical analysis

Statistical analysis was performed using the SPSS 22.0 software, and chi-square or Fisher’s exact tests calculated the significance between categorical data. A p-value of <0.05 was considered statistically significant.

3 RESULTS

3.1 The characteristics of DDX41 mutations

A total of 29 DDX41 variants were identified, including 22 germline variants and seven somatic variants. The germline variants were classified as causal (n = 8) and uncertain significance (n = 14) (Table S2); the latter were excluded in this assay. Seven causal variants (K102Rfs*32, S104F, L193P, Q210*, R282C, R323H, R471W) have not been reported previously. All of the germline variants were located on or upstream of the DEAD domain. Somatic DDX41 variants occurred throughout the whole coding region in ALL, but in the myeloid neoplasm, 80% of them were hotspot R525H (Figure 1A).

3.2 DDX41 mutations in myeloid neoplasm

DDX41 mutations were identified in 0.8% of patients (six AML, one chronic myelomonocytic leukemia [CMML] with myeloid neoplasm. The median age was 59 years (range, 28–78 years), and 57% were male. None of the patients had del 5/5q. In six patients with AML, four cases (66.7%) were arising from previous MDS; one case had a history of thrombocytopenia for 2 years before diagnosed with AML. Two patients had a family history of anemia and leukemia, respectively. Germline nonsense and missense mutation were respectively detected in three patients with AML, five of which acquired a somatic DDX41 mutation (4 R525H, 1 T227M), the remaining one concomitant with five somatic gene mutations (CSF3R, IDH2, RUNX1, SH2B3, WT1) (Figure 1B). The median age of patients with germline missense was lower than that with germline nonsense mutations (37 years vs. 70 years, p = 0.100).

3.3 DDX41 mutations in ALL and AA

DDX41 mutations were identified in 0.9% of the ALL cohort (1.0% of B-ALL (n = 6), 0.6% of T-ALL (n = 1)). The median age was 9 years (range, 4–27 years), and 56% were male. None of the patients had a family history of hematological malignancy and del 5/5q. Somatic (four missenses, one nonsense) and germline DDX41 mutations (two missenses) were respectively detected in five and two patients, which appeared to be mutually exclusive because no biallelic mutations were detected (Figure 1B).

One case of AA in this cohort was observed with DDX41 mutations. He was diagnosed with AA at eight years old and treated with cyclosporin A for five years. Then he developed a drop in blood cell count, abdominal pain, and dark-colored urine. Flow cytometry showed that the paroxysmal nocturnal hemoglobinuria (PNH) clone size in red blood cells was 77.2%, and in granulocyte was 96.7%. DDX41 germline mutation (R159*, variant allele frequency [VAF] 50%) and PIGA mutations (c.1188+1G > A, VAF 36%; L110Cfs*13, VAF 14%) were identified. The diagnosis was modified to AA-PNH syndrome. His parents are young (36 years) and healthy, without a family history of tumors.

3.4 Prognosis of patients with DDX41 mutations

Among six patients with AML, two received treatment, and the rest were abandoned after diagnosis. One relapsed after hematopoietic stem cell transplantation (HSCT) and received the second HSCT; the other one received 10 courses of chemotherapy and showed continuous no remission, then abandoned. Among five patients with ALL carried somatic DDX41 mutation, three relapsed and the median RFS was 21 months; four received HSCT (Table 1). Only one of ALL patients with germline DDX41 mutation was available to follow-up, which was continued to be minimal residual disease positive and then received HSCT. The patient with AA-PNH syndrome eventually received HSCT due to persistent, recurrent abdominal pain and dark-colored urine, and the need for platelet and red blood cell transfusion. At the time of this writing, the disease-free survival was 9 months after transplantation.

