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Advances in predisposition to bone marrow failure and hematopoietic neoplasms

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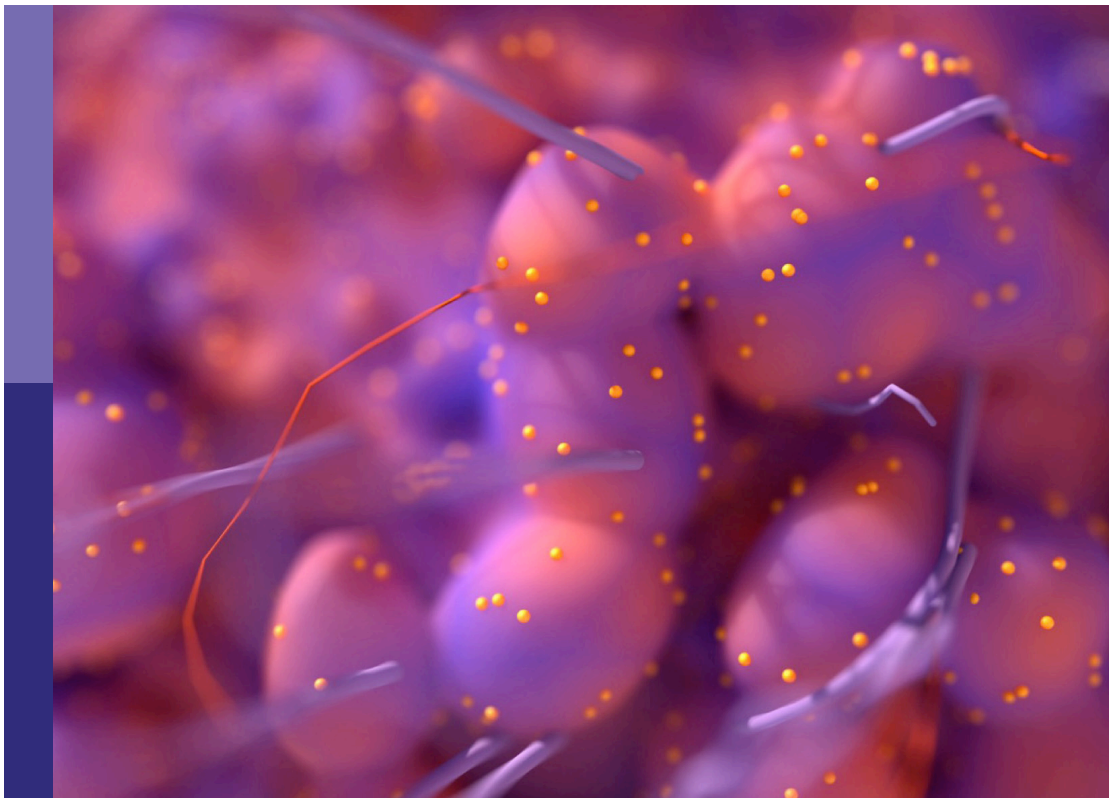

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Advances in predisposition to bone marrow failure and hematopoietic neoplasms

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Editorial: Advances in predisposition to bone marrow failure and hematopoietic neoplasms

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Editorial on the Research Topic

Advances in predisposition to bone marrow failure and hematopoietic neoplasms

1 Introduction

Recent advances in genomic techniques have increasingly associated germline predisposition to hematopoietic malignancies (1). Inherited bone marrow failure syndromes (IBMFS) including Fanconi anemia (FA), Diamond-Blackfan anemia (DBA), Schwachman Diamond syndrome (SDS), and telomere biology disorders (TBD) are recognized as distinct hereditary blood disorders associated with a higher risk of developing a hematologic neoplasm. This list also includes the newly characterized group of myeloid neoplasms with bone marrow failure (BMF), caused by mutations in *GATA2*, *CEBPA*, *DDX41*, *RUNX1*, *ANKDR26*, *ETV6*, *SAMD9*, *SAMD9L*, and *ERCC6L2* (2–4). The progression from BMF to malignancy is a continuum influenced by both germline and additional acquired genetic events (5). Extensive studies on both pediatric and adult patient cohorts have illustrated certain somatic alterations to be associated with unfavorable clinical outcomes and decreased overall survival, underscoring the importance of early detection for effective patient management and therapeutic intervention (6–12). Once malignant transformation has occurred, hematopoietic stem cell transplantation (HSCT) often represents the only curative approach specifically in the setting of a preexisting germline predisposition, and presents with many challenges including of donor selection, conditioning regimen related toxicities, infections and post-HSCT complications (13, 14). Hence to augment the current treatment strategies, ongoing research efforts are focused on delineating the molecular dysregulation associated with BMF and leukemia progression to inform novel druggable pathways (15). Additionally,

novel gene-editing approaches for gene correction are being explored as potential strategies to enable early interception prior to development of a hematologic neoplasm (16, 17).

The Research Topic titled "*Advances in Predisposition to Bone Marrow Failure and Hematopoietic Neoplasms*" covers many aspects of this increasingly appreciated clinical and basic scientific field (Figure 1). This editorial serves as a spotlight to encompass these contributions, aiming to inspire further advancements and collaboration in the field.

2 Composition

Based on the scientific focus, 10 carefully selected articles were published as part of this Research Topic collection and are categorized to the following:

2.1 Diagnosis

With the interplay of germline and somatic genetics being increasingly recognized as a critical factor for the biology of various hematopoietic malignancies, adequate detection of both is critical. Towards that, in a review, Godley described the combination of germline genetic testing with tumor-based profiling in blood cancers for accurate risk assessment and treatment planning. The original research article by Perani et al. complimentarily highlights the importance of using appropriate biological samples to confirm germline variants, to enable adequate treatment planning early on. They compared sequencing data from bone marrow, blood, saliva, skin fibroblasts and hair follicles in a group of 29 patients and 44 relatives. The study revealed limitations in saliva testing due to tumor cell infiltration, while hair follicle DNA extraction showed potential as an alternative to skin biopsy.

2.2 Genetics

Over 100 genes have been associated with inherited BMF, and continued research is needed to fully understand their role in disease development and progression. Feurstein conducted a comprehensive review on four recently identified BMF syndromes involving genes *ERCC6L2*, *MECOM*, *DNAJC21*, and *ADH5/ALDH2*. Additionally, a new gene, called Replication protein A1 (*RPA1*) has recently been identified to cause TBD. In a brief research report, Sharma et al. found significant enrichment of novel and ultra-rare germline RPA1 variants in solid tumors, brain cancer, and hematological malignancies, suggesting a potential link between *RPA1* variants and predisposition to pediatric cancer.

2.3 Therapy

Treating BMF and hematopoietic malignancies that arise in the background of an underlying germline predisposition syndrome remains a complex and evolving topic. The mini-review by Bhoopalan et al. discusses important considerations for DBA patients and caregivers when deciding on HSCT. Factors like age, transfusion dependence, steroid response, and iron overload should be considered for eligibility. Gene correction by lentiviral vectors with *GATA1* gene overexpression or CRISPR/Cas9are promising alternative strategies. However, practical obstacles like limited access to stem cells, long-term efficacy and safety need to be addressed through further research and clinical trials. The second mini-review by Toya et al. specifically delves into adult-onset hereditary myeloid malignancies (HMM). The review highlights the challenges related to diagnosis, optimal treatment strategies, uncertainties regarding the timing and indication for HSCT, the risk of donor cell leukemia, and the absence of a recommended conditioning regimen for HMMs, emphasizing the need for further research to improve patient management in this area.

2.4 Pathomechanism

An important aspect of this Research Topic is exploring dysregulated molecular pathways in BMF and blood cancers for potential targeted therapy. This topic is covered by three systematic reviews and one original research article. The first review by Shinriki and Matsui provides an overview of *DDX41* mutations, which are found in approximately 2-5% of acute myeloid leukemia and myelodysplastic syndrome (MDS) patients. The review explores the role of DDX41 in various processes such as RNA splicing, DNA sensing in innate immunity, R-loop regulation, ribosome biogenesis, and translation. It also highlights the need for alternative treatments

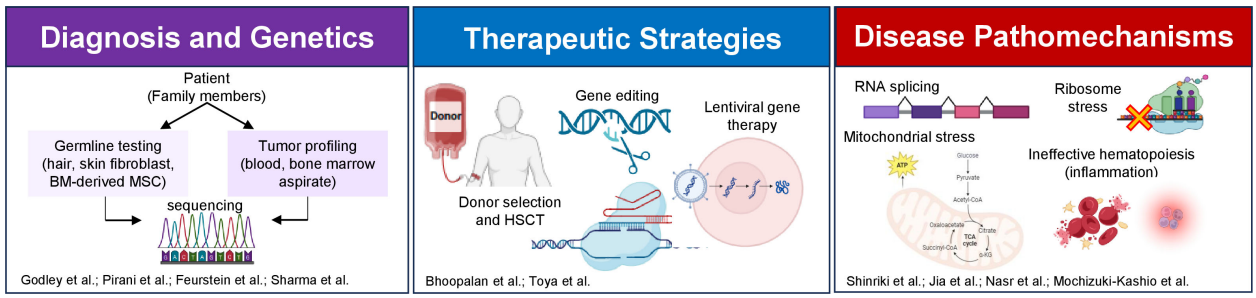

FIGURE 1
An overview of the Research Topic articles focusing on diagnosis, genetics, therapy and pathomechanism of predisposition to bone marrow failure and hematopoietic neoplasms. The figure is created using BioRender. BM, bone marrow; MSC, mesenchymal stem cells; HSCT, hematopoietic stem cell transplantation.

utilizing synthetic lethality, particularly for elderly patients who have difficulty with traditional cytotoxic chemotherapy. The second review by Jia and Gu discusses *PAX5* gene alterations and their impact on B-cell acute lymphoblastic leukemia. The third review by Nasr and Filippi highlights the emerging role of mitochondria in the development of BMF and MDS, emphasizing the impact of abnormal mitochondrial metabolism, dynamics and reactive oxygen species on ineffective hematopoiesis. In the final article of this Research Topic, Mochizuki-Kashio et al. conducted an original research exploring the effects of replication stress (RS) on mitochondrial function in a *Fancd2*-deficient FA mice model. The study revealed RS to affect mitochondrial activity and mitophagy in *Fancd2*-deficient fetal hematopoietic stem cells (HSCs) and adult bone marrow HSCs, pointing to mitochondrial metabolism defect in FA pathophysiology.

3 Conclusions and perspectives

The risk of malignancy in both inherited and acquired BMF disorders is shaped by disease specific genetics and associated cellular changes. This Research Topic has significantly raised awareness of the current advances in the diagnosis, molecular understanding and development of therapeutic strategies of BMF syndromes. Although progress has been made in identifying modifiable risk factors and developing targeted therapies, there is still significant work to do. To advance diagnosis, treatment, and patient outcomes in terms of overall survival and quality of life, it is crucial to integrate basic research with "omics" studies conducted on well-annotated clinical samples. This collaborative approach will pave the way for improvements in managing BMF and delivering better care to patients.

Author contributions

SS: Writing – original draft, Writing – review & editing. SA: Writing – review & editing. MM-K: Writing – review & editing. LW: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Gurbuxani S, Hochman MJ, DeZern AE, Shimamura A. The times, they are A-changing: the impact of next-generation sequencing on diagnosis, classification, and prognostication of myeloid Malignancies with focus on myelodysplastic syndrome, AML, and germline predisposition. *Am Soc Clin Oncol Educ Book* (2023) 43:e390026. doi: 10.1200/EDBK_390026

2. Furutani E, Shimamura A. Germline genetic predisposition to hematologic Malignancy. *J Clin Oncol* (2017) 35:1018–28. doi: 10.1200/JCO.2016.70.8644

3. Klco JM, Mullighan CG. Advances in germline predisposition to acute leukaemias and myeloid neoplasms. *Nat Rev Cancer* (2021) 21:122–37. doi: 10.1038/s41568-020-00315-z

4. Sahoo SS, Kozyra EJ, Wlodarski MW. Germline predisposition in myeloid neoplasms: Unique genetic and clinical features of GATA2 deficiency and SAMD9/SAMD9L syndromes. *Best Pract Res Clin Haematol* (2020) 33:101197. doi: 10.1016/j.beha.2020.101197

5. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up. *Haematologica* (2018) 103:30–9. doi: 10.3324/haematol.2017.178111

6. Sahoo SS, Pastor VB, Goodings C, Voss RK, Kozyra EJ, Szvetnik A, et al. Clinical evolution, genetic landscape and trajectories of clonal hematopoiesis in SAMD9/SAMD9L syndromes. *Nat Med* (2021) 27:1806–17. doi: 10.1038/s41591-021-01511-6

7. Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med* (2011) 365:1384–95. doi: 10.1056/NEJMoa1103283

8. Graubert TA, Shen D, Ding L, Okeyo-Owuor T, Lunn CL, Shao J, et al. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. *Nat Genet* (2011) 44:53–7. doi: 10.1038/ng.1031

9. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* (2014) 28:241–7. doi: 10.1038/leu.2013.336

10. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* (2011) 478:64–9. doi: 10.1038/nature10496

11. Makishima H, Yoshizato T, Yoshida K, Sekeres MA, Radivoyevitch T, Suzuki H, et al. Dynamics of clonal evolution in myelodysplastic syndromes. *Nat Genet* (2017) 49:204–12. doi: 10.1038/ng.3742

12. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* (2017) 129:424–47. doi: 10.1182/blood-2016-08-733196

13. Peffault de Latour R. Transplantation for bone marrow failure: current issues. *Hematol Am Soc Hematol Educ Program* (2016) 2016:90–8. doi: 10.1182/asheducation-2016.1.90

14. Mehta PA, Rotz SJ, Majhail NS. Unique challenges of hematopoietic cell transplantation in adolescent and young adults with hematologic Malignancies. *Biol Blood Marrow Transplant* (2018) 24:e11–9. doi: 10.1016/j.bbmt.2018.09.011

15. Groarke EM, Calado RT, Liu JM. Cell senescence and Malignant transformation in the inherited bone marrow failure syndromes: Overlapping pathophysiology with therapeutic implications. *Semin Hematol* (2022) 59:30–7. doi: 10.1053/j.seminhematol.2022.01.003

16. Ferrari G, Thrasher AJ, Aiuti A. Gene therapy using haematopoietic stem and progenitor cells. *Nat Rev Genet* (2021) 22:216–34. doi: 10.1038/s41576-020-00298-5

17. Bueren JA, Quintana-Bustamante O, Almarza E, Navarro S, Rio P, Segovia JC, et al. Advances in the gene therapy of monogenic blood cell diseases. *Clin Genet* (2020) 97:89–102. doi: 10.1111/cge.13593

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Unique role of DDX41, a DEAD-box type RNA helicase, in hematopoiesis and leukemogenesis

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In myeloid malignancies including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), patient selection and therapeutic strategies are increasingly based on tumor-specific genetic mutations. Among these, mutations in *DDX41*, which encodes a DEAD-box type RNA helicase, are present in approximately 2–5% of AML and MDS patients; this disease subtype exhibits a distinctive disease phenotype characterized by late age of onset, tendency toward cytopenia in the peripheral blood and bone marrow, a relatively favorable prognosis, and a high frequency of normal karyotypes. Typically, individuals with a loss-of-function germline *DDX41* variant in one allele later acquire the p.R525H mutation in the other allele before overt disease manifestation, suggesting that the progressive decrease in DDX41 expression and/or function is involved in myeloid leukemogenesis.RNA helicases play roles in many processes involving RNA metabolism by altering RNA structure and RNA-protein interactions through ATP-dependent helicase activity. A single RNA helicase can play multiple cellular roles, making it difficult to elucidate the mechanisms by which mutations in *DDX41* are involved in leukemogenesis. Nevertheless, multiple DDX41 functions have been associated with disease development. The enzyme has been implicated in the regulation of RNA splicing, nucleic acid sensing in the cytoplasm, R-loop resolution, and snoRNA processing.Most of the mutated RNA splicing-related factors in MDS are involved in the recognition and determination of 3' splice sites (SS), although their individual roles are distinct. On the other hand, DDX41 is likely incorporated into the C complex of the spliceosome, which may define a distinctive disease phenotype. This review summarizes the current understanding of how DDX41 is involved in this unique myeloid malignancy.

KEYWORDS

acute myeloid leukemia, myelodysplastic syndromes, RNA helicase, DDX41, R-loop, RNA splicing, nucleic acid sensor

Introduction

Recent advances in comprehensive genomic analysis for malignancies including hematopoietic tumors has elucidated most of the driver gene mutations involved in the disease development or progression (1, 2). Analysis of a large number of samples has also led to the identification of low-frequency mutations that had previously been overlooked. With regard to hematopoietic malignancies, it is now clear that low-frequency germline mutations may drive pathology in tumors that were previously thought to arise *via* unknown mechanisms (3, 4). Based on these findings, the WHO classification of myeloid malignancies was updated in 2016 to introduce the concept of disease classification based on somatic and germline gene mutations (5). The discovery in 2015 that *DDX41* mutations are found in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) is relevant to this revision. In brief, Polprasert et al. performed a comprehensive genetic analysis of families with suspected inherited myeloid malignancies without known mutations such as *RUNX1*, *CEBPA*, and *GATA2* and isolated *DDX41* as a new disease-associated gene (4). This was the first example of mutation of an RNA helicase-encoding gene in hematopoietic malignancies; the *DDX41* mutations are found in both MDS and *de novo* AML cases that does not exhibit non-hematopoietic phenotypes, and are generally characterized by the absence of marked thrombocytopenia before overt disease manifestation.

As will be discussed later, *DDX41* encodes a DEAD-box type RNA helicase, which plays important roles in biological processes related to RNA metabolism. DDX41 performs these roles by converting RNA structure and changing interactions between RNA and proteins in an ATP-dependent manner (6–8). Several recent large clinical studies, including a prospective investigation, have established the clinical characteristics of myeloid malignancies with *DDX41* mutations (9–18). Of note, heterozygous germline *DDX41* variants cause mild defects in hematopoiesis; subsequent acquisition of a somatic mutation in the remaining wild-type allele at a different location from that of germline variants results in biallelic alteration, which is a requirement for a disease-developing driver mutation (Figure 1) (9, 10).

Although RNA helicase mutations have been reported sporadically in a number of malignancies, examples of driver mutations are quite limited. Two examples are mutation of *DHX15* in core binding factor leukemia (19, 20) and mutation or splice variants of *DHX34* in AML (3, 21). Largely however, the significance of *DDX41* mutations in disease pathogenesis remains poorly understood. In this review, we will present the clinical features of hematopoietic malignancies associated with *DDX41* mutations and discuss the molecular functions of DDX41.

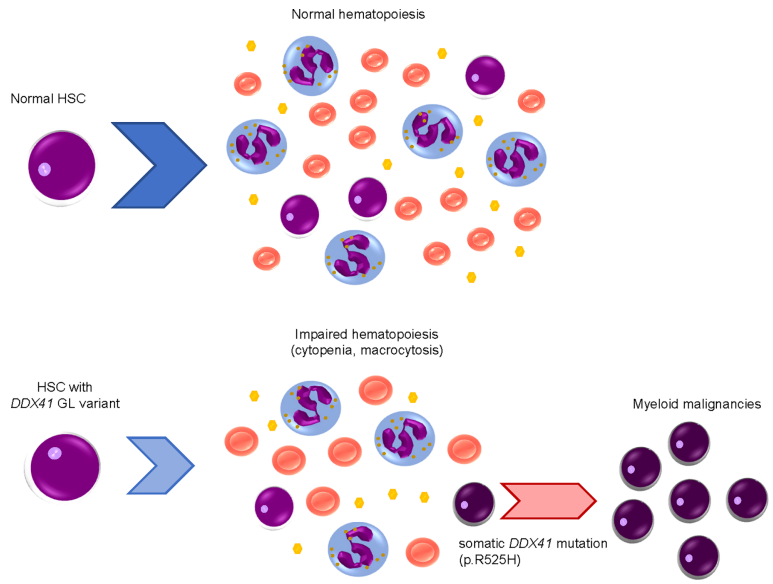

FIGURE 1
Development of myeloid malignancies by acquisition of DDX41 mutations in a stepwise manner.Hematopoietic stem cells (HSCs) with a heterozygous germline (GL) *DDX41* variant have mildly impaired hematopoiesis, including cytopenia and macrocytosis. Somatic *DDX41* mutation in the remaining wildtype allele later emerges within HSCs with a GL *DDX41* variant, which will lead to overt disease development.

Clinical features of myeloid malignancies with *DDX41* mutations

DDX41 mutations occur at a rate of 2 to 5% in AML (9, 10). Most affected patients are in their 60s and are therefore not markedly different to non-*DDX41* mutant sporadic AML cases with regard to peak age of disease diagnosis. The male-to-female ratio is around 3:1, suggesting a male predominance of the disease (22), but the reason for this is unclear. There does not appear to be a bias toward a specific AML subtype, but the disease is often characterized by low peripheral WBC counts and bone marrow hypoplasia, and a less differentiated tumor cell phenotype. However, there have been no reports of specific mutations in other genes that could contribute to these features of *DDX41* mutant AML (9). *DDX41* mutations are also observed in acute erythroid leukemia and lymphoid malignancies (23–25). Although solid tumors are sometimes found in patients with hematological malignancies and germline *DDX41* variants (26), an association between *DDX41* variants and onset of non-hematological maligancies has not been definitively shown.

As mentioned in the introduction, individuals with a germline *DDX41* variant acquire a somatic mutation before overt disease manifestation. The exact rate at which individuals with germline variants develop hematopoietic malignancies is still uncertain. However, 50–88% of MDS/AML patients with germline *DDX41* variants develop disease with somatic mutation (18). The fact that somatic *DDX41* mutations are the most frequent concomitant mutation with germline *DDX41* variants demonstrates that *DDX41* alterations are clearly linked to the disease etiology. The most frequent germline variant in the gene is p.D140fs, followed by p.M1I (27). Although the genomic positions at which germline variants occur may vary by race (28–31), the wide range of nonsense and frameshift mutations, especially in the N-terminal portion of the gene, strongly suggests that germline *DDX41* variants are loss-of-function type mutations. On the other hand, somatic mutations are highly concentrated in p.R525H, and less prevalently in p.G530D (14, 15). These somatic mutations are located within the helicase domain where ATP interacts with DDX41 (32), suggesting that somatic mutations interfere with the ATP-dependent helicase activity of the enzyme. Indeed, our previous study showed lower ATPase activity of the helicase domain with the p.R525H mutation (33). The reasons underlying the differential position of germline variants versus somatic mutations are not clear. However, the p.R525H mutation likely generates a hypomorphic protein that retains RNA-binding activity but has low helicase activity, which inhibits RNA and RNA/protein conformational conversion. Individuals with a germline variant sometimes manifest cytopenia and macrocytosis in the peripheral blood, and are thus likely to be diagnosed with idiopathic cytopenia of undetermined significance (ICUS) (34). This suggests that a 50% reduction of DDX41 expression or function affects hematopoiesis to some degree, but that this level does not impair the enzyme sufficiently to cause myeloid malignancies.

Recently, a large prospective study for AML with germline *DDX41* variants revealed that the response to conventional chemotherapy for the patients is relatively good, although relapse at 3 years post-treatment is comparable to that of patients with wild-type DDX41 (18). In addition, germline testing for *DDX41* before conducting allogeneic hematopoietic stem cell transplantation (27) should be conducted to reduce the potential risk of donor-derived leukemia (35–38).

As will be discussed later, AML cells with *DDX41* mutations tend to display an excessive DNA damage response, which may be due to the accumulation of DNA replication stress. On this basis, treatment of the disease with ATR inhibitors has been suggested (39), but to our knowledge, no clinical trials have yet been conducted. A few case reports suggested that lenalidomide may be efficacious in MDS with *DDX41* mutations, which could be related to the localization of *DDX41* in chr.5q35, which is likely to be deleted in the 5q- subset of patients (40, 41). However, no clinical trials have been conducted to test this hypothesis, possibly due to the relatively small number of patients available.

Molecular function of DDX41

'Helicase' is the general term for enzymes that alter the tertiary structure of nucleic acids (both DNA and RNA) and proteins in this class are categorized into the SF1 to SF6 superfamilies (42). The SF2 superfamily is the largest, and contains the DEAD-box type RNA helicases, of which DDX41 is a member. There are 41 and 25 DEAD-box type RNA helicases in humans and budding yeast (*Saccharomyces cerevisiae*), respectively. They play multiple celluar roles including those involved with transcription, RNA splicing, ribosome biogenesis, and translation (32, 43, 44). RNA helicases are also regulators of genome stability (45). Single RNA helicases often play multiple roles, posing a challenge with regard to elucidation of the disease-relevant activities of the enzymes. DEAD-box type RNA helicases are named after a motif consisting of Asp-Glu-Ala-Asp (D-E-A-D) amino acids within RecA-like domain 1; they are often comparatively discussed with DEAH-box type RNA helicases (46). Although these RNA helicases both unwind RNA duplex or alter RNA-protein interactions *via* their ATPase activities, the molecular mechanisms employed to carry out this function are not shared by the two groups (47). Specifically, DEAD-box type RNA helicases recognize and unwind short RNA duplexes in an ATP-dependent manner, while DEAH-box type helicases form a tunnel through the RecA, Winged-helix (WH), Helix-bundle (HB) and Oligosaccharide-binding (OB) domains at their C-termini, where they translocate on the RNA by gripping the RNA in the tunnel. DEAH-box helicases may also alter the structure of RNA or the spliceosome by winding up RNA in a 'winch-like' manner (46). In

the following sections, we discuss the roles of DDX41 that have been proposed in the literature to date.

RNA splicing

Comprehensive analysis for factors that constitute the spliceosome at each phase of RNA splicing have suggested that DDX41 is a component of splicing C complex (48, 49). RNA splicing occurs through two consecutive trans-esterification reactions (Figure 2A) (50); in the first process, the 2'-OH of adenine at the branch site attacks the 5' splice site (SS) and cleaves the RNA, forming a 2',5'-phosphodiester bond to create an intron lariat. This structure is called the C complex. In the second process, the 3'-OH at the 3' end of the free 5' exon attacks the 3'SS and cleaves the RNA, forming a phosphodiester bond between the 3'-OH terminus of the 5'-exon and the 5'-P terminus of the 3'-exon, leaving the intron lariat in the vicinity of the ligated exons. This structure is the P complex. RNA splicing involves five types of small nucleolar RNPs (snRNPs)

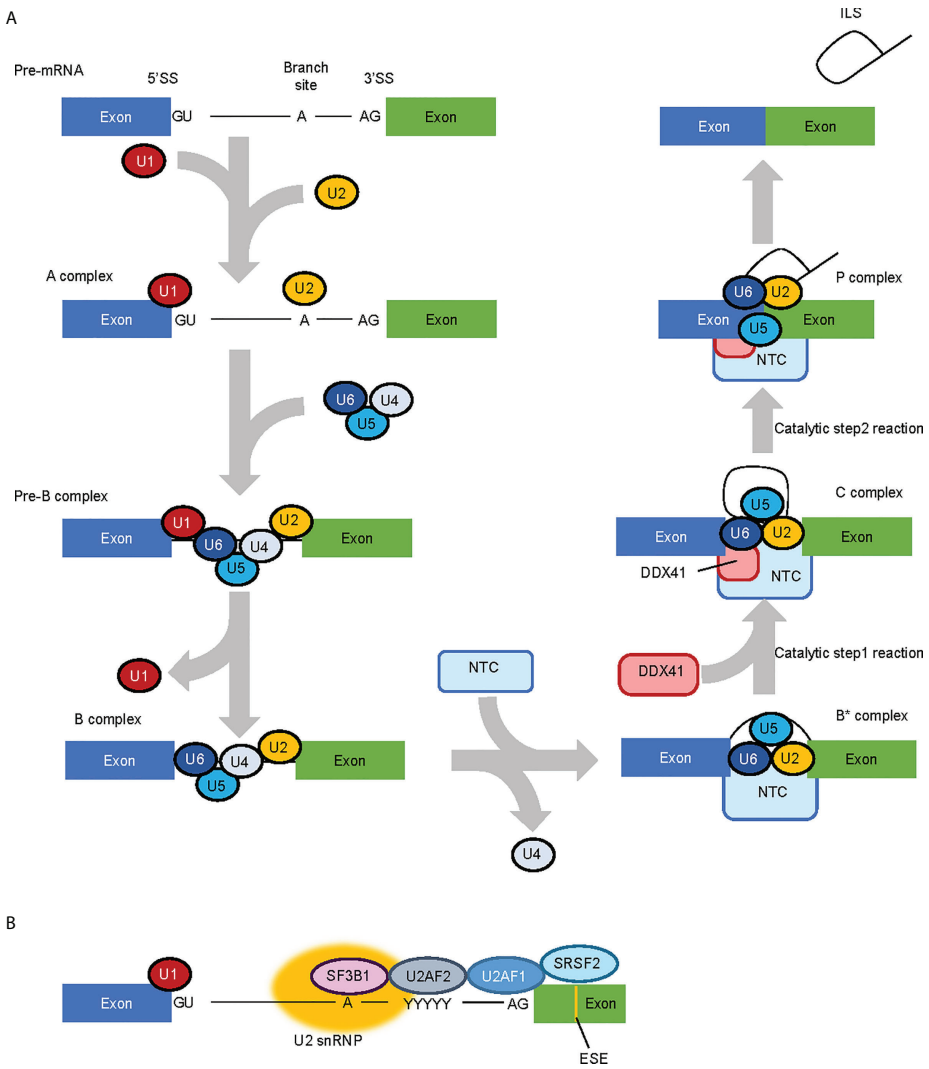

FIGURE 2
RNA splicing process and factors involved in the process. **(A)** Simplified RNA splicing process. The 5' splice site (SS) and branch site of transcribed pre-mRNA are first recognized by U1 snRNP and U2 snRNP, respectively. U4/U6.U5 tri-snRNP is then recruited while U1 snRNP and U4 snRNP are released in a stepwise manner. The NineTeen Complex (NTC), consisting of 7 core NTC proteins and 14 NTC-associated proteins, is recruited to (and regulates the conformaiotn of) the spliceosome. In the catalytic Step 1 reaction, the 2'-OH of adenine in the branch site attacks the 5'SS and cleaves the RNA to form an intron lariat, and in the Step 2 reaction, the 3'-OH at the 3' end of the free 5' exon attacks the 3'SS and cleaves the RNA. DDX41 is likely recruited to the spliceosome at the C complex phase. **(B)** MDS-related RNA splicing factors. SF3B1 is involved in branch site recognition, U2AF1 in 3' SS recognition and SRSF2 in exonic splicing enhancer (ESE) recognition, respectively. Therefore, most of the mutations in MDS-related RNA splicing factors are concentrated in factors involved in 3'SS recognition.

(U1, U2, U4, U5 and U6 snRNP) and more than 100 proteins, which are required to carry out the structural transformation of pre-mRNA and spliceosome in a co-ordinated manner (50, 51).

Beyond DDX41, mutations in genes that encode RNA splicing-related factors implicated in myeloid malignancies are observed in about 40–60% of MDS patients (52, 53); of note, frequently mutated genes (namely, *SF3B1*, *SRSF2* and *U2AF1*) all encode factors involved in the recognition of the 3'SS (Figure 2B) (54). However, the nature of the RNA splicing aberrations are specific to each mutated splicing factor, rather than being shared between them all (55). DDX41 is incorporated into the spliceosome at the C complex when the SS has already been determined (49). Therefore, the role of DDX41 in RNA splicing will be largely different to that of typical MDS-related splicing factors. In fact, Li et al. showed that 21 of 176 cases with germline *DDX41* variants (with or without somatic *DDX41* mutations), and 2 of 19 cases with somatic *DDX41* mutations alone had mutations in at least one of the genes encoding typical MDS-related RNA splicing factors (SF3B1, SRSF2, U2AF1, U2AF2 and ZRSR2) with a variant allele frequency of 3% or more (9). Although clonal heterogeneity must be considered when discussing the co-existing mutations, these observations indicate that germline *DDX41* mutations are not necessarily mutually exclusive with mutations in these RNA splicing factors. Thus, the RNA splicing processes regulated by DDX41 may be distinct from those that are modulated by the other splicing factors.

Deletion of the *Caenorhabditis elegans* gene *sacy-1* (an ortholog of *DDX41*) has been reported to alter 3'SS selectivity (56). In contrast, little is known about the precise role of DDX41 in RNA splicing, although exon skipping was a major change feature of cells derived from AML patients with *DDX41* mutations (4), and splicing changes were also observed in hematopoietic progenitor cells from *Ddx41*-deficient mice, with exon skipping and retained introns being the major alterations (57).

Recognition of nucleic acids from intracellular pathogens and induction of innate immune response by DDX41

In 2011, DDX41 was reported as a sensor that recognizes nucleic acids released from pathogens that invade the cytosol (58); the authors of this study found that knockdown of DDX41 diminished the induction of IFN-β following poly (dA:dT) stimulation. A subsequent study suggested that DDX41 recognizes cyclic di-guanosine monophosphate (c-di-GMP) (59), which is a cyclic di-nucleotide produced from two molecules of GTP by diguanylate cyclase that is widely used in bacteria as a second messenger for signal transduction (60). Upon recognition of these nucleic acids, DDX41 interacts with the adaptor molecule STING, which in turn triggers a STING-dependent innate immune response. DDX41 function is regulated by phosphorylation by Bruton's tyrosine kinase (BTK), which mediates the interaction of DDX41 with STING by phosphorylating Y414 of DDX41 (61). The degradation of DDX41 is regulated by poly-ubiquitination (62); DDX41 interacts with the SPRY-PRY domain of TRIM21, an E3 ubiquitin ligase, *via* the DEADc domain, and TRIM21 appears to promote degradation of DDX41 *via* K48-linked polyubiquitination of K9 and K115.

These reports suggest a role for DDX41 in promoting the innate immune response (Figure 3) (63). However, it remains unclear whether this function contributes to hematopoietic malignancies. Since germline variants of *DDX41* are likely loss-of-function, an assumption is that the immune response will be attenuated in cells expressing these variants. However, the opposite has also been reported, as the loss of DDX41 can induce R-loop formation (64, 65), which in turn leads to an inflammatory state in the cells. This is discussed in the next section.

DDX41 has a nuclear localization signal (NLS) at its N-terminus, and recent studies revealed that it is predominantly detected in the nucleus (66). However, there are at least two forms of DDX41 (33); one is a full length 70 kDa protein translated from the first methionine, and the other is a shorter 50 kDa form translated from the second methionine that lacks the NLS and is localized in the cytoplasm. It is possible that the shorter form is involved in nucleic acid sensing in the cytoplasm. On the other hand, there are two reports of DDX41 shuttling between the cytoplasm and nucleus (66, 67). In this context, our collaborators recently found that K9 acetylation of the NLS within DDX41 promotes its transport to the cytoplasm (68). They also suggested a possible mechanism by which the p.R525H mutant of DDX41 activates the innate immune response despite its attenuated helicase activity, as follows. RNA helicases generally have ATP-independent strand annealing activity, in addition to ATP-dependent strand unwinding activity. Since the p.R525H mutant exhibits less unwinding activity but retains annealing activity, it would effectively increase the amount of double-stranded cytosolic DNA available for activation of the STING-TBK1 pathway. The extent to which this influences hematopoietic malignancies remains unclear, as germline *DDX41* variants would not be expected to exhibit this selective retention of function.

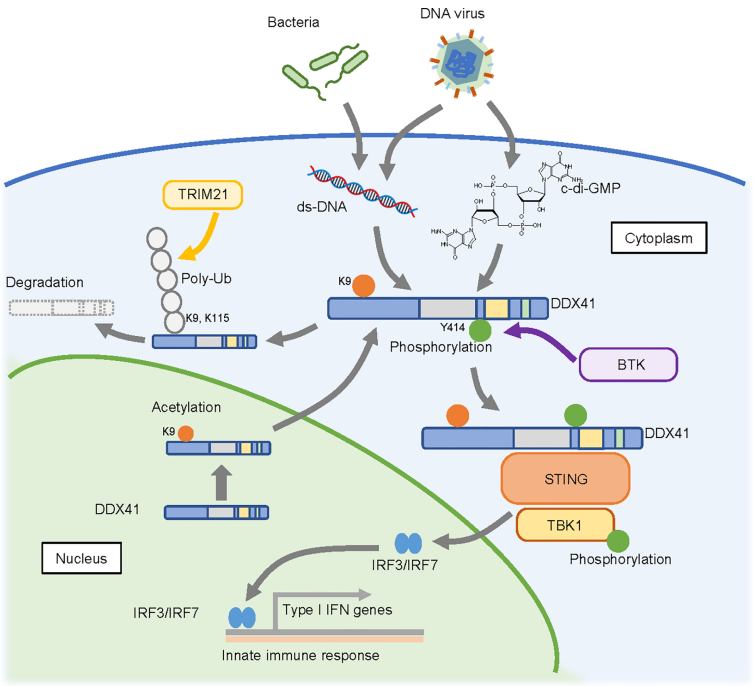

FIGURE 3
Possible cytosolic function of DDX41 as a nucleic acid sensor. DDX41 is exported to the cytoplasm in a nuclear localizing signal (NLS)-acetylation dependent manner and is activated by BTK-mediated phosphorylation. It senses double-strand DNA or c-di-GMP released from pathogens that invade the cytoplasm, and activates innate immune reactions through the STING-TBK1 axis. DDX41 is also reportedly degraded by TRIM21 mediated poly-ubiquitination (poly-Ub).

R-loop regulation by DDX41 limits DNA damage response signaling

An increase in R-loop formation occurs in MDS regardless of the mutation spectrum present in tumor cells (69, 70). R-loops are structures on genomic DNA consisting of DNA : RNA hybrids and single stranded DNA displaced from the paired strand (71) (Figure 4); they are involved in physiological processes such as transcription termination, immune globulin class-switching, mitochondrial DNA replication, and the DNA repair response (72). However, excessive accumulation of R-loops is associated with various pathological conditions, causing impaired transcriptional elongation and genomic instability. The first paper to report the involvement of R-loops in MDS showed that RNA splicing changes were exclusive to cells expressing different splicing factor mutants, while an increased DNA damage response and DNA replication stress were commonly observed (69). Although little is known about the process by which mutations in genes encoding RNA splicing factors lead to R-loop formation, SRSF2 can promote initiation of transcriptional elongation by releasing P-TEFb, a complex that activates RNA polymerase II (Pol II), by liberating it from an inhibitory factor (73). The presence of SRSF2 mutations in MDS cells may inhibit this effect and impair the pause-release of Pol II, which would render cells prone to R-loop formation (69). However, no mechanistic links between R-loop accumulation and mutations in genes encoding other MDS-related RNA splicing factors have been proposed. Further studies that will provide clearer insight are thus clearly warranted.

Two independent papers suggested that DDX41 regulates R-loop formation (39, 64). Expression of a Ddx41 loss-of-function mutant in zebrafish induces R-loop formation, along with the upregulation of inflammatory signals *via* the STING-TBK1 axis (65). The induction of inflammation upon DDX41 loss is somewhat contradictory to the aforementioned theory that DDX41 positively regulates inflammatory signals (63), but consistent with the fact that MDS and AML cells are often in an inflammatory state due to intrinsic production of inflammatory cytokines (74). As for the role of DDX41 in the regulation of R-loops, it is proposed that DDX41 directly resolves DNA : RNA hybrids (39). A comprehensive RNA-DNA proximity proteomics approach in the vicinity of R-loops identified several helicases, and showed that DDX41 knockdown induces DNA damage signaling. Since DDX41 can unwind DNA

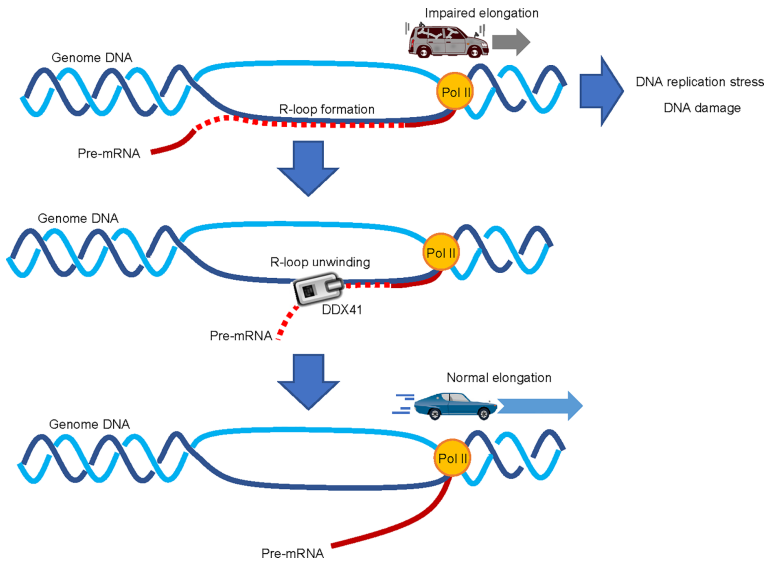

FIGURE 4
Involvement of DDX41 in R-loop resolution. Abnormal accumulation of R-loops impairs transcriptional elongation by RNA polymerase II (Pol II), which leads to increased replication stress and DNA damage; DDX41 has been proposed to directly unwind DNA : RNA hybrids to resolve R-loops.

: RNA hybrids *in vitro* (68), it is plausible that DDX41 may indeed play a direct role in R-loop resolution. However, the mechanism by which DDX41 specifically resolves R-loops formed close to the transcription initiation site is largely unknown. While other molecules such as SENATAXIN, BLM, FANCM and WRN can unwind DNA : RNA hybrids (75), we await the outcome of future studies to delineate the specific role of DDX41 in the suppression of R-loop formation.

Increased R-loop formation is associated with activation of the DNA damage response (76). This includes activation of both Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3-related (ATR) pathways, which respond to double- and single-strand breaks, respectively (76). In particular, the ATR pathway has been implicated in the response to and resolution of replication stress caused by R-loop-induced collisions between transcription and replication. R-loops are also likely to induce double-strand breaks, which would be consistent with ATM activation. However, the mechanism by which this occurs is unclear, as the ATM pathway is activated by R-loops even in non-replicating cells (77). In any case, the ATM and ATR pathways could be potential therapeutic targets for tumor cells with accumulated R-loops. Indeed, the efficacy of Chk1 inhibitors for MDS with *SF3B1* mutations, as well as ATR inhibitors for those with *U2AF1* variants have been demonstrated (70, 78). Since hematopoietic malignancies with *DDX41* mutations also likely have increased levels of R-loops, inhibition of ATR/ATM may be a therapeutic option also for this disease.

Involvement of DDX41 in ribosome biogenesis and translation

Our group has previously shown that DDX41 is involved in pre-ribosomal RNA (rRNA) processing (33). The study was inspired by findings from another group that (like many other nucleolar proteins) showed knockdown of DDX41 affected pre-rRNA processing (79). In ribosomal RNA synthesis in mammals, the 28S, 5.8S, and 18S rRNAs are transcribed together by RNA polymerase I to form 47S pre-rRNA, and 5S rRNA is transcribed by polymerase III; these pre-rRNAs undergo stepwise cleavage and trimming to produce mature rRNAs. In addition, small nucleolar ribonucleoproteins (snoRNPs) composed of small nucleolar RNAs (snoRNAs) and proteins undergo 2'-O-methylation and uridine isomerization (pseudouridylation) of rRNA (80). Finally, numerous ribosomal proteins that are bound to pre-rRNA promote the assembly of 60S and 40S ribosomal subunits. In our previous study, we revealed that the expression of p.R525H increased unprocessed 47S pre-rRNA and impaired ribosome biogenesis as a result. Although we have not yet identified the exact role that DDX41 plays in pre-rRNA processing process, we have found that the loss of DDX41 function causes a defect in pre-rRNA biogenesis and disrupts the balance of ribosome synthesis, thus leading to cell cycle arrest and apoptosis (33). Additionally, Chlon et al. reported that introduction of DDX41 mutation disrupted snoRNA processing, thereby leading to impaired ribosome function (81). snoRNAs are short RNAs of 60–300 nucleotides that localize in the nucleolus

and are classified into two major groups (82). The first group contains Box C/D snoRNAs, which determine the position of 2'-O-methylation; the second is comprised of Box H/ACA snoRNAs, which are responsible for pseudouridylation. Each snoRNA interacts with proteins to form a small nucleolar ribonucleoprotein (snoRNP). For Box H/ACA snoRNA that are localized to the intronic regions of pre-mRNA, snoRNP-associated proteins first interact with the snoRNA region in the pre-mRNA, then intron lariats excised from the mRNA are processed into snoRNP (83). Cells deficient for DDX41 have impaired snoRNA processing (i.e., impaired isolation of snoRNA regions from introns), and this reduces ribosome biogenesis (81). Since RNA splicing is closely associated with snoRNA production, this study is noteworthy because it demonstrates a novel role for DDX41 in linking RNA splicing and ribosome biosynthesis. Abnormal expression of snoRNAs is implicated in disease phenotypes and drug resistance in hematopoietic malignancies and solid tumors (84–86), suggesting that snoRNAs play a significant role in the development of malignancies.

However, this discovery simultaneously raises another question regarding the role that DDX41 plays in snoRNA processing. Although reduced expression of DDX41 impairs intron removal in certain host genes, it is still unclear how it confers such splicing selectivity. Considering that RNA splicing factors play a role in snoRNA processing, as shown for IBP160 (87), it is possible that DDX41 in the splicing C complex is directly involved in snoRNA processing. Alternatively, snoRNPs and the spliceosome may interact *via* DDX41.

Conclusion

Due to the limited variety and frequency of hematopoietic malignancies with gene mutations encoding RNA helicases, the link between these enzymes and oncogenesis has historically been unclear. However, this link has been partially elucidated by recent studies. Cells with *DDX41* mutations are more likely to exhibit a DNA damage phenotype that renders them more prone to apoptosis than to proliferation, at least prior to overt disease development. DDX41 may play a role in late process of RNA splicing, and mutant-related defects would affect the transcription and DNA replication that normally progress in concert with RNA splicing. This hypothesis may explain the unique phenotypes of this disease. Minor clones with biallelic *DDX41* mutations emerged from those with a germline variant and define the disease phenotype (81). However, no explanation has been provided for how such minor clones could contribute to the disease; it might be possible that cells with biallelic mutations create an inflammatory state through R-loop accumulation, or *via* other yet-unidentified mechanisms.

Myeloid malignancies with DDX41 variants respond relatively well to standard therapies using anthracycline and cytarabine with or without gemtuzumab ozogamicin for induction and consolidation treatments, or to hypomethylating agents for patients not eligible for intensive therapy. However, the disease is more likely to occur in the elderly and it is often difficult to administer cytotoxic chemotherapy to these patients. Therefore, treatments based on the concept of synthetic lethality with molecular targeted agents that inhibit DNA damage response pathways or RNA splicing processes could be a reasonable and promising approach.

Author contributions

SS and HM wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Hou HA, Tien HF. Genomic landscape in acute myeloid leukemia and its implications in risk classification and targeted therapies. *J BioMed Sci* (2020) 27:81. doi: 10.1186/s12929-020-00674-7

2. Palomo L, Meggendorfer M, Hutter S, Twardziok S, Ademà V, Fuhrmann I, et al. Molecular landscape and clonal architecture of adult myelodysplastic/myeloproliferative neoplasms. *Blood* (2020) 136:1851–62. doi: 10.1182/blood.2019004229

3. Rio-Machin A, Vulliamy T, Hug N, Walne A, Tawana K, Cardoso S, et al. The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. *Nat Commun* (2020) 11:1044. doi: 10.1038/s41467-020-14829-5

4. 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) 27:658–70. doi: 10.1016/j.ccell.2015.03.017

5. 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) 127:2391–405. doi: 10.1182/blood-2016-03-643544

6. Andreou AZ. DDX41: a multifunctional DEAD-box protein involved in pre-mRNA splicing and innate immunity. *Biol Chem* (2021) 402:645–51. doi: 10.1515/hsz-2020-0367

7. Jiang Y, Zhu Y, Qiu W, Liu YJ, Cheng G, Liu ZJ, et al. Structural and functional analyses of human DDX41 DEAD domain. *Protein Cell* (2017) 8:72–6. doi: 10.1007/s13238-016-0351-9

8. Cheah JJC, Hahn CN, Hiwase DK, Scott HS, Brown AL. Myeloid neoplasms with germline DDX41 mutation. *Int J Hematol* (2017) 106:163–74. doi: 10.1007/s12185-017-2260-y

9. Li P, Brown S, Williams M, White TA, Xie W, Cui W, et al. The genetic landscape of germline DDX41 variants predisposing to myeloid neoplasms. *Blood* (2022)140:716–55. doi: 10.1182/blood.2021015135

10. Churpek JE, Smith-Simmer K. DDX41-associated familial myelodysplastic syndrome and acute myeloid leukemia. In: *GeneReviews*. Seattle: University of Washington (2021). p. 1993–2022.

11. Li P, White T, Xie W, Cui W, Peker D, Zeng G, et al. AML with germline DDX41 variants is a clinicopathologically distinct entity with an indolent clinical course and favorable outcome. *Leukemia* (2021) 36:664–74. doi: 10.1038/s41375-021-01404-0

12. Alkhateeb HB, Nanaa A, Viswanatha D, Foran JM, Badar T, Sproat L, et al. Genetic features and clinical outcomes of patients with isolated and comutated DDX41-mutated myeloid neoplasms. *Blood Adv* (2022) 6:528–32. doi: 10.1182/bloodadvances.2021005738

13. Goyal T, Tu ZJ, Wang Z, Cook JR. Clinical and pathologic spectrum of DDX41-mutated hematolymphoid neoplasms. *Am J Clin Pathol* (2021) 156:829–38. doi: 10.1093/ajcp/aqab027

14. Qu S, Li B, Qin T, Xu Z, Pan L, Hu N, et al. Molecular and clinical features of myeloid neoplasms with somatic DDX41 mutations. *Br J Haematol* (2021) 192:1006–10. doi: 10.1111/bjh.16668

15. 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) 134:1441–4. doi: 10.1182/blood.2019000909

16. Maciejewski JP, Padgett RA, Brown AL, Müller-Tidow C. DDX41-related myeloid neoplasia. *Semin Hematol* (2017) 54:94–7. doi: 10.1053/j.seminhematol.2017.04.007

17. Cardoso SR, Ryan G, Walne AJ, Ellison A, Lowe R, Tummala H, et al. Germline heterozygous DDX41 variants in a subset of familial myelodysplasia and acute myeloid leukemia. *Leukemia* (2016) 30:2083–6. doi: 10.1038/leu.2016.124

18. Duployez N, Largeaud L, Duchmann M, Kim R, Rieunier J, Lambert J, et al. Prognostic impact of DDX41 germline mutations in intensively treated acute myeloid leukemia patients: an ALFA-FILO study. *Blood* (2022) 40:756–68. doi: 10.1182/blood.2021015328

19. Opatz S, Bamopoulos SA, Metzeler KH, Herold T, Ksienzyk B, Bräundl K, et al. The clinical mutatome of core binding factor leukemia. *Leukemia* (2020) 34:1553–62. doi: 10.1038/s41375-019-0697-0

20. Faber ZJ, Chen X, Gedman AL, Boggs K, Cheng J, Ma J, et al. The genomic landscape of core-binding factor acute myeloid leukemias. *Nat Genet* (2016) 48:1551–6. doi: 10.1038/ng.3709

21. Rivera OD, Mallory MJ, Quesnel-Vallières M, Chatrikhi R, Schultz DC, Carroll M, et al. Alternative splicing redefines landscape of commonly mutated genes in acute myeloid leukemia. *Proc Natl Acad Sci U.S.A.* (2021) 118:e2014967118. doi: 10.1073/pnas.2014967118

22. 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) 94:757–66. doi: 10.1002/ajh.25486

23. Iacobucci I, Wen J, Meggendorfer M, Choi JK, Shi L, Pounds SB, et al. Genomic subtyping and therapeutic targeting of acute erythroleukemia. *Nat Genet* (2019) 51:694–704. doi: 10.1038/s41588-019-0375-1

24. Lewinsohn M, Brown AL, Weinel LM, Phung C, Rafidi G, Lee MK, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. *Blood* (2016) 127:1017–23. doi: 10.1182/blood-2015-10-676098

25. Shin WY, Yoon SY, Park R, Kim JA, Song HH, Bang HI, et al. A novel bi-alleleic DDX41 mutations in b-cell lymphoblastic leukemia: Case report. *BMC Med Genomics* (2022) 15:46. doi: 10.1186/s12920-022-01191-2

26. Singhal D, Hahn CN, Feurstein S, Wee LYA, Moma L, Kutyna MM, 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) 35:3245–56. doi: 10.1038/s41375-021-01246-w

27. Bannon SA, Routbort MJ, Montalban-Bravo G, Mehta RS, Jelloul FZ, Takahashi K, et al. Next-generation sequencing of DDX41 in myeloid neoplasms leads to increased detection of germline alterations. *Front Oncol* (2020) 10:582213. doi: 10.3389/fonc.2020.582213

28. Fazio F, Quintini M, Carmosino I, Matteucci C, Miulli E, Pellanera F, et al. New dead/H-box helicase gene (ddx41) mutation in an Italian family with recurrent leukemia. *Leuk Lymph* (2021) 62:2280–3. doi: 10.1080/10428194.2021.1910689

29. Polprasert C, Takeda J, Niparuck P, Rattanathammethee T, Pirunsarn A, Suksusut A, et al. Novel DDX41 variants in Thai patients with myeloid neoplasms. *Int J Hematol* (2020) 111:241–6. doi: 10.1007/s12185-019-02770-3

30. Király AP, Kállay K, Gángó A, Kellner Á, Egyed M, Szőke A, et al. Familial acute myeloid leukemia and myelodysplasia in Hungary. *Pathol Oncol Res* (2018) 24:83–8. doi: 10.1007/s12253-017-0216-4

31. Choi EJ, Cho YU, Hur EH, Jang S, Kim N, Park HS, et al. Unique ethnic features of DDX41 mutations in patients with idiopathic cytopenia of undetermined significance, myelodysplastic syndrome, or acute myeloid leukemia. *Haematologica* (2022) 107:510–8. doi: 10.3324/haematol.2020.270553

32. Linder P, Jankowsky E. From unwinding to clamping - the DEAD box RNA helicase family. *Nat Rev Mol Cell Biol* (2011) 12:505–16. doi: 10.1038/nrm3154

33. 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) 44:745–54.e4. doi: 10.1016/j.exphem.2016.04.017

34. Choi EJ, Cho YU, Hur EH, Park HS, Choi Y, Lee JH, et al. Clinical implications and genetic features of clonal cytopenia of undetermined significance compared to lower-risk myelodysplastic syndrome. *Br J Haematol* (2022) 198:703–12. doi: 10.1111/bjh.18273

35. Gibson CJ, Kim HT, Zhao L, Murdock HM, Hambley B, Ogata A, et al. Donor clonal hematopoiesis and recipient outcomes after transplantation. *J Clin Oncol* (2022) 40:189–201. doi: 10.1200/jco.21.02286

36. Kobayashi S, Kobayashi A, Osawa Y, Nagao S, Takano K, Okada Y, et al. Donor cell leukemia arising from preleukemic clones with a novel germline DDX41 mutation after allogenic hematopoietic stem cell transplantation. *Leukemia* (2017) 31:1020–2. doi: 10.1038/leu.2017.44

37. Williams L, Doucette K, Karp JE, Lai C. Genetics of donor cell leukemia in acute myelogenous leukemia and myelodysplastic syndrome. *Bone Marrow Transplant* (2021) 56:1535–49. doi: 10.1038/s41409-021-01214-z

38. 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) 31:520–2. doi: 10.1038/leu.2016.310

39. Mosler T, Conte F, Longo GMC, Mikicic I, Kreim N, Möckel MM, et al. R-loop proximity proteomics identifies a role of DDX41 in transcription-associated genomic instability. *Nat Commun* (2021) 12:7314. doi: 10.1038/s41467-021-27530-y

40. Abou Dalle I, Kantarjian H, Bannon SA, Kanagal-Shamanna R, Routbort M, Patel KP, et al. Successful lenalidomide treatment in high risk myelodysplastic syndrome with germline DDX41 mutation. *Am J Hematol* (2020) 95:227–9. doi: 10.1002/ajh.25610

41. Negoro E, Radivoyevitch T, Polprasert C, Adema V, Hosono N, Makishima H, et al. Molecular predictors of response in patients with myeloid neoplasms treated with lenalidomide. *Leukemia* (2016) 30:2405–9. doi: 10.1038/leu.2016.228

42. Fairman-Williams ME, Guenther UP, Jankowsky E. SF1 and SF2 helicases: family matters. *Curr Opin Struct Biol* (2010) 20:313–24. doi: 10.1016/j.sbi.2010.03.011

43. Pan C, Russell R. Roles of DEAD-box proteins in RNA and RNP folding. *RNA Biol* (2010) 7:667–76. doi: 10.4161/rna.7.6.13571

44. Ali MAM. The DEAD-box protein family of RNA helicases: sentinels for a myriad of cellular functions with emerging roles in tumorigenesis. *Int J Clin Oncol* (2021) 26:795–825. doi: 10.1007/s10147-021-01892-1

45. Cargill M, Venkataraman R, Lee S. DEAD-box RNA helicases and genome stability. *Genes (Basel)* (2021) 12:1471. doi: 10.3390/genes12101471

46. De Bortoli F, Espinosa S, Zhao R. DEAH-box RNA helicases in pre-mRNA splicing. *Trends Biochem Sci* (2021) 46:225–38. doi: 10.1016/j.tibs.2020.10.006

47. Gilman B, Tijerina P, Russell R. Distinct RNA-unwinding mechanisms of DEAD-box and DEAH-box RNA helicase proteins in remodeling structured RNAs and RNPs. *Biochem Soc Trans* (2017) 45:1313–21. doi: 10.1042/bst20170095

48. Wahl MC, Will CL, Lührmann R. The spliceosome: Design principles of a dynamic RNP machine. *Cell* (2009) 136:701–18. doi: 10.1016/j.cell.2009.02.009

49. Cvitkovic I, Jurica MS. Spliceosome database: a tool for tracking components of the spliceosome. *Nucleic Acids Res* (2013) 41:D132–41. doi: 10.1093/nar/gks999

50. Wilkinson ME, Charenton C, Nagai K. RNA Splicing by the spliceosome. *Annu Rev Biochem* (2020) 89:359–88. doi: 10.1146/annurev-biochem-091719-064225

51. Will CL, Lührmann R. Spliceosome structure and function. *Cold Spring Harb Perspect Biol* (2011) 3:a003707. doi: 10.1101/cshperspect.a003707

52. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* (2014) 28:241–7. doi: 10.1038/leu.2013.336

53. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* (2011) 478:64–9. doi: 10.1038/nature10496

54. Anczuków O, Krainer AR. Splicing-factor alterations in cancers. *RNA* (2016) 22:1285–301. doi: 10.1261/rna.057919.116

55. Hershberger CE, Moyer DC, Adema V, Kerr CM, Walter W, Hutter S, et al. Complex landscape of alternative splicing in myeloid neoplasms. *Leukemia* (2021) 35:1108–20. doi: 10.1038/s41375-020-1002-y

56. Tsukamoto T, Gearhart MD, Kim S, Mekonnen G, Spike CA, Greenstein D. Insights into the involvement of spliceosomal mutations in myelodysplastic disorders from analysis of SACY-1/DDX41 in caenorhabditis elegans. *Genetics* (2020) 214:869–93. doi: 10.1534/genetics.119.302973

57. Ma J, Mahmud N, Bosland MC, Ross SR. DDX41 is needed for pre- and postnatal hematopoietic stem cell differentiation in mice. *Stem Cell Rep* (2022) 17:879–93. doi: 10.1016/j.stemcr.2022.02.010

58. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol* (2011) 12:959–65. doi: 10.1038/ni.2091

59. Parvatiyar K, Zhang Z, Teles RM, Ouyang S, Jiang Y, Iyer SS, et al. The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. *Nat Immunol* (2012) 13:1155–61. doi: 10.1038/ni.2460

60. Krasteva PV, Giglio KM, Sondermann H. Sensing the messenger: the diverse ways that bacteria signal through c-di-GMP. *Protein Sci* (2012) 21:929–48. doi: 10.1002/pro.2093

61. Lee KG, Kim SS, Kui L, Voon DC, Mauduit M, Bist P, et al. Bruton's tyrosine kinase phosphorylates DDX41 and activates its binding of dsDNA and STING to initiate type 1 interferon response. *Cell Rep* (2015) 10:1055–65. doi: 10.1016/j.celrep.2015.01.039

62. Zhang Z, Bao M, Lu N, Weng L, Yuan B, Liu YJ. The E3 ubiquitin ligase TRIM21 negatively regulates the innate immune response to intracellular double-stranded DNA. *Nat Immunol* (2013) 14:172–8. doi: 10.1038/ni.2492

63. Jiang Y, Zhu Y, Liu ZJ, Ouyang S. The emerging roles of the DDX41 protein in immunity and diseases. *Protein Cell* (2017) 8:83–9. doi: 10.1007/s13238-016-0303-4

64. Weinreb JT, Ghazale N, Pradhan K, Gupta V, Potts KS, Tricomi B, et al. Excessive r-loops trigger an inflammatory cascade leading to increased HSPC production. *Dev Cell* (2021) 56:627–40. doi: 10.1016/j.devcel.2021.02.006

65. Weinreb JT, Gupta V, Sharvit E, Weil R, Bowman TV. Ddx41 inhibition of DNA damage signaling permits erythroid progenitor expansion in zebrafish. *Haematologica* (2021) 107:644–54. doi: 10.3324/haematol.2020.257246

66. Ma JX, Li JY, Fan DD, Feng W, Lin AF, Xiang LX, et al. Identification of DEAD-box RNA helicase DDX41 as a trafficking protein that involves in multiple innate immune signaling pathways in a zebrafish model. *Front Immunol* (2018) 9:1327. doi: 10.3389/fimmu.2018.01327

67. Abdul-Ghani M, Hartman KL, Ngsee JK. Abstrakt interacts with and regulates the expression of sorting nexin-2. *J Cell Physiol* (2005) 204:210–8. doi: 10.1002/jcp.20285

68. Singh RS, Vidhyasagar V, Yang S, Arna AB, Yadav M, Aggarwal A, et al. DDX41 is required for cGAS-STING activation against DNA virus infection. *Cell Rep* (2022) 39:110856. doi: 10.1016/j.celrep.2022.110856

69. Chen L, Chen JY, Huang YJ, Gu Y, Qiu J, Qian H, et al. The augmented r-loop is a unifying mechanism for myelodysplastic syndromes induced by high-risk splicing factor mutations. *Mol Cell* (2018) 69:412–25.e6. doi: 10.1016/j.molcel.2017.12.029

70. Singh S, Ahmed D, Dolatshad H, Tatwavedi D, Schulze U, Sanchi A, et al. SF3B1 mutations induce r-loop accumulation and DNA damage in MDS and leukemia cells with therapeutic implications. *Leukemia* (2020) 34:2525–30. doi: 10.1038/s41375-020-0753-9

71. Allison DF, Wang GG. R-loops: formation, function, and relevance to cell stress. *Cell Stress* (2019) 3:38–46. doi: 10.15698/cst2019.02.175

72. Niehrs C, Luke B. Regulatory r-loops as facilitators of gene expression and genome stability. *Nat Rev Mol Cell Biol* (2020) 21:167–78. doi: 10.1038/s41580-019-0206-3

73. Ji X, Zhou Y, Pandit S, Huang J, Li H, Lin CY, et al. SR proteins collaborate with 7SK and promoter-associated nascent RNA to release paused polymerase. *Cell* (2013) 153:855–68. doi: 10.1016/j.cell.2013.04.028

74. Soyfer EM, Fleischman AG. Inflammation in myeloid malignancies: From bench to bedside. *J Immunother Precis Oncol* (2021) 4:160–7. doi: 10.36401/jipo-21-3

75. Rinaldi C, Pizzul P, Longhese MP, Bonetti D. Sensing r-Loop-Associated DNA damage to safeguard genome stability. *Front Cell Dev Biol* (2020) 8:618157. doi: 10.3389/fcell.2020.618157

76. Barroso S, Herrera-Moyano E, Muñoz S, García-Rubio M, Gómez-González B, Aguilera A. The DNA damage response acts as a safeguard against harmful DNA-RNA hybrids of different origins. *EMBO Rep* (2019) 20:e47250. doi: 10.15252/embr.201847250

77. Tresini M, Warmerdam DO, Kolovos P, Snijder L, Vrouwe MG, Demmers JA, et al. The core spliceosome as target and effector of non-canonical ATM signalling. *Nature* (2015) 523:53–8. doi: 10.1038/nature14512

78. Nguyen HD, Leong WY, Li W, Reddy PNG, Sullivan JD, Walter MJ, et al. Spliceosome mutations induce r loop-associated sensitivity to ATR inhibition in myelodysplastic syndromes. *Cancer Res* (2018) 78:5363–74. doi: 10.1158/0008-5472.can-17-3970

79. Tafforeau L, Zorbas C, Langhendries JL, Mullineux ST, Stamatopoulou V, Mullier R, et al. The complexity of human ribosome biogenesis revealed by systematic nucleolar screening of pre-rRNA processing factors. *Mol Cell* (2013) 51:539–51. doi: 10.1016/j.molcel.2013.08.011

80. Weinstein LB, Steitz JA. Guided tours: from precursor snoRNA to functional snoRNP. *Curr Opin Cell Biol* (1999) 11:378–84. doi: 10.1016/s0955-0674(99)80053-2

81. Chlon TM, Stepanchick E, Hershberger CE, Daniels NJ, Hueneman KM, Kuenzi Davis A, et al. Germline DDX41 mutations cause ineffective hematopoiesis and myelodysplasia. *Cell Stem Cell* (2021) 28:1966–81. doi: 10.1016/j.stem.2021.08.004

82. Wajahat M, Bracken CP, Orang A. Emerging functions for snoRNAs and snoRNA-derived fragments. *Int J Mol Sci* (2021) 22:10193. doi: 10.3390/ijms221910193

83. Kufel J, Grzechnik P. Small nucleolar RNAs tell a different tale. *Trends Genet* (2019) 35:104–17. doi: 10.1016/j.tig.2018.11.005

84. Lin LM, Pan Q, Sun YM, Wang WT. Small nucleolar RNA is potential as a novel player in leukemogenesis and clinical application. *Blood Sci* (2021) 3:122–31. doi: 10.1097/bs9.0000000000000091

85. Liang J, Wen J, Huang Z, Chen XP, Zhang BX, Chu L. Small nucleolar RNAs: Insight into their function in cancer. *Front Oncol* (2019) 9:587. doi: 10.3389/fonc.2019.00587

86. Fazi F, Fatica A. Regulation of ribosome function by RNA modifications in hematopoietic development and leukemia: It is not only a matter of m(6)A. *Int J Mol Sci* (2021) 22:4755. doi: 10.3390/ijms22094755

87. Hirose T, Ideue T, Nagai M, Hagiwara M, Shu MD, Steitz JA. A spliceosomal intron binding protein, IBP160, links position-dependent assembly of intron-encoded box C/D snoRNP to pre-mRNA splicing. *Mol Cell* (2006) 23:673–84. doi: 10.1016/j.molcel.2006.07.011

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Adult-onset hereditary myeloid malignancy and allogeneic stem cell transplantation

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Hereditary myeloid malignancies, especially in adults or elderly persons, had been considered quite rare before the next-generation sequencing era; however, increased usage of clinical sequencing has revealed much higher prevalence of inherited myeloid malignancies. *DDX41* and various pathogenic germline mutations have newly been recognized as the cause of adult-onset familial leukemia and myeloid malignancies. Although germline predisposition to myeloid neoplasms had been categorized as a provisional entity in the World Health Organization classification of hematopoietic neoplasms in 2016, methodology for the identification of hereditary myeloid malignancies has not been fully established yet. In addition, many unresolved problems, such as epidemiology, the exact pathogenic mechanisms, and ideal treatment strategy, including indications of allogeneic hematopoietic stem cell transplantation, still remain. Related donor selection for stem cell transplant is a particularly sensitive issue due to the possibility of germline mutation of the candidate relatives and the risk of donor cell leukemia after transplantation. Here, we reviewed the current evidence regarding epidemiology, diagnosis, mechanisms of progression, and transplantation strategy for hereditary myeloid malignancies.

KEYWORDS

hereditary myeloid malignancy, germline mutation, genetic testing, allogeneic stem cell transplantation, donor cell leukemia

Introduction

Hereditary myeloid malignancies (HMMs) are myeloid neoplasms that arise in individuals with germline mutations associated with increased risk of myeloid malignancies. The first report of HMMs were published in 1861, although the causality remained unclear (1); HMMs had been considered an extremely rare disease

for a long time. In 1999, germline *RUNX1* mutation was identified, for the first time, as the genetic background of familial platelet disorder with predisposition to myeloid malignancy (FPD-MM). Subsequent advent of next-generation sequencing (NGS) era unraveled HMMs as much more manifold and common diseases than considered earlier (2–6). In 2016, HMMs were defined as "myeloid neoplasms with germline predisposition" in the revised fourth edition of the World Health Organization (WHO) classification of myeloid neoplasms (7), and were renamed as "myeloid neoplasms associated with germline predisposition" in the fifth edition of WHO classification (8). Candidate genes associated with HMMs, such as *GATA2*, *CEBPA*, *ETV6*, *ANKRD26*, *SAMD9*, and *SAMD9L*, have been discovered subsequently (9). Recent reports have shown that approximately 5–10% of patients with hematological malignancy carry a germline variant (10). However, the penetrance is not 100% in HMMs, since not all patients with a germline variant develop hematological malignancy. Moreover, *de novo* germline variant may occur where an obvious family history could be lacking. Some cases with HMMs are often elderly-onset, especially in individuals with germline *DDX41* mutations (11). Therefore, the accurate and prompt diagnosis of HMMs is often challenging.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative intervention for HMMs. However, the optimal strategy for HSCT, including indication, timing, donor selection, conditioning regimen, and toxicity management has not been fully elucidated yet. Although many guidelines recommend HSCT for HMMs, data regarding transplant outcome are highly limited (12). In this manuscript, we reviewed the pathophysiology, clinical characteristics, and recent evidence regarding HSCT for HMMs. The review focused on adult-onset HMMs, which often get involved with solitary adult myeloid malignancies in clinical practice. Some child-onset hereditary disorders are beyond the scope of the current review, and the relevant guidelines should be perused in that regard.

Clinical features corresponding to each mutation

In general, HMMs are classified into three groups (Table 1), namely (1) myeloid neoplasms with germline predisposition and pre-existing platelet disorder (*RUNX1*, *ANKRD26*, and *ETV6*), (2) myeloid neoplasms with germline predisposition and potential organ dysfunction (*GATA2*, *SAMD9*, *SAMD9L*, etc.), and (3) myeloid neoplasms with germline predisposition without a preexisting platelet disorder or organ dysfunction (*CEBPA* and *DDX41*). Four representative genes responsible for adult-onset HMMs, namely *RUNX1*, *GATA2*, *CEBPA*, and *DDX41*, are described in detail below.

RUNX1

RUNX1 is a critical transcription factor for hematopoiesis; *RUNX1* mutation is recurrently detected and is a poor prognostic marker in myeloid malignancies (24). FPD-MM is the first HMMs whose genetic causality was recognized in 1999 (20). A germline *RUNX1* mutation is associated with predisposition to mainly acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) while some cases with various lymphoid malignancies have also been reported (25–28). FPD-MM accounts for 8–30% of AML cases with *RUNX1* mutations (29–33).

Chronic thrombocytopenia and/or bleeding tendency is a characteristic of FPD-MM pedigree. Some pedigrees may be

TABLE 1 Major subtypes of myeloid neoplasms associated with germline predisposition[8].

Germline variant	Major hematologic disorder	Other characteristics
Myeloid neoplasms with germline predisposition without a preexisting platelet disorder or organ dysfunction		
CEBPA[13, 14]	AML	None
DDX41[15-17]	MDS, AML	None
TP53[18, 19]	ALL, myeloid neoplasms	Cancer predisposition
Myeloid neoplasms with germline predisposition and pre-existing platelet disorder		
RUNX1[13, 20]	MDS, AML	Thrombocytopenia, decreased platelet function
ANKRD26[9, 21]	AML, MDS, CML	Thrombocytopenia, decreased platelet function
ETV6[18, 21]	ALL, MDS, AML	Thrombocytopenia, decreased platelet function
Myeloid neoplasms with germline predisposition and potential organ dysfunction		
GATA2[13, 22]	MDS, AML, BMF	Monocytopenia, B lymphocytopenia, lymphoedema, pulmonary alveolar proteinosis, congenital deafness
SAMD9[18, 23]	MDS, AML, BMF	Infection, growth restriction, adrenal hypoplasia, genital phenotypes, enteropathy
SAMD9L[18]	MDS, AML, BMF	Ataxia, systemic autoinflammatory diseases

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BMF, bone marrow failure; CML, chronic myeloid leukemia; MDS, myelodysplastic syndromes.

considered as immune thrombocytopenic purpura. Approximately 40% of the family members with blood relationship develop myeloid malignancy during their lifetime (34), although significant heterogeneity is evident across families (35). Churpek et al. had reported that cumulative risk of developing clonal hematopoiesis by 50 years of age is > 80% (36). Heterozygous *RUNX1* germline mutation alone is not sufficient for leukemogenesis in this context, and additionally acquired mutations, such as gene mutations in *ASXL1*, *FLT3*, *WT1* and *CDC25C*, may also be important for malignant transformation (37–39).

GATA2

MonoMAC syndrome is characterized by monocytopenia, B lymphocytopenia, pulmonary alveolar proteinosis (PAP), and frequent *M. avium* complex infection. Patients with Emberger syndrome exhibit primary lymphoedema, cutaneous warts, and sensorineural deafness. Dendritic cell, monocyte, and B- and NK-lymphoid deficiencies occur with DCML deficiency. The patients frequently develop familial MDS/AML, although the pathogenesis remains to be clarified. In 2011, Hahn et al. revealed that loss-of-function germline GATA-binding protein 2 (*GATA2*) mutations are associated with all the above-mentioned syndromes (22). Currently, the syndromes are collectively called *GATA2* deficiency. Opportunistic viral and mycobacterial infections are frequently observed in patients with *GATA2* deficiency. Interestingly, interindividual variation of clinical phenotypes is significant even among patients with the same germline mutation (21).

GATA2 deficiency is the most common cause of childhood MDS, and monosomy 7 and *ASXL1* mutation are often accompanied by the development of MDS and AML in cases with germline *GATA2* mutation (40). Donadieu et al. analyzed 79 patients with *GATA2* deficiency and reported 92% of them to develop some symptom at 40 years of age (41).

CEBPA

Germline mutations in CCAAT enhancer binding protein alpha (*CEBPA*) that predispose an individual to AML were first reported in 2004 (42). Among the patients with AML having *CEBPA* mutations, approximately 7–11% had those of germline origin (43, 44). Individuals with a germline *CEBPA* mutation do not have specific phenotype before leukemia onset. In general, the onset age of leukemic progression in patients with germline *CEBPA* mutations is lower than that in patients with germline *RUNX1* and *GATA2* mutations (13). Importantly, *CEBPA* germline mutations are autosomal dominant inheritance and the penetrance of AML is reported to be nearly 100% (14).

Most patients with familial AML having germline *CEBPA* mutations have been reported to possess both the N-terminal germline mutation and the C-terminal acquired mutation (14, 45). N-terminal mutations are known to generate p30 isoforms, which have dominant negative effect (46). Most sporadic AML cases with *CEBPA* mutations were reported to possess C-terminal mutations or both (14), suggesting the importance of secondary C-terminal somatic mutations.

DDX41

Germline mutations in DEAD/H-box helicase gene (*DDX41*) were reported for the first time in 2015 (15). Despite being a recent discovery, *DDX41* mutation is the most common genetic predisposition to MDS/AML, representing 1–5% of myeloid malignancies (11, 16, 17). Many HMMs cases with germline *DDX41* mutations were accompanied by somatic *DDX41* mutations (16, 47). In addition, remarkable ethnic deviation was found in the mutation sites in *DDX41*; however, second hit was predominantly R525H, irrespective of ethnicity (47–49). *TP53* and *ASXL1* somatic mutations are recurrently detected (48), although the prognostic impact of concomitant mutations is limited (50).

AML with germline *DDX41* mutations has unique clinical characteristics, such as approximately 3:1 male predominance, frequent absence of family history, and indolent clinical course (18, 50, 51). Makishima et al. reported that the age of progression in individuals with germline *DDX41* mutation was solely greater than 40 years, and penetrance of pathogenic *DDX41* germline variants was estimated to be 38.5% at the age of 85. In addition, they also suggested outstanding efficacy of hypomethylating agents in patients with HMMs having *DDX41* mutations (52).

Diagnosis

Considering the low prevalence of hematological neoplasms, family history of hematological disorder is a key circumstantial evidence of HMMs, and mutation analysis should be done; however, it should be noted that lack of family history cannot deny the possibility of HMMs completely (53). Patients with signs/symptoms indicative of HMMs and those younger than 50 years should undergo genetic testing (12). However, considering the frequent absence of family history and the elderly onset in patients with germline *DDX41* mutations (51), genetic tests can be also applied for patients older than 50 years old.

Genetic testing of non-hematological tissue is the standard option to confirm the diagnosis of HMMs, and the most authorized method is a culture of fibroblasts obtained from a skin biopsy (54, 55). Although the technique can avoid

contamination of blood cells, it is complicated, and buccal swab, nails, or hair roots are often used as alternatives (21).

In addition, if NGS panel in clinical sequencing detects a suspected mutation with variant allele frequency close to 50 or 100%, further testing should be done. Mutations remaining in hematologic complete remission (CR) also suggested the possibility of germline origin (37), although clonal hematopoiesis (CH) can as well persist (56, 57). The NGS panel approaches are able to efficiently identify single nucleotide variants and small insertion/deletion in target region, but can overlook copy number variants or loss of heterozygosity (58–60). Especially when the patient could be a candidate for HSCT, early diagnosis of HMMs is important, since the diagnosis can affect donor selection, as described below.

First-line treatment for patients with myeloid malignancies

There is mostly no evidence about first-line therapy about HMMs and the optimal treatment strategies for HMMs have not been sufficiently established. However, in general, patients with HMMs, who developed myeloid malignancies, are treated as the patients with sporadic myeloid malignancies, i.e., intensive chemotherapy for fit patients with AML and using demethylating agents for patients with MDS (21). Targeted therapy can also be administered when adequate molecular target is available, although specific data for HMMs are not yet available. Whether chemosensitivity in patients with HMMs is different from that in sporadic cases is unclear, except that CR rate of patients with AML having germline *DDX41* mutations after induction chemotherapy was higher than that of patients with wild-type *DDX41* (94% vs. 69%) (16). More vigorous studies to explore the suitable initial treatment strategies are warranted.

Indication and timing of transplantation

The suitable indication and timing of HSCT is still unclear. However, in general, long-term remission can only be obtained by allogeneic HSCT, and HSCT should be considered soon after diagnosis of myeloid malignancy, except for cases with germline *CEBPA* mutations (61).

For patients with AML having a germline *CEBPA* mutation, HSCT in CR1 is not routinely recommended because of modest survival outcome after chemotherapy and the risk of morbidity and mortality after HSCT (12, 21, 45). However, the appropriateness of refraining from HSCT in CR1 has not been sufficiently validated yet, because prospective and/or controlled studies are still lacking due to the rarity of the disease. Considering the high relapse rate and/or secondary leukemia development after chemotherapy (14), availability of a suitable donor, low dose intensity/density of chemotherapy due to side effects, and development of safer HSCT strategy may rationalize HSCT in CR1. In addition, prognostic impact of the mutation site has been reported in sporadic AML with *CEBPA* mutation (62, 63). Clinical consequences of *CEBPA* mutation site in AML with germline mutation should be clarified, although it is difficult due to the remarkable rarity of C-terminal *CEBPA* germline mutation.

Prophylactic HSCT before transformation is another strategy, but it is not commonly performed due to the risk of morbidity and mortality. However, different from other HMMs, *GATA2* deficiency can be treated with HSCT before transformation. Although optimum timing and indication of HSCT in *GATA2* deficiency is still unclear, frequent/severe infection and serious organ damage, such as pulmonary function, can be a trigger for launching HSCT (64). Norwegian nationwide survey revealed that approximately 80% of patients with symptomatic *GATA2* deficiency need HSCT (65).

In cases with a germline variant but without cytopenia and/or dysplasia, bone marrow should be assessed at the time of diagnosis, and be followed every six months or every year (53).

Donor selection and risk of donor cell leukemia

HSCT from a donor with predisposition to myeloid malignancy should be withheld due to the risk of donor cell leukemia (DCL) (66–68). DCL is a rare post-HSCT complication and cumulative incidence has been estimated to be approximately 0.16-0.70% 15 years after HSCT (69, 70). The exact incidence of DCL in HMMs setting is unknown, but Williams et al. reviewed 19 DCL cases with genetic sequencing results and at least three of them had germline predisposing mutations (71); it suggested significant proportion of DCLs to have been derived from donors with germline mutation.

Related donor candidates of patients with HMMs should undergo genetic testing as well as HLA typing; importantly, genetic counseling before testing is also essential (12). When only a matched related donor with predisposing germline mutation is available and the relapse risk without HSCT is very high, there is no consensus about necessary and sufficient condition of the donor because the quantitative risk of DCL has not been clarified.

Genetic testing of a donor might add fuel to the problems. Gibson et al. had shown donor CH to be associated with improved recipient survival due to reduced relapse risk (70). They also showed donor *DNMT3A*-CH to be associated with lower relapse risk and superior survival only when post-

transplant cyclophosphamide (PT-CY) GVHD prophylaxis was not used. The situation is more complicated, since Crysandt et al. suggested germline predisposition to be extended to polymorphisms (21). Inamoto et al. had reported the association between donor/recipient polymorphisms and the risk of sclerotic GVHD after HSCT (72). Should a donor candidate test HLA, germline predisposition, CH, and polymorphisms? The obligation of the bona fide donor is large, and exploration of the "perfect" donor can reduce donor availability. Suitable biomarkers for quantitative evaluation of the relevant risks are highly required.

Among alternative donors, comparison between umbilical cord blood (UCB) and haploidentical donor remains a matter of debate (73). In most patients with HMMs, little evidence exists about alternative donor selection. However, in patients with *GATA2* deficiency, Grossman et al. reported poor outcome of UCB transplantation (73) and Nichols-Vinueza et al. reported excellent outcome of haploidentical transplantation with PT-CY GVHD prophylaxis (74). At least in HSCT for *GATA2* deficiency, haploidentical transplantation with PT-CY may be more suitable compared with UCB transplant.

Conditioning regimen

There is no recommended conditioning regimen yet specific for patients with HMMs. In general, adult patients with HMMs are younger than patients with sporadic myeloid malignancies, and myeloablative conditioning can be applied in more cases than in sporadic cases. However, reduced intensity conditioning is preferred in patients with *GATA2* deficiency due to comorbidities such as concomitant infections and PAP (73).

High-dose total body irradiation (TBI)-regimens are avoided in cases with germline DNA damage response gene mutations due to the risk of second cancer after HSCT. For example, in patients with Fanconi anemia, non-TBI- or low dose-TBI (4 Gy)-containing regimens are usually employed (75). Although some categories of HMMs, such as Fanconi anemia and Li-Fraumeni syndrome, indicate high-risk feature (19, 76), the risk of second cancer after HSCT in most patients with HMMs is unclear, and further accumulation of cases would be necessary to answer these questions.

Complications after transplantation

In patients with myeloid neoplasms and germline predisposition without potential organ dysfunction (i.e., patients with germline mutation in *RUNX1*, *DDX41*, *CEBPA* and so on), no specific complication after HSCT has been reported till date. However, there is no prospective as well as large-scale retrospective study which focus on transplant complications in patients with HMMs; therefore, we have limited data about post-HSCT complications in HMMs and future studies in these settings are necessary.

Adult patients with germline predisposition and potential organ dysfunction practically refer to patients with *GATA2* deficiency, since nearly all patients with *SAMD9*/*SAMD9L* germline mutations, i.e. MIRAGE syndrome and Ataxia pancytopenia syndrome, are diagnosed and treated in their childhood (23). For HSCT in patients with *GATA2* deficiency, variable comorbidities could complicate the transplantation procedures. For example, many patients with *GATA2* deficiency suffer from nontuberculous mycobacteria (NTM) and human papillomavirus (HPV) infections in their clinical courses. However, previous reports had suggested that reactivation of NTM during HSCT could be quite rare under appropriate antimycobacterial therapy up to at least three months after transplantation (73). Drug interaction between calcineurin inhibitors (CIs) and anti-NTM drugs might affect the concentration of CIs (77), and hepatic toxicity due to anti-NTM drugs should also be noted. Reactivation of HPV after HSCT seems to be also rare (73). Patients with *GATA2* deficiency frequently develop PAP, which can cause severe respiratory failure. Early HSCT may be better for lower post-HSCT morbidity and mortality in patients with PAP, although pulmonary dysfunction can also be corrected to some extent after HSCT (73, 78).

Nichols-Vinueza et al. reported the outcome of 59 HSCT recipients with *GATA2* deficiency and compared transplant outcome based on GVHD prophylaxis (PT-CY vs. tacrolimus and methotrexate-based prophylaxis). PT-CY resulted in a significantly reduced incidence of grade III–IV acute GVHD (0 vs. 32%) and moderate-to-severe chronic GVHD (9 vs. 42%) without increase of relapse (0 vs. 5.2%) compared to that in patients who were administered tacrolimus and methotrexate-based prophylaxis (74). PT-CY may be a promising HSCT strategy in patients with *GATA2* deficiency, although the exact mechanism of deteriorating GVHD is still unclear. Hofmann et al. comparatively analyzed the transplant outcomes of pediatric patients with *GATA2* deficiency with those of patients without, and revealed that neurologic toxicities (six of 15 cases) and thrombotic events (eight out of 15 cases) were more common in patients with *GATA2* deficiency, although the underlying mechanism is uncertain (79). The risk of acute and chronic GVHD was not different based on the existence of germline *GATA2* mutation, and second malignancy was rare with a median follow up of nearly six years after HSCT.

Transplantation outcome

Most reports about HMMs are case reports and case series, and large-scale prospective study of HSCT outcome for HMMs

is lacking. Even retrospective analysis is highly limited due to its rarity (Table 2).

Since the prevalence of patients with germline *DDX41* mutations is relatively high among patients with HMMs, retrospective comparison of patients with and without germline *DDX41* mutations using large-scale data of some clinical trials was recently reported by Duployez et al.; 191 patients with AML having *DDX41* germline mutations and 1,604 *DDX41* wild-type patients with AML were compared (16). Interestingly, AML with germline *DDX41* mutation displayed a specific relapse kinetics, with a lower short-term relapse rate and higher late relapse risk. Some previous reports had suggested apparently favorable outcomes in AML with germline *DDX41* mutations (11, 51, 80); however, long-term follow up may be necessary to evaluate the genuine prognosis of AML with germline *DDX41* mutations. Regarding the role of HSCT, Duployez et al. showed that HSCT in first CR for AML with germline *DDX41* mutation may contribute to the suppression of late relapse, although OS was not significantly different (16).

Transplantation outcomes in *GATA2* deficiency have been prospectively analyzed in some clinical trials (73, 74, 78), perhaps because the unique clinical features promoted participation in clinical trials. Many of the studies on *GATA2* deficiency included tens of patients, mostly children, adolescents, or young adults. In children and adolescents, OS and disease-free survival (DFS) after HSCT in bone marrow failure, MDS, or AML in patients with *GATA2* deficiency were 65% and 51%, and those were comparable to the values in patients without *GATA2* deficiency (81). Other studies also reported good survival rate after HSCT; four- or five-year OS was 78–93% (64, 74). However, reported HSCT outcomes for *GATA2* deficiency should be interpreted with caution, since the proportion of MDS with high-risk features, such as monosomy 7, and severity of concomitant organ dysfunction can affect the outcomes.

Transplant outcomes for FPD-MM, AML with germline *CEBPA* mutations, and other HMMs have not been reported yet, and HSCT outcomes of patients with sporadic relevant mutations still need to be extrapolated. Waidhauser et al. analyzed 674 patients with AML, who underwent HSCT in first CR (*RUNX1* mutation; positive 183, negative 491). Two-year overall survival (OS) was not significantly different between *RUNX1* mutation-positive and -negative (67.7 vs 66.1%, p = 0.7). Considering the poor prognosis of AML with *RUNX1* mutation (33), HSCT in first CR was considered to overcome the unfavorability. In patients with AML having somatic biallelic *CEBPA* mutations and having underwent HSCT, five-year OS

TABLE 2 Previous reports about HSCT for HMMs.

Author	Germline mutation	Patient number	Hematologic disorder	Median age at HSCT	Donor source	GVHD prophylaxis	Conditioning	Grade III–IV aGVHD	Moderate–severe cGVHD	Outcome
Grossman (73)	*GATA2*	14	MDS 12 CMML 1 EBV-LPD 1	33	MR-PB 4 UR-PB 4 UCB 4 HaploBM 2	TAC+SIR (MR/UR, UCB) PT-CY (Haplo)	Flu+TBI 2Gy (MR/UR) Flu+CY+TBI 2Gy (UCB) Flu+CY+TBI 2Gy (Haplo)	21%	NA	8/14 (57%) are alive at a median follow-up of 3.5 years (range, 12 months to 5 y)
Parta (64)	*GATA2*	22	AML 2 MDS 20	26	MR-BM 1 MR-PB 1 UR-BM 6 UR-PB 7 HaploBM 7	TAC+MTX (MR/UR) PT-CY (Haplo)	BU-based regimen (4 days)	MR/UR 26% Haplo PT-CY 0%	MR/UR 46% Haplo PT-CY 28%	2-y OS 86%
Nichols-Vinueza (74)	*GATA2*	59	AML 2 CMML 1 MDS 39 Other 17	Mean 28.4	MR/UR TAC/MTX 19 MR/UR PT-CY 23 Haplo PT-CY 17		Flu+BU (MR/UR) Flu+CY+BU+TBI 4Gy (Haplo)	TAC/MTX 32% MR/UR PT-CY 0% Haplo PT-CY 6%	TAC/MTX 42% MR/UR PT-CY 9% Haplo PT-CY 24%	TAC/MTX 4-y OS 78.9% MR/UR PT-CY 4-y OS 82.2% Haplo PT-CY 4-y OS 93.3%
Duployez (16)	*DDX41*	Mut 35 WT 288	AML	Mut 61 WT 54	NA	NA	NA	NA	NA	Mut 5-y relapse 16% WT 5-y relapse 31%
Alkhateeb (80)	*DDX41*	12	MDS/AML	NA	NA	NA	NA	NA	NA	2-y OS 87%

aGVHD, acute graft-versus-host disease; BU, busulfan; cGVHD, chronic graft-versus-host disease; CY, cyclophosphamide; Flu, fludarabine; Haplo, haploidentical; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndromes; MR, matched related; MTX, methotrexate; Mut, germline mutationNA; NA, Not available; OS, overall survival; PT-CY, posttransplant-cyclophosphamide; SIR, sirolimus; TAC, tacrolimus; TBI, total body irradiation; UR, unrelated; WT, wild-type; y, year.

was 71.8%, which was not significantly different from that in patients with AML having somatic biallelic *CEBPA* mutations and receiving consolidation chemotherapy alone (82).

Concluding remarks

We summarized the concurrent available reports about HSCT in patients with HMMs. Although HMMs have recently been noticed more widely, there is too little evidence about recipients as well as donors. Increasing awareness of HMMs would ensure better management by physicians, which could surely improve patient outcomes.

Author contributions

TT wrote the manuscript. HH and YH critically revised the paper and approved the final version. ND supervised the study. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Biermer A. Ein fall Von leukamie. *Arch Pathol Anat* (1861) 20:552–4. doi: 10.1007/BF02360192

2. Takaoka K, Koya J, Yoshimi A, Toya T, Kobayashi T, Nannya Y, et al. Nationwide epidemiological survey of familial myelodysplastic Syndromes/Acute myeloid leukemia in Japan: A multicenter retrospective study. *Leuk Lymphoma* (2020) 61(7):1688–94. doi: 10.1080/10428194.2020.1734595

3. Yoshimi A, Toya T, Nannya Y, Takaoka K, Kirito K, Ito E, et al. Spectrum of clinical and genetic features of patients with inherited platelet disorder with suspected predisposition to hematological malignancies: A nationwide survey in Japan. *Ann Oncol* (2016) 27(5):887–95. doi: 10.1093/annonc/mdw066

4. Holme H, Hossain U, Kirwan M, Walne A, Vulliamy T, Dokal I. Marked genetic heterogeneity in familial Myelodysplasia/Acute myeloid leukaemia. *Br J Haematol* (2012) 158(2):242–8. doi: 10.1111/j.1365-2141.2012.09136.x

5. Yasuda T, Sanada M, Nishijima D, Kanamori T, Iijima Y, Hattori H, et al. Clinical utility of target capture-based panel sequencing in hematological malignancies: A multicenter feasibility study. *Cancer Sci* (2020) 111(9):3367–78. doi: 10.1111/cas.14552

6. Martin ES, Ferrer A, Mangaonkar AA, Khan SP, Kohorst MA, Joshi AY, et al. Spectrum of hematological malignancies, clonal evolution and outcomes in 144 Mayo clinic patients with germline predisposition syndromes. *Am J Hematol* (2021) 96(11):1450–60. doi: 10.1002/ajh.26321

7. 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. doi: 10.1182/blood-2016-03-643544

8. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the world health organization classification of haematolymphoid tumours: Myeloid and Histiocytic/Dendritic neoplasms. *Leukemia* (2022) 36 (7):1703–19. doi: 10.1038/s41375-022-01613-1

9. Roloff GW, Drazer MW, Godley LA. Inherited susceptibility to hematopoietic malignancies in the era of precision oncology. *JCO Precis Oncol* (2021) 5:107–22. doi: 10.1200/PO.20.00387

10. Kim B, Yun W, Lee ST, Choi JR, Yoo KH, Koo HH, et al. Prevalence and clinical implications of germline predisposition gene mutations in patients with acute myeloid leukemia. *Sci Rep* (2020) 10(1):14297. doi: 10.1038/s41598-020-71386-z

11. Sebert M, Passet M, Raimbault A, Rahme 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. doi: 10.1182/blood.2019000909

12. Baliakas P, Tesi B, Wartiovaara-Kautto U, Stray-Pedersen A, Friis LS, Dybedal I, et al. Nordic Guidelines for germline predisposition to myeloid neoplasms in adults: Recommendations for genetic diagnosis, clinical management and follow-up. *Hemasphere* (2019) 3(6):e321. doi: 10.1097/HS9.0000000000000321

13. Brown AL, Hahn CN, Scott HS. Secondary leukemia in patients with germline transcription factor mutations (RUNX1, GATA2, CEBPA). *Blood* (2020) 136(1):24–35. doi: 10.1182/blood.2019000937

14. Tawana K, Wang J, Renneville A, Bodor C, Hills R, Loveday C, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. *Blood* (2015) 126(10):1214–23. doi: 10.1182/blood-2015-05-647172

15. 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. doi: 10.1016/j.ccell.2015.03.017

16. Duployez N, Largeaud L, Duchmann M, Kim R, Rieunier J, Lambert J, et al. Prognostic impact of DDX41 germline mutations in intensively treated acute myeloid leukemia patients: An ALFA-FILO study. *Blood* (2022) 140(7):756–68. doi: 10.1182/blood.2021015328

17. Bannon SA, Routbort MJ, Montalban-Bravo G, Mehta RS, Jelloul FZ, Takahashi K, et al. Next-generation sequencing of DDX41 in myeloid neoplasms leads to increased detection of germline alterations. *Front Oncol* (2020) 10:582213. doi: 10.3389/fonc.2020.582213

18. Klco JM, Mullighan CG. Advances in germline predisposition to acute leukaemias and myeloid neoplasms. *Nat Rev Cancer* (2021) 21(2):122–37. doi: 10.1038/s41568-020-00315-z

19. Hendrickson PG, Luo Y, Kohlmann W, Schiffman J, Maese L, Bishop AJ, et al. Radiation therapy and secondary malignancy in Li-fraumeni syndrome: A hereditary cancer registry study. *Cancer Med* (2020) 9(21):7954–63. doi: 10.1002/cam4.3427

20. Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet* (1999) 23(2):166–75. doi: 10.1038/13793

21. Crysandt M, Brings K, Beier F, Thiede C, Brummendorf TH, Jost E. Germ line predisposition to myeloid malignancies appearing in adulthood. *Expert Rev Hematol* (2018) 11(8):625–36. doi: 10.1080/17474086.2018.1494566

22. Hahn CN, Chong CE, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet* (2011) 43(10):1012–7. doi: 10.1038/ng.913

23. Narumi S, Amano N, Ishii T, Katsumata N, Muroya K, Adachi M, et al. SAMD9 mutations cause a novel multisystem disorder, MIRAGE syndrome, and

are associated with loss of chromosome 7. *Nat Genet* (2016) 48(7):792–7. doi: 10.1038/ng.3569

24. Hayashi Y, Harada Y, Harada H. Myeloid neoplasms and clonal hematopoiesis from the Runx1 perspective. *Leukemia* (2022) 36(5):1203–14. doi: 10.1038/s41375-022-01548-7

25. Nishimoto N, Imai Y, Ueda K, Nakagawa M, Shinohara A, Ichikawa M, et al. T Cell acute lymphoblastic leukemia arising from familial platelet disorder. *Int J Hematol* (2010) 92(1):194–7. doi: 10.1007/s12185-010-0612-y

26. Li Y, Yang W, Devidas M, Winter SS, Kesserwan C, Yang W, et al. Germline RUNX1 variation and predisposition to childhood acute lymphoblastic leukemia. *J Clin Invest* (2021) 131(17):e147898. doi: 10.1172/JCI147898

27. Sood R, Kamikubo Y, Liu P. Role of RUNX1 in hematological malignancies. *Blood* (2017) 129(15):2070–82. doi: 10.1182/blood-2016-10-687830

28. Toya T, Yoshimi A, Morioka T, Arai S, Ichikawa M, Usuki K, et al. Development of hairy cell leukemia in familial platelet disorder with predisposition to acute myeloid leukemia. *Platelets* (2014) 25(4):300–2. doi: 10.3109/09537104.2013.818636

29. Ernst MPT, Kavelaars FG, Lowenberg B, Valk PJM, Raaijmakers M. RUNX1 germline variants in RUNX1-mutant AML: How frequent? *Blood* (2021) 137(10):1428–31. doi: 10.1182/blood.2020008478

30. Simon L, Spinella JF, Yao CY, Lavallee VP, Boivin I, Boucher G, et al. High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. *Blood* (2020) 135(21):1882–6. doi: 10.1182/blood.2019003357

31. Drazer MW, Kadri S, Sukhanova M, Patil SA, West AH, Feurstein S, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. *Blood Adv* (2018) 2(2):146–50. doi: 10.1182/bloodadvances.2017013037

32. Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. *Leukemia* (2016) 30(11):2282. doi: 10.1038/leu.2016.207

33. Mendler JH, Maharry K, Radmacher MD, Mrozek K, Becker H, Metzeler KH, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and microrna expression signatures. *J Clin Oncol* (2012) 30(25):3109–18. doi: 10.1200/JCO.2011.40.6652

34. Owen C, Barnett M, Fitzgibbon J. Familial myelodysplasia and acute myeloid leukaemia–a review. *Br J Haematol* (2008) 140(2):123–32. doi: 10.1111/j.1365-2141.2007.06909.x

35. Brown AL, Arts P, Carmichael CL, Babic M, Dobbins J, Chong CE, et al. RUNX1-mutated families show phenotype heterogeneity and a somatic mutation profile unique to germline predisposed AML. *Blood Adv* (2020) 4(6):1131–44. doi: 10.1182/bloodadvances.2019000901

36. Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, et al. Genomic analysis of germ line and somatic variants in familial Myelodysplasia/Acute myeloid leukemia. *Blood* (2015) 126(22):2484–90. doi: 10.1182/blood-2015-04-641100

37. Yoshimi A, Toya T, Kawazu M, Ueno T, Tsukamoto A, Iizuka H, et al. Recurrent CDC25C mutations drive malignant transformation in Fpd/Aml. *Nat Commun* (2014) 5:4770. doi: 10.1038/ncomms5770

38. Forster A, Decker M, Schlegelberger B, Ripperger T. Beyond pathogenic RUNX1 germline variants: The spectrum of somatic alterations in RUNX1-familial platelet disorder with predisposition to hematologic malignancies. *Cancers (Basel)* (2022) 14(14):3431. doi: 10.3390/cancers14143431

39. Ripperger T, Steinemann D, Gohring G, Finke J, Niemeyer CM, Strahm B, et al. A novel pedigree with heterozygous germline RUNX1 mutation causing familial MDS-related AML: Can these families serve as a multistep model for leukemic transformation? *Leukemia* (2009) 23(7):1364–6. doi: 10.1038/leu.2009.87

40. Wlodarski MW, Hirabayashi S, Pastor V, Stary J, Hasle H, Masetti R, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood* (2016) 127(11):1387–97. doi: 10.1182/blood-2015-09-669937

41. Donadieu J, Lamant M, Fieschi C, de Fontbrune FS, Caye A, Ouachee M, et al. Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients. *Haematologica* (2018) 103(8):1278–87. doi: 10.3324/haematol.2017.181909

42. Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. *N Engl J Med* (2004) 351(23):2403–7. doi: 10.1056/NEJMoa041331

43. Pabst T, Eyholzer M, Haefliger S, Schardt J, Mueller BU. Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia. *J Clin Oncol* (2008) 26(31):5088–93. doi: 10.1200/JCO.2008.16.5563

44. Taskesen E, Bullinger L, Corbacioglu A, Sanders MA, Erpelinck CA, Wouters BJ, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: Further evidence for CEBPA double mutant aml as a distinctive disease entity. *Blood* (2011) 117(8):2469–75. doi: 10.1182/blood-2010-09-307280

45. Su L, Shi YY, Liu ZY, Gao SJ. Acute myeloid leukemia with CEBPA mutations: Current progress and future directions. *Front Oncol* (2022) 12:806137. doi: 10.3389/fonc.2022.806137

46. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/Enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. *Nat Genet* (2001) 27(3):263–70. doi: 10.1038/85820

47. Choi EJ, Cho YU, Hur EH, Jang S, Kim N, Park HS, et al. Unique ethnic features of DDX41 mutations in patients with idiopathic cytopenia of undetermined significance, myelodysplastic syndrome, or acute myeloid leukemia. *Haematologica* (2022) 107(2):510–8. doi: 10.3324/haematol.2020.270553

48. 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. doi: 10.1002/ajh.25486

49. Makishima H. [Genomic aberrations in myelodysplastic syndromes and related disorders]. *Rinsho Ketsueki* (2019) 60(6):600–9. doi: 10.11406/rinketsu.60.600

50. Li P, Brown S, Williams M, White T, Xie W, Cui W, et al. The genetic landscape of germline DDX41 variants predisposing to myeloid neoplasms. *Blood* (2022) 140(7):716–55. doi: 10.1182/blood.2021015135

51. Li P, White T, Xie W, Cui W, Peker D, Zeng G, et al. Aml with germline DDX41 variants is a clinicopathologically distinct entity with an indolent clinical course and favorable outcome. *Leukemia* (2022) 36(3):664–74. doi: 10.1038/s41375-021-01404-0

52. Makishima H, Saiki R, Nannya Y, Korotev S, Gurnari C, Takeda J, et al. P741: DDX41 mutations define a unique subtype of myeloid neoplasms. *HemaSphere* (2022) 6:636–7. doi: 10.1097/01.HS9.0000845848.97346.3e

53. Schlegelberger B, Mecucci C, Wlodarski M. Review of guidelines for the identification and clinical care of patients with genetic predisposition for hematological malignancies. *Fam Cancer* (2021) 20(4):295–303. doi: 10.1007/s10689-021-00263-z

54. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* (2017) 129(4):424–47. doi: 10.1182/blood-2016-08-733196

55. Churpek JE, Lorenz R, Nedumgottil S, Onel K, Olopade OI, Sorrell A, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic Syndrome/Acute leukemia predisposition syndromes. *Leuk Lymphoma* (2013) 54(1):28–35. doi: 10.3109/10428194.2012.701738

56. Morita K, Kantarjian HM, Wang F, Yan Y, Bueso-Ramos C, Sasaki K, et al. Clearance of somatic mutations at remission and the risk of relapse in acute myeloid leukemia. *J Clin Oncol* (2018) 36(18):1788–97. doi: 10.1200/JCO.2017.77.6757

57. Tanaka T, Morita K, Loghavi S, Wang F, Furudate K, Sasaki Y, et al. Clonal dynamics and clinical implications of postremission clonal hematopoiesis in acute myeloid leukemia. *Blood* (2021) 138(18):1733–9. doi: 10.1182/blood.2020010483

58. Tawana K, Brown AL, Churpek JE. Integrating germline variant assessment into routine clinical practice for myelodysplastic syndrome and acute myeloid leukaemia: Current strategies and challenges. *Br J Haematol* (2022) 196(6):1293–310. doi: 10.1111/bjh.17855

59. Engvall M, Karlsson Y, Kuchinskaya E, Jornegren A, Mathot L, Pandzic T, et al. Familial platelet disorder due to germline exonic deletions in RUNX1: A diagnostic challenge with distinct alterations of the transcript isoform equilibrium. *Leuk Lymphoma* (2022) 1-10. doi: 10.1080/10428194.2022.2067997

60. Feurstein S, Drazer M, Godley LA. Germline predisposition to hematopoietic malignancies. *Hum Mol Genet* (2021) 30(20):R225–R35. doi: 10.1093/hmg/ddab141

61. Kohlmann W, Schiffman JD. Discussing and managing hematologic germ line variants. *Blood* (2016) 128(21):2497–503. doi: 10.1182/blood-2016-06-716704

62. Wakita S, Sakaguchi M, Oh I, Kako S, Toya T, Najima Y, et al. Prognostic impact of CEBPA bZIP domain mutation in acute myeloid leukemia. *Blood Adv* (2022) 6(1):238–47. doi: 10.1182/bloodadvances.2021004292

63. Tarlock K, Lamble AJ, Wang YC, Gerbing RB, Ries RE, Loken MR, et al. CEBPA-bZIP mutations are associated with favorable prognosis in *de novo* AML: A report from the children's oncology group. *Blood* (2021) 138(13):1137–47. doi: 10.1182/blood.2020009652

64. Parta M, Shah NN, Baird K, Rafei H, Calvo KR, Hughes T, et al. Allogeneic hematopoietic stem cell transplantation for GATA2 deficiency using a busulfan-based regimen. *Biol Blood Marrow Transplant* (2018) 24(6):1250–9. doi: 10.1016/j.bbmt.2018.01.030

65. Jorgensen SF, Buechner J, Myhre AE, Galteland E, Spetalen S, Kulseth MA, et al. A nationwide study of GATA2 deficiency in Norway-the majority of patients have undergone allo-HSCT. *J Clin Immunol* (2022) 42(2):404–20. doi: 10.1007/s10875-021-01189-y

66. Galera P, Hsu AP, Wang W, Droll S, Chen R, Schwartz JR, et al. Donor-derived MDS/AML in families with germline GATA2 mutation. *Blood* (2018) 132 (18):1994–8. doi: 10.1182/blood-2018-07-861070

67. 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. doi: 10.1038/leu.2016.310

68. Kobayashi S, Kobayashi A, Osawa Y, Nagao S, Takano K, Okada Y, et al. Donor cell leukemia arising from preleukemic clones with a novel germline DDX41 mutation after allogenic hematopoietic stem cell transplantation. *Leukemia* (2017) 31(4):1020–2. doi: 10.1038/leu.2017.44

69. Kato M, Yamashita T, Suzuki R, Matsumoto K, Nishimori H, Takahashi S, et al. Donor cell-derived hematological malignancy: A survey by the Japan society for hematopoietic cell transplantation. *Leukemia* (2016) 30(8):1742–5. doi: 10.1038/leu.2016.23

70. Gibson CJ, Kim HT, Zhao L, Murdock HM, Hambley B, Ogata A, et al. Donor clonal hematopoiesis and recipient outcomes after transplantation. *J Clin Oncol* (2022) 40(2):189–201. doi: 10.1200/JCO.21.02286

71. Williams L, Doucette K, Karp JE, Lai C. Genetics of donor cell leukemia in acute myelogenous leukemia and myelodysplastic syndrome. *Bone Marrow Transplant* (2021) 56(7):1535–49. doi: 10.1038/s41409-021-01214-z

72. Inamoto Y, Martin PJ, Flowers ME, Lee SJ, Carpenter PA, Warren EH, et al. Genetic risk factors for sclerotic graft-Versus-Host disease. *Blood* (2016) 128 (11):1516–24. doi: 10.1182/blood-2016-05-715342

73. Grossman J, Cuellar-Rodriguez J, Gea-Banacloche J, Zerbe C, Calvo K, Hughes T, et al. Nonmyeloablative allogeneic hematopoietic stem cell transplantation for GATA2 deficiency. *Biol Blood Marrow Transplant* (2014) 20 (12):1940–8. doi: 10.1016/j.bbmt.2014.08.004

74. Nichols-Vinueza DX, Parta M, Shah NN, Cuellar-Rodriguez JM, Bauer TRJr., West RR, et al. Donor source and post-transplantation cyclophosphamide influence outcome in allogeneic stem cell transplantation for GATA2 deficiency. *Br J Haematol* (2022) 196(1):169–78. doi: 10.1111/bjh.17840

75. Sakaguchi H, Yoshida N. Recent advances in hematopoietic cell transplantation for inherited bone marrow failure syndromes. *Int J Hematol* (2022) 116(1):16–27. doi: 10.1007/s12185-022-03362-4

76. Savage SA, Walsh MF. Myelodysplastic syndrome, acute myeloid leukemia, and cancer surveillance in fanconi anemia. *Hematol Oncol Clin North Am* (2018) 32(4):657–68. doi: 10.1016/j.hoc.2018.04.002

77. Ibrahim RB, Abella EM, Chandrasekar PH. Tacrolimus-clarithromycin interaction in a patient receiving bone marrow transplantation. *Ann Pharmacother* (2002) 36(12):1971–2. doi: 10.1345/aph.1C117

78. van Lier YF, de Bree GJ, Jonkers RE, Roelofs J, Ten Berge IJM, Rutten CE, et al. Allogeneic hematopoietic cell transplantation in the management of GATA2 deficiency and pulmonary alveolar proteinosis. *Clin Immunol* (2020) 218:108522. doi: 10.1016/j.clim.2020.108522

79. Hofmann I, Avagyan S, Stetson A, Guo D, Al-Sayegh H, London WB, et al. Comparison of outcomes of myeloablative allogeneic stem cell transplantation for pediatric patients with bone marrow failure, myelodysplastic syndrome and acute myeloid leukemia with and without germline GATA2 mutations. *Biol Blood Marrow Transplant* (2020) 26(6):1124–30. doi: 10.1016/j.bbmt.2020.02.015

80. Alkhateeb HB, Nanaa A, Viswanatha D, Foran JM, Badar T, Sproat L, et al. Genetic features and clinical outcomes of patients with isolated and comutated DDX41-mutated myeloid neoplasms. *Blood Adv* (2022) 6(2):528–32. doi: 10.1182/bloodadvances.2021005738

81. Bortnick R, Wlodarski M, de Haas V, De Moerloose B, Dworzak M, Hasle H, et al. Hematopoietic stem cell transplantation in children and adolescents with GATA2-related myelodysplastic syndrome. *Bone Marrow Transplant* (2021) 56 (11):2732–41. doi: 10.1038/s41409-021-01374-y

82. Ahn JS, Kim JY, Kim HJ, Kim YK, Lee SS, Jung SH, et al. Normal karyotype acute myeloid leukemia patients with CEBPA double mutation have a favorable prognosis but no survival benefit from allogeneic stem cell transplant. *Ann Hematol* (2016) 95(2):301–10. doi: 10.1007/s00277-015-2540-7

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PAX5 alterations in B-cell acute lymphoblastic leukemia

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PAX5, a master regulator of B cell development and maintenance, is one of the most common targets of genetic alterations in B-cell acute lymphoblastic leukemia (B-ALL). *PAX5* alterations consist of copy number variations (whole gene, partial, or intragenic), translocations, and point mutations, with distinct distribution across B-ALL subtypes. The multifaceted functional impacts such as haploinsufficiency and gain-of-function of PAX5 depending on specific variants have been described, thereby the connection between the blockage of B cell development and the malignant transformation of normal B cells has been established. In this review, we provide the recent advances in understanding the function of PAX5 in orchestrating the development of both normal and malignant B cells over the past decade, with a focus on the *PAX5* alterations shown as the initiating or driver events in B-ALL. Recent large-scale genomic analyses of B-ALL have identified multiple novel subtypes driven by *PAX5* genetic lesions, such as the one defined by a distinct gene expression profile and *PAX5* P80R mutation, which is an exemplar leukemia entity driven by a missense mutation. Although altered *PAX5* is shared as a driver in B-ALL, disparate disease phenotypes and clinical outcomes among the patients indicate further heterogeneity of the underlying mechanisms and disturbed gene regulation networks along the disease development. In-depth mechanistic studies in human B-ALL and animal models have demonstrated high penetrance of *PAX5* variants alone or concomitant with other genetic lesions in driving B-cell malignancy, indicating the altered *PAX5* and deregulated genes may serve as potential therapeutic targets in certain B-ALL cases.

KEYWORDS

***PAX5*, *PAX5* alterations, B cell development, B-cell acute lymphoblastic leukemia, driver genetic lesions, B-ALL subtype, PAX5alt, *PAX5* P80R**

Background

B lymphocytes are known for generating countless high-affinity antibodies against foreign pathogens. The development of B cells starts in the bone marrow, where the hematopoietic stem cells hierarchically differentiate into fate-restricted progenitors, eventually giving rise to immature B cells heading to the spleen, where B cells further differentiate into mature B cells.

This process is highly elaborate and orchestrated by a combination of intracellular mechanisms and external stimuli. Among them, PAX5 (paired box 5), or BSAP (B-cell-specific activator protein), is the pivotal transcription factor commanding the B cell development. It has been well-established that PAX5 is not only required to initiate B-lineage commitment but also essential for the maintenance of B cell identity by repressing signature genes of other lineages during the whole differentiation process.

Accompanied by the gradually uncovered mechanisms of PAX5 in normal B lymphopoiesis, extensive studies have revealed that deregulated PAX5 activities by somatic or germline alterations may lead to B-cell malignancies. Recent advances in genome-wide assays have greatly accelerated the discovery of genomic variants in B-cell acute lymphoblastic leukemia (B-ALL). SNP microarray and DNA sequencing of large cohorts of pediatric and adult B-ALL samples revealed diverse genetic lesions, of which *PAX5* was ranked the most frequently altered gene being detected in around one-third of B-ALL cases (Table 1) (1, 2, 6). The prevalence of *PAX5* alterations has been continually emphasized in different cohorts of B-ALL (3, 5, 7, 9, 10, 12, 15), with two B-ALL subtypes even defined by *PAX5* genetic lesions and distinct gene expression profiles (GEPs) (16). *PAX5* genetic lesions are heterogeneous, including deletions, rearrangements, sequence mutations, and focal intragenic amplifications (iAmp), which lead to the haploinsufficiency or gain-of-function of PAX5 depending on specific variants (1, 2, 8, 16). Rather than secondary events, increasing evidence from recent multi-omics and mechanistic studies has demonstrated that *PAX5* alterations can function as the initiating genetic lesions for B-ALL. In this review, we summarize recent advances in understanding the function of PAX5 in both normal and malignant B cells, with a focus on the *PAX5* alterations as founder events in B-ALL.

The function of PAX5 in B cell development

The multifaceted roles of PAX5 in B cell development and differentiation have been gradually unveiled. In blood cells, *PAX5* expression is exclusively restricted to the B lineage,

TABLE 1 *PAX5* alterations in B-ALL.

Cohort	Platform	Comments on *PAX5* alteration	Ref.
242 pediatric ALL	SNP array	*PAX5* gene is the most frequent target of somatic mutation, being altered in 31.7% of cases.	(1)
40 pediatric ALL	SNP array	Cell cycle and B cell related genes, including *PAX5*, are the most frequent mutated genes.	(2)
304 ALL samples	SNP array	Deletion of *PAX5* in 51% BCR::ABL1 cases, of which 95% have a deletion of *IKZF1*.	(3)
61 pediatric B-ALL (diagnosis & relapse)	SNP array	Around 50% of B-ALL have CNAs in genes known to regulate B-lymphoid development, especially in *PAX5* and *IKZF1* genes.	(4)
399 pediatric ALL	SNP array	7 cases harbor *PAX5* fusions.	(5)
221 pediatric B-ALL (high-risk)	SNP array, GEP array, target sequencing	*PAX5* CNA is involved in 31.7% of patients; P80R is the most frequent mutation.	(6)
466 pediatric ALL	FISH	*PAX5* rearrangements occur in 2.5% of B-ALL.	(7)
117 adult B-ALL	FISH, qPCR, target sequencing	*PAX5* is mutated in 34% of adult B-ALL. P80R is the most frequent point mutation. *PAX5* deletion is a secondary event.	(8)
153 adult and pediatric B-ALL with 9p abnormalities	SNP array, FISH	*PAX5* has internal rearrangements in 21% of the cases. Malignant cells carrying *PAX5* fusion genes displayed a simple karyotype.	(9)
89 Ph^+ B-ALL	SNP array	*PAX5* genomic deletions were identified in 29 patients (33%). In all cases, the deletion was heterozygous.	(10)
Two B-ALL families	WES, SNP array	Germline *PAX5* G183S confers susceptibility to B-ALL.	(11)
116 B-ALL	MLPA	5 cases with *PAX5* intragenic amplifications were identified.	(12)
One B-ALL family	SNP array	A third B-ALL family carrying germline G183S mutation.	(13)
798 adult B-ALL	GEP array, SNP array, RNA-seq	38% of Ph-like B-ALL have *PAX5* alterations. Enrichment of CNA of *IKZF1*, *PAX5*, *EBF1*, and *CDKN2A/B* observed in the Ph-like subtype.	(14)
79 B-ALL with *PAX5* iAmp	MLPA, FISH, SNP array	*PAX5* iAmp defines a novel, relapse-prone subtype of B-ALL with a poor outcome.	(15)
1,988 B-ALL	RNA-seq, WGS, WES, SNP array	Detailed description of *PAX5* alterations in B-ALL. Defined the PAX5alt and PAX5 P80R subtypes.	(16)
110 pediatric B-others	RNA-seq, WES, SNP array	*PAX5* fusions, iAmp and P80R mutations are mutually exclusive, altogether accounting for 20% of the B-other group. *PAX5* P80R is associated with a specific gene expression signature.	(17)
250 B-ALL	DNA methylation array, WES, RNA-seq	16 patients with P80R grouped into an individual subgroup with biallelic *PAX5* alterations.	(18)
1,028 pediatric B-ALL	SNP array	20 cases of *PAX5* P80R with intermediate or poor outcome compared to the rest of this cohort.	(19)
One B-ALL family	WES, RNA-seq	*PAX5* R38H germline mutation was identified in a family with B-ALL.	(20)

CNA, copy number alterations; GEP, gene expression profile; WGS, whole genome sequencing; WES, whole exome sequencing; FISH, fluorescence in situ hybridization; MLPA, multiplex ligation-dependent probe amplification; RNA-seq, whole transcriptome sequencing.

beginning from the early pre-pro B cells and maintained through the whole process of B cell development (Figure 1) (27). Expression levels of *PAX5* are correlated with B cell developmental stages (28). During terminal differentiation from mature B cells to plasma cells, physiological down-regulation of *PAX5* is observed (29). This repression is not necessary for plasma cell development but essential for optimal IgG production (30). Constitutive deletion of *Pax5* in mice failed to produce mature B cells owing to a complete arrest of B lymphopoiesis at an early pro-B stage in the bone marrow (31). In contrast, B cell development is blocked at an earlier stage even before the appearance of $B220^+$ progenitors in the fetal liver, suggesting different roles of PAX5 in fetal and postnatal B lymphopoiesis (31, 32). Without *Pax5*, pro-B cells retain lineage-promiscuous capacity that can differentiate into other lineages upon stimulation with proper cytokines (32–34). Therefore, PAX5 is not only required for B lymphopoiesis initiation but also continuously required for its maintenance (34). Conditional inactivated *Pax5* expression from pro-B to mature B cell stages leads to down-regulation of B-cell-specific genes and preferential loss of mature B cells, indicating that PAX5 is essential for maintaining the identity of B cells during late B lymphopoiesis (35). Further investigation of deleting *Pax5* in immature B cells in the spleen results in the loss of B-1a, marginal zone, and germinal center B cells as well as plasma cells (22). Finally, *Pax5*-deficient follicular B cells fail to proliferate due to the inhibition of PI3K signaling *via* PTEN up-regulation (22).

PAX5 safeguards the development of B cells by tailoring the gene expression profile towards the B-lineage program. On one hand, it up-regulates the expression of B-cell-specific genes such as *CD19* and *BLNK* (36). On the other hand, it down-regulates lineage-inappropriate genes such as *FLT3* and *CCL3* to suppress alternative lineage choices (Figure 1) (33, 37). Both activation and repression require its continuous expression (37). ChIP analysis revealed that through binding to promoters and enhancers, PAX5 directly regulates 44% of previous identified PAX5-activated genes and 24% of repressed genes (38). For PAX5 activated genes, it can induce active chromatin marks at their regulatory elements (36, 38). For example, PAX5 activates *HCK* transcription by inducing active chromatin in the *HCK* promoter in MYD88-mutated lymphoma cells (21). Conversely, it can also modify the chromatin state of its repressed genes by eliminating active histone marks (38).

PAX5 is part of a complex network of transcription factors orchestrating B cell development, including IKZF1, E2A, EBF1, and RUNX1 (23, 39, 40). Together with IKZF1, PAX5 functions as a metabolic gatekeeper to restrict glucose and energy supply. Heterozygous deletion of *Pax5* releases this restriction and increases glucose uptake and ATP levels (23). Furthermore, PAX5 is found in a physiological complex together with IKZF1 and RUNX1. Specifically, over 65% of PAX5 binding sites identified in mouse pre-B cells are overlapped with regions bound by either IKZF1, RUNX1, or both, suggesting that they are part of a regulatory network sharing a multitude of target genes (40). In addition, PAX5 and EBF1 are actively involved in a reciprocal positive regulatory loop (41, 42), yet with opposing roles in *Myc* regulation through binding to the *Myc* promoter (24). They also cooperatively regulate IL-7 signaling and folate metabolism (25).

A signature feature of B cells is the recombination of V_HDJ_H segments to generate a functional *immunoglobulin heavy chain (IgH)* gene for B cell receptor and antibody coding. PAX5 contributes to the diversity of the antibody repertoire by balancing distal-proximal V_H gene choices. The first insight into the V_HDJ_H recombination role of PAX5 was provided by the observation that, in *Pax5*-deleted mouse pro-B and pre-B-I (large pre-B) cells, recombination of distal but not proximal V_H

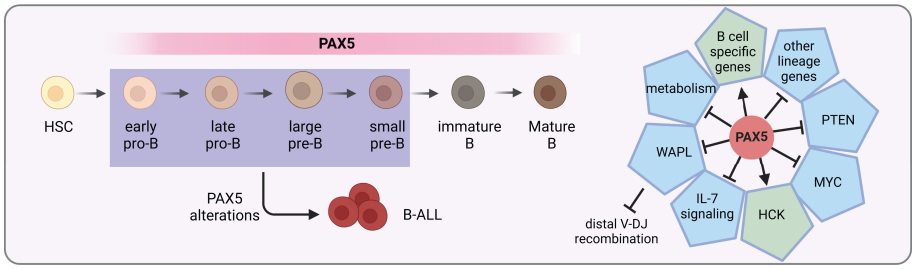

FIGURE 1
PAX5 functions in B cell development. *PAX5* is expressed during the whole B cell developmental stages. It activates the expression of B cell specific genes, while at the same time represses the expression of other lineage genes to initiate and maintain the B cell identity. In MYD88-driven B-cell lymphomas, it activates the pro-survival kinase HCK (21). In addition, it activates PI3K signaling *via* PTEN inhibition to stimulate follicular B cell proliferation (22). Furthermore, PAX5 safeguards leukemic transformation by limiting glucose and energy supply, inhibiting IL-7 signaling as well as *MYC* expression (23–25). Finally, PAX5 also has an essential role in the V(D)J recombination of the *IgH* locus by repressing *WAPL* expression (26). PAX5 alterations with compromised activity can lead to developmental arrest of B cells, which are commonly seen in B-ALL.

genes was dramatically compromised (43, 44). Further experiments uncovered that PAX5 can mediate spatial organization of the *Igh* locus to balance the accessibility of distal and proximal V_H genes (45). The mystery of this spatial regulation remained unsolved for more than a decade. Recently, it was found that PAX5 specifically inhibits the expression of *WAPL*, which encodes an architectural protein that releases the cohesin complex from chromatin (Figure 1) (26). With decreased levels of WAPL protein, chromatin loops are able to extrude for a longer distance to spatially connect the distal V_H genes with the recombination center during the loop extrusion process (26). Thus, PAX5 fulfills a master regulator role for B lymphopoiesis by, but not limited to, inducing B-lineage commitment, maintaining B cell identity, and regulating V_HDJ_H recombination.

Copy-number alterations of *PAX5* in B-ALL

Deletion is the most frequent form of copy number alteration of *PAX5* in B-ALL (1, 6, 8, 16). *PAX5* deletions usually affect only one allele, with either no expression or expression of truncated proteins lacking functional domains, resulting in loss of function of this allele (1). These monoallelic *PAX5* deletions in B-ALL are observed on different scales, from as focal as deletion of exons within *PAX5* gene body, to as large as loss of 9p arm or whole chromosome 9 where *PAX5* gene is located (1). In B-ALL, *PAX5* deletions are commonly concurrent with complete loss of *CDKN2A/B* genes, which encode key cell cycle regulators also situated in chromosome 9p (8, 46). Notably, *PAX5* deletions are associated with complex karyotype which is thought to be a secondary or late event, indicating the requirement of other oncogenic lesions to cause overt malignant transformation (1, 8). In support of this notion, *PAX5* deletions were found in over 50% of BCR::ABL1 and 18% of TCF3::PBX1 B-ALL cases (3, 8, 10), and were enriched in Ph-like B-ALL patients as well (14).

Studies using mouse models showed that haploinsufficiency of *Pax5* caused by monoallelic deletion exerted susceptibility of B cell transformation. Mice with heterozygous loss of *Pax5* show normal B cell development and never develop leukemia (47). But with the acquisition of other oncogenic events, they can spontaneously develop B-lineage leukemia. For example, $Pax5^{+/-}$ cooperated with STAT5 activation can initiate B-ALL with full penetrance in mice (47). Furthermore, compound heterozygous mutations in *Pax5* and *Ebf1* dramatically increase ALL frequency in mice (48), which is associated with the hyperactivation of the IL-7 signaling pathway (25). When synergized with *BCR::ABL1* in hematopoietic stem cells, $Pax5^{+/-}$ gives rise to B-ALL with shorter latencies and high incidence compared to *BCR::ABL1* alone (49). This synergistic effect may explain the frequent *PAX5* deletions in BCR::ABL1 B-ALL cases (3, 10). Finally, in $Pax5^{+/-}$ mice, *Jak3* mutations following postnatal infections can also act as a secondary hit for leukemic transformation (50).

The indispensable role of *PAX5* in B-lineage maintenance also explains the monoallelic but not biallelic deletions of *PAX5* observed in B-ALL cases. Complete loss of *Pax5* in mice resulted in the lack of B cells, growth retardation, and premature death (31). When *Pax5* deficiency is restricted to mature B cells to circumvent premature death, these cells dedifferentiate back into uncommitted progenitors and develop aggressive progenitor cell tumors instead of B-ALL (51). Therefore, *PAX5* deletions often disrupt only one allele and act as cooperating events in B-ALL.

Translocations

Translocations resulting in *PAX5* rearrangements occur in around 2.5% of pediatric and 1% of adult B-ALL patients (8). The majority of the rearrangements produce chimeric genes encoding proteins that retain the DNA-binding paired box domain and nuclear localization signal of PAX5, but with C-terminal domains adopted from the fusion partners (7) (Figure 2). A variety of partner genes, including transcription factors, structural proteins, and signal transducers, have been identified to fuse with the *PAX5* gene (7, 16).

With the intact paired domain, these fusion proteins are thought to bind DNA and act as dominant-negative proteins to interfere the wild-type (WT) PAX5 activities (1, 52–54). Notably, PAX5 protein consisting of only the paired domain cannot compete with full-length PAX5 for DNA binding *in vivo* (55), suggesting that the C terminal of PAX5 may contribute to DNA binding through unknown mechanisms. Indeed, PAX5 fusions with different partners display distinct DNA binding and gene regulation activities which should be examined case by case (56). In general, transient reporter assays revealed that PAX5 fusions functioned as a dominant-negative regulator for WT PAX5 through binding to PAX5-target sequences (5, 52). Some of the fusions, such as PAX5::C20S and PAX5::ETV6, showed stable DNA binding activity through forming oligomers due to the presence of oligomerization domains of the fusion partners (54, 56). As one exception, PAX5::PML barely showed DNA-binding activity but interfered with PAX5 regulatory activity through association with PAX5 proteins (53).

In contrast to deletions, *PAX5* fusions are commonly observed in leukemic cells displaying a relatively normal karyotype, indicating that they are founder lesions in leukemogenesis (9). In addition, *PAX5* fusions, except *PAX5::JAK2* and *PAX5::ZCCHC7*, are observed in over 30% of PAX5alt B-ALL, a recently reported subtype defined by various *PAX5* alterations and a distinct gene expression profile (16). Consistently, *PAX5* rearranged with *ETV6*, *ELN*, and *PML* were verified to be the primary oncogenic drivers in transgenic mice (55, 57, 58). PAX5::ETV6, the most recurrent PAX5 fusion

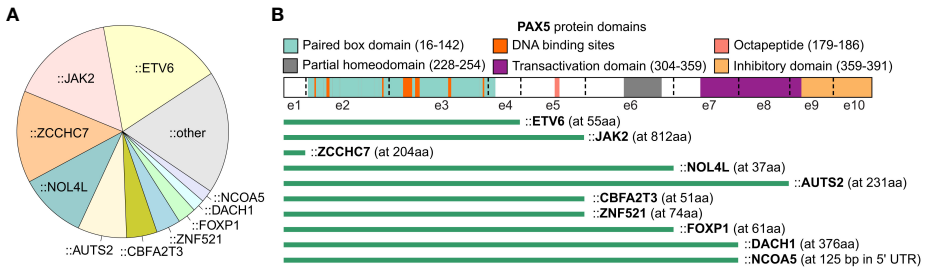

FIGURE 2
PAX5 rearrangements in B-ALL. The summary of PAX5 rearrangements is based on the result from the 1,988 B-ALL cohort (16). **(A)** Distribution of PAX5 fusion partners. The fusion partners observed in at least 2 B-ALL cases are annotated in the pie chart, and the singletons are merged into the "other" group. **(B)** Scheme of PAX5 rearrangements with recurrent partner genes. The most common isoform of each fusion is shown. The green bars indicate the remaining part of the PAX5 protein. The starting amino acid (aa) of the fusion partners are annotated in parentheses. All the rearrangements reserve the paired box DNA binding domain of PAX5, except the fusions with *ZCCHC7*, a proximal gene commonly fused with *PAX5* by focal deletion.

in B-ALL, contains three domains that contribute to DNA binding behavior, which are the paired box and helix-loop-helix domain of PAX5, and the DNA binding domain of ETV6 (16, 56, 59, 60). PAX5::ETV6 regulates 68% of PAX5-target genes in an opposing manner when transduced into murine B cells. This opposite dominant effect might be responsible for impaired B cell development (61). When knocked-in into the mouse *Pax5* locus, PAX5::ETV6 blocked B cell development at the pro-B to pre-B transition but was insufficient to promote leukemogenesis (55). However, when crossed with *Cdkn2a/b* deletion mice, B-lineage leukemia was developed at full penetrance with frequent loss of the remaining WT *Cdkn2a/b* allele (55). Comparing to *PAX5*::*ETV6*, *PAX5*::*ELN* acts as a more potent initiating event to induce leukemia, with frequent acquisition of secondary mutations in *Ptpn11*, *Jak3*, and *Kras* genes in mice (58). Different from the transient expression *in vitro*, the PAX5 fusions expressed in murine models do not generally antagonize the WT PAX5 function but activate independent biological pathways to establish the molecular basis required for leukemic transformation (55, 58). This discrepancy may be explained by either different protein levels or distinct regulatory mechanisms between transient and *in vivo* expressed proteins (58).

PAX5::*JAK2* rearrangement exerts a distinct gene expression signature in B-ALL and is exclusively found in the Ph-like subtype (16, 62). It consists of the paired domain of *PAX5* and the kinase domain of *JAK2* (7). In contrast to cytoplasmatic localization of other JAK2 fusions such as BCR::JAK2 and ETV6::JAK2, PAX5::JAK2 protein is localized in nucleus and binds the PAX5 targets (62). It simultaneously deregulates PAX5-target genes while activating JAK/STAT signaling in the nucleus (62). In a constitutive knock-in mouse model, PAX5::JAK2 rapidly induced aggressive B-ALL without acquisition of other cooperating mutations (63), which unequivocally implicated that PAX5::JAK2 functions as dual hits, which are PAX5 haploinsufficiency and constitutively active kinase activity, to drive leukemogenesis (63).

There's a rare translocation that does not produce chimeric protein but juxtaposes the *IGH* Eμ enhancer to proximity of the *PAX5* promoter, leading to dysregulation of *PAX5* expression. This translocation is found in a subset of B cell non-Hodgkin's lymphoma cases (64, 65). When reconstructed by insertion of a *PAX5* mini gene into the mouse *Igh* locus, these mice develop aggressive T-lymphoblastic lymphomas instead of B-ALL, probably because of the expression of *PAX5* throughout the lymphoid system as a germline mutation rather than as somatic mutations in patients (66). It also reflects the potential caveats of using mouse models to mimic the B-lineage malignancies induced by PAX5 alterations (67).

Intragenic amplification (iAmp)

PAX5 intragenic amplifications (*PAX5*-iAmp) were reported in different B-ALL cohorts at an incidence of 0.5-1.4% (1, 15–17, 68). B-ALL cases with *PAX5*-iAmp lacked stratifying genetic markers and were mutually exclusive from other risk-stratifying alterations (12, 15). Transcriptome sequencing (RNA-seq) revealed that they formed a tight cluster in unsupervised hierarchical cluster analysis (17) and can be grouped into the PAX5alt subtype (16). Interestingly, *PAX5*-iAmp frequently harbor *CDKN2A/B* homozygous loss and trisomy 5 (15, 17). The preservation of *PAX5*-iAmp in matched diagnosis and relapse samples, as well as GEP clustering in the PAX5alt subtype, indicates that it may act as a driver lesion in B-ALL (15, 16).

Whether *PAX5*-iAmp can encode structurally mutant PAX5 proteins or loss of function is still unknown. For most cases, the amplifications encompass exons 2 to 5, which encode the DNA-binding and octapeptide domains of PAX5 (15, 16, 68). Efforts

have been taken to delineate the copy number of the amplified region, including chromosomal microarray analysis and multiplex ligation dependent probe amplification-based testing (15, 68). Recently, optical genomic mapping, a direct visualization method, has been applied to 3 *PAX5*-iAmp cases and found that they have an extra 4-5 copies of exons 2 to 5 inserted *in situ* in direct orientation (68) (Figure 3). Considering the amplified paired domain by *PAX5*-iAmp, the increased copies of the DNA-binding region may alter the binding to PAX5-target genes, thus leading to dysregulated B cell differentiation and transformation. Further functional studies are still needed to address the specific role of *PAX5*-iAmp in B-ALL.

Alternative splicing and different isoforms

Alternative splicing of *PAX5* gene has been found during normal B cells development. By using two distinct promoters, *PAX5* can generate two different isoforms (*PAX5A* and *PAX5B*) that share the same exons 2-10 but with different exon 1 encoding N-terminal 15 or 14 amino acids, respectively (64). *Pax5A* is exclusively expressed in B cells, while *Pax5B* is active in all *Pax5*-expressing tissues such as the nervous system, testis, and B-lineage cells (64). In humans, five additional alternative isoforms have been detected in normal human B cells generated by the exclusion of exons 7, 8 and/or 9, which encode the C-terminal transactivation domain (69). The ability to induce CD19-promoter-based reporter expression by various isoforms was significantly influenced by the changes in the C-terminal domain (69). In mouse models, three additional isoforms of *Pax5* due to alternative splicing have been detected during B cells development. These isoforms arise from the exclusion of exon 2 and/or 3' region, encoding proteins lacking part of the DNA-binding and/or the transcriptional regulatory domains, which are assumed to participate in stage-specific regulation of B cell maturation (70).

In multiple myeloma, a plasma cell disorder, diverse *PAX5* isoforms have been identified accompanied with low levels of the WT PAX5 expression (71). These noncanonical isoforms are incapable of generating functional PAX5 proteins, which may drive proliferating B cells to prematurely differentiate into plasma cells (71). In B-ALL, alternative *PAX5* isoforms missing exon 2, exons 8-9, or exon 5 have been reported (72, 73). However, considering the frequent *PAX5* intragenic deletions in B-ALL (1, 16), some of the alternative isoforms found in B-ALL might be attributed to focal deletions instead of alternative splicing.

Point mutations

Point mutations are the second most common *PAX5* variants observed in B-ALL (7%~10%) (1, 6, 8). In 203 nonsilent *PAX5* mutations identified from 1,988 B-ALL cases (16), around three quarters are missense mutations enriched in the DNA-binding domain and are predicted to impair DNA binding by structural modelling, whereas disruptive mutations such as frameshift and nonsense are often found in the transcriptional regulatory domain (1, 16). The paired domain is a bipartite DNA-binding domain consisting of two subdomains (NTD and CTD). Each subdomain contains a helix-turn-helix motif which binds to major grooves of the DNA helix contributing to the overall binding affinity (74).

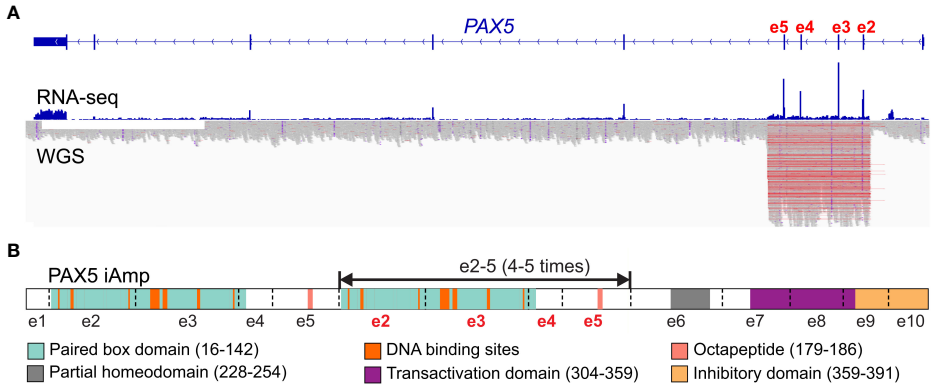

FIGURE 3
Scheme of *PAX5* intragenic amplification (*PAX5* iAmp). **(A)** *PAX5* iAmp detected by RNA-seq and whole genome sequencing (WGS (16)). The most frequently affected exons are e2-5, which are shown with increased expression levels (by RNA-seq) compared to the adjacent exons. The amplified region on the genomic level is reflected by abruptly elevated depth from WGS, which corroborates the affected exons identified from RNA-seq. **(B)** *PAX5* iAmp leads to an extended isoform of *PAX5*. Using optical genomic mapping, 4-5 extra copies of *PAX5* e2-5 were determined in the *PAX5* iAmp cases. The tandem multiplication of PAX5 paired box DNA binding domain may change its binding activity, thus altering its transcription program and disrupting B cell differentiation (68).

Although both contribute to DNA binding, NTD determines the specificity of binding with its affinity 10 times higher than the CTD (75, 76). Coincidentally, mutations within the paired domain tend to enrich in NTD compared to CTD (52.7% to 10.3%, respectively) (16). Supporting the prevalence and importance of paired domain mutations, a study used chemical and retroviral strategies to induce random mutagenesis in *Pax5*$^{+/-}$ mice. For the 13 induced *Pax5* mutations acting as cooperating lesions for B-ALL, 12 are in the paired domain (67).

PAX5 P80R, a substitution located in the paired domain, was identified as the most frequent sequence mutation of *PAX5* (1, 6, 8). B-ALL patients with the *PAX5* P80R mutation are classified as a novel subtype defined by this missense mutation and a highly distinct GEP (16, 77). This subtype is characterized by biallelic alterations of *PAX5*, homozygous deletion of *CDKN2A/B*, and hotspot activating mutations of RAS signaling (Figure 4) (16, 18). The biallelic alterations of *PAX5* are achieved by deletions, copy-neutral loss of heterozygosity, or deleterious mutations on the other allele of *PAX5* (16). Gene set enrichment analysis (GSEA) revealed dysregulation of B-cell-specific genes, suggesting that PAX5 P80R decreases the regulatory activity of PAX5 (16), probably through altering its DNA binding pattern (1). Consistently, PAX5 P80R blasts were arrested at the pre-pro-B stage (16), with T-cell antigen CD2 expressed in half of the patients (78). *PAX5* P80R B-ALL subtype, together with *DUX4r* and *ZNF384r* subtypes, frequently undergo monocytic switch (79). The patients of this subtype were reported with various levels of risk in different cohorts (16, 18, 19, 80). Therefore, further evaluation of the clinical significance of this novel subtype is still needed. The oncogenic role of PAX5 P80R has been demonstrated using constitutive knock-in mouse models. Both homozygous and heterozygous *Pax5* P80R transgenic mice developed B-lineage leukemia with almost complete penetrance. Analysis of mouse leukemia from the *Pax5* P80R heterozygous mice revealed the disruption of the remaining *Pax5* WT allele by deletion or frameshift mutations, which recapitulated the loss of *PAX5* WT allele in patient samples (16).

Non-silent *PAX5* mutations are observed in over 30% of the PAX5alt group compared to less than 5% of the other B-ALL cases (16). Over 40% of *PAX5* mutations within PAX5alt subtype are hemizygous due to loss of the *PAX5* WT allele. The two most recurrent mutations enriched in this subtype are R38H and R140L, which are located in the NTD and CTD of the DNA binding domain, respectively. Notably, 10 of 11 R140L mutations were found co-occurrent with R38H in the same patients (16). RNA-seq of one familial B-ALL case observed that these two mutations were detected on different alleles (20). PAX5alt cases with *PAX5* mutations (except R38H and R140L) are commonly observed with loss of the remaining *PAX5* WT allele and total deletion of *CDKN2A/B* genes. They are also enriched with RAS signaling pathway mutations as cooperating events (Figure 4) (16). Besides *PAX5* mutations, *PAX5* rearrangements and intragenic amplifications were also reported as signature genetic lesions of the PAX5alt group (16). The prognosis of this subtype is significantly worse than *PAX5* P80R (16), especially in adult cases (78). Within the PAX5alt subtype, patients with *IKZF1* deletions were observed with even worse prognosis (81). In conclusion, the large collection of *PAX5*

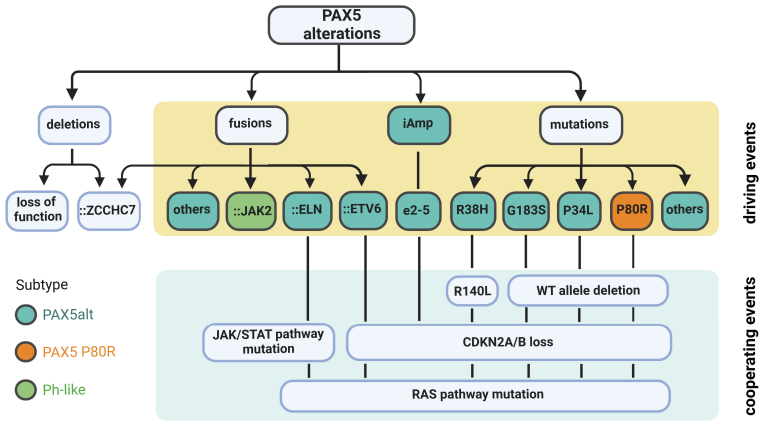

FIGURE 4
Summary of *PAX5* alterations in B-ALL. Deletion is the most common type of *PAX5* alteration in B-ALL, but generally considered as a secondary driver event. Focal deletion can lead to the concatenation of PAX5 to its adjacent gene ZCCHC7, a genetic lesion frequently observed in Ph-like and other B-ALL subtypes. *PAX5* fusions, iAmp (most commonly targeting exon 2 to 5 (e2-5)), and point mutations are highly enriched in the PAX5alt subtype (16). *PAX5* fusions and iAmp of e2-5 driven B-ALL harbor biallelic *CDKN2A/B* loss and RAS or JAK/STAT pathway mutations (16, 55, 58). *PAX5*::*JAK2*, a signature fusion of the Ph-like subtype, can act as a dual hit for B-ALL without cooperating lesions (63). *PAX5*-mutation-driven cases normally have deletion of the remaining WT allele and total loss of *CDKN2A/B*. They also frequently acquire mutations in the RAS signaling pathway. *PAX5* P80R mutation defines an independently subtype with a distinct GEP (16).

point mutations in B-ALL with unique gene expression features implies that besides P80R, certain *PAX5* mutations may also act as initiating driver events. Further direct experimental evidence is needed to test this hypothesis.

Germline variants and B-ALL susceptibility

PAX5 germline variants have been identified in multiple familial B-ALL studies. Although rare, the existence of certain familial B-ALL cases provided compelling evidence that *PAX5* germline variants can induce B-ALL. The first evidence came from a heterozygous germline variant *PAX5* G183S, affecting the octapeptide domain of PAX5, found in three unrelated B-ALL kindreds with incomplete penetrance (11) (13). All affected cases exhibited chromosome 9p deletion that removed the *PAX5* WT allele and caused homozygous deletion of *CDKN2A/B* (Figure 4). Functional and gene expression analysis of the *PAX5* G183S mutation demonstrated that it significantly reduced transcriptional activity of PAX5 (11). The finding of *PAX5* WT allele deletion in *PAX5* G183S cases suggests that a complete disruption of WT *PAX5* is essential for B cell developmental arrest mediated by G183S (11, 13).

Further evidence came from another family with a high incidence of B-ALL affecting all three children, which harbored a *PAX5* R38H germline variant (20). This variant was inherited from one of the parents who didn't develop leukemia, suggesting that additional lesions are required for full transformation. Consistently, all affected children gained mutations in the remaining *PAX5* WT allele. Specifically, two of them independently developed R140L mutation, which is commonly concomitant with R38H in sporadic B-ALL, while the remaining one had a *PAX5* frameshift mutation at Y371. In addition, all three children had *CDKN2A/B* homozygous loss and RAS signaling pathway mutations (Figure 4) (20). When transduced into murine *Pax5*$^{-/-}$ cells, *PAX5* R38H failed to regulate PAX5-target genes, suggesting that R38H impaired normal PAX5 function (20). Comparing to *PAX5* G183S germline variant, *PAX5* R38H is associated with an older onset, but both shared the feature of disrupting the PAX5 WT allele and *CDKN2A/B* genes (11, 13, 20). Taken together, these findings strengthen the conclusion that *PAX5* germline variants can confer strong B-ALL susceptibility and are associated with specific additional genetic lesions to initiate overt B-ALL.

Therapeutic potential of *PAX5* alterations

As the most frequent genetic lesions in B-ALL, *PAX5* alterations have been demonstrated to impair B cell differentiation and give rise to overt leukemia with the acquisition of cooperating genetic lesions. In addition, ongoing *PAX5* deficiency is required for maintaining the lymphoblastic status of the malignant B cells *in vivo* (82). Based on these findings, strategies such as re-activating the differentiation potential of the malignant B cells to circumvent the developmental blockage may provide new therapeutic entry points. Indeed, restoring *Pax5* through Tet-Off the transgenic shPax5 in a mouse B-ALL model (driven by *Pax5* knockdown and constitutively active Stat5) enables differentiation and immunophenotypic maturation by reshaping the B cell development program, leading to durable disease remission (82). Remarkably, even brief *Pax5* restoration in B-ALL cells causes rapid cell cycle exit and disables their leukemia-initiating capacity (82). In addition, reconstitution of *PAX5* in B-ALL patient samples carrying *PAX5* deletions can restore an energy nonpermissive state, leading to energy crisis and cell death (23). Interestingly, forced expression of *PAX2* or *PAX8*, the two most closely related paralogs of *PAX5*, resulted in growth inhibition of the REH cell line, which carries a heterozygous *PAX5* A322fs frameshift mutation. These two paralogs complement the haploinsufficiency of *PAX5* in B-ALL cells by modulating PAX5-target genes and restoring B cell differentiation (83). Therefore, approaches that can by-pass the differentiation blockage resulting from PAX5 haploinsufficiency may lead to novel therapeutic approaches for this group of B-ALL, including but not limited to *PAX5* restoration and *PAX5* paralog activation.

The deregulated networks triggered by *PAX5* variants may offer other therapeutic strategies as well. For example, PAX5 deficiency can lead to upregulated metabolic genes and consequently increased glucose uptake and energy metabolism, which are essential for leukemic transformation (23, 49). Specifically, glucocorticoid receptor NR3C1, glucose-feedback sensor TXNIP, and cannabinoid receptor CNR2 were identified as central effectors of energy supply restriction in B cells. In addition, agonists against CNR2 and TXNIP synergized with glucocorticoids to exacerbate B-cell-intrinsic ATP depletion and restored the energy barrier against B-cell malignancy (23). Furthermore, *Pax5* heterozygosis can enhance the expression of inflammatory cytokine interleukin−6 (IL−6), which then promote proliferation of leukemia cells. Genetic down-regulation or pharmacologic inhibition of IL−6 is beneficial to leukemic cell clearance (84). In addition, as mentioned above, *PAX5*-variant-related leukemia is commonly associated with aberrant activation of the kinase pathways such as JAK/STAT and RAS signaling. On one hand, treatment with kinase inhibitors resulted in increased apoptosis of leukemic cells (50, 85). On the other hand, considering the requirement of converging genetic lesions into one principal pathway for leukemia initiation, pharmacological reactivation of suppressed divergent pathways may also provide a powerful barrier to leukemic transformation (86). Finally, since the majority of B-ALL subtypes are observed with distinct GEPs, the subtype-specific biomarkers may serve as targets for developing tailored

therapies. Notably, the *MEGF10* gene was exclusively overexpressed in the *PAX5* P80R B-ALL subtype, which may serve as a biomarker as well as a potential therapeutic target for this subtype (16).

Discussion

Recent genomic and transcriptomic analysis of B-ALL has largely advanced our understanding of PAX5 and its altered isoforms in regulating normal B cell development and driving malignant transformation (Table 1). It has been demonstrated that genetic alterations of *PAX5* in B-ALL commonly lead to a reduction in rather than a total loss of PAX5 activity. These observations suggest that a certain level of PAX5 activity is required in B-ALL to maintain B cell identity and sustain clonal expansion but is insufficient to execute normal B cell differentiation. Therefore, the remaining *PAX5* WT allele must be ablated by either deletions or deleterious mutations to achieve this haploinsufficiency threshold. The process of acquiring additional genetic lesions in *PAX5*-altered B-ALL and the underlying mechanisms are intriguing but still largely unknown. The mechanisms of V(D)J recombination, as well as class-switch recombination and somatic hypermutation in B cell development might be exploited to generate these oncogenic lesions.

In-depth investigation of the oncogenic roles of germline and somatic *PAX5* variants is still largely unavailable. Reconstruction of genetic lesions in mouse models to recapitulate the corresponding human disease is widely applied to approach this goal. However, cautions should be taken considering the potential phenotypical discrepancies between human diseases and mouse models. For example, the *Igh::Pax5* mice develop T-ALL instead of B-ALL observed in patients, which might be attributed to the germline nature of the fusion gene in mice (66). Moreover, the *Pax5*::*Jak2* mouse model generates a more aggressive leukemia through loss of the *Pax5* WT allele caused by uniparental disomy of the *Pax5*::*Jak2* allele, but the *PAX5* WT allele is normally retained in human *PAX5*::*JAK2* leukemia (63). Finally, *Pax5* G183S is insufficient for malignant transformation in a transgenic mouse model (16), although it has been found as a germline variant associated with strong susceptibility to human B-ALL (11, 13). Nonetheless, mouse models still play a critical role for investigating the function of *PAX5* variants in leukemic transformation.

In summary, genome-wide technologies have greatly refined the molecular diagnosis of B-ALL, at the same time leading to the discovery of diverse *PAX5* alterations as primary or secondary events in B cell transformation. In conjunction with the advanced understanding of PAX5 in B cell development, it will provide an objective basis for a better diagnosis and treatment of B-ALL.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* (2007) 446(7137):758–64. doi: 10.1038/nature05690

2. Kuiper RP, Schoenmakers EF, van Reijmersdal SV, Hehir-Kwa JY, van Kessel AG, van Leeuwen FN, et al. High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. *Leukemia* (2007) 21(6):1258–66. doi: 10.1038/sj.leu.2404691

3. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of ikaros. *Nature* (2008) 453(7191):110–4. doi: 10.1038/nature06866

4. Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science* (2008) 322(5906):1377–80. doi: 10.1126/science.1164266

5. Kawamata N, Ogawa S, Zimmermann M, Niebuhr B, Stocking C, Sanada M, et al. Cloning of genes involved in chromosomal translocations by high-resolution

single nucleotide polymorphism genomic microarray. *Proc Natl Acad Sci U.S.A.* (2008) 105(33):11921–6. doi: 10.1073/pnas.0711039105

6. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med* (2009) 360 (5):470–80. doi: 10.1056/NEJMoa0808253

7. Nebral K, Denk D, Attarbaschi A, Konig M, Mann G, Haas OA, et al. Incidence and diversity of PAX5 fusion genes in childhood acute lymphoblastic leukemia. *Leukemia* (2009) 23(1):134–43. doi: 10.1038/leu.2008.306

8. Familiades J, Bousquet M, Lafage-Pochitaloff M, Bene MC, Beldjord K, De Vos J, et al. PAX5 mutations occur frequently in adult b-cell progenitor acute lymphoblastic leukemia and PAX5 haploinsufficiency is associated with BCR-ABL1 and TCF3-PBX1 fusion genes: a GRAALL study. *Leukemia* (2009) 23(11):1989–98. doi: 10.1038/leu.2009.135

9. Coyaud E, Struski S, Prade N, Familiades J, Eichner R, Quelen C, et al. Wide diversity of PAX5 alterations in b-ALL: a groupe francophone de cytogenetique hematologique study. *Blood* (2010) 115(15):3089–97. doi: 10.1182/blood-2009-07-234229

10. Iacobucci I, Lonetti A, Paoloni F, Papayannidis C, Ferrari A, Storlazzi CT, et al. The PAX5 gene is frequently rearranged in BCR-ABL1-positive acute lymphoblastic leukemia but is not associated with outcome. a report on behalf of the GIMEMA acute leukemia working party. *Haematologica* (2010) 95(10):1683–90. doi: 10.3324/haematol.2009.020792

11. Shah S, Schrader KA, Waanders E, Timms AE, Vijai J, Miething C, et al. A recurrent germline PAX5 mutation confers susceptibility to pre-b cell acute lymphoblastic leukemia. *Nat Genet* (2013) 45(10):1226–31. doi: 10.1038/ng.2754

12. Ofverholm I, Tran AN, Heyman M, Zachariadis V, Nordenskjold M, Nordgren A, et al. Impact of IKZF1 deletions and PAX5 amplifications in pediatric b-cell precursor ALL treated according to NOPHO protocols. *Leukemia* (2013) 27(9):1936–9. doi: 10.1038/leu.2013.92

13. Auer F, Ruschendorf F, Gombert M, Husemann P, Ginzel S, Izraeli S, et al. Inherited susceptibility to pre b-ALL caused by germline transmission of PAX5 c.547G>A. *Leukemia* (2014) 28(5):1136–8. doi: 10.1038/leu.2013.363

14. Roberts KG, Gu Z, Payne-Turner D, McCastlain K, Harvey RC, Chen IM, et al. High frequency and poor outcome of Philadelphia chromosome-like acute lymphoblastic leukemia in adults. *J Clin Oncol* (2017) 35(4):394–401. doi: 10.1200/JCO.2016.69.0073

15. Schwab C, Nebral K, Chilton L, Leschi C, Waanders E, Boer JM, et al. Intragenic amplification of PAX5: a novel subgroup in b-cell precursor acute lymphoblastic leukemia? *Blood Adv* (2017) 1(19):1473–7. doi: 10.1182/bloodadvances.2017006734

16. Gu Z, Churchman ML, Roberts KG, Moore I, Zhou X, Nakitandwe J, et al. PAX5-driven subtypes of b-progenitor acute lymphoblastic leukemia. *Nat Genet* (2019) 51(2):296–307. doi: 10.1038/s41588-018-0315-5

17. Zaliova M, Stuchly J, Winkowska L, Musilova A, Fiser K, Slamova M, et al. Genomic landscape of pediatric b-other acute lymphoblastic leukemia in a consecutive European cohort. *Haematologica* (2019) 104(7):1396–406. doi: 10.3324/haematol.2018.204974

18. Bastian L, Schroeder MP, Eckert C, Schlee C, Tanchez JO, Kampf S, et al. PAX5 biallelic genomic alterations define a novel subgroup of b-cell precursor acute lymphoblastic leukemia. *Leukemia* (2019) 33(8):1895–909. doi: 10.1038/s41375-019-0430-z

19. Jung M, Schieck M, Hofmann W, Tauscher M, Lentes J, Bergmann A, et al. Frequency and prognostic impact of PAX5 p.P80R in pediatric acute lymphoblastic leukemia patients treated on an AIEOP-BFM acute lymphoblastic leukemia protocol. *Genes Chromosomes Cancer* (2020) 59(11):667–71. doi: 10.1002/gcc.22882

20. Duployez N, Jamrog LA, Fregona V, Hamelle C, Fenwarth L, Lejeune S, et al. Germline PAX5 mutation predisposes to familial b-cell precursor acute lymphoblastic leukemia. *Blood* (2021) 137(10):1424–8. doi: 10.1182/blood.2020005756

21. Liu X, Chen JG, Munshi M, Hunter ZR, Xu L, Kofides A, et al. Expression of the prosurvival kinase HCK requires PAX5 and mutated MYD88 signaling in MYD88-driven b-cell lymphomas. *Blood Adv* (2020) 4(1):141–53. doi: 10.1182/bloodadvances.2019000947

22. Calderon L, Schindler K, Malin SG, Schebesta A, Sun Q, Schwickert T, et al. Pax5 regulates b cell immunity by promoting PI3K signaling *via* PTEN down-regulation. *Sci Immunol* (2021) 6(61). doi: 10.1126/sciimmunol.abg5003

23. Chan LN, Chen Z, Braas D, Lee JW, Xiao G, Geng H, et al. Metabolic gatekeeper function of b-lymphoid transcription factors. *Nature* (2017) 542 (7642):479–83. doi: 10.1038/nature21076

24. Somasundaram R, Jensen CT, Tingvall-Gustafsson J, Ahsberg J, Okuyama K, Prasad M, et al. EBF1 and PAX5 control pro-b cell expansion via opposing regulation of the myc gene. *Blood* (2021) 137(22):3037–49. doi: 10.1182/blood.2020009564

25. Ramamoorthy S, Kometani K, Herman JS, Bayer M, Boller S, Edwards-Hicks J, et al. EBF1 and Pax5 safeguard leukemic transformation by limiting IL-7 signaling, myc expression, and folate metabolism. *Genes Dev* (2020) 34(21-22):1503–19. doi: 10.1101/gad.340216.120

26. Hill L, Ebert A, Jaritz M, Wutz G, Nagasaka K, Tagoh H, et al. Wapl repression by Pax5 promotes V gene recombination by igh loop extrusion. *Nature* (2020) 584(7819):142–7. doi: 10.1038/s41586-020-2454-y

27. Barberis A, Widenhorn K, Vitelli L, Busslinger M. A novel b-cell lineage-specific transcription factor present at early but not late stages of differentiation. *Genes Dev* (1990) 4(5):849–59. doi: 10.1101/gad.4.5.849

28. Simmons S, Knoll M, Drewell C, Wolf I, Mollenkopf HJ, Bouquet C, et al. Biphenotypic b-lymphoid/myeloid cells expressing low levels of Pax5: potential targets of BAL development. *Blood* (2012) 120(18):3688–98. doi: 10.1182/blood-2012-03-414821

29. Lin KI, Angelin-Duclos C, Kuo TC, Calame K. Blimp-1-dependent repression of pax-5 is required for differentiation of b cells to immunoglobulin m-secreting plasma cells. *Mol Cell Biol* (2002) 22(13):4771–80. doi: 10.1128/MCB.22.13.4771-4780.2002

30. Liu GJ, Jaritz M, Wohner M, Agerer B, Bergthaler A, Malin SG, et al. Repression of the b cell identity factor Pax5 is not required for plasma cell development. *J Exp Med* (2020) 217(11). doi: 10.1084/jem.20200147

31. Urbanek P, Wang ZQ, Fetka I, Wagner EF, Busslinger M. Complete block of early b cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP. *Cell* (1994) 79(5):901–12. doi: 10.1016/0092-8674(94)90079-5

32. Nutt SL, Heavey B, Rolink AG, Busslinger M. Commitment to the b-lymphoid lineage depends on the transcription factor Pax5. *Nature* (1999) 401 (6753):556–62. doi: 10.1038/44076

33. Rolink AG, Nutt SL, Melchers F, Busslinger M. Long-term *in vivo* reconstitution of T-cell development by Pax5-deficient b-cell progenitors. *Nature* (1999) 401(6753):603–6. doi: 10.1038/44164

34. Mikkola I, Heavey B, Horcher M, Busslinger M. Reversion of b cell commitment upon loss of Pax5 expression. *Science* (2002) 297(5578):110–3. doi: 10.1126/science.1067518

35. Horcher M, Souabni A, Busslinger M. Pax5/BSAP maintains the identity of b cells in late b lymphopoiesis. *Immunity* (2001) 14(6):779–90. doi: 10.1016/S1074-7613(01)00153-4

36. Schebesta A, McManus S, Salvagiotto G, Delogu A, Busslinger GA, Busslinger M. Transcription factor Pax5 activates the chromatin of key genes involved in b cell signaling, adhesion, migration, and immune function. *Immunity* (2007) 27(1):49–63. doi: 10.1016/j.immuni.2007.05.019

37. Delogu A, Schebesta A, Sun Q, Aschenbrenner K, Perlot T, Busslinger M. Gene repression by Pax5 in b cells is essential for blood cell homeostasis and is reversed in plasma cells. *Immunity* (2006) 24(3):269–81. doi: 10.1016/j.immuni.2006.01.012

38. McManus S, Ebert A, Salvagiotto G, Medvedovic J, Sun Q, Tamir I, et al. The transcription factor Pax5 regulates its target genes by recruiting chromatin-modifying proteins in committed b cells. *EMBO J* (2011) 30(12):2388–404. doi: 10.1038/emboj.2011.140

39. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of b cell identity and function. *Nat Immunol* (2007) 8(5):463–70. doi: 10.1038/ni1454

40. Okuyama K, Strid T, Kuruvilla J, Somasundaram R, Cristobal S, Smith E, et al. PAX5 is part of a functional transcription factor network targeted in lymphoid leukemia. *PloS Genet* (2019) 15(8):e1008280. doi: 10.1371/journal.pgen.1008280

41. Decker T, Pasca di Magliano M, McManus S, Sun Q, Bonifer C, Tagoh H, et al. Stepwise activation of enhancer and promoter regions of the b cell commitment gene Pax5 in early lymphopoiesis. *Immunity* (2009) 30(4):508–20. doi: 10.1016/j.immuni.2009.01.012

42. Roessler S, Gyory I, Imhof S, Spivakov M, Williams RR, Busslinger M, et al. Distinct promoters mediate the regulation of Ebf1 gene expression by interleukin-7 and Pax5. *Mol Cell Biol* (2007) 27(2):579–94. doi: 10.1128/MCB.01192-06

43. Nutt SL, Urbanek P, Rolink A, Busslinger M. Essential functions of Pax5 (BSAP) in pro-b cell development: Difference between fetal and adult b lymphopoiesis and reduced V-to-DJ recombination at the IgH locus. *Genes Dev* (1997) 11(4):476–91. doi: 10.1101/gad.11.4.476

44. Hesslein DG, Pflugh DL, Chowdhury D, Bothwell AL, Sen R, Schatz DG. Pax5 is required for recombination of transcribed, acetylated, 5' IgH V gene segments. *Genes Dev* (2003) 17(1):37–42. doi: 10.1101/gad.1031403

45. Fuxa M, Skok J, Souabni A, Salvagiotto G, Roldan E, Busslinger M. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. *Genes Dev* (2004) 18(4):411–22. doi: 10.1101/gad.291504

46. Kim M, Choi JE, She CJ, Hwang SM, Shin HY, Ahn HS, et al. PAX5 deletion is common and concurrently occurs with CDKN2A deletion in b-lineage acute lymphoblastic leukemia. *Blood Cells Mol Dis* (2011) 47(1):62–6. doi: 10.1016/j.bcmd.2011.04.003

47. Heltemes-Harris LM, Willette MJ, Ramsey LB, Qiu YH, Neeley ES, Zhang N, et al. Ebf1 or Pax5 haploinsufficiency synergizes with STAT5 activation to initiate acute lymphoblastic leukemia. *J Exp Med* (2011) 208(6):1135–49. doi: 10.1084/jem.20101947

48. Prasad MA, Ungerback J, Ahsberg J, Somasundaram R, Strid T, Larsson M, et al. Ebf1 heterozygosity results in increased DNA damage in pro-b cells and their synergistic transformation by Pax5 haploinsufficiency. *Blood* (2015) 125(26):4052–9. doi: 10.1182/blood-2014-12-617282

49. Martin-Lorenzo A, Auer F, Chan LN, Garcia-Ramirez I, Gonzalez-Herrero I, Rodriguez-Hernandez G, et al. Loss of Pax5 exploits Sca1-BCR-ABL(p190) susceptibility to confer the metabolic shift essential for pB-ALL. *Cancer Res* (2018) 78(10):2669–79. doi: 10.1158/0008-5472.CAN-17-3262

50. Martin-Lorenzo A, Hauer J, Vicente-Duenas C, Auer F, Gonzalez-Herrero I, Garcia-Ramirez I, et al. Infection exposure is a causal factor in b-cell precursor acute lymphoblastic leukemia as a result of Pax5-inherited susceptibility. *Cancer Discovery* (2015) 5(12):1328–43. doi: 10.1158/2159-8290.CD-15-0892

51. Cobaleda C, Jochum W, Busslinger M. Conversion of mature b cells into T cells by dedifferentiation to uncommitted progenitors. *Nature* (2007) 449 (7161):473–7. doi: 10.1038/nature06159

52. Bousquet M, Broccardo C, Quelen C, Meggetto F, Kuhlein E, Delsol G, et al. A novel PAX5-ELN fusion protein identified in b-cell acute lymphoblastic leukemia acts as a dominant negative on wild-type PAX5. *Blood* (2007) 109 (8):3417–23. doi: 10.1182/blood-2006-05-025221

53. Kurahashi S, Hayakawa F, Miyata Y, Yasuda T, Minami Y, Tsuzuki S, et al. PAX5-PML acts as a dual dominant-negative form of both PAX5 and PML. *Oncogene* (2011) 30(15):1822–30. doi: 10.1038/onc.2010.554

54. Kawamata N, Pennella MA, Woo JL, Berk AJ, Koeffler HP. Dominant-negative mechanism of leukemogenic PAX5 fusions. *Oncogene* (2012) 31(8):966–77. doi: 10.1038/onc.2011.291

55. Smeenk L, Fischer M, Jurado S, Jaritz M, Azaryan A, Werner B, et al. Molecular role of the PAX5-ETV6 oncoprotein in promoting b-cell acute lymphoblastic leukemia. *EMBO J* (2017) 36(6):718–35. doi: 10.15252/embj.201695495

56. Fortschegger K, Anderl S, Denk D, Strehl S. Functional heterogeneity of PAX5 chimeras reveals insight for leukemia development. *Mol Cancer Res* (2014) 12(4):595–606. doi: 10.1158/1541-7786.MCR-13-0337

57. Imoto N, Hayakawa F, Kurahashi S, Morishita T, Kojima Y, Yasuda T, et al. B cell linker protein (BLNK) is a selective target of repression by PAX5-PML protein in the differentiation block that leads to the development of acute lymphoblastic leukemia. *J Biol Chem* (2016) 291(9):4723–31. doi: 10.1074/jbc.M115.637835

58. Jamrog L, Chemin G, Fregona V, Coster L, Pasquet M, Oudinet C, et al. PAX5-ELN oncoprotein promotes multistep b-cell acute lymphoblastic leukemia in mice. *Proc Natl Acad Sci U.S.A.* (2018) 115(41):10357–62. doi: 10.1073/pnas.1721678115

59. Cazzaniga G, Daniotti M, Tosi S, Giudici G, Aloisi A, Pogliani E, et al. The paired box domain gene PAX5 is fused to ETV6/TEL in an acute lymphoblastic leukemia case. *Cancer Res* (2001) 61(12):4666–70.

60. Strehl S, Konig M, Dworzak MN, Kalwak K, Haas OA. PAX5/ETV6 fusion defines cytogenetic entity dic(9;12)(p13;p13). *Leukemia* (2003) 17(6):1121–3. doi: 10.1038/sj.leu.2402923

61. Fazio G, Cazzaniga V, Palmi C, Galbiati M, Giordan M, te Kronnie G, et al. PAX5/ETV6 alters the gene expression profile of precursor b cells with opposite dominant effect on endogenous PAX5. *Leukemia* (2013) 27(4):992–5. doi: 10.1038/leu.2012.281

62. Schinnerl D, Fortschegger K, Kauer M, Marchante JR, Kofler R, Den Boer ML, et al. The role of the janus-faced transcription factor PAX5-JAK2 in acute lymphoblastic leukemia. *Blood* (2015) 125(8):1282–91. doi: 10.1182/blood-2014-04-570960

63. Jurado S, Fedl AS, Jaritz M, Kostanova-Poliakova D, Malin SG, Mullighan CG, et al. The PAX5-JAK2 translocation acts as dual-hit mutation that promotes aggressive b-cell leukemia *via* nuclear STAT5 activation. *EMBO J* (2022) 41(7):e108397. doi: 10.15252/embj.2021108397

64. Busslinger M, Klix N, Pfeffer P, Graninger PG, Kozmik Z. Deregulation of PAX-5 by translocation of the emu enhancer of the IgH locus adjacent to two alternative PAX-5 promoters in a diffuse large-cell lymphoma. *Proc Natl Acad Sci U.S.A.* (1996) 93(12):6129–34. doi: 10.1073/pnas.93.12.6129

65. Poppe B, De Paepe P, Michaux L, Dastugue N, Bastard C, Herens C, et al. PAX5/IGH rearrangement is a recurrent finding in a subset of aggressive b-NHL with complex chromosomal rearrangements. *Genes Chromosomes Cancer* (2005) 44(2):218–23. doi: 10.1002/gcc.20214

66. Souabni A, Jochum W, Busslinger M. Oncogenic role of Pax5 in the T-lymphoid lineage upon ectopic expression from the immunoglobulin heavy-chain locus. *Blood* (2007) 109(1):281–9. doi: 10.1182/blood-2006-03-009670

67. Dang J, Wei L, de Ridder J, Su X, Rust AG, Roberts KG, et al. PAX5 is a tumor suppressor in mouse mutagenesis models of acute lymphoblastic leukemia. *Blood* (2015) 125(23):3609–17. doi: 10.1182/blood-2015-02-626127

68. Jean J, Kovach AE, Doan A, Oberley M, Ji J, Schmidt RJ, et al. Characterization of PAX5 intragenic tandem multiplication in pediatric b-lymphoblastic leukemia by optical genome mapping. *Blood Adv* (2022) 6 (11):3343–6. doi: 10.1182/bloodadvances.2021006328

69. Robichaud GA, Nardini M, Laflamme M, Cuperlovic-Culf M, Ouellette RJ. Human pax-5 c-terminal isoforms possess distinct transactivation properties and are differentially modulated in normal and malignant b cells. *J Biol Chem* (2004) 279(48):49956–63. doi: 10.1074/jbc.M407171200

70. Zwollo P, Arrieta H, Ede K, Molinder K, Desiderio S, Pollock R. The pax-5 gene is alternatively spliced during b-cell development. *J Biol Chem* (1997) 272 (15):10160–8. doi: 10.1074/jbc.272.15.10160

71. Borson ND, Lacy MQ, Wettstein PJ. Altered mRNA expression of Pax5 and blimp-1 in b cells in multiple myeloma. *Blood* (2002) 100(13):4629–39. doi: 10.1182/blood.V100.13.4629

72. Sadakane Y, Zaitsu M, Nishi M, Sugita K, Mizutani S, Matsuzaki A, et al. Expression and production of aberrant PAX5 with deletion of exon 8 in b-lineage acute lymphoblastic leukaemia of children. *Br J Haematol* (2007) 136(2):297–300. doi: 10.1111/j.1365-2141.2006.06425.x

73. Peinert S, Carney D, Prince HM, Januszewicz EH, Seymour JF. Altered mRNA expression of PAX5 is a common event in acute lymphoblastic leukaemia. *Br J Haematol* (2009) 146(6):685–6. doi: 10.1111/j.1365-2141.2009.07806.x

74. Czerny T, Schaffner G, Busslinger M. DNA Sequence recognition by pax proteins: bipartite structure of the paired domain and its binding site. *Genes Dev* (1993) 7(10):2048–61. doi: 10.1101/gad.7.10.2048

75. Perez-Borrajero C, Okon M, McIntosh LP. Structural and dynamics studies of Pax5 reveal asymmetry in stability and DNA binding by the paired domain. *J Mol Biol* (2016) 428(11):2372–91. doi: 10.1016/j.jmb.2016.04.004

76. Perez-Borrajero C, Heinkel F, Gsponer J, McIntosh LP. Conformational plasticity and DNA-binding specificity of the eukaryotic transcription factor Pax5. *Biochemistry* (2021) 60(2):104–17. doi: 10.1021/acs.biochem.0c00737

77. Li JF, Dai YT, Lilljebjorn H, Shen SH, Cui BW, Bai L, et al. Transcriptional landscape of b cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases. *Proc Natl Acad Sci U.S.A.* (2018) 115(50):E11711–20. doi: 10.1073/pnas.1814397115

78. Paietta E, Roberts KG, Wang V, Gu Z, Buck G, Pei D, et al. Molecular classification improves risk assessment in adult BCR-ABL1-negative b-ALL. *Blood* (2021) 138(11):948–58. doi: 10.1182/blood.2020010144

79. Novakova M, Zaliova M, Fiser K, Vakrmanova B, Slamova L, Musilova A, et al. DUX4r, ZNF384r and PAX5-P80R mutated b-cell precursor acute lymphoblastic leukemia frequently undergo monocytic switch. *Haematologica* (2020) 106(8):2066–75. doi: 10.3324/haematol.2020.250423

80. Passet M, Boissel N, Sigaux F, Saillard C, Bargetzi M, Ba I, et al. PAX5 P80R mutation identifies a novel subtype of b-cell precursor acute lymphoblastic leukemia with favorable outcome. *Blood* (2019) 133(3):280–4. doi: 10.1182/blood-2018-10-882142

81. Li Z, Lee SHR, Chin WHN, Lu Y, Jiang N, Lim EH, et al. Distinct clinical characteristics of DUX4- and PAX5-altered childhood b-lymphoblastic leukemia. *Blood Adv* (2021) 5(23):5226–38. doi: 10.1182/bloodadvances.2021004895

82. Liu GJ, Cimmino L, Jude JG, Hu Y, Witkowski MT, McKenzie MD, et al. Pax5 loss imposes a reversible differentiation block in b-progenitor acute lymphoblastic leukemia. *Genes Dev* (2014) 28(12):1337–50. doi: 10.1101/gad.240416.114

83. Hart MR, Anderson DJ, Porter CC, Neff T, Levin M, Horwitz MS. Activating PAX gene family paralogs to complement PAX5 leukemia driver mutations. *PloS Genet* (2018) 14(9):e1007642. doi: 10.1371/journal.pgen.1007642

84. Isidro-Hernandez M, Mayado A, Casado-Garcia A, Martinez-Cano J, Palmi C, Fazio G, et al. Inhibition of inflammatory signaling in Pax5 mutant cells mitigates b-cell leukemogenesis. *Sci Rep* (2020) 10(1):19189. doi: 10.1038/s41598-020-76206-y

85. Cazzaniga V, Bugarin C, Bardini M, Giordan M, te Kronnie G, Basso G, et al. LCK over-expression drives STAT5 oncogenic signaling in PAX5 translocated BCP-ALL patients. *Oncotarget* (2015) 6(3):1569–81. doi: 10.18632/oncotarget.2807

86. Chan LN, Murakami MA, Robinson ME, Caeser R, Sadras T, Lee J, et al. Signalling input from divergent pathways subverts b cell transformation. *Nature* (2020) 583(7818):845–51. doi: 10.1038/s41586-020-2513-4

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Acquired and hereditary bone marrow failure: A mitochondrial perspective

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The disorders known as bone marrow failure syndromes (BMFS) are life-threatening disorders characterized by absence of one or more hematopoietic lineages in the peripheral blood. Myelodysplastic syndromes (MDS) are now considered BMF disorders with associated cellular dysplasia. BMFs and MDS are caused by decreased fitness of hematopoietic stem cells (HSC) and poor hematopoiesis. BMF and MDS can occur *de novo* or secondary to hematopoietic stress, including following bone marrow transplantation or myeloablative therapy. *De novo* BMF and MDS are usually associated with specific genetic mutations. Genes that are commonly mutated in BMF/MDS are in DNA repair pathways, epigenetic regulators, heme synthesis. Despite known and common gene mutations, BMF and MDS are very heterogenous in nature and non-genetic factors contribute to disease phenotype. Inflammation is commonly found in BMF and MDS, and contribute to ineffective hematopoiesis. Another common feature of BMF and MDS, albeit less known, is abnormal mitochondrial functions. Mitochondria are the power house of the cells. Beyond energy producing machinery, mitochondrial communicate with the rest of the cells *via* triggering stress signaling pathways and by releasing numerous metabolite intermediates. As a result, mitochondria play significant roles in chromatin regulation and innate immune signaling pathways. The main goal of this review is to investigate BMF processes, with a focus mitochondria-mediated signaling in acquired and inherited BMF.

KEYWORDS

bone marrow failure (BMF), mitochondria, TGF beta, innate immune signaling, myelodysplastic disorder (MDS)

Introduction

Ineffective hematopoiesis leading to the absence of one or more hematopoietic lineages in the peripheral blood represents broad and heterogeneous blood disorders comprised of bone marrow failure (BMF) and myelodysplastic (MDS) syndromes. Patients with BMF or MDS suffer from a severe reduction of one or more hematopoietic lineages in the peripheral blood, which is life-threatening (1–4) BMF can be inherited or acquired. The most common inherited BMFs include Fanconi anemia, Shwachman-Diamond syndrome, congenital amegakaryocytic thrombocytopenia, and reticular dysgenesis. Other inherited BMF are X-linked recessive dyskeratosis congenita and Blackfan-Diamond Anemia (5–7). MDS, which are now classified as acquired disorders that resemble BMF with a variety of cell dysplastic features, may occur *de novo* or secondary to BMF. MDS are classified in several groups based on established clinical and histopathological features, as defined by the World Health Organization: MDS with single lineage dysplasia, MDS with ring sideroblasts (MDS-RS), MDS with multilineage dysplasia, MDS with excess blasts (MDS-EB), MDS with isolated del(5q). BMF/MDS can also appear after allogenic or autologous hematopoietic stem cell transplantation (HSCT) (8, 9), as well as after myeloablative chemotherapy (10), and are called therapy-related BMF/MDS. Some patients develop secondary MDS/AML within 6 years of autologous HSCT (11). Although BMF/MDS are very heterogeneous, the same genomic mutations are frequently found in MDS patients such as mutations in genes related to RNA splicing (SF3B1, SRSF2, U2F1, ZRSR2), DNA methylation (TET2, DNMT3A, IDH1/IDH2), chromatin modification (ASXL1, EZH2), transcription regulation (RUNX1, BCOR), and DNA repair control (p53). These observations suggest that additional environmental factors largely contribute to disease development. Substantial clinical data have shown that hyperactivity of inflammatory cytokines, including TNFα, IL-6, and transforming growth factor–β (TGFβ), directly contribute to hematopoietic failure in BMF/MDS.1 (12–14), Chronic inflammation and enhanced innate immune signaling are also recognized as contributing factors of inefficient hematopoiesis (15–17). Interestingly, several evidence suggest that disruption of mitochondria is another preponderant factor in BMF/MDS development. Abnormal mitochondria have been linked to both acquired and hereditary BMF (18–24). Patients with MDS have transcriptional, morphological and functional dysregulation of their mitochondria, according to several studies (25–28). Some of these defects are the direct consequences of abnormal expression of nuclear-encoded mitochondrial genes. Others could arise in response to stress or the inflammatory milieu. The functions of mitochondria, which supply energy and metabolic activity in response to cellular demand (29), go well beyond energy production. Mitochondria communicate with the rest of the cell through activation of signaling pathways and control a broad range of cellular functions such as apoptosis, iron metabolism and heme production. In addition, mitochondria participate in the generation of metabolite intermediates, acetyl-CoA and S-Adenosyl-Methionine (SAM), used for epigenetic remodeling, as well as those for *de novo* biosynthetic processes, including nucleotides and fatty acids. Finally, mitochondria control the cellular response to stress, including inflammation stress. This review will discuss the emerging role of mitochondria as driver of *de novo* or secondary BMF/MDS. It will discuss the potential mechanism, direct or indirect, of how abnormal mitochondrial functions contribute to ineffective hematopoiesis.

MDS with sideroblasts are mitochondrial diseases

Mitochondria are home of heme synthesis

The first step in heme biosynthesis takes place into mitochondria and involves the condensation of succinyl-CoA and glycine to form δ-aminolevulinic acid (ALA) in the mitochondrial matrix. This reaction is catalyzed by ALA synthase (ALAS). There are two isoforms of ALAS, ALAS1 and ALAS2, which is found exclusively in erythroid cells. ALA is exported to the cytosol *via* SLC25A38 and ABCB10 where it is converted to coproporphyrinogen III (CPgenIII). CPgenIII is imported back into mitochondria, where it is converted to protoporphyrinogen IX by coproporphyrinogen oxidase (CPOX). Then, protoporphyrinogen IX is oxidized to protoporphyrin IX (PPIX) by protoporphyrinogen oxidase (PPOX). Finally, ferrous iron is incorporated into PPIX to form heme in the mitochondrial matrix, a reaction catalyzed by ferrochelatase (FECH) (30). T the expression of both Alas2 and *FECH* is controlled by iron, thus linking the regulation of heme biosynthesis in erythroid cells to the availability of iron. Iron is acquired by differentiating erythroid progenitors *via* transferrin receptor 1 (TfR1)-mediated endocytosis and transferred to mitochondria for heme synthesis *via* mitoferrin1 (*MFRN1*) and mitoferrin2 (*MFRN2*), expressed in erythroid and non-erythroid tissues, respectively. The generation of globin and heme levels in erythroid precursors is balanced by a cell membrane heme exporter known as feline leukemia virus subgroup C receptor 1 (FLVCR1). Flvcr1b, an isoform of Flvcr1 that is present in mitochondria, facilitates heme efflux into the cytoplasm (31).

MDS with RS: A mitochondrial disorder affecting the erythroid lineage

Sideroblastic anemia, congenital or acquired, are associated with MDS and are characterized by the presence of ring

sideroblasts, which result from decreased heme production and excess iron deposit within mitochondria of erythroid cells (32). Mutations in genes related to heme synthesis are drivers of MDS-RS. Mutation in ALAS2 reduces protoporphyrin causing an accumulation of iron in mitochondria and subsequently cell death. Mutations in Ala carriers, *ABCB7 and* in Slc25a38, also reduces heme synthesis and causes MDS-RS. Germline mutation in the Glutaredoxin 5 [*GLRX5]* gene causes iron overload and is associated with sideroblastic-like microcytic anaemia. GLRX5 is a mitochondrial protein, which is involved in the biogenesis of iron-sulfur clusters.

The splicing factor SF3B1 is the most commonly mutated genes in MDS with the disease phenotype with ring sideroblasts (33, 34). SF3B1 Splicing factor 3b, together with splicing factor 3a and a 12S RNA unit, forms the U2 small nuclear ribonucleoproteins complex (U2 snRNP) and binds pre-mRNA upstream of the intron's branch site in a sequence independent manner. SF3B1 mutations cause abnormal mitochondrial iron absorption and ineffective erythropoiesis (35). Initial studies found that SF3B1-mutant erythroblasts displayed larger quantities of mitochondria. When co-mutation with EZH2 occurs, mitochondrial membrane potential is abnormal and ROS are increased; likely driving cell death (1). The exact molecular mechanism behind the abnormal mitochondrial functions and iron deposition is being uncovered. Mutated-SF3B1 notably targets expression of genes involved in mitochondrial heme synthesis such as *PPOX*, *TMEM14C and Abcb7*, causing a block in protoporphyrin synthesis (36–39). Interestingly, SFB1 mutation confers proliferation advantage to the clone. In a remarkable study by Hsu (40), the clonal evolution of MDS was studied using iPSCs reprogrammed from patient samples and shows that the initial mutation is t (4, 12), followed by mutations in SF3B1, EZH2, and del(5q), in that sequence.

Another study described the importance of the Retinoblastoma protein (pRb) gene, a crucial cell cycle regulator that controls the transition from the G1 to the S phase, in mitochondrial functions in erythroid cells. Deletion of Rb in erythroid cells caused poor erythropoiesis with dysplastic features due to abnormal mitochondrial biogenesis and cell cycle exit. Erythroid-specific deletion of pRb led to decreased expression of mitochondria-related genes, a reduction in mitochondrial membrane potential and a change in the ROS produced by the mitochondria. Expression of critical oxidative phosphorylation genes such Ndufa1 (complex 1, OXPHOS), Atp5s (ATP synthesis), and Cox7b (electron transfer), expression of the mitochondrial biogenesis gene PGC1b, of the mitochondrial antioxidant Prdx3, which is crucial for maintaining the balance of (ROS), as well as ALAS2, and ABCB were all decreased. In this Rb-deficiency mouse model, overexpressing PGC1b was sufficient to normalize the RBC counts, underscoring the critical role of mitochondria in the pathogenesis of the disease (27).

Finally, decreased FLVCR1 levels or increased expression of aberrant alternative splicing of FLVCR1 transcript are seen in DBA patients and a cellular model of DBA (41, 42). Downregulation of FLVCR1a and FLVCR1b results in an increase in oxidative stress, cell cycle arrest at G0/G1, and apoptosis due to heme accumulation. This is yet another illustration of how a flaw in mitochondrial homeostasis can result in ineffective erythropoiesis and BMF. Germline mutations in other genes, such as *PUS1*, *YARS2*, *SLC19A2* and *TRNT1*, as well as mitochondrial DNA deletions, have been identified in distinct forms of inherited sideroblastic anemias (32) (Figure 1).

Mutations in genes that alter the generation of mitochondrially – produced metabolites cause MDS

Mitochondrial functions and epigenetic regulation are tightly linked in several ways. One way is through the tricarboxylic acid (TCA) cycle – a major mitochondrial metabolic pathway. The TCA cycle produces several intermediate metabolites, citrate, alpha-ketoglutarate (α-KG), itaconate, succinate, fumarate, malate and oxaloacetate, through a series of enzymatic reactions. When the TCA metabolites are coupled with the mitochondrial electron transport chain, TCA intermediates are used for subsequent metabolic reactions through oxidative phosphorylation (OXPHOS) to generate ATP. TCA metabolites also serve in non-metabolic signaling roles. For example, itaconate, succinate, fumarate have all been shown to alter the innate immune response. In addition, the TCA metabolites are directly involved in epigenetic regulation. Succinate and fumarate can directly inhibit the activity of histone or DNA demethylase. Alpha-KG is needed for the activity of DNA demethylase. Acetyl-CoA serve as donor group of histone acetylation; S-Adenosyl-Methionine (SAM) which serve as donor group for DNA or histone methylation is generated through a complex interaction between the mitochondrial one-carbon folate pathway and the methionine cycle (43). The tight connection between mitochondrial metabolism and chromatin regulation is one component of the preponderant, yet ill-understood, role of mitochondria in MDS pathogenesis. Mutations in the TCA enzyme IDHs (*IDH1-IDH2)* occur in about 7% of MDS cases, with *IDH2* mutations being more frequent (about 4.5%) than *IDH1* mutations (about 2.5%). (44) *IDH2* mutations are particularly enriched in the RAEB subtype of MDS. IDH1/2 catalyzes the oxidation of isocitrate to oxalosuccinate within the TCA cycle, which is followed by decarboxylation of the carboxyl group beta to the ketone to form α-KG. This reaction also generates NADPH. 44 Mutations in IDH1/2 thus by impacting α-KG production alter the activity of metabolic enzymes that depend on α-KG availability, such as the DNA demethylase Tet2.

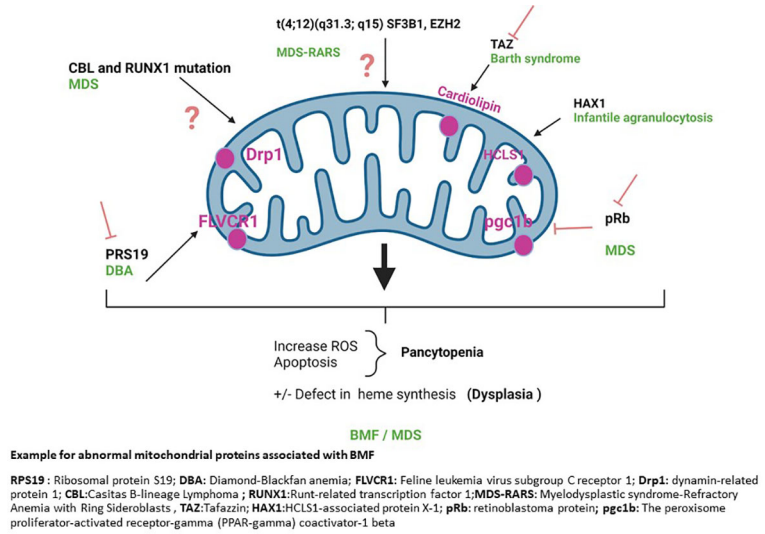

FIGURE 1
Example for abnormal mitochondrial proteins associated with BMF. RPS19, Ribosomal protein S19; DBA, Diamond-Blackfan anemia; FLVCR1, Feline leukemia virus subgroup C receptor 1; Drp1, dynamin-related protein 1; CBL, Casitas B-lineage Lymphoma; RUNX1, Runt-related transcription factor 1; MDS-RARS, Myelodysplastic syndrome-Refractory Anemia with Ring Sideroblasts, TAZ, Tafazzin; HAX1, HCLS1-associated protein X-1; pRb, retinoblastoma protein; pgc1b, The peroxisome proliferator-activated receptor-gamma (PPAR-gamma) coactivator-1 beta.

Tet2 primarily catalyzes the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Mutations in Tet2 are also associated with clonal hematopoiesis, increased risk of MDS progression, and poor prognosis in AML (45). Interestingly, IDH2 *mutations* are mutually exclusive with *TET2* and *SF3B1* mutations, but are frequently associated with *SRSF2* mutations. Other known mutations in epigenetic regulators that are associated with clonal hematopoiesis and MDS are found in the DNA methylase DNMT3a (46). DNMT3A, catalyzes the methylation of CpG dinucleotides in genomic DNA, which is dependent on SAM availability. Hence, stressors that alter mitochondrial functions could easily contribute to disease development and participate in disease heterogeneity in a given genetic background. Any abnormality in mitochondrial functions that would cause abnormal production of SAM or aKG would also alter Tet2 or DNMT3a functions and could drive MDS pathogenesis without the need for somatic mutation in specific genes (see below).

Because of the link between mitochondria and epigenetics, altered mitochondrial metabolism is a common characteristics of MDS/BMF that drives disease phenotype. Interestingly, in addition to exhibiting specific metabolic alterations that result from the genetic context, MDS have a common abnormal metabolic signature. Elegant studies from the Huang's lab showed that hypoxia-inducible factor 1α (HIF1A) transcriptional signature is generally activated in MDS patient bone marrow stem/progenitors, in major MDS-associated mutations (*Dnmt3a, Tet2, Asxl1, Runx1*, and *Mll1*). 48 Remarkably, using inducible activation of HIF1A signaling mouse model, they show that HIF1A is sufficient to induce dysplastic and cytopenic MDS phenotypes. On the other hand, both genetic and chemical inhibition of HIF1A signaling rescues MDS phenotypes in a mouse model of MDS, indicating that elevated HIF1A is necessary for MDS phenotype. Therefore, metabolic changes associated with HIF1A are central pathobiologic mediators of MDS. Two other important observations were that HIF1A signature is also associated with enrichment in several inflammatory/immune response–related pathways. Plus, it renders a state of pseudohypoxia and mitochondrial dysfunction in which expressions of nuclear-encoded mitochondrial genes, notably the electron transport chain complex II that is normally important for OXPHOS, are downregulated. In this context, metabolites of the TCA cycle, aKG, succinate, fumarate and malate, accumulate – thus further altering cellular functions (47).

Abnormal mitochondrial dynamics contributes to MDS

Mitochondria are very dynamic organelles, whose numbers and organization can vary greatly. (48–50) The mitochondrial network can be organized into interconnected and fused filaments, or into fragmented and smaller unit (48). Mitochondrial fusion is controlled by mitofusins 1 and 2 (Mfn1 and Mfn2) and optic atrophy 1 and 3 (Opa1, Opa3). Mitochondrial fission is regulated by dynamin-related protein 1 (Drp1) and fission protein 1 (Fis1) (48–50). Mitochondrial

dynamism is important to adapt cells to energy demand. When energy demand is high, mitochondria are fused and mitochondrial oxidative phosphorylation [OXPHOS] is favored. Mitochondrial fusion also enables 'mixing' mitochondrial membrane proteins for repair mechanisms. A recent study describes the role of aberrant mitochondrial fission as driver of MDS. They found a MDS patient harboring a mutation in both the E3 ubiquitin-protein ligase CBL gene and the transcription factor RUNX1 gene. In a mouse model of *CBL* exon deletion with RUNX1 mutants, that recapitulated clinically relevant MDS phenotypes, HSC and progenitor cells exhibited excessive mitochondrial fragmentation that was caused by enhanced activity of the mitochondrial fission regulator DRP1. The subsequent elevation in ROS production and inflammatory signals promoted the development of dysplasia and impaired granulopoiesis (18). Other studies have reported abnormal mitochondrial structure, or abnormal mitochondrial biogenesis and mitophagy in BMF, notably in FA. In this case, they found that FA genes are required for selective autophagy, which removes unwanted cytoplasmic contents including mitochondria such that FA gene deficiency results in impaired virophagy and antiviral host defense, decreased Parkin-mediated mitophagy, and increased mitochondrial ROS-dependent inflammasome activation (51). Loss of FA-gene-mediated selective autophagy may contribute to the pathophysiology of FA-gene associated diseases.

Role of reactive oxygen species in developing BMF/MDS

One common characteristic of BMF/MDS is the higher susceptibility of myeloid progenitors to apoptosis. Excessive myeloid cell apoptosis contributes to peripheral cytopenias even when the bone marrow is hypercellular. Numerous variables, including internal or external reactive oxygen species (ROS), can cause apoptosis. ROS comprise radical and non-radical molecules (52), and are often released as a byproduct of oxidative phosphorylation or during mitochondrial stress conditions. To counteract ROS, HSC express enzymatic and nonenzymatic defensive mechanisms, such as superoxide dismutase, glutathione peroxidase, myeloperoxidase, Vitamins C, E, and reduced glutathione (GSH) (53). When compared to controls, MDS patients have significantly higher levels of intracellular peroxides in lymphocytes, erythroid precursors, monocytes and granulocytes, as well as a considerably lower superoxide/peroxides ratio and GSH levels, resulting in oxidative stress and subsequent macromolecule and organelle damages (52, 54).

Chronic oxidative stress is also found in FA cells due to increased DNA damage. This is associated with mitochondrial damage and OXPHOS dysfunction. In fact, spontaneous mitochondrial fragmentation occurs in FA cells that leads to change in mitochondrial distribution, shape, and integrity (55, 56). HSPCs deficient in the FA protein Fancd2 rely on increased mitochondrial translation for survival and proliferation (57). The changes in mitochondrial structure are also accompanied by changes in metabolism. FA cells exhibit lower OXPHOS, increased glycolytic flux and decreased glutaminolysis (23). We know that a balance between glycolysis and OXPHOS is necessary for HSPC differentiation. Quiescent HSC rely mostly on anaerobic glycolysis and lysosomal functions for their energy needs. HSC activation and commitment to differentiation are associated with increased aerobic glycolysis, mitochondrial activation and increased OXPHOS. Hence, abnormal mitochondrial function in FA cells could substantially impact the ability of HSPC to differentiate, further contributing the FA pathogenesis (23). Similarly, a study focused on SBDS gene that affects ribosome biogenesis, mitotic spindle assembly, chemotaxis, and ROS generation, shows that lower expression of SBDS causes defective mitochondria and elevated ROS (58) (Figure 1).

Other mitochondrial abnormalities linked to inherited BMF

Mitochondrial abnormalities are found in a variety of hematologic phenotypes. The Pearson syndrome (24, 59), is a multisystem mitochondrial respiratory chain disorder caused by a single large scale mitochondrial DNA deletion. Patients present with pancytopenia sideroblastic anemia and exocrine pancreatic insufficiency. The Barth syndrome, which presents with neutropenia in addition to musculoskeletal defects and cardiomyopathy, is another mitochondrial disorder (22, 60).The Barth syndrome is caused by a mutation in the gene TAZ. TAZ encodes for the mitochondrial phospholipid transacylase tafazzin. TAZ controls the production of tetralinoleoyl cardiolipin, a mitochondrial membrane-specific lipid. When tafazzin is knocked down by shRNA in mice, tetralinoleoyl cardiolipin levels are drastically reduced, and monolysocardiolipins accumulate in mitochondria (61).The aberrant buildup of monolysocardiolipins causes mitochondrial dysfunction (62) leading to enhanced mitochondrial membrane potential breakdown, abnormal cytochrome c release, caspase-3 activation (63) and cellular death, including in myeloid precursors and neutrophils (64). Similarly, in the Kostmann disease also known as infantile agranulocytosis (severe congenital neutropenia) nonsense mutations in mitochondrial-associated antiapoptotic protein (HCLS1) lead to premature stop codons, loss of function, and frequently, total loss of protein expression, causing acute neutropenia that is often accompanied by neurologic and cognition impairments (65, 66). Finally, BMF and MDS have been linked to mutations in mitochondrial DNA (67) (Figure 1).

It has long been known that mitochondria can be taken up by cells and can transfer from cell to cell, *in vivo* or *in vitro*. Because of this, mitochondrial replacement or supplementation in cells is being proposed as novel therapeutic approach of mitochondrial diseases. A recent study shows that mitochondrial augmentation in human CD34+ cells from healthy donors or patients with mitochondrial DNA disorder can prove beneficial (68). The group described a method of ex vivo transfer of HSPC with normal exogenous mitochondria, that they termed mitochondrial augmentation therapy (MAT). They show that MAT can improve mitochondrial content and oxygen consumption of healthy and diseased HSPCs. Importantly, they used xenotransplant in immunodeficient NSGS mice to show that MAT confers HSPCs from a patient with an mtDNA disorder superior human engraftment (68).

Secondary BMF/MDS following hematopoietic stress from bone marrow transplantation: Importance of abnormal mitochondrial functions

Secondary MDS and AML are becoming more widely known as late complications of stem cell transplantation (69). The incidence of treatment-related MDS and AML is between 5 percent and 20 percent 5-10 years after an autologous stem cell transplantation (ASCT) (10). Among all cancers, MDS development occurred in 35% of non-Hodgkin's lymphoma patients who receive ASCT (70). In a Japanese study, 1.38% lymphoma patients receiving ASCT developed secondary myeloid dysplasia 3 years after transplant; 0.37% lymphoma patients receiving allogeneic SCT developed secondary MDS 3 years later (71). The causes and mechanisms behind the development of secondary BMF/MDS are multiple and could be cell intrinsic or arising from damage in the bone marrow microenvironment.

One clear cell intrinsic mechanism is linked to the *therapy* of the original disorder. There is strong evidence that alkylating anti-leukemic drugs, or whole body irradiation (TBI) used pre-transplantation cause chromosomal damage that can result in MDS/AML (72).

Increased inflammation following treatment could also be a contributing factor. Inflammatory factors are altered after BMT. Among those factors is the transforming growth factor beta (TGFβ). TGFβ is known to suppress cellular growth and to contribute to ineffective hematopoiesis (73–77). The allogeneic and autologous stem cell transplantation conditioning protocols decrease TGFβ production (78, 79). After roughly 7 weeks of bone marrow repopulation, the plasma level of TGFβ returns to normal (80, 81). Interestingly, this is seen in mouse model as well. Research from our lab has demonstrated that while TGFβ levels are lower in the bone marrow microenvironment, TGFβ protein and signaling are enhanced in HSPC after bone marrow transplantation. In this context, TGFβ acts through p38MAPK to impair HSC self-renewal and cause ineffective hematopoiesis after bone marrow transplantation (13). Increased inflammatory cytokines and associated inflammatory signals are a common characteristic of BMD/BMF. TGFβ plasma levels are elevated in hematopoietic cells of patients with myelodysplastic syndromes MDS (82–84). TGFβ signaling is also elevated in FA patients (85, 86). In a Fanca-deficient mouse model, challenge with polyinosinic:polycytidylic acid (pIC) leads to changing DNA repairing system *via* enhanced TGFβ signaling and causes BMF due to increased DNA mutations (75). TGFβ signaling inhibition restored hematopoiesis in this mouse model. TGFβ is also elevated in Shwachman-Bodian-Diamond Condition (SBDS) and Diamond Blackfan anemia (DBA) 2. Blocking the TGFβ pathway using a small molecule inhibitor or a TGF-family ligand trap can ameliorate the inefficient erythropoiesis also found in SDS or Diamond Blackfan anemia patients (87–89).

Overall, the role of inflammation in BMF or MDS development is now established. Toll-like receptors (TLRs) or their signaling effectors are often overexpressed in MDS samples compared to healthy controls, enhancing a type I interferon response through NFkB, MAPK, and IRF3 (12, 90, 91). The inflammasome is elevated in BMF/MDS patients and contributes to ineffective hematopoiesis (92–94). The inflammasome is a multiprotein complex composed of the sensor of damage associated molecular patterns (DAMPs), ie NLRP3 Nod-like receptor, an adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1 causing the release of interleukin-1b (IL-1b) and IL-18 and cell death by pyroptosis (95). Inflammatory milieu and cell death it creates contribute to pancytopenia and ineffective hematopoiesis. In addition, inflammation could provide a selective advantage to mutated HSC clones, as seen in a model of Dnmt3a-loss of functions in which chronic infection drives clonal expansion of the Dnmt3a-mutant clones *via* INFy (96). Therefore, increased inflammation and/or inflammatory cytokines following transplantation or hematopoietic stress could be a factor contributing to secondary BMF/MDS.

Viral infections activate DNA and RNA sensing pathways to trigger innate immune signaling pathways that converge on an interferon response. The DNA-sensing pathway, cGAS/STING, activates NFkB, IRF3 to clear the viral infection. In response to viral RNA, the innate immune response starts with cytosolic viral RNA sensor retinoic acid inducible gene-I (RIG-I). Then, RIG-I engages the adaptor protein MAVS (Mitochondrial AntiViral Signaling). In turn, MAVS, which is anchored onto mitochondria, triggers a sequence of signaling that converge onto NFkB, IRF3 or the inflammasome (97–99). These pathways have been involved in MDS, directly or indirectly. DDX41 can activate cGAS/STING; mutation in DDX41 are associated with MDS (100). It is important to note that numerous viruses have been connected to the formation of MDS, including CMV (101), HTLV-1 (102), parvovirus B19 (103), and HHV-6 (104). In a

very interesting retrospective study on lymphoma patients who developed secondary AML following HSCT indicates that at the time of stem cell transplantation, 1% of patients who received auto-SCT and 5% of patients who received allo-SCT had infections (71). The hypothesis that MDS could start as a viral infection was suggested more than 20 years ago (105). The infection could trigger dysregulated cytokine production in the BM microenvironment, providing optimal growth support to stem cells harboring a mutation (8, 9, 106, 107)., as recent studies are now demonstrating (96).

Stress-induced abnormal mitochondria. Interestingly, abnormal expression of nuclear-encoded mitochondrial genes is a predicting factor of therapy-related MDS (108). Consistent with this, we have shown that HSC keep abnormal mitochondria after BMT, indicating that the stress of BMT permanently alters mitochondria and HSC functions (109). The mechanisms causing alteration in mitochondrial functions in HSC following transplantation involves deregulation in mitochondrial dynamism and decreased expression of mitochondrial genes (109). Inability to remove abnormal mitochondria could contribute to secondary BMF/MDS in multiple ways. Abnormal mitochondria likely cause abnormal metabolism, including abnormal TCA cycle and OXPHOS that could mimic IDH or Tet2 mutation phenotypes. Abnormal mitochondria could contribute to secondary BMF/MDS *via* activation of innate immune signaling. Indeed, mitochondria serve as platform of innate immune signaling. Activation of numerous innate immune pathways occurs at the plasma membrane of mitochondria and depends on mitochondrial regulation (110, 111). For instance, activation of the inflammasome can depend on ROS production from stressed mitochondria (112). Viral infections, as seen above, lead to MAVS activation (97–99). MAVS activation requires mitochondrial polarization (i.e., established mitochondria membrane potential [MMP]) and is enhanced by mitochondrial fusion (111). Conversely, termination of the innate immune response is mediated by removal of mitochondria *via* mitophagy (113–115). Finally, mitochondrial stress is often accompanied by an abnormal release of mitochondrial DNA, which could activate DNA sensing pathways and subsequently innate immune signaling. Hence, abnormal mitochondria could be a mediator of inflammation following transplantation directly or in the context of added infection, and thus create an inflammatory a context for a mutated clone to expand.

The case for combinatorial effects: possible interactions between dysregulated TGFB, defective mitochondria and innate immune pathways as causal factors of secondary BMF/MDS. TGFβ upregulation and mitochondria abnormality occur in tandem in many BMFs. The source or consequence of this relationship is not completely clear. Our group recently reported the possible link between overexpression of TGFβ and mitochondria in the development of BMF/MDS (106). Using an TGFβ overexpressing mouse model, we demonstrated that elevated TGFβ signaling alone is not sufficient to cause BMF or MDS. However, elevated TGFβ signaling plus challenge with the double-stranded RNA pIC cause chronic pancytopenia, bone marrow dysplasia, increased hematopoietic stem and progenitor cell pools, which are phenotypes to human BMF. We further showed that elevated TGFβ plus pIC challenge alters mitochondrial functions with an elevated mitochondrial membrane potential and mitochondrial content. The gene expression profile of HSC shows persistent changes in the transcription profile in HSC from overexpressed TGFβ mice challenged with pIC that includes nuclear-encoded mitochondrial genes. Only overexpressed TGFβ HSC had higher expression of mrpl46 and other genes essential for the regulation of mitochondrial translation following pIC stress. This phenotype was associated with elevated levels of reactive oxygen species, and caspase-1 activation (106). Our findings imply that bone marrow failure with dysplastic features can occur without a prior genetic damage when chronic enhanced TGFβ signaling changes the acute immune response to pIC. Because pIC triggers an innate immune response mimicking a viral response, TGFβ may alter the innate immune pathways by modifying mitochondrial response, thus leading to development of an environment favored for BMF/MDS initiation and progression. These findings suggest a combinatorial effect between TGFβ and mitochondrial-mediated innate immune pathways could contribute to secondary BMF/MDS (Figure 2).

Conclusions and future directions

The formation of BMF involves numerous crucial interrelated factors, including genetics, proteomics, nutrition, cellular signaling, metabolism, and interaction between the HSC and other stromal cells. Mitochondria are emerging as important factors in the pathogenesis of BMF/MDS. How exactly mitochondria contribute to BMF/MDS remain to be analyzed in detail. We need to further understand the potential effects of damaged mitochondria on BMF/MDS development, including the potential consequences on HSC metabolism in disease context and how metabolic changes contribute to disease development. Examining this will need to be done both in the context of *de novo* BMF/MDS and secondary BMF/MDS. How the stress of bone marrow transplantation, with or without viral infection, alters mitochondrial functions is another area of interest. The fact that mitochondria serve as platform of innate immune signaling is very intriguing and will need to be examined in detail, as abnormal mitochondria could represent an important mechanism of hyperinflammation associated with BMF/MDS. Finally, the development of mitochondrial transfer or metabolic reprogramming through metabolite addition could be complimentary to current therapeutics, and need to be carefully evaluated. Therefore, a fuller understanding of interplay between mitochondrial functions and inflammation is essential for both our fundamental understanding of HSC biology and BMF/MDS pathogenesis as well as for the developmental of novel therapies.

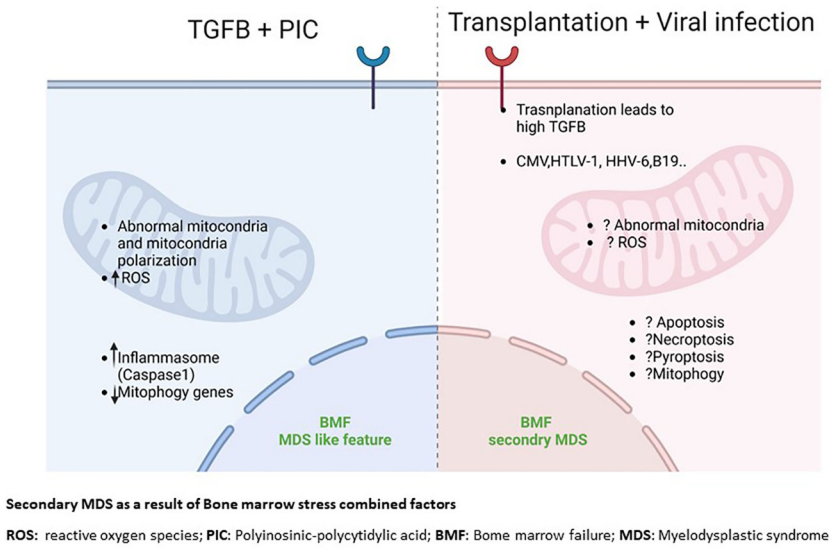

FIGURE 2
Secondary MDS as a result of Bone marrow stress combined factors. ROS, reactive oxygen species; PIC, Polyinosinic-polycytidylic acid; BMF, Bome marrow failure; MDS, Myelodysplactic syndrome.

It will be important to systematically investigate the role of mitochondrial functions and associated metabolism in BMF/MDS.

Author contributions

WN and M-DF equally contributed to writing the manuscript. M-DF edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Bagby GC, Fleischman AG. The stem cell fitness landscape and pathways of molecular leukemogenesis. *Front Biosci (Schol Ed).* (2011) 3:487–500. doi: 10.2741/s167

2. Leguit RJ, van den Tweel JG. The pathology of bone marrow failure. *Histopathology.* (2010) 57(5):655–70. doi: 10.1111/j.1365-2559.2010.03612.x

3. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev* (2010) 24(3):101–22. doi: 10.1016/j.blre.2010.03.002

4. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood.* (2006) 108(8):2509–19. doi: 10.1182/blood-2006-03-010777

5. Nepal M, Che R, Ma C, Zhang J, Fei P. FANCD2 and DNA damage. *Int J Mol Sci* (2017) 18(8):1804. doi: 10.3390/ijms18081804

6. Da Costa L, Leblanc T, Mohandas N. Diamond-blackfan anemia. *Blood.* (2020) 136(11):1262–73. doi: 10.1182/blood.2019000947

7. Tsai FD, Lindsley RC. Clonal hematopoiesis in the inherited bone marrow? Failure syndromes. *Blood.* (2020) 136(14):1615–22. doi: 10.1182/blood.2019000990

8. Friedberg JW, Neuberg D, Stone RM, Alyea E, Jallow H, LaCasce A, et al. Outcome in patients with myelodysplastic syndrome after autologous bone marrow transplantation for non-hodgkin's lymphoma. *J Clin Oncol* (1999) 17 (10):3128–35. doi: 10.1200/JCO.1999.17.10.3128

9. Stone RM, Neuberg D, Soiffer R, Takvorian T, Whelan M, Rabinowe SN, et al. Myelodysplastic syndrome as a late complication following autologous bone marrow transplantation for non-hodgkin's lymphoma. *J Clin Oncol* (1994) 12 (12):2535–42. doi: 10.1200/JCO.1994.12.12.2535

10. Brown JR, Yeckes H, Friedberg JW, Neuberg D, Kim H, Nadler LM, et al. Increasing incidence of late second malignancies after conditioning with cyclophosphamide and total-body irradiation and autologous bone marrow transplantation for non-hodgkin's lymphoma. *J Clin Oncol* (2005) 23(10):2208–14. doi: 10.1200/JCO.2005.05.158

11. Krishnan A, Bhatia S, Slovak ML, Arber DA, Niland JC, Nademanee A, et al. Predictors of therapy-related leukemia and myelodysplasia following autologous transplantation for lymphoma: an assessment of risk factors. *Blood* (2000) 95 (5):1588–93. doi: 10.1182/blood.V95.5.1588.005k38_1588_1593

12. Barreyro L, Chlon TM, Starczynowski DT. Chronic immune response dysregulation in MDS pathogenesis. *Blood* (2018) 132(15):1553–60. doi: 10.1182/blood-2018-03-784116

13. Hinge A, Xu J, Javier J, Mose E, Kumar S, Kapur R, et al. p190-b RhoGAP and intracellular cytokine signals balance hematopoietic stem and progenitor cell self-renewal and differentiation. *Nat Commun* (2017) 8:14382. doi: 10.1038/ncomms14382

14. Pietras EM, Mirantes-Barbeito C, Fong S, Loeffler D, Kovtonyuk LV, Zhang S, et al. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. *Nat Cell Biol* (2016) 18(6):607–18. doi: 10.1038/ncb3346

15. Leimkühler NB, Schneider RK. Inflammatory bone marrow microenvironment. *Hematology/the Educ Program Am Soc Hematol Am Soc Hematol Educ Program* (2019) 2019(1):294–302. doi: 10.1182/hematology.2019000045

16. Mekinian A, Grignano E, Braun T, Decaux O, Liozon E, Costedoat-Chalumeau Nc , et al. Systemic inflammatory and autoimmune manifestations associated with myelodysplastic syndromes and chronic myelomonocytic leukaemia: a French multicentre retrospective study. *Rheumatol (Oxford).* (2016) 55(2):291–300. doi: 10.1093/rheumatology/kev294

17. Trowbridge JJ, Starczynowski DT. Innate immune pathways and inflammation in hematopoietic aging, clonal hematopoiesis, and MDS. *J Exp Med* (2021) 218(7):e20201544. doi: 10.1084/jem.20201544

18. Aoyagi Y, Hayashi Y, Harada Y, Choi K, Matsunuma N, Sadato D, et al. Mitochondrial fragmentation triggers ineffective hematopoiesis in myelodysplastic syndromes. *Cancer Discovery* (2022) 12(1):250–69. doi: 10.1158/2159-8290.CD-21-0032

19. Cappelli E, Ravera S, Vaccaro D, Cuccarolo P, Bartolucci M, Panfoli I, et al. Mitochondrial respiratory complex I defects in fanconi anemia. *Trends Mol Med* (2013) 19(9):513–4. doi: 10.1016/j.molmed.2013.07.008

20. Cui X, Wang J, Cai Z, Wang J, Liu K, Cui S, et al. Complete sequence analysis of mitochondrial DNA and telomere length in aplastic anemia. *Int J Mol Med* (2014) 34(5):1309–14. doi: 10.3892/ijmm.2014.1898

21. Kim HR, Shin MG, Kim MJ, Kim HJ, Shin JH, Suh SP, et al. Mitochondrial DNA aberrations of bone marrow cells from patients with aplastic anemia. *J Korean Med Sci* (2008) 23(6):1062–7. doi: 10.3346/jkms.2008.23.6.1062

22. Beecher G, Fleming MD, Liewluck T. Hereditary myopathies associated with hematological abnormalities. *Muscle Nerve.* (2022) 65(4):374–90. doi: 10.1002/mus.27474

23. Cappelli E, Cuccarolo P, Stroppiana G, Miano M, Bottega R, Cossu V, et al. Defects in mitochondrial energetic function compels fanconi anaemia cells to glycolytic metabolism. *Biochim Biophys Acta Mol Basis Dis* (2017) 1863(6):1214–21. doi: 10.1016/j.bbadis.2017.03.008

24. Wild KT, Goldstein AC, Muraresku C, Ganetzky RD. Broadening the phenotypic spectrum of Pearson syndrome: Five new cases and a review of the literature. *Am J Med Genet A.* (2020) 182(2):365–73. doi: 10.1002/ajmg.a.61433

25. Filanovsky K, Haran M, Mirkin V, Braester A, Shevetz O, Stanevsky A, et al. Peripheral blood cell mitochondrial dysfunction in myelodysplastic syndrome can be improved by a combination of coenzyme Q10 and carnitine. *Mediterr J Hematol Infect Dis* (2020) 12(1):e2020072. doi: 10.4084/MJHID.2020.072

26. Schildgen V, Wulfert M, Gattermann N. Impaired mitochondrial gene transcription in myelodysplastic syndromes and acute myeloid leukemia with myelodysplasia-related changes. *Exp Hematol* (2011) 39(6):666–75.e661. doi: 10.1016/j.exphem.2011.03.007

27. Sen T, Jain M, Gram M, Mattebo A, Soneji S, Walkley CR, et al. Enhancing mitochondrial function *in vivo* rescues MDS-like anemia induced by pRb deficiency. *Exp Hematol* (2020) 88:28–41. doi: 10.1016/j.exphem.2020.06.006

28. Ward GA, McGraw KL, Abbas-Aghababazadeh F, Meyer BS, McLemore AF, Vincelette ND, et al. Oxidized mitochondrial DNA released after inflammasome activation is a disease biomarker for myelodysplastic syndromes. *Blood Adv* (2021) 5(8):2216–28. doi: 10.1182/bloodadvances.2020003475

29. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. *Cell.* (2012) 148(6):1145–59. doi: 10.1016/j.cell.2012.02.035

30. Chiabrando D, Vinchi F, Fiorito V, Mercurio S, Tolosano E. Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes. *Front Pharmacol* (2014) 5:61. doi: 10.3389/fphar.2014.00061

31. Chiabrando D, Marro S, Mercurio S, Giorgi C, Petrillo S, Vinchi F, et al. The mitochondrial heme exporter FLVCR1b mediates erythroid differentiation. *J Clin Invest.* (2012) 122(12):4569–79. doi: 10.1172/JCI62422

32. Ducamp S, Fleming MD. The molecular genetics of sideroblastic anemia. *Blood.* (2019) 133(1):59–69. doi: 10.1182/blood-2018-08-815951

33. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature.* (2011) 478(7367):64–9. doi: 10.1038/nature10496

34. Malcovati L, Papaemmanuil E, Ambaglio I, Elena C, Galli A, Della Porta MG, et al. Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. *Blood.* (2014) 124(9):1513–21. doi: 10.1182/blood-2014-03-560227

35. Malcovati L, Cazzola M. Recent advances in the understanding of myelodysplastic syndromes with ring sideroblasts. *Br J Haematol* (2016) 174 (6):847–58. doi: 10.1111/bjh.14215

36. Clough CA, Pangallo J, Sarchi M, Ilagan JO, North K, Bergantinos R, et al. Coordinated missplicing of TMEM14C and ABCB7 causes ring sideroblast formation in SF3B1-mutant myelodysplastic syndrome. *Blood.* (2022) 139 (13):2038–49. doi: 10.1182/blood.2021012652

37. Dolatshad H, Pellagatti A, Fernandez-Mercado M, Yip BH, Malcovati L, Attwood M, et al. Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndrome hematopoietic stem and progenitor cells. *Leukemia.* (2015) 29(5):1092–103. doi: 10.1038/leu.2014.331

38. Dolatshad H, Pellagatti A, Liberante FG, Llorian M, Repapi E, Steeples V, et al. Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes. *Leukemia.* (2016) 30 (12):2322–31. doi: 10.1038/leu.2016.149

39. Ochi T, Fujiwara T, Ono K, Suzuki C, Nikaido M, Inoue D, et al. Exploring the mechanistic link between SF3B1 mutation and ring sideroblast formation in myelodysplastic syndrome. *Sci Rep* (2022) 12(1):14562. doi: 10.1038/s41598-022-18921-2

40. Hsu J, Reilly A, Hayes BJ, Clough CA, Konnick EQ, Torok-Storb B, et al. Reprogramming identifies functionally distinct stages of clonal evolution in myelodysplastic syndromes. *Blood.* (2019) 134(2):186–98. doi: 10.1182/blood.2018884338

41. Rey MA, Duffy SP, Brown JK, Kennedy JA, Dick JE, Dror Y, et al. Enhanced alternative splicing of the FLVCR1 gene in diamond blackfan anemia disrupts FLVCR1 expression and function that are critical for erythropoiesis. *Haematologica.* (2008) 93(11):1617–26. doi: 10.3324/haematol.13359

42. Mercurio S, Aspesi A, Silengo L, Altruda F, Dianzani I, Chiabrando D. Alteration of heme metabolism in a cellular model of diamond-blackfan anemia. *Eur J Haematol* (2016) 96(4):367–74. doi: 10.1111/ejh.12599

43. Martinez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun* (2020) 11(1):102. doi: 10.1038/s41467-019-13668-3

44. DiNardo CD, Jabbour E, Ravandi F, Takahashi K, Daver N, Routbort M, et al. IDH1 and IDH2 mutations in myelodysplastic syndromes and role in disease progression. *Leukemia.* (2016) 30(4):980–4. doi: 10.1038/leu.2015.211

45. Bowman RL, Levine RL. TET2 in normal and malignant hematopoiesis. *Cold Spring Harb Perspect Med* (2017) 7(8):a026518. doi: 10.1101/cshperspect.a026518

46. Ewalt M, Galili NG, Mumtaz M, Churchill M, Rivera S, Borot F, et al. DNMT3a mutations in high-risk myelodysplastic syndrome parallel those found in acute myeloid leukemia. *Blood Cancer J* (2011) 1(3):e9. doi: 10.1038/bcj.2011.7

47. Hayashi Y, Zhang Y, Yokota A, Yan X, Liu J, Choi K, et al. Pathobiological pseudohypoxia as a putative mechanism underlying myelodysplastic syndromes. *Cancer Discovery* (2018) 8(11):1438–57. doi: 10.1158/2159-8290.CD-17-1203

48. Cerveny KL, Tamura Y, Zhang Z, Jensen RE, Sesaki H. Regulation of mitochondrial fusion and division. *Trends Cell Biol* (2007) 17(11):563–9. doi: 10.1016/j.tcb.2007.08.006

49. Picard M, Shirihai OS, Gentil BJ, Burelle Y. Mitochondrial morphology transitions and functions: implications for retrograde signaling? *Am J Physiol Regul Integr Comp Physiol* (2013) 304(6):R393–406. doi: 10.1152/ajpregu.00584.2012

50. Roy M, Reddy PH, Iijima M, Sesaki H. Mitochondrial division and fusion in metabolism. *Curr Opin Cell Biol* (2015) 33:111–8. doi: 10.1016/j.ceb.2015.02.001

51. Sumpter RJr., Sirasanagandla S, Fernandez AF, Wei Y, Dong X, Franco L, et al. Fanconi anemia proteins function in mitophagy and immunity. *Cell.* (2016) 165(4):867–81. doi: 10.1016/j.cell.2016.04.006

52. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* (2003) 552(Pt 2):335–44. doi: 10.1113/jphysiol.2003.049478

53. Gonçalves AC, Alves V, Silva T, Carvalho C, Oliveira CR, Sarmento-Ribeiro AB. Oxidative stress mediates apoptotic effects of ascorbate and dehydroascorbate in human myelodysplasia cells *in vitro*. *Toxicol In Vitro.* (2013) 27(5):1542–9. doi: 10.1016/j.tiv.2013.03.009

54. Yang Y, Karakhanova S, Hartwig W, D'Haese JG, Philippov PP, Werner J, et al. Mitochondria and mitochondrial ROS in cancer: Novel targets for anticancer therapy. *J Cell Physiol* (2016) 231(12):2570–81. doi: 10.1002/jcp.25349

55. Capanni C, Bruschi M, Columbaro M, Cuccarolo P, Ravera S, Dufour C, et al. Changes in vimentin, lamin A/C and mitofilin induce aberrant cell organization in fibroblasts from fanconi anemia complementation group a (FA-a) patients. *Biochimie.* (2013) 95(10):1838–47. doi: 10.1016/j.biochi.2013.06.024

56. Ravera S, Vaccaro D, Cuccarolo P, Columbaro M, Capanni C, Bartolucci M, et al. Mitochondrial respiratory chain complex I defects in fanconi anemia complementation group a. *Biochimie.* (2013) 95(10):1828–37. doi: 10.1016/j.biochi.2013.06.006

57. Chatla S, Du W, Wilson AF, Meetei AR, Pang Q. Fancd2-deficient hematopoietic stem and progenitor cells depend on augmented mitochondrial translation for survival and proliferation. *Stem Cell Res* (2019) 40:101550. doi: 10.1016/j.scr.2019.101550

58. Henson AL, JBt M, Alard P, Wattenberg MM, Liu JM, Ellis SR. Mitochondrial function is impaired in yeast and human cellular models of shwachman diamond syndrome. *Biochem Biophys Res Commun* (2013) 437 (1):29–34. doi: 10.1016/j.bbrc.2013.06.028

59. Pearson HA, Lobel JS, Kocoshis SA, Naiman JL, Windmiller J, Lammi AT, et al. A new syndrome of refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic dysfunction. *J Pediatr* (1979) 95 (6):976–84. doi: 10.1016/S0022-3476(79)80286-3

60. Steward CG, Groves SJ, Taylor CT, Maisenbacher MK, Versluys B, Newbury-Ecob RA, et al. Neutropenia in barth syndrome: characteristics, risks, and management. *Curr Opin hematology.* (2019) 26(1):6–15. doi: 10.1097/MOH.0000000000000472

61. Acehan D, Vaz F, Houtkooper RH, James J, Moore V, Tokunaga C, et al. Cardiac and skeletal muscle defects in a mouse model of human barth syndrome. *J Biol Chem* (2011) 286(2):899–908. doi: 10.1074/jbc.M110.171439

62. Clarke SL, Bowron A, Gonzalez IL, Groves SJ, Newbury-Ecob R, Clayton N, et al. Barth syndrome. *Orphanet J Rare Dis* (2013) 8:23. doi: 10.1186/1750-1172-8-23

63. Makaryan V, Kulik W, Vaz FM, Allen C, Dror Y, Dale DC, et al. The cellular and molecular mechanisms for neutropenia in barth syndrome. *Eur J Haematol* (2012) 88(3):195–209. doi: 10.1111/j.1600-0609.2011.01725.x

64. Kuijpers TW, Maianski NA, Tool AT, Becker K, Plecko B, Valianpour F, et al. Neutrophils in barth syndrome (BTHS) avidly bind annexin-V in the absence of apoptosis. *Blood.* (2004) 103(10):3915–23. doi: 10.1182/blood-2003-11-3940

65. Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer AA, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). *Nat Genet* (2007) 39(1):86–92. doi: 10.1038/ng1940

66. Schäffer AA, Klein C. Genetic heterogeneity in severe congenital neutropenia: how many aberrant pathways can kill a neutrophil? *Curr Opin Allergy Clin Immunol* (2007) 7(6):481–94. doi: 10.1097/ACI.0b013e3282f1d690

67. Katada S, Mito T, Ogasawara E, Hayashi J, Nakada K. Mitochondrial DNA with a large-scale deletion causes two distinct mitochondrial disease phenotypes in mice. *G3 (Bethesda).* (2013) 3(9):1545–52. doi: 10.1534/g3.113.007245

68. Jacoby E, Ben Yakir-Blumkin M, Blumenfeld-Kan S, Brody Y, Meir A, Melamed-Book N, et al. Mitochondrial augmentation of CD34(+) cells from healthy donors and patients with mitochondrial DNA disorders confers functional benefit. *NPJ Regener Med* (2021) 6(1):58. doi: 10.1038/s41536-021-00167-7

69. Amigo ML, del Cañizo MC, Rios A, Garcia MA, Caballero MD, Martin A, et al. Diagnosis of secondary myelodysplastic syndromes (MDS) following autologous transplantation should not be based only on morphological criteria used for diagnosis of *de novo* MDS. *Bone Marrow Transplant.* (1999) 23(10):997–1002. doi: 10.1038/sj.bmt.1701757

70. Metheny L, Callander NS, Hall AC, Zhang MJ, Bo-Subait K, Wang HL, et al. Allogeneic transplantation to treat therapy-related myelodysplastic syndrome and acute myelogenous leukemia in adults. *Transplant Cell Ther* (2021) 27 (11):923.e921–923.e912. doi: 10.1016/j.jtct.2021.08.010

71. Yamasaki S, Suzuki R, Hatano K, Fukushima K, Iida H, Morishima S, et al. Therapy-related acute myeloid leukemia and myelodysplastic syndrome after hematopoietic cell transplantation for lymphoma. *Bone Marrow Transplant.* (2017) 52(7):969–76. doi: 10.1038/bmt.2017.52

72. Armitage JO, Carbone PP, Connors JM, Levine A, Bennett JM, Kroll S. Treatment-related myelodysplasia and acute leukemia in non-hodgkin's lymphoma patients. *J Clin Oncol* (2003) 21(5):897–906. doi: 10.1200/JCO.2003.07.113

73. Hu HH, Chen DQ, Wang YN, Feng YL, Cao G, Vaziri ND, et al. New insights into TGF-β/Smad signaling in tissue fibrosis. *Chem Biol Interact* (2018) 292:76–83. doi: 10.1016/j.cbi.2018.07.008

74. Blank U, Karlsson S. The role of smad signaling in hematopoiesis and translational hematology. *Leukemia.* (2011) 25(9):1379–88. doi: 10.1038/leu.2011.95

75. Larsson J, Karlsson S. The role of smad signaling in hematopoiesis. *Oncogene.* (2005) 24(37):5676–92. doi: 10.1038/sj.onc.1208920

76. Vaidya A, Kale VP. TGF-β signaling and its role in the regulation of hematopoietic stem cells. *Syst Synth Biol* (2015) 9(1-2):1–10. doi: 10.1007/s11693-015-9161-2

77. Hinge A, Filippi MD. Deconstructing the complexity of TGFβ signaling in hematopoietic stem cells: Quiescence and beyond. *Curr Stem Cell Rep* (2016) 2 (4):388–97. doi: 10.1007/s40778-016-0069-x

78. Coomes SM, Moore BB. Pleiotropic effects of transforming growth factor-β in hematopoietic stem-cell transplantation. *Transplantation.* (2010) 90(11):1139–44. doi: 10.1097/TP.0b013e3181efd018

79. Liem LM, Fibbe WE, van Houwelingen HC, Goulmy E. Serum transforming growth factor-beta1 levels in bone marrow transplant recipients correlate with blood cell counts and chronic graft-versus-host disease. *Transplantation.* (1999) 67 (1):59–65. doi: 10.1097/00007890-199901150-00009

80. Coomes SM, Wilke CA, Moore TA, Moore BB. Induction of TGF-beta 1, not regulatory T cells, impairs antiviral immunity in the lung following bone marrow transplant. *J Immunol* (2010) 184(9):5130–40. doi: 10.4049/jimmunol.0901871

81. Panoskaltsis-Mortari A, Taylor PA, Yaeger TM, Wangensteen OD, Bitterman PB, Ingbar DH, et al. The critical early proinflammatory events associated with idiopathic pneumonia syndrome in irradiated murine allogeneic recipients are due to donor T cell infusion and potentiated by cyclophosphamide. *J Clin Invest.* (1997) 100(5):1015–27. doi: 10.1172/JCI119612

82. Zhou L, McMahon C, Bhagat T, Alencar C, Yu Y, Fazzari M, et al. Reduced SMAD7 leads to overactivation of TGF-beta signaling in MDS that can be reversed by a specific inhibitor of TGF-beta receptor I kinase. *Cancer Res* (2011) 71(3):955–63. doi: 10.1158/0008-5472.CAN-10-2933

83. Zhou L, Nguyen AN, Sohal D, Ying Ma J, Pahanish P, Gundabolu K, et al. Inhibition of the TGF-beta receptor I kinase promotes hematopoiesis in MDS. *Blood.* (2008) 112(8):3434–43. doi: 10.1182/blood-2008-02-139824

84. Bhagat TD, Zhou L, Sokol L, Kessel R, Caceres G, Gundabolu K, et al. miR-21 mediates hematopoietic suppression in MDS by activating TGF-β signaling. *Blood.* (2013) 121(15):2875–81. doi: 10.1182/blood-2011-12-397067

85. Río P, Bueren JA. TGF-β: a master regulator of the bone marrow failure puzzle in fanconi anemia. *Stem Cell Investig* (2016) 3:75. doi: 10.21037/sci.2016.09.17

86. Zhang H, Kozono DE, O'Connor KW, Vidal-Cardenas S, Rousseau A, Hamilton A, et al. TGF-β inhibition rescues hematopoietic stem cell defects and bone marrow failure in fanconi anemia. *Cell Stem Cell* (2016) 18(5):668–81. doi: 10.1016/j.stem.2016.03.002

87. Ashley RJ, Yan H, Wang N, Hale J, Dulmovits BM, Papoin J, et al. Steroid resistance in diamond blackfan anemia associates with p57Kip2 dysregulation in erythroid progenitors. *J Clin Invest.* (2020) 130(4):2097–110. doi: 10.1172/JCI132284

88. Suragani RN, Cadena SM, Cawley SM, Sako D, Mitchell D, Li R, et al. Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. *Nat Med* (2014) 20(4):408–14. doi: 10.1038/nm.3512

89. Joyce CE, Saadatpour A, Ruiz-Gutierrez M, Bolukbasi OV, Jiang L, Thomas DD, et al. TGFβ signaling underlies hematopoietic dysfunction and bone marrow failure in shwachman-diamond syndrome. *J Clin Invest.* (2019) 129(9):3821–6. doi: 10.1172/JCI125375

90. Starczynowski DT, Kuchenbauer F, Wegrzyn J, Rouhi A, Petriv O, Hansen CL, et al. MicroRNA-146a disrupts hematopoietic differentiation and survival. *Exp Hematol* (2011) 39(2):167–78.e164. doi: 10.1016/j.exphem.2010.09.011

91. Varney ME, Niederkorn M, Konno H, Matsumura T, Gohda J, Yoshida N, et al. Loss of tifab, a del(5q) MDS gene, alters hematopoiesis through derepression of toll-like receptor-TRAF6 signaling. *J Exp Med* (2015) 212(11):1967–85. doi: 10.1084/jem.20141898

92. Basiorka AA, McGraw KL, Eksioglu EA, Chen X, Johnson J, Zhang L, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. *Blood.* (2016) 128(25):2960–75. doi: 10.1182/blood-2016-07-730556

93. Sallman DA, Cluzeau T, Basiorka AA, List A. Unraveling the pathogenesis of MDS: The NLRP3 inflammasome and pyroptosis drive the MDS phenotype. *Front Oncol* (2016) 6:151. doi: 10.3389/fonc.2016.00151

94. Wagner PN, Shi Q, Salisbury-Ruf CT, Zou J, Savona MR, Fedoriw Y, et al. Increased Ripk1-mediated bone marrow necroptosis leads to myelodysplasia and bone marrow failure in mice. *Blood.* (2019) 133(2):107–20. doi: 10.1182/blood-2018-05-847335

95. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol* (2019) 19(8):477–89. doi: 10.1038/s41577-019-0165-0

96. Hormaechea-Agulla D, Matatall KA, Le DT, Kain B, Long X, Kus P, et al. Chronic infection drives Dnmt3a-loss-of-function clonal hematopoiesis via IFNgamma signaling. *Cell Stem Cell* (2021) 8(8):1428–42. doi: 10.1016/j.stem.2021.03.002

97. Hou F, Sun L, Zheng H, Skaug B, Jiang QX, Chen ZJ. MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell.* (2011) 146(3):448–61. doi: 10.1016/j.cell.2011.06.041

98. Subramanian N, Natarajan K, Clatworthy MR, Wang Z, Germain RN. The adaptor MAVS promotes NLRP3 mitochondrial localization and inflammasome activation. *Cell.* (2013) 153(2):348–61. doi: 10.1016/j.cell.2013.02.054

99. Zhou X, You F, Chen H, Jiang Z. Poly(C)-binding protein 1 (PCBP1) mediates housekeeping degradation of mitochondrial antiviral signaling (MAVS). *Cell Res* (2012) 22(4):717–27. doi: 10.1038/cr.2011.184

100. Jiang Y, Zhu Y, Liu ZJ, Ouyang S. The emerging roles of the DDX41 protein in immunity and diseases. *Protein Cell* (2017) 8(2):83–9. doi: 10.1007/s13238-016-0303-4

101. Raza A. Initial transforming event in myelodysplastic syndromes may be viral: Case for cytomegalovirus. *Med Oncol* (1998) 15(3):165–73. doi: 10.1007/BF02821935

102. Karlic H, Möstl M, Mucke H, Pavlova B, Pfeilstöcker M, Heinz R. Association of human T-cell leukemia virus and myelodysplastic syndrome in a central European population. *Cancer Res* (1997) 57(21):4718–21.

103. Urban C, Lackner H, Muller E, Benesch M, Strenger V, Sovinz P, et al. Stem cell transplantation in 6 children with parvovirus B19- induced severe aplastic anaemia or myelodysplastic syndrome. *Klin Padiatr.* (2011) 223(6):332–4. doi: 10.1055/s-0031-1287839

104. Kagialis-Girard S, Durand B, Mialou V, Pagès MP, Galambrun C, Bertrand Y, et al. Human herpesvirus 6 infection and transient acquired myelodysplasia in children. *Pediatr Blood Cancer.* (2006) 47(5):543–8. doi: 10.1002/pbc.20667

105. Raza A. Hypothesis: myelodysplastic syndromes may have a viral etiology. *Int J hematology.* (1998) 68(3):245–56. doi: 10.1016/S0925-5710(98)00051-6

106. Javier J, Hinge A, Bartram J, Xu J, Filippi MD. Transforming growth factor-β signaling modifies the hematopoietic acute inflammatory response to drive bone marrow failure. *Haematologica.* (2022) 107(6):1323–34. doi: 10.3324/haematol.2020.273292

107. Engels EA. Infectious agents as causes of non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev* (2007) 16(3):401–4. doi: 10.1158/1055-9965.EPI-06-1056

108. Li L, Li M, Sun C, Francisco L, Chakraborty S, Sabado M, et al. Altered hematopoietic cell gene expression precedes development of therapy-related myelodysplasia/acute myeloid leukemia and identifies patients at risk. *Cancer Cell* (2011) 20(5):591–605. doi: 10.1016/j.ccr.2011.09.011

109. Hinge A, He J, Bartram J, Javier J, Xu J, Fjellman E, et al. Asymmetrically segregated mitochondria provide cellular memory of hematopoietic stem cell replicative history and drive HSC attrition. *Cell Stem Cell* (2020) 26(3):420–30.e426. doi: 10.1016/j.stem.2020.01.016

110. Breda CNS, Davanzo GG, Basso PJ, Saraiva Camara NO, Moraes-Vieira PMM. Mitochondria as central hub of the immune system. *Redox Biol* (2019) 26:101255. doi: 10.1016/j.redox.2019.101255

111. Kim SJ, Ahn DG, Syed GH, Siddiqui A. The essential role of mitochondrial dynamics in antiviral immunity. *Mitochondrion.* (2018) 41:21–7. doi: 10.1016/j.mito.2017.11.007

112. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature.* (2011) 469(7329):221–5. doi: 10.1038/nature09663

113. Kim SH, Shin HJ, Yoon CM, Lee SW, Sharma L, Dela Cruz CS, et al. PINK1 inhibits multimeric aggregation and signaling of MAVS and MAVS-dependent lung pathology. *Am J Respir Cell Mol Biol* (2021) 64(5):592–603. doi: 10.1165/rcmb.2020-0490OC

114. Sun X, Sun L, Zhao Y, Li Y, Lin W, Chen D, et al. MAVS maintains mitochondrial homeostasis *via* autophagy. *Cell Discovery* (2016) 2:16024. doi: 10.1038/celldisc.2016.24

115. Xu SS, Xu LG, Yuan C, Li SN, Chen T, Wang W, et al. FKBP8 inhibits virus-induced RLR-VISA signaling. *J Med Virol* (2019) 91(3):482–92. doi: 10.1002/jmv.25327

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Prioritization of patients for germline testing based on tumor profiling of hematopoietic malignancies

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Germline predisposition to hematopoietic malignancies is more common than previously appreciated, with several clinical guidelines advocating for cancer risk testing in an expanding pool of patients. As molecular profiling of tumor cells becomes a standard practice for prognostication and defining options for targeted therapies, recognition that germline variants are present in all cells and can be identified by such testing becomes paramount. Although not to be substituted for proper germline cancer risk testing, tumor-based profiling can help prioritize DNA variants likely to be of germline origin, especially when they are present on sequential samples and persist into remission. Performing germline genetic testing as early during patient work-up as possible allows time to plan allogeneic stem cell transplantation using appropriate donors and optimize post-transplant prophylaxis. Health care providers need to be attentive to the differences between molecular profiling of tumor cells and germline genetic testing regarding ideal sample types, platform designs, capabilities, and limitations, to allow testing data to be interpreted as comprehensively as possible. The myriad of mutation types and growing number of genes involved in germline predisposition to hematopoietic malignancies makes reliance on detection of deleterious alleles using tumor-based testing alone very difficult and makes understanding how to ensure adequate testing of appropriate patients paramount.

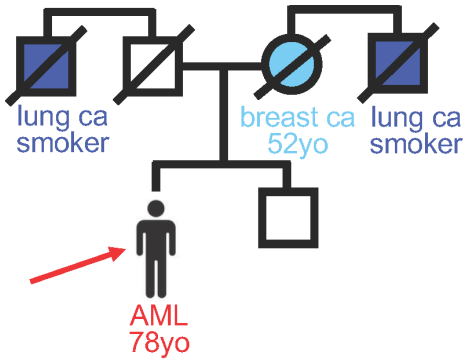
KEYWORDS

germline predisposition, tumor profiling, molecular profiling, hematopoietic malignancies, cancer risk

1 Introduction

1.1 Opening case

A 78 year-old (yo) man was diagnosed with acute myeloid leukemia during a work-up for his worsening fatigue (Figure 1). His family history was significant for his mother, who had been diagnosed with breast cancer at 52yo, and two uncles, who were smokers, with lung cancer. Cytogenetic analysis from the bone marrow at diagnosis revealed a normal karyotype,



		Diagnosis	Remission
VAFs	*DNMT3A* R882H	36%	3%
	TP53 R248Q	64%	45%

FIGURE 1
Persistence of deleterious variants in genes known to confer germline risk to hematopoietic malignancies suggests germline status. Top, Pedigree of an individual diagnosed with acute myeloid leukemia (AML) at 78 years old (yo), indicated by the red arrow. Circles, women; squares, men. Strike-out line indicates deceased individual at the time of pedigree generation. Ca, cancer. Bottom, Variant allele frequency (VAF) of deleterious variants at diagnosis (left) versus remission (right).

and molecular profiling demonstrated two DNA mutations: A *DNMT3A* mutation encoding R882H was present at a variant allele frequency (VAF) of 36%, and a *TP53* variant encoding R248Q was present at a VAF of 64%. The patient received standard induction chemotherapy with 7 days of cytarabine and 3 days of daunorubicin and achieved a clinical remission. Molecular testing from the remission bone marrow biopsy showed a decrease in the *DNMT3A* variant to 3%, but the *TP53* variant VAF remained high at 45%. Because the patient was in a clinical remission, with one DNA mutation decreasing to levels consistent with clonal hematopoiesis, the *TP53* variant VAF remaining about 50% suggested that this was a germline allele. The patient's treating physician counseled the patient about this finding, and the patient chose to have a skin biopsy for germline genetic testing. The *TP53* variant was confirmed to be germline based on testing from DNA derived from cultured skin fibroblasts, and the patient was counseled about cancer risks associated with Li-Fraumeni Syndrome. Cascade testing for family members and cancer surveillance strategies were put into place for the patient and his affected family members.

2 Germline predisposition testing for patients with hematopoietic malignancies

Germline predisposition to hematopoietic malignancies (HMs) is being recognized increasingly (1, 2), as classification schemes (3, 4) and clinical guidelines (5–10) advocate for germline genetic testing for individuals with hematopoietic malignancies. Over time, such predisposition testing is being recommended for a larger and larger group of people. Currently, germline risk testing is advised for those with certain physical features (5, 11), and/or a: personal history of two or more cancers; personal history of a HM diagnosed at a much younger age than average [*e.g.*, MDS at <40yo]; personal history of a HM along with a family history of: another HM/prolonged cytopenia/or other hematologic abnormality (*e.g.*, macrocytosis) or diagnosis of a non-hematopoietic tumor in an individual < 50 yo within two generations of the proband; and/or molecular testing of tumor cells showing a deleterious variant in a gene known to confer a hereditary hematopoietic malignancy (HHM) at a VAF consistent with germline inheritance (1, 2, 5–8). VAFs in the range of 30-60% are generally considered typical for germline allele status, but this value can change depending on the testing platform and/or any copy number variants (CNVs) that may be present in the tissue being tested (7, 8, 12, 13). Other centers also prioritize those with excessive toxicity from chemo- radiotherapy for germline predisposition testing (11). Although germline predisposition testing is recommended for those diagnosed at particularly young ages as noted above, such testing should be considered in all patients with HMs regardless of age (14, 15). Certain cytogenetic and molecular abnormalities detected in tumor cells may also provide clues as to an underlying germline predisposition, including (i) the presence of two mutations within a gene known to confer inherited risk, such as *RUNX1* or *CEBPA*, one of which is actually a germline mutation; (ii) the presence of monosomy 7, which may suggest a deleterious germline variant in *SAMD9/SAMD9L* or *GATA2*; or (iii) a hypermutator tumor phenotype, which may indicate a germline alteration in a mismatch repair gene (16) or *MBD4* (17, 18).

Germline cancer syndromes were initially described by clinicians who naturally focused on extreme personal and family histories (5, 19). Thus, classic descriptions of these conditions were almost always too narrowly defined, as the Opening Case illustrates. The actual tumor spectrum of germline cancer disorders, like Li-Fraumeni syndrome, is likely much broader than first described (5, 19). As diagnosis becomes based more on molecular techniques rather than history and physical examination, we may identify more subtle cancer histories and/or physical findings associated with these classic cancer predisposition disorders.

3 Prioritizing DNA variants for germline testing identified in molecular profiling data from hematopoietic tumors

Because germline variants are present in all of the cells within a person's body, malignant cells also contain that individual's germline alleles (6–8, 12). Molecular profiling assays may be DNA- or RNA-based (13). In the case of platforms that use DNA derived from tumor biopsies, detected DNA variants may be derived from admixed normal cells and/or from germline alleles (12). Unfortunately, often, deleterious DNA variants are assumed to be somatic in nature and interpreted as such (20–22). Clinical reports may indicate that a particular variant could be germline in nature, but busy clinicians may not read these caveats closely or understand the distinction between germline and somatic alleles.

Importantly, the clinical classification of variants detected by tumor-based profiling are based on their assumed somatic nature, which may differ if the variant is actually germline (2, 8, 20–22). The impact of DNA changes is context dependent, and therefore, a germline allele, which is present in all tissues, may have different effects compared to a somatic allele, which is present only in a tumor (23, 24). For this reason, germline and somatic variant curations are distinct (23, 25). Recognizing this, it is our practice to review all DNA variants called in genes known to confer risk for HHMs from molecular testing of malignant hematopoietic tumor cells with the goal of identifying those that could be germline (6–8, 21). Particular gene alleles are overwhelmingly likely to be germline (Table 1), and identifying these in tumor cells quickly singles out these individuals for counselling regarding the likely germline nature of the allele (6, 21). When DNA variants likely to be germline are found in tumor-based sequencing assays, we notify the clinic physician in charge of the patient and urge genetic counselling and testing. Some centers have established parallel pipelines that assess for somatic and germline variants simultaneously by sequencing DNA from tumor cells and buccal swabs as well as tumor-derived RNA (13).

As indicated in Table 1, based on our clinical experience at The University of Chicago, there are two genes in which certain variants are overwhelmingly likely to be germline: *CHEK2* and *DDX41*. When these variants are detected even in a single sample, they are typically seen at germline-range VAFs, and treating physicians are notified of the likely germline nature of the variants. We advocate genetic counselling, with recommendation for determination of germline status using either (i) testing of DNA from cultured skin fibroblasts or hair bulbs, both of which are equivalent to germline, or (ii) segregation of the variant within the family (Figure 2). Identifying a DNA variant in two related individuals is sufficient to determine germline status, and occasionally, this approach is more feasible than testing through skin biopsy and fibroblast culture. We advise against assuming that these variants are germline and do not provide clinical recommendations unless these variants have been confirmed to be germline. Importantly, founder mutations exist in other cancer predisposing genes, such as *BRCA1/2* and *TP53*, which may also confer risk to HMs as well as solid tumors. For this reason, care should be given to review all DNA variants in cancer predisposing genes when tumor based molecular profiling is performed.

Molecular profiling in patients with HMs is often conducted sequentially over time to document remission status. In these cases, serial sampling over time is an excellent means of prioritizing patients for germline testing (12), as outlined by the Opening Case. Gene variants that persist over time despite changes in disease status, especially those that remain in germline-range VAF from diagnosis through clinical remission, are likely to be germline, and again identify individuals who deserve genetic counseling and testing (12). However, we need to be cautious and interpret variants within the clinical context.

TABLE 1 Gene alleles that are commonly germline.

	Alleles that are overwhelmingly likely to be germline
CHEK2 NM 007194.4	• c.470T>C, p.1200T • c.1100delC, p.T367fs • c.1283C>T, p.S428F
DDXI NM 016222.3	• truncating variants • c.3G>A, p.M1?

4 Case 2: After allogeneic hematopoietic stem cell transplantation (HSCT), molecular profiling can identify donor-derived germline variants

The pre-transplant work-up of a patient with acute myeloid leukemia revealed two deleterious *DDX41* variants: one encoding the truncating D140fs variant with a VAF of 49%, and the other encoding the R525H variant at a VAF of 9% (Figure 3). Because the D140fs variant has always been seen as a germline variant (Table 1), the patient was counseled and proper germline testing confirmed its germline status. The transplant team decided to proceed with an allogeneic HSCT from an unrelated donor. The post-transplant day +30 bone marrow biopsy was performed, and several studies were performed in parallel: engraftment analysis [to determine the degree of donor chimerism], which showed that >95% of bone marrow cells were donor-derived; and molecular profiling [to ensure molecular remission from leukemia], which identified the CHEK2 I200T variant at a VAF of 51%, an allele which is overwhelmingly likely to be germline (Table 1).

5 Sensitivity around molecular testing after HSCT

In Case 2, the patient and his treating team did not have consent from the unrelated donor to know their germline genetic testing result, nor was there a mechanism to share this information with the donor. It is important to recognize that this situation is not unique to unrelated donors. Since all people have deleterious germline DNA variants, all donor types (*e.g.*, related, unrelated, umbilical cord) have the capacity to introduce such alleles into a transplant recipient. Sequential testing in which engraftment analysis is performed first

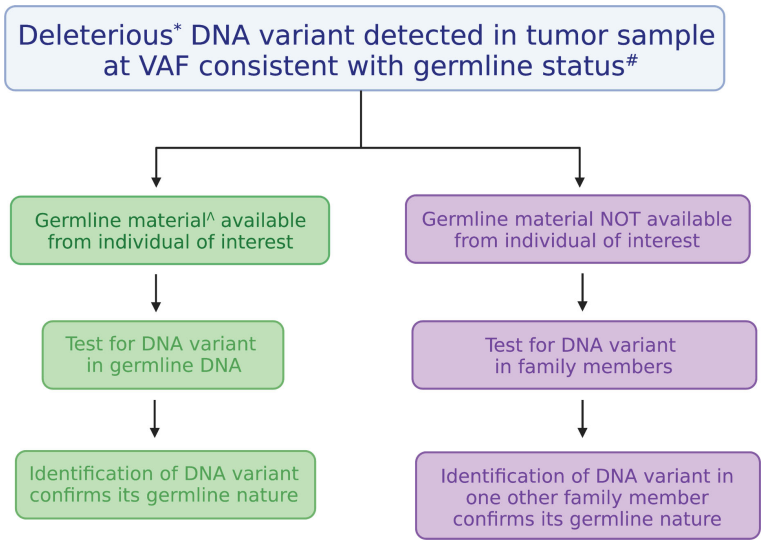


FIGURE 2
Algorithm for testing the germline nature of a DNA variant identified in a tumor. Testing the germline nature of a DNA variant begins with recognizing a deleterious DNA variant found in a gene known to confer cancer risk at a variant allele frequency (VAF) consistent with a germline allele. Testing can be performed using another DNA sample derived from a tissue considered equivalent to the germline (on left, in green) or through familial segregation (on right, in purple). Figure was generated with BioRender. *Deleterious variants are those classified as pathogenic or likely pathogenic. #VAFs from 30-60% are generally considered to be consistent with germline status, but they can be as high as 100% depending on chromosome gains or losses. ^DNA derived from cultured skin fibroblasts, hair bulbs, or bone marrow-derived mesenchymal stromal cells are considered equivalent to germline samples.

and informs subsequent testing and reporting could have avoided this situation. If chimerism were tested first and the sample were noted to be overwhelmingly donor-derived, then reporting of molecular profiling could have noted that fact, at a minimum, or ideally, been restricted to leukemia-associated somatic variants. Limiting post-transplant clinical reports to detailing the presence or absence of malignancy-associated somatic DNA variants (*i.e.*, in this case, the DDX41 R525H allele) is appropriate, but requires coordination across clinical laboratories. Ideally, test reports should also clearly indicate that by the nature of this type of testing, germline variants of donor or

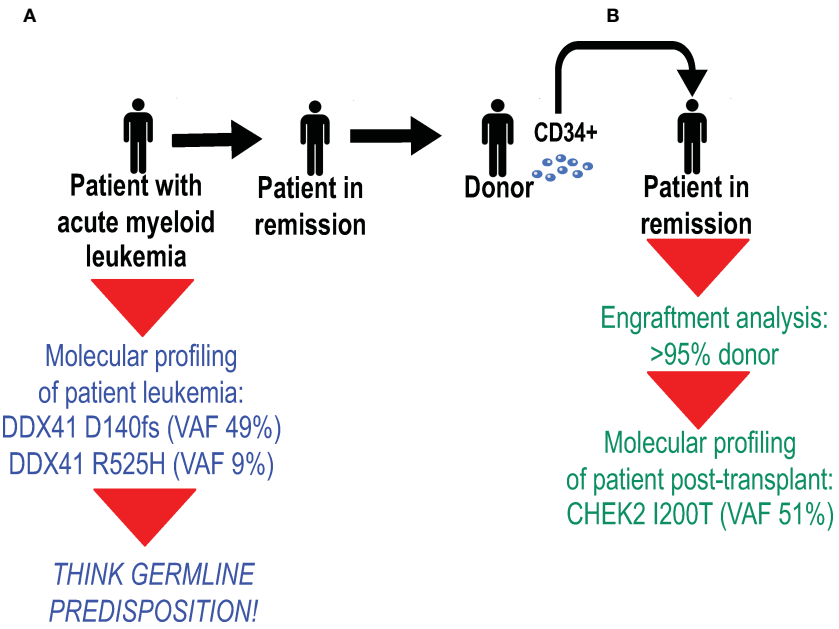


FIGURE 3
Molecular profiling has the capacity to identify donor-derived germline variants after allogeneic hematopoietic stem cell transplantation (HSCT). **(A)** Molecular profiling of a patient with acute myeloid leukemia revealed two deleterious DDX41 variants (shown in blue): the D140fs variant at a variant allele frequency (VAF) of 49%, and the R525H variant at a VAF of 9%. Because the D140fs variant has always been seen as a germline variant (Table 2), the patient had proper germline testing, which confirmed its germline status. **(B)** After allogeneic HSCT from an unrelated donor, a day 30 bone marrow biopsy was performed. Studies performed on bone marrow cells (shown in green) included engraftment analysis, which showed that >95% of bone marrow cells were donor-derived, and molecular profiling identified the CHEK2 I200T variant at a VAF of 51%.

host origin could be missed. Providers and patients receiving these reports need to know this information, as it could be clinically relevant over time for the patient or a related donor.

6 Cautions in using tumor profiling data

Although tumor-based molecular studies can identify some deleterious germline variants, these platforms should not be used in place of proper germline testing (Table 2). First, the ideal sample type for each test is distinct. For tumor-based testing, a sample containing tumor cells must be used. Therefore, peripheral blood with circulating malignant cells; involved bone marrow, lymph node(s), and/or cerebrospinal fluid; or any other tissue (*e.g.*, extramedullary hematopoiesis, myeloid sarcoma) containing such cells can be used to generate DNA. In most of these cases, normal cells are also present, with the quantity dictated by the degree of tumor burden. In contrast, germline testing is performed ideally using tissues that are equivalent to germline. Most clinical laboratories accept DNA derived from cultured skin fibroblasts, and some accept DNA generated from non-hematopoietic hair bulbs or bone marrow-derived mesenchymal stromal cells (MSCs), which are easily cultured from a bone marrow aspirate.

The distinction between the use of hematopoietic versus non-hematopoietic tissue for proper germline predisposition testing is of paramount importance. Use of non-hematopoietic tissue is critical for germline risk assessment, because hematopoietic tissue undergoes somatic reversion relatively easily compared to other tissues, like skin fibroblasts (19). For some deleterious germline variants, like those in *SAMD9* and *SAMD9L*, somatic reversion is a common mode of escape hematopoiesis (19). In these cases, correction in hematopoietic tissues occurs commonly, and therefore testing for these alleles in hematopoietic tissues fails to reveal the underlying germline defect. Somatic reversion has been documented for nearly all of the genes that confer germline susceptibility to HHMs. Thus, if hematopoietic tissue is used for germline genetic testing and a negative result is obtained, this may be a false negative result, and one cannot be confident that a deleterious variant is not present (19).

Traditionally, germline genetic testing has used peripheral blood or saliva/buccal swab for testing. However, we now recognize how frequent clonal hematopoiesis and therefore somatic mutation occurs within the hematopoietic compartment, which complicates DNA variant interpretation. For example, there have now been many cases of "mosaic *TP53* mutations" being confirmed to be due to CH (26). CH is itself a form of somatic mosaicism but one that arises well after embryogenesis and generally during adulthood. Therefore, we avoid germline genetic testing from hematopoietic tissues.

Thus, for these reasons, we recommend germline testing using DNA derived from tissues considered equivalent to germline (*e.g.*, cultured skin fibroblasts, hair bulbs, or bone marrow derived MSCs) (1, 6, 8, 12, 19, 27). When DNA variants are identified within germline range from DNA derived from these tissues, the result can be immediately interpreted as germline and is immediately relevant when considering relatives as allogeneic HSC donors (6). Some retrospective studies have determined germline status through sharing of the allele in relatives (15) or the presence of an allele at a VAF consistent with germline status obtained from hematopoietic tissue in clinical remission (28), but we do not advocate such approaches in general for clinical germline predisposition testing due to the concerns of somatic reversion and somatic mosaicism, as discussed above. When germline genetic testing is incorporated into the initial assessment of patients with HMs, results are often available when that individual and their family members are being evaluated for allogeneic HSCT and for optimal post-transplant prophylaxis (29).

The assay designs used in tumor-based molecular profiling versus germline testing are also quite distinct. Acquired mutations in HMs typically occur in gene exons, and therefore, platform designs tend to

TABLE 2 Contrasting tumor-based versus germline testing.

	Tumor-based Testing	Germline Testing
Sample type	• peripheral blood • bone marrow • lymph node • CSF • any sample with hematopoietic tumor cells	• cultured skin fibroblasts • hair bulbs • bone marrow-derived mesenchymal stromal cells
Benefits	Sample is likely already being collected for other tests.	Result is confirmed germline and can be interpreted as such. Result is immediately relevant when considering relatives as allogeneic hematopoietic stem cell donors.
Cautions/ caveats	Hematopoietic tissues undergo somatic reversion easily, so the absence of a finding does not give assurance that there is no deleterious germline variant Non-coding regions of genes are typically not covered by these assays. CNVs are typically not covered by these assays.	Time from sample collection to result can take up to three months, which can complicate planning future therapy, including allogeneic stem cell transplantation.
Platforms	Generally cover genes/exons where deleterious germline variants can be found. Are typically designed to detect SNVs and are capable of detecting large CNVs, but are insensitive to small CNVs. Coverage depth is in the hundreds-thousands depth to allow detection of small clone sizes.	Generally are designed to cover genes/exons as well as non-coding regions (*e.g.*, prornoters and enhancers) where deleterious germline variants can be found. Are capable of identifying SNVs and CNVs. Current platforms need to be flexible to accommodate the predisposition genes that continue to be discovered. Coverage depth 30-50-fold is sufficient to detect germline-range VAFs.
Specific alleles	Often, the same allele (*e.g.*, in *TP53*, *RUNX1*, and *CEBPA*, among others) can be somatic or germline.	Specific alleles (*e.g.*, in *CHEK2* and *DDX41*) are overwhelmingly likely to be germline.

CNV, copy number variant; CSF, cerebrospinal fluid; indel, insertion/deletion; SNV, single nucleotide variant; VAF, variant allele frequency.

be capture-based amplification assays with hundreds-thousands fold depth to allow detection of small clone sizes (30). Also, these assays are typically designed to detect single nucleotide variants (SNVs), but can sometimes detect large copy number variants (CNVs). In contrast, germline variants exist as SNVs in exons and regulatory regions like promoters and enhancers, the latter being poorly covered generally in exon-based panels, as well as CNVs of various sizes. Tumor-based panels generally are not designed to detect small CNVs, and therefore, are incapable of detecting them.

In contrast, germline genetic testing platforms are designed to cover the mutation types and genomic elements where those variants occur (8), often performed as augmented whole exome sequencing (aWES) in which primers designed to capture non-coding regions are added to an exome platform, and bioinformatic pipelines capable of identifying CNVs from such data are used. Alternatively, germline platforms may combine aWES with microarray analysis or multiplex ligation amplification (MLPA) to detect CNVs (6). Some advocate performing whole genome sequencing (WGS) initially, which facilitates CNV detection, but the current cost of running and storing such data are prohibitive for most clinical centers (6, 31). Importantly, some platforms separate SNV from CNV testing, so careful attention must be paid at the time of test ordering to ensure that testing is comprehensive for both SNVs and CNVs. Generally, a sequencing depth of about 30-50X is sufficient to detect germline genetic variants. Finally, germline cancer predisposition genes continue to be discovered, especially for hematopoietic malignancies, and therefore, clinical testing platforms need to be flexible to accommodate the increasing number of genes recognized to confer risk.

7 Conclusion

The increasing use of molecular profiling of tumor cells for prognostication and therapy decisions affords the opportunity to identify DNA variants that are germline in nature and confer risk to hematopoietic, and potentially other, cancers. These panel-based tests do not substitute for proper germline genetic testing, which relies on platform designs that accommodate a growing list of cancer predisposition genes and a myriad of mutation types and rely on DNA that is equivalent to germline. Therefore, when a deleterious DNA variant is identified at an allele frequency consistent with the germline, especially when it is observed consistently across time and during remission, it should be considered as potentially germline in nature. Once prioritized for germline testing, individuals can undergo assessment in time for future treatments, such as allogeneic HSCT, which often involves relatives as the donor stem cell source.

Author contributions

LG wrote this article and designed the tables and figures and is accountable for its contents of the work.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Trottier AM, Godley LA. Inherited predisposition to haematopoietic malignancies: Overcoming barriers and exploring opportunities. *Br J Haematol* (2021) 194:663–76. doi: 10.1111/bjh.17247

2. Aleem A, Haque AR, Roloff GW, Griffiths EA. Application of next-generation sequencing-based mutational profiling in acute lymphoblastic leukemia. *Curr Hematol Malig Rep* (2021) 16:394–404. doi: 10.1007/s11899-021-00641-5

3. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. *Blood* (2022) 140:1200–28. doi: 10.1182/blood.2022015850

4. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the world health organization classification of haematolymphoid tumours: Myeloid and Histiocytic/Dendritic neoplasms. *Leukemia* (2022) 36:1703–19. doi: 10.1038/s41375-022-01613-1

5. Ripperger T, Bielack SS, Borkhardt A, Brecht IB, Burkhardt B, Calaminus G, et al. Childhood cancer predisposition syndromes-a concise review and recommendations by the cancer predisposition working group of the society for pediatric oncology and hematology. *Am J Med Genet Part A* (2017) 173:1017–37. doi: 10.1002/ajmg.a.38142

6. Baliakas P, Tesi B, Wartiovaara-Kautto U, Stray-Pedersen AR, Friis LS, Dybedal I, et al. Nordic Guidelines for germline predisposition to myeloid neoplasms in adults: Recommendations for genetic diagnosis, clinical management and follow-up. *Hemasphere* (2019) 3:e321. doi: 10.1097/HS9.0000000000000321

7. Palomo L, Ibáñez M, Abáigar M, Vázquez I, Álvarez S, Cabezón M, et al. Spanish Guidelines for the use of targeted deep sequencing in myelodysplastic syndromes and chronic myelomonocytic leukaemia. *Br J Haematol* (2020) 188:605–22. doi: 10.1111/bjh.16175

8. Schlegelberger B, Mecucci C, Wlodarski M. Review of guidelines for the identification and clinical care of patients with genetic predisposition for hematological malignancies. *Familial Cancer* (2021) 20:295–303. doi: 10.1007/s10689-021-00263-z

9. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* (2022) 140:1345–77. doi: 10.1182/blood.2022016867

10. Greenberg PL, Stone RM, Al-Kali A, Bennett JM, Borate U, Brunner AM, et al. NCCN guidelines® insights: Myelodysplastic syndromes, version 3.2022. *J Natl Compr Cancer Netw* (2022) 20:106–17. doi: 10.6004/jnccn.2022.0009

11. Jongmans MC, Loeffen JL, Waanders E, Hoogerbrugge PM, Ligtenberg MJ, Kuiper RP, et al. Recognition of genetic predisposition in pediatric cancer patients: An easy-to-use selection tool. *Eur J Med Genet* (2016) 59:116–25. doi: 10.1016/j.ejmg.2016.01.008

12. Kraft IL, Godley LA. Identifying potential germline variants from sequencing hematopoietic malignancies. *Blood* (2020) 136:2498–506. doi: 10.1182/blood.2020006910

13. Fukuhara S, Oshikawa-Kumade Y, Kogure Y, Shingaki S, Kariyazono H, Kikukawa Y, et al. Feasibility and clinical utility of comprehensive genomic profiling of hematological malignancies. *Cancer Sci* (2022) 113:2763–77. doi: 10.1111/cas.15427

14. Fenwarth L, Caulier A, Lachaier E, Goursaud L, Marceau-Renaut A, Fournier E, et al. Hereditary predisposition to acute myeloid leukemia in older adults. *Hemasphere* (2021) 5:e552. doi: 10.1097/HS9.0000000000000552

15. Feurstein S, Trottier AM, Estrada-Merly N, Pozsgai M, McNeely K, Drazer MW, et al. Germ line predisposition variants occur in myelodysplastic syndrome patients of all ages. *Blood* (2022) 140:2533–48. doi: 10.1182/blood.2022015790

16. Bhattacharya P, Patel TN. Microsatellite instability and promoter hypermethylation of DNA repair genes in hematologic malignancies: a forthcoming direction toward diagnostics. *Hematology* (2018) 23:77–82. doi: 10.1080/10245332.2017.1354428

17. Sanders MA, Chew E, Flensburg C, Zeilemaker A, Miller SE, Al Hinai AS, et al. MBD4 guards against methylation damage and germ line deficiency predisposes to clonal hematopoiesis and early-onset AML. *Blood* (2018) 132:1526–34. doi: 10.1182/blood-2018-05-852566

18. Palles C, West HD, Chew E, Galavotti S, Flensburg C, Grolleman JE, et al. Germline MBD4 deficiency causes a multi-tumor predisposition syndrome. *Am J Hum Genet* (2022) 109:953–60. doi: 10.1016/j.ajhg.2022.03.018

19. Feurstein S, Drazer M, Godley LA. Germline predisposition to hematopoietic malignancies. *Hum Mol Genet* (2021) 30:R225–35. doi: 10.1093/hmg/ddab141

20. Lee LA, Arvai KJ, Jones D. Annotation of sequence variants in cancer samples: Processes and pitfalls for routine assays in the clinical laboratory. *J Mol Diagnost* (2015) 17:339–51. doi: 10.1016/j.jmoldx.2015.03.003

21. Drazer MW, Kadri S, Sukhanova M, Patil SA, West AH, Feurstein S, et al. Prognostic tumor sequencing panels frequently identify germline variants associated with hereditary hematopoietic malignancies. *Blood Adv* (2018) 2:146–50. doi: 10.1182/bloodadvances.2017013037

22. Trottier AM, Cavalcante de Andrade Silva M, Li Z, Godley LA. Somatic mutation panels: Time to clear their names. *Cancer Genet* (2019) 235-236:84–92. doi: 10.1016/j.cancergen.2019.04.065

23. Walsh MF, Ritter DI, Kesserwan C, Sonkin D, Chakravarty D, Chao E, et al. Integrating somatic variant data and biomarkers for germline variant classification in cancer predisposition genes. *Hum Mutat* (2018) 39:1542–52. doi: 10.1002/humu.23640

24. Krysiak K, Danos AM, Saliba J, McMichael JF, Coffman AC, Kiwala S, et al. CIViCdb 2022: Evolution of an open-access cancer variant interpretation knowledgebase. *Nucleic Acids Res* (2023) 51:D1230–41. doi: 10.1093/nar/gkac979

25. Ritter DI, Rao S, Kulkarni S, Madhavan S, Offit K, Plon SE. A case for expert curation: An overview of cancer curation in the clinical genome resource (ClinGen). *Cold Spring Harb Mol Case Stud* (2019) 5:a004739. doi: 10.1101/mcs.a004739

26. Batalini F, Peacock EG, Stobie L, Robertson A, Garber J, Weitzel JN, et al. Li-Fraumeni syndrome: not a straightforward diagnosis anymore-the interpretation of pathogenic variants of low allele frequency and the differences between germline PVs, mosaicism, and clonal hematopoiesis. *Breast Cancer Res* (2019) 21:107. doi: 10.1186/s13058-019-1193-1

27. Padron E, Ball MC, Teer JK, Painter JS, Yoder SJ, Zhang C, et al. Germ line tissues for optimal detection of somatic variants in myelodysplastic syndromes. *Blood* (2018) 131:2402–5. doi: 10.1182/blood-2018-01-827881

28. Fenwarth L, Duployez N, Marceau-Renaut A, Chahla WA, Ducassou S, Gandemer V, et al. Germline pathogenic variants in transcription factors predisposing to pediatric acute myeloid leukemia: results from the French ELAM02 trial. *Haematologica* (2021) 106:908–12. doi: 10.3324/haematol.2020.248872

29. Saygin C, Roloff GW, Hahn CN, Chhetri R, Gill SI, Elmariah H, et al. Allogeneic hematopoietic stem cell transplant outcomes in adults with inherited myeloid malignancies. *Blood Adv* (2022). doi: 10.1182/bloodadvances.2022008172

30. Kadri S, Long BC, Mujacic I, Zhen CJ, Wurst MN, Sharma S, et al. Clinical validation of a next-generation sequencing genomic oncology panel *via* cross-platform benchmarking against established amplicon sequencing assays. *J Mol Diagnost* (2017) 19:43–56. doi: 10.1016/j.jmoldx.2016.07.012

31. Duncavage EJ, Schroeder MC, O'Laughlin M, Wilson R, MacMillan S, Bohannon A, et al. Genome sequencing as an alternative to cytogenetic analysis in myeloid cancers. *N Engl J Med* (2021) 384:924–35. doi: 10.1056/NEJMoa2024534

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Hereditary predisposition to malignant myeloid hemopathies: Caution in use of saliva and guideline based on our experience

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Background: Predisposition to myeloid malignancies is a field at the border of hematology and genetics. Knowledge in this domain has so rapidly increased that WHO defined in 2016 the new "Myeloid Neoplasms with Germline Predisposition" category of tumors. High throughput sequencing is frequently performed in tumors either for diagnosis or prognosis, but this approach may identify potential germline variants that have to be confirmed on non-infiltrated tissues.

Method: In this study, we systematically compared NGS data from genetic analysis performed on all sample types (bone marrow, blood, saliva, skin fibroblasts and hair follicles) in 29 patients, and 44 of their relatives (blood and saliva).

Results: We showed that saliva was usable for relatives, but only for 24% (7/29) of our patients. Most of patients' saliva were either "non-contributive" (14/29 *i.e.*, 48% because clearly or probably infiltrated) or "inconclusive" (8/29 corresponding to 28%).

Conclusion: The recommendations for the use of saliva we present here focus on the importance of collecting saliva during remission when possible. Moreover, we propose hair follicles as an alternative to skin biopsy, that remains the gold standard especially in case of allogenic hematopoietic stem cells transplantation. Technological progresses have revolutionized the diagnosis of predisposition to solid or hematological malignancies, and it is very likely that new techniques will help to manage the familial predisposition in the future.

KEYWORDS

myeloid hemopathies, predisposition, saliva, *DDX41*, genetic counseling

1 Introduction

Hematopoiesis is the biological lifelong process that produces all blood cells from a very small contingent of multipotent stem cells located in the bone marrow. That differentiation is closely regulated by numerous factors (1). Germline (GL) mutations in genes involving this pathway increase the risk of developing hemopathies, leading World Health Organization (2) and International Consensus Classification (3) to define the myeloid neoplasms (MN) "with germline predisposition" subtype.

Among these genes, recent literature shows an association between germline *DEAD-Box Helicase 41 (DDX41)* mutations and familial MN, including myelodysplastic syndromes (MDS) (4, 5). Recent study of Makishima et al. (6) and the review of Kim et al. (7) characterized *DDX41*-mutated MN, detailing their high frequency among MN with germline predisposition (about 80%), specific clinical outcomes (high progression rate to acute myeloid leukemia: AML) and high penetrance of GL traits of *DDX41* (about 50% by the age of 90), that even tend to consider them as a specific subset of MN.

MDS is an heterogenous group of clonal myeloid disorders. They are usually suspected in presence of unexplained persistent peripheral blood cytopenias, but diagnosis requires a bone marrow assessment. The presence of dysplasias on cellular morphological examination is a hallmark of the diagnosis (8).

Three groups of factors can help to define the prognosis of MDS: cytogenetics aberrations, bone marrow blast percentage and depth of cytopenia. They are combined in the Revised International Prognosis Scoring System (IPSS-R) widely used in clinical routine. It assesses the risk of secondary evolution AML (9). Nevertheless, studies of genomic alterations in tumors become important in the diagnostic process, not only in solid tumors but also in hematological malignancies (HM). Based on National Comprehensive Cancer Network (NCCN) constatations (10), Bernard et al. (11) recently proposed an adaptation of IPPS-R, called IPSS-M score (M for molecular), including molecular abnormalities of 31 genes.

In this context, High Throughput Sequencing (HTS) of gene panel involved in HM is frequently performed in early stage of MDS. In some cases, this approach on bone marrow sample may identify potentially germline variants. Confirmation of their germline nature on another biological tissue becomes therefore essential for appropriate clinical management and genetic counseling for relatives.

Due to diffusion of hematopoietic tumoral cells in peripheral blood, saliva appears to be a more appropriate biological sample for germline studies, and its collection is not invasive. Nevertheless, literature reports risks of saliva's contamination by tumoral blood cells (12, 13); and eventuality that could impede the discrimination between germinal and acquired mutations (14). To our knowledge, no recent study is available to drive geneticists' routine practice.

The aim of this study is to report our local experience and propose recommendations for use of saliva in case of suspicion of familial hemopathy (FH). Here, we compared HTS data from saliva, bone marrow, and other tissues (blood, cultured fibroblasts from skin biopsy, or hair follicle) collected for diagnosis of FH or follow-up. Data from patients as well as those collected in their relatives were also used to build the guideline we propose.

2 Material and method

2.1 Patients and relatives' inclusion

We extracted from our database all analysis performed between 2020 and 2022 on DNA isolated from saliva (n=78). Patients were distinguished from relatives through a systematic review of medical history and molecular analysis performed in our laboratory (Supplementary Figure S1). We excluded 5 patients because HTS data at diagnosis were performed elsewhere and/or unavailable. Finally, 29 index-cases and 44 relatives were included. Absence of hemopathy in relatives was verified before sample collection, with standard blood count and clinical examination.

All individuals received a genetic counselling from a geneticist of Limoges University Hospital Center. They all provided informed consent for molecular studies, and study was approved by local ethic committee (registration number: 584-2022-240).

2.2 Molecular studies

We isolated DNA with Maxwell RSC instrument (Promega, Madison, WI, USA) using the appropriate kit for each sample type according to the supplier's recommendations. DNA was extracted from saliva (collected with ORAGEN DNA Kit, DNA Genotek, Kanata, Canada) with the Stabilized Saliva DNA kit (Promega); from whole blood on EDTA or 5M cell pellets with the Simply DNA Blood kit (Promega); and from hair follicle (10 follicles minimum) with the Tissue and Hair kit (Promega). We only extracted DNA manually from fibroblasts with the PureGene kit (QIAGEN, Hilden, Germany), according to manufacturer's recommendations. We cultured fibroblasts after microdissection of skin biopsy in incubator (37°C, 5% CO2), with Chang Medium (Clinisciences, Nanterre, France).

We prepared amplicon library using an Ion AmpliSeq custom panel (ThermoFisher, Waltham, MA, USA) targeting 17 diagnostic and prognostic genes (list in Table S1). HTS was then performed using the Ion S5 Sequencing System (ThermoFisher). Alignment and variant calling were made with Torrent Suite Software (ThermoFisher), and annotation with VEP and Ensembl. Copy Number Variations (CNV) were detected with CovCopCan (15) and OncoCNV (16). We classified SNV according ACMG recommendations. Sensitivity of SNV with this method is greater than 99% for minimal depth of 100X and analysis detects CNV if present in greater than 30% of total sample cells.

Sanger method was performed to sequence gene regions with depth <100X as well as mutation specific analysis in patents and their relatives. We performed amplification with Taq Purple Mix (Ozyme, ST CYR L'ECOLE, France), purification of PCR products with ExoSap (Applied biosystems, Waltham, MA, USA) and

sequencing with BigDye Terminator V1.1 (Applied Biosystems) on 3130xL or 3500 xL Genetic Analyzer (Applied Biosystems).

2.3 Contribution of saliva collected

We systematically reviewed results of HTS analysis in all samples (bone marrow at diagnosis and for follow-up, blood, skin fibroblasts, hair follicle and saliva) for each patient. Variant Allele Frequencies (VAF) of pathogenic (class 5) and likely pathogenic (class 4) variants was compared between samples. We considered that saliva was « not contributive » for germline studies when somatic mutations present at diagnosis were also detected in saliva regardless of their VAF, suggesting an infiltration by tumoral cells. Conversely not infiltrated saliva were qualified as "contributive", and if it was not possible to affirm the absence of infiltration saliva were "inconclusive". Moreover, for patients treated with allogenic hematopoietic stem cell (HSC) transplantation, we studied the presence or absence and zygosity of Single Nucleotide Polymorphism (SNP) to assess donor's contribution to HTS results in patients.

3 Results

3.1 Patients and mutations

Between May 2020 and May 2022, 29 patients and 44 relatives (of 14 patients) were included in the study. Majority of them were males (respectively 21 in the group "patient", and 20 in the group "relatives"), but ages were similar (59 years ± 14 *versus* 53 years ± 15 respectively). HTS was performed for diagnosis and prognosis in patients with various types of myeloid disorders (Table 1). Myelodysplastic syndromes (all types) were the most frequent (18/29, 62%).

Patients received genetic counselling for suspicion of germline mutation of 6 genes: *CBL* (n=1), *DDX41* (n=22), *GATA2* (n=1), *KRAS* (n=1), *RUNX1* (n=2), and *TP53* (n=2). Among them, *DDX41* was thus the most frequently mutated, but only five pathogenic mutations were detected: G173R (n=12), A270V (n=1), E268Dfs*36 (n=3), L283Cfs*21 (n=4) and S363del (n=2). All of them were already reported (6, 17, 18).

3.2 Saliva contribution

Clinical data and sequencing results of patients and their relatives are presented in Table S2. Results obtained with Sanger sequencing on saliva and blood in relatives were concordant. We thus concluded that saliva was contributive and usable in clinical routine for them, after verification of absence of hemopathy.

3.2.1 Contributive saliva samples

In the group of 29 patients, saliva samples were contributive in 7 patients, corresponding to 24% of our cohort (patients ID 2, 9, 11, 14, 24, 28 and 29). All samples were collected at remission (after allogenic HSC transplantation or chemotherapy) or during treatment, and HTS did not detect somatic mutation in saliva. Among them, only patient ID 11 beneficiated of Sanger sequencing of *DDX41* to search for S363del mutation, although somatic mutations were present at diagnosis (*IDH2* R140Q and *DNMT3A* R882H). We however considered that saliva as "contributive", because *IDH2* and *DNMT3A* mutations were absent of NGS performed on bone marrow for follow-up one month later, suggesting that saliva was not infiltrated when it was collected.

TABLE 1 Myeloid disorders of patients included in the study.

Myeloid Disorder			Sex		Age mean (± SD)
			Male	Female	
MDS	MDS *ns*	3	2	1	63 (50-75)
	MDS-EB *ns*	1	0	1	77
	MDS-EB1	8	7	1	64 (55-73)
	MDS-EB2	5	4	1	62 (56-67)
	RCMD	1	1	0	57
	Total	18	14	4	63 (55-71)
AML	AML *ns*	6	4	2	60 (45-75)
	AML1	1	1	0	46
	AML2	2	1	1	38 (35-41)
	Total	9	6	3	59 (44-74)
Other	CMML	2	1	1	59 (44-75)
Total		29	21	8	59 (45-73)

MDS, Myelodysplastic Syndrome; ns, not specified; EB, Excess Blasts; RCMD, Refractory Cytopenia with Multilineage Dysplasia; AML, Acute Myeloid Leukemia; CMML, Chronic Myelomonocytic Leukemia.

3.2.2 Non-contributive saliva samples

Ten samples (34%) were clearly (patients ID 1, 6, 10, 13, 16, 25 and 27) or probably (ID 3, 8 and 23) infiltrated by tumoral cells. Infiltration was demonstrated by presence of somatic mutations detected at diagnosis in saliva or in samples collected for follow-up (blood or bone marrow). Concerning patient ID 8 and 23, saliva infiltration was highly probable, because collected during a progressing disease (ID 8) or before a transplantation of HSC and out of remission (ID23). Among them, three patients (ID 13, 23 and 27) were treated with allogenic HSC transplantation, but their saliva samples were collected before, which did not bias our interpretations (no contribution of donor to results in these patients).

Surprisingly, four other saliva samples were also not-contributive even though collected after allogenic HSC transplantation (patients ID 4, 12, 21 and 22). Thus, the total of not-contributive saliva was 48% (14 among 29). Conclusions for those patients required more investigations so we reviewed presence or absence of SNP detected by NGS in the different samples available.

- Patient 4: two *DDX41* mutations were present at diagnosis: E268Dfs*21 (VAF=51,2%) and G530D (VAF=3,5%). Bone marrow collected for relapse despite an extra-familial allogenic HSC transplantation, found same mutations (VAF= 1.9% and 1.6% respectively) and the new *IDH1* R132C mutation (VAF= 3.5%). In saliva collected 5 months later, we detected *DDX41* E268Dfs*28 mutation, with a VAF of 15.8% consistent with an incomplete chimerism (Figure 1). We confirmed that hypothesis with comparison of SNP which showed that 9 SNP detected in saliva and bone marrow at relapse were absent of bone marrow collected at diagnosis (Table S3). Finally, we could affirm the germline nature of the *DDX41* E268Dfs*28 mutation with NGS performed on DNA isolated from skin fibroblasts (VAF=50%, and absence of the 9 SNP from the donor).
- Patient 12: difficulties of interpretation were comparable to situation of case 4, but this patient received two allogenic HSC transplantations: the first with a relative and the second with an unrelated donor. The *DDX41* G173R detected at diagnosis and relapse after the first transplantation was not detected on blood and saliva after the second graft. HTS performed on skin fibroblasts and hair follicle confirmed the germline nature of *DDX41* G173R mutation (VAF=54% and 48.8% respectively). Comparisons of SNP with skin (data on request) showed that i) the relapse was developed on transplant (different SNP profile between skin and bone marrow at relapse); and ii) only DNA of unrelated donor is present in saliva, corresponding to a 100% donor chimerism (different SNP profile between skin and saliva).
- Patients 21 and 22: Situation for these patients was quite different of previous patients. *DDX41* G173R mutation was still present after one (patient 21) or two (patient 22) transplantations, but with a very low VAF in saliva (4% and 5% respectively), suggesting an incomplete chimerism. We confirmed this hypothesis with SNP studies (Table S3) which showed presence of some patient's SNPs (detected in skin) in saliva with a low VAF. NGS performed on skin fibroblasts confirmed germline nature of *DDX41* G173R mutation in these patients.

3.2.3 Inconclusive saliva samples

The eight remaining samples (28%) were considered as "inconclusive" because it was not possible to conclude with the available data (patients ID 5, 7, 15, 17, 18, 19, 20 and 26). None of these saliva samples were collected at remission, and bone marrow at diagnosis showed presence of clonal and subclonal mutations. Our local workflow initially considers only Sanger sequencing on saliva to detect supposed germline mutations. Majority of these eight cases (ID15, 17, 19, 20 and 23) were characterized by a *DDX41* acquired mutation in minor proportion of cells in bone marrow (VAF ranged from 3 to 16%), thus probably not detectable by Sanger in saliva. Therefore, even if only supposed germline mutation was targeted by Sanger, absence of acquired mutation could not be verified and tumor infiltration in saliva cannot be definitively excluded. Two cases are nevertheless interesting:

- Patient 5: Bone marrow at diagnosis showed two mutations of *DDX41*: G173R (VAF=53%) and a R525H (VAF=20%), associated with a *TET2* mutation (VAF=98%). Bone marrow collected for follow-up after allogenic HSC transplantation with a relative showed acquisition of a SNP from the donor (homozygous *SETBP1*: Y1303S) and presence of *DDX41* G173R mutation. This patient next presented a relapse of his disease after transplantation, with *DDX41* G173R and R525H mutation, and presence of *SETBP1* homozygous Y1303S. Sanger sequencing was performed on saliva collected two months later, which showed presence of *DDX41* G173R. Thus, it was not possible to affirm that saliva was not contaminated by tumoral cells (*DDX41* R525H not targeted), and we could not conclude if mutation detected in saliva was carried by patient or brought by the transplantation with a carrier relative (*SETBP1* SNP not targeted by Sanger).
- Patient 18: Bone marrow at diagnosis show presence of *RUNX1* E223* (VAF=45%; depth=5925X) and *SRSF2* P95H (VAF=46%, depth=483X) mutations. NGS performed on saliva collected two months later (and without any treatment) detected only *RUNX1* E223* mutation (VAF=42%, depth=361X). Due to low sequencing depth on saliva, it is probable that even if *SRSF2* P95H mutation was present at heterozygous state, bioinformatic pipeline would have filtrated this mutation (probable depth <50X). Thus, saliva was considered as potentially infiltrated, so "inconclusive". Unfortunately, patient died of endocarditis before we could collect hair follicles or skin fibroblast, so germline nature of *RUNX1* E223* mutation could not be verified.

FIGURE 1
IGV view of DDX41 E268Dfs*21 mutation in different sample type of patient 4. **(A)** Saliva (VAF = 15.8%); **(B)** Skin fibroblasts (VAF=50%); **(C)** Bone marrow at diagnosis (VAF= 51.2%); **(D)** = bone marrow at relapse (VAF=1.9%). VAF of the mutation on saliva showed a chimerism, and germline nature is proven by analysis of skin fibroblasts.

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4 Discussion

Predisposition to myeloid disorders is a recent field of genetics, especially since the definition of a specific category of tumors by WHO in 2016 (19). This definition of "Myeloid Neoplasms with Germline Predisposition" category (2, 3) was based on several studies, perfectly summarized in the review of Klco and Mulligan in 2021 (20).

Due to absence of strong recommendations, strategy for diagnosis of predisposition to myeloid malignancies was highly variable between our patients. Some of them benefited of Sanger sequencing on saliva, other ones of HTS. That was one of the weaknesses of our study. For some cases, only saliva samples collected at remission were informative (24% of our cohort), and retrospectively, obtaining more samples at remission would probably reduce the rate of inconclusive saliva samples.

Saliva was preferably studied because its collection is less invasive than skin biopsy. However, we showed that in case of allogenic HSC transplantations, analysis on DNA from skin fibroblasts are required. These results are concordant with methodology used by other authors, such as Churpek et al. (21) and Li et al. (22) who used either skin biopsy, T-lymphocytes from blood, or buccal swab to affirm germline nature of the detected variants. Our initial workflow thus retrospectively appeared unappropriated to patients that are being evaluated for HM predisposition. It was centered on Sanger sequencing of saliva samples, similarly to patients with a suspected hereditary predisposition to solid tumors.

Most saliva samples collected from affected patients (48%) showed obvious signs of infiltration by tumoral cells. In these cases, mutational profiles (mutations and VAF) were comparable between medullary and salivary compartments. Moreover, analysis performed on blood (patient ID4 and 25) even showed an infiltration higher in saliva than in blood; and case of patient ID13 interestingly showed different mutations in saliva compared to bone marrow at diagnosis, which could be compatible with sub-clonal mutations not detectable in medullary compartment.

In order to resolve the problem of infiltration in saliva, we could test extraction of DNA from hair follicle in the 4 most recently included patients (ID1, 10, 12 and 14). Results were encouraging, as they confirmed the results obtained on the skin for patient ID12 and avoided skin biopsy for the others (ID1, 10 and 14). These results need to be confirmed on larger cohort of patients and could even be used *a posteriori* to conclude on the saliva samples that we considered as "unconclusive" (28% of our patients).

Concerning the relatives, we showed that saliva was usable, after verification of absence of hemopathy signs by standard blood count and clinical examination. In some cases, detection of mutation in relatives proved the germline nature of mutation detected at diagnosis in index case. For example, Sanger sequencing performed on patient ID6's son (ID37) to determine his eligibility as a donor for HSC allograft, showed that he carried the heterozygous *DDX41* L283Cfs*21 mutation. Consequently, his status was known at 32 years old, while HTS performed in bone marrow at diagnosis and in his father' saliva showed identical results, suggesting a significant infiltration of saliva by tumoral cells. Thus, segregation of mutation in this family proved germline nature of *DDX41* variant detected in patient ID6. In the context of intrafamilial allogenic HSC transplantations, early testing of relatives appears to be ethically acceptable but is more debatable out of those situations, considering the penetrance of *DDX41* traits, which is negligible until 40 years of age (6).

In our Institution, fibroblasts are not cultured by hematology biologists, but in the Genetics unit. Availability of skin biopsies for patients followed up by hematologists is thus conditioned by another unit, with its own cell culture activity. Consequently, and despite the small cohort of patient available, our local experience led us to define an optimized algorithm for the diagnosis of hereditary predisposition to HM, to limit the use of skin biopsy to patients whom it is indispensable. (Figure 2).

This algorithm, easily usable in clinical routine, focuses on the importance of collecting patients' samples at remission if possible. Due to difficulties of interpretations encountered in patients treated with allograft, the use of saliva should be limited to relatives (Sanger sequencing) or patients at remission (HTS) without antecedent of

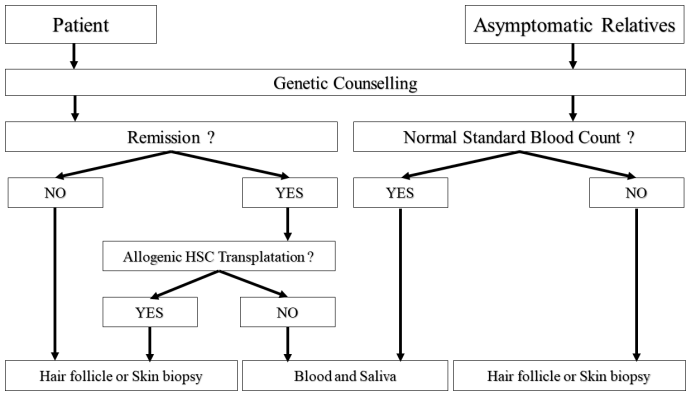

FIGURE 2
Algorithm proposed for diagnostic. HSC = Hematopoietic Stem Cells. Relatives with abnormal standard blood count should be considered as patients. Conversely, patients at remission without HSC transplantation can be analyzed as asymptomatic relatives.

transplantation, even if the donor was unrelated to patient. In case where saliva is not usable, or when patients' samples cannot be collected at remission, we suggest the use of hair follicles that are less invasive to collect, but biopsy remains the gold standard until a larger cohort confirms our results.

5 Conclusion

Our study showed that HTS is an efficient tool for detecting tumoral cells in patients' saliva samples or assessing donor contribution in case of HSC transplantation. In addition, we showed that saliva can be used in the field of predisposition to malignant myeloid hemopathies, but cautions are needed to ensure proper interpretation of the results.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Comité d'éthique du CHU de Limoges. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AP: Planned the experiment, analyzed the results, and wrote the manuscript. SB: Technical and biological validation of the results, and critical revision of the manuscript. DR: Technical and biological validation of the results, and proofread the manuscript. JC: Technical and biological validation of the results, and proofread the manuscript. BD: Patients and relatives genetic counselling, and proofread the manuscript. PT: Patients clinical diagnostic and follow-up. SG: patients clinical diagnostic and follow-up. LV: Patients and relatives genetic counselling. MR: patients and relatives genetic counselling. JF: Proofread the manuscript. CY: Critical revision of the manuscript. NG: Technical and biological validation of the results, and critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1120829/full#supplementary-material

References

1. Bao EL, Cheng AN, Sankaran VG. The genetics of human hematopoiesis and its disruption in disease. *EMBO Mol Med* (2019) 11(8). doi: 10.15252/emmm.201910316

2. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the world health organization classification of haematolymphoid tumors: Myeloid and Histiocytic/Dendritic neoplasms. *Leukemia* (2022) 36):1703–19. doi: 10.1038/s41375-022-01613-1

3. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphological, clinical, and genomic data. *Blood* (2022) 11):1200–28. doi: 10.1182/blood.2022015850

4. 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. doi: 10.1016/j.ccell.2015.03.017

5. Lewinsohn M, Brown AL, Weinel LM, Phung C, Rafidi G, Lee MK, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. *Blood* (2016) 127(8):1017–23. doi: 10.1182/blood-2015-10-676098

6. Makishima H, Saiki R, Nannya Y, Korotev S, Gurnari C, Takeda J, et al. Germ line DDX41 mutations define a unique subtype of myeloid neoplasms. *Blood* (2023) 141 (5):534–49. doi: 10.1182/blood.2022018221

7. Kim K, Ong F, Sasaki K. Current understanding of *DDX41* mutations in myeloid neoplasms. *Cancers (Basel)* (2023) 2):344. doi: 10.3390/cancers15020344

8. Garcia-Manero G, Chien KS, Montalban-Bravo G. Myelodysplastic syndromes: 2021 update on diagnosis, risk stratification and management. *Am J Hematol* (2020) 95 (11):1399–420. doi: 10.1002/ajh.25950

9. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood* (2012) 120(12):2454–65. doi: 10.1182/blood-2012-03-420489

10. Greenberg PL, Stone RM, Al-Kali A, Barta SK, Bejar R, Bennett JM, et al. Myelodysplastic syndromes, version 2.2017, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* (2017) 15(1):60–87. doi: 10.6004/jnccn.2017.0007

11. Bernard E, Tuechler H, Greenberg PL, Hasserjian RP, Arango Ossa JE, Nannya Y, et al. Molecular international prognostic scoring system for myelodysplastic syndromes. *NEJM Evid* (2022) 1(7). doi: 10.1056/EVIDoa2200008

12. Thiede C, Prange-Krex G, Freiberg-Richter J, Bornhäuser M, Ehninger G. Buccal swabs but not mouthwash samples can be used to obtain pretransplant DNA fingerprints from recipients of allogeneic bone marrow transplants. *Bone Marrow Transplant* (2000) 25:575–7. doi: 10.1038/sj.bmt.1702170

13. Endler G, Greinix H, Winkler K, Mitterbauer G, Mannhalter C. Genetic fingerprinting in mouthwashes of patients after allogeneic bone marrow transplantation. *Bone Marrow Transplant* (1999) 24:95–8. doi: 10.1038/sj.bmt.1701815

14. Garcia-Gisbert N, Camacho L, Fernández-Ibarrondo L, Fernández-Rodriguez C, Longarón R, Gibert J, et al. Analysis of saliva samples and cluster of differentiation 3 (CD3)+ lymphocytes as a source of germline DNA in myeloproliferative neoplasms. *Br J Haematol* (2020) 189(5):e204–7. doi: 10.1111/bjh.16624

15. Derouault P, Chauzeix J, Rizzo D, Miressi F, Magdelaine C, Bourthoumieu S, et al. CovCopCan: An efficient tool to detect copy number variation from amplicon sequencing data in inherited diseases and cancer. *PloS Comput Biol* (2020) 16(2). doi: 10.1111/bjh.16624

16. Boeva V, Popova T, Lienard M, Toffoli M, Kamal M, Le Tourneau C, et al. Multi-factor data normalization enables the detection of copy number aberrations in amplicon sequencing data. *Bioinformatics* (2014) 30(24):3443–50. doi: 10.1093/bioinformatics/btu436

17. 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. doi: 10.1182/blood.2019000909

18. Bannon SA, Routbort MJ, Montalban-Bravo G, Mehta RS, Jelloul FZ, Takahashi K, et al. Next-generation sequencing of DDX41 in myeloid neoplasms leads to increased detection of germline alterations. *Front Oncol* (2021) 10:582213. doi: 10.3389/fonc.2020.582213

19. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz JM, 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. doi: 10.1182/blood-2016-03-643544

20. Klco JM, Mullighan CG. Advances in germline predisposition to acute leukemias and myeloid neoplasms. *Nat Rev Cancer* (2021) 21(2):122–37. doi: 10.1038/s41568-020-00315-z

21. Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood* (2015) 126(22):2484–90. doi: 10.1182/blood-2015-04-641100

22. Li ST, Wang J, Wei R, Shi R, Adema V, Nagata Y, et al. Rare germline variant contributions to myeloid malignancy susceptibility. *Leukemia* (2020) 34(6):1675–8. doi: 10.1038/s41375-019-0701-8

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Replication stress increases mitochondrial metabolism and mitophagy in FANCD2 deficient fetal liver hematopoietic stem cells

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Fanconi Anemia (FA) is an inherited bone marrow (BM) failure disorder commonly diagnosed during school age. However, in murine models, disrupted function of FA genes leads to a much earlier decline in fetal liver hematopoietic stem cell (FL HSC) number that is associated with increased replication stress (RS). Recent reports have shown mitochondrial metabolism and clearance are essential for long-term BM HSC function. Intriguingly, impaired mitophagy has been reported in FA cells. We hypothesized that RS in FL HSC impacts mitochondrial metabolism to investigate fetal FA pathophysiology. Results show that experimentally induced RS in adult murine BM HSCs evoked a significant increase in mitochondrial metabolism and mitophagy. Reflecting the physiological RS during development in FA, increase mitochondria metabolism and mitophagy were observed in FANCD2-deficient FL HSCs, whereas BM HSCs from adult FANCD2-deficient mice exhibited a significant decrease in mitophagy. These data suggest that RS activates mitochondrial metabolism and mitophagy in HSC.

KEYWORDS

Hematopoietic stem cell, FANCD2, replication stress, mitochondria metabolism, mitophagy, fetal liver

Introduction

FA is an inherited BM failure with hematologic disease onset around school age (1). Yet, murine models and studies on FA patients have revealed that genetic mutation of FA genes affects embryonic HSC function (2–4). FA HSCs exhibit a decrease in number in the fetal liver where HSCs should rapidly expand. Compared to predominantly quiescent adult BM HSCs, FL HSCs are highly proliferative around E13.5 and show a strong RS response. We previously showed that FA FL HSCs exhibited significantly greater RS compared to wild-type (WT) FL HSCs due to delayed recovery from replication fork collapse (5).

Recently, we and others have highlighted mitochondrial metabolism as a critical component for maintenance in adult quiescent HSCs (6, 7). Mitochondrial membrane potential (MMP) is lower in HSCs than in progenitor cells but HSCs with large mitochondrial mass exhibit high stem cell potential (8). Due to their proliferation, FL HSCs exhibit higher mitochondrial activity compared to adult HSCs (9). Mitochondria oxidative phosphorylation and TCA cycle-associated gene expression are also higher in FL HSCs compared to adult BM HSCs. Others previously reported that mitochondrial metabolism and mitochondria clearance by mitophagy was reduced in FA cells (10–12). Moreover, HSC number in FA mice were rescued by treatment with the mitochondrial complex I inhibitor, metformin (13).

To understand how mitochondrial metabolism affects FA pathophysiology, we hypothesized that RS during FL HSC expansion modulates mitochondrial metabolism. Using pharmacologically induced RS, we observed temporary alterations in MMP and increased mitophagy. We then analyzed FL and BM HSCs from Fancd2 knockout (Fancd2 KO) mice. Strikingly, MMP and mitophagy were elevated in FANCD2-deficient FL HSCs. In contrast, mitophagy was downregulated in adult BM HSC. Taken together these observations indicate that the pathophysiological RS in FANCD2-deficient fetal HSCs boosts mitochondrial metabolism and mitophagy.

Material and methods

Animal husbandry

C57/BL6 background Fancd2 KO mice (14) were kindly gifted by Dr. Markus Grompe (OHSU). mitoDendra mice were obtained from JAX (#018397). All animal experiments were approved by TWMU animal experiment committee.

Flowcytometry (FACS) analysis

HSC immunophenotyping analysis was conducted using antibodies for EPCR, CD150, CD48 (HM48-1), c-Kit (2B8), Sca-1 (D7, excluded when analyzing 5-FU treated mice) and Lineage (Gr-1 (RB6-8C5), Ter119 (Ter-119), B220 (RA3-6B2), CD4 (RM4-5), CD8 (53-6.72), Mac-1 (M/70) excluded when analyzing FL HSCs). MMP was measured with tetramethylrhodamine (TMRE) 200nM and used 488nm lasor/585-642nm detector. mtROS was measured with MitoSoxRed (M36008, Thermo Fisher) and used 488nm lasor/585-642nm detector. Lysosome acidification was detected with Lysotracker Red (LTR, L7528, Thermo Fisher) and used 638nm lasor/660-720nm detector. Mitophagy activity was detected with Mitophagy detection kit (mtPH, MD1, Dojindo) and used 488nm lasor/780-860nm detector.

To perform intracellular staining, cells were fixed and penetrated using IntraPrep (A07803 Beckman) and stained with anti-p4EBP (T37/46, CST2855) 1:200.

All data were measured by CytoFlex FACS analyzer (Beckman Coulter). Analysis is performed with FlowJo software.

O-propargyl-puromycin (OPP) protein synthesis assay

Newly synthesized protein was measured. E13.5 FL cells or Mom's BM were cultured with 10mM O-propargyl-puromycin (OPP) in IMDM 10%FBS for 40min, harvested and staind antibodies of HSC (CD150-PE-Cy7, CD48-AF700, c-Kit-BV421, Sca1-PE-Cy7, Lineages with PerCpCy5.5). After washed, cells are fixed and penetrated using IntraPrep (A07803 Beckman). By using Click-iT Plus OPP Alexa Fluor 488 (AF488) Protein Synthesis Assay Kit (C10456, Thermo Fisher), stained OPP with AF488 and subsequent to FACS to measure the intensity (used 488nm lasor/525-540nm detector).

Immunofluorescence and imaging flow cytometer (IFM) analysis

HSC (CD150+ CD48- Lin- Sca-1+ c-Kit+) or HSPCs (Lin- Sca-1+ c-Kit+) were sorted by FACS AriaII or III (BD) and fixed with 4%PFA. Cells were permeabilized with 0.5% Triton for 15min and stained with anti-Tomm20 (ab78547 or ab289670) 1:200 or anti-ssDNA (18731, IBL) 1:50. Secondary stains were performed with anti-rabbit-AF488 1:1000 or anti-rat-AF488 1:1000. DAPI 1:1000 or 7-AAD 1:200 were used for nuclear staining. Data correction was performed with Mark-II (Amnis) and analysis was performed with IDEAs software (Amnis).

5-FU treatment

Mice were intraperitoneally injected 5-FU with one shot (200mg/kg body weight).

Ex vivo HSC culture

HSCs were sorted by FACS Aria II or III (BD) and subjected to ex vivo culture. 2000 HSCs per 1 well of 96-well dish are cultured with SF-03 medium (Sekisui) supplemented with 100ng/ml SCF (455-MC, R&D), 100ng/ml TPO (488-TO, R&D) and 0.1% BSA. Aphidicolin was diluted with DMSO for 50mg/ml and used as 1:5000-10000(final concentration 50-100ng/ml).

CFU assay

HSCs were sorted by FACS Aria II or III (BD