4 DISCUSSION

Previous studies have reported that DDX41 mutations were identified in 3% of families with suspected inherited HMs [2]. In 3.1% of Han Chinese patients with myeloid neoplasms [4], in 5.5% of Thai patients with myeloid neoplasms [3]. In this cohort, DDX41 mutations were found only in 0.8% of patients with myeloid neoplasms. It might be related to the composed of mainly primary AML patients in this cohort. The median age of this cohort is 28 years, which is lower than the mean age of onset (62 years) of HMs that have been reported [2]. However, our results also support that most AML cases who were carrying DDX41 mutations have an MDS history and are older.
**FIGURE 1**  (A) Schematic diagram of DDX41 mutations in hematological disease. Germline mutations are in the upper part and somatic mutations in the lower part. The number of dots represents the case number of the mutation observed in this cohort. The color of the dots indicates the mutation type: black is a truncating, and green is a missense mutation. The variants in red color were detected in ALL. (B) The DDX41 and concomitant mutations in each patient with myeloid neoplasms, ALL, and AA. Row in the graph represents individual mutated genes, and columns represent individual patients.
| Number | Age (years) | Gender | Diagnosis            | Cytogenetics                                      | Fusion gene | DDX41 mutation | Somatic (VAF) | Treatment       | Status       | Time of OS (m) | Time of DFS (m) |
|--------|-------------|--------|----------------------|--------------------------------------------------|-------------|----------------|--------------|----------------|--------------|---------------|----------------|
| 1      | 70          | M      | AML-MRC              | Normal karyotype                                 | Negative    | Anemia         | R159*       | R525H (17)     | Palliative   | Deceased      | 33             |               |
| 2      | 78          | F      | Primary AML          | Normal karyotype                                 | Negative    | No             | Q210*       | R525H (10)     | Palliative   | Loss to follow-up | –             | –             |
| 3      | 59          | M      | AML-MRC              | Normal karyotype                                 | Negative    | No             | Y259C       | T227M (14)     | Palliative   | Alive         | 9              |               |
| 4      | 68          | M      | AML-MRC              | Normal karyotype                                 | Negative    | No             | Q41*        | R525H (3)      | Palliative   | Loss to follow-up | –             | –             |
| 5      | 37          | M      | AML-MRC              | 46,XY,add(9)(p22)[20]                             | Negative    | No             | P258L       | R525H (15)     | Chemotherapy, HSCT | Deceased      | 74             | 35             |
| 6      | 28          | F      | primary AML          | 46,XX,-12der(22)t(12;22)                          | Negative    | Leukemia       | R282C       | Chemotherapy   | Deceased      | 31             |               |
| 7      | 56          | F      | CMML                 | 46,XX(t11;19)[q23;p13.1][2]/46,XX[37]             | MLL-ELL     | No             | K102Rfs+3   | Chemotherapy   | Alive         | 11             |               |
| 8      | 9           | F      | B-ALL                | 48,XY,+del(1)(p12p21), del(4),+8,add(9)(p24),+t(121)[p21q11.2],+22[20] | Negative    | No             | P258L       | Not available  | Loss to follow-up | –             | –             |
| 9      | 6           | F      | B-ALL                | Normal karyotype                                 | BCR-ABL1    | No             | R339C       | Chemotherapy, HSCT | Alive         | 19             |               |
| 10     | 7           | M      | B-ALL                | Normal karyotype                                 | TEL-AML1    | No             | S104F (35)  | Chemotherapy, HSCT | Alive         | 71             | 28             |
| 11     | 9           | M      | B-ALL                | 46,XY,t(17;19)[q22;p13.3][4]/46,XY(7]             | E2A-HLF     | No             | L193P (48)  | Chemotherapy, CAR-T, HSCT | Alive         | 17             | –             |
| 12     | 4           | F      | B-ALL                | Normal karyotype                                 | Negative    | No             | R323H (43)  | Chemotherapy   | Alive         | 25             |               |
| 13     | 16          | M      | B-ALL                | Normal karyotype                                 | E2A-PBX1    | No             | R471W (6)   | Chemotherapy, CAR-T, HSCT | Deceased      | 25             | 12             |
| 14     | 27          | M      | T-ALL                | 46,XY,der(1)[t1;1][p36.1;q25], del(4)[q23],del(6)[p23],inv(7)[q21q2] | Negative    | No             | R369* (40)  | Chemotherapy, HSCT | Alive         | 54             | 21             |

Abbreviations: AML-MRC, AML with myelodysplasia-related changes; DFS, disease-free survival; F, female; M, male; m, months; OS, overall survival; VAF, variant allele frequency.
There are a few reports of DDX41 mutations in lymphoid malignancies; it was speculated that dysregulation of the innate immune response might be linked to lymphoid malignancy [2]. However, it has not been reported in ALL. We identified DDX41 mutations in 0.9% of patients with ALL, and the characterize was distinct from myeloid tumors; with a young age of onset, somatic and germline DDX41 variants appeared to be mutually exclusive, the majority (80.0%) of the somatic variants were located on or upstream of the DEAD domain. It suggested that the pathogenesis of DDX41 mutations in ALL may differ from myeloid tumors, which merits further study.

Compared with other myeloid neoplasm predisposition syndromes, patients with germline DDX41 mutations are older at the time of presentation [10,11]. Loss of function (LOF) germline DDX41 mutations is by far most prevalent. However, the age of onset at different mutation sites may differ, as suggested by some reports. Missense germline mutations in the helicase C domain of DDX41 have been observed to cause an earlier onset disease than those with LOF mutations [2]. Consistent with previous reports, we also observed that patients with a missense germline DDX41 mutation have a lower onset age than nonsense germline mutation carriers (37 years vs. 70 years). The notable difference from the previous is that the missense mutation we observed is located at the N-terminal DEAD-box domain. It indicates that patients with DDX41 missense mutations have an early age of onset, which is irrelevant to the domain where it occurs.

The p. D140fs or p.M1I variants were previously reported as the most common germline DDX41 mutations in the Caucasian population [2,5]. However, it is rare in East and Southeast Asian populations [3,4], neither was detected in this cohort. It is indicating that distinct ethnically associated with recurrent germline mutations. Approximately 50% of patients with germline DDX41 mutations also harbor somatic mutations in the other allele as a double hit event during progression to MDS or AML [5,12] with the missense mutation p.R525H being most common [13,14]. However, we and others have observed that individuals with single germline DDX41 mutations also progressed to hematological tumors [15]. In this cohort, we found one patient with AML concomitant with CSF3R, IDH2, RUNX1, and WT1 mutations, one patient with CMML concurrent with the MLL-ELL fusion gene. We speculate that these mutations or fusion genes may interact with germline DDX41 variants in the development of myeloid neoplasms.

In conclusion, we first reported the DDX41 mutations in ALL and AA, which have distinct characteristics, thereby expanding the mutation and phenotypic spectrum of DDX41 mutations. Moreover, the genotype-phenotype correlations regarding DDX41 mutations should be clarified more specifically in the future.

ACKNOWLEDGMENTS
We thank all the patients in this study. We acknowledge all the technicians working in the Laboratory Medicine Division of Hebei Yanda Lu Daopei Hospital.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Hongxing Liu designed the research and critically revised the paper. Yang Zhang performed the research and wrote the paper. Fang Wang and Xue Chen supervised clinical and experimental findings. Hong Liu, Xiaoliang Wang, Jiaqi Chen, and Xiaoli Ma analyzed the data. Panxiang Cao performed bioinformatics analysis. All the authors read and approved the final version of the manuscript.

ETHICS APPROVAL
This study was approved by the Institutional Review Board and Ethical Committee of the Hebei Yanda Lu Daopei Hospital.

PATIENT CONSENT STATEMENT
Written informed consent was obtained from all the patients.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID
Yang Zhang https://orcid.org/0000-0001-5794-5185
Panxiang Cao https://orcid.org/0000-0002-6432-1773

REFERENCES
1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
2. Lewinsohn M, Brown AL, Weinel LM, Phung C, Rafidi G, Lee MK, et al. Novel germline DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. Blood. 2016;127(4):1017–23.
3. Polprasert C, Takeda J, Niparuck P, Rattanathammethe T, Pirunsarn A, Sukussut A, et al. Novel DDX41 variants in Thai patients with myeloid neoplasms. Int J Hematol. 2020;111(2):241–6.
4. Qu S, LiB, Qin T, XU Z, Pan L, Hu N, et al. Molecular and clinical features of myeloid neoplasms with somatic DDX41 mutations. Brit J Haematol. 2020;192(6):1006–10.
5. Polprasert C, Schulze I, Sekeres MA, Makishima H, Przychodzen B, Hosono N, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. Cancer Cell. 2015;27(5):658–70.
6. Sébert M, Passet M, Raimbault A, Rahmé R, Raffoux E, Sicre De Fontbrune F, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. Blood. 2019;134(17):1441–4.
7. Diness BR, Risom L, Frandsen TL, Hansen B, Andersen MK, Schmiegelow K, et al. Putative new childhood leukemia cancer predisposition syndrome caused by germline bi-allelic missense mutations in DDX41. Genes Chromosomes Cancer. 2018;57(12):670–4.
8. Zhang Y, Wang F, Chen X, Zhang Yu, Wang M, Liu H, et al. CSF3R mutations are frequently associated with abnormalities of RUNX1, CBFB, CEBPA, and NPM1 genes in acute myeloid leukemia. Cancer. 2018;124(16):3329–38.
9. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–23.
10. Weinberg OK, Kuo F, Calvo KR. Germline predisposition to hematolymphoid neoplasia. Am J Clin Pathol. 2019;152(3):258–76.
11. Bannon S, Dinardo C. Hereditary predispositions to myelodysplastic syndrome. Int J Mol Sci. 2016;17(6):838.
12. Maciejewski JP, Padgett RA, Brown AL, Müller-Tidow C. DDX41-related myeloid neoplasia. Semin Hematol. 2017;54(2):94–7.
13. Kadono M, Kanai A, Nagamachi A, Shinriki S, Kawata J, Iwato K, et al. Biological implications of somatic DDX41 p.R525H mutation in acute myeloid leukemia. Exp Hematol. 2016;44(8):745–54.e4.
14. Quesada AE, Routbort MJ, Dinardo CD, Bueso-Ramos CE, Kanagal-Shamanna R, Khoury JD, et al. DDX41 mutations in myeloid neoplasms are associated with male gender, TP53 mutations and high-risk disease. Am J Hematol. 2019;94(7):757–66.
15. Berger G, Van Den Berg E, Sikkema-Raddatz B, Abbott KM, Sinke RJ, Bungener LB, et al. Re-emergence of acute myeloid leukemia in donor cells following allogeneic transplantation in a family with a germline DDX41 mutation. Leukemia. 2017;31(2):520–2.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Zhang Y, Wang F, Chen X, Liu H, Wang X, Chen J, et al. Next-generation sequencing reveals the presence of DDX41 mutations in acute lymphoblastic leukemia and aplastic anemia. eJHaem. 2021;1–6.
https://doi.org/10.1002/jha2.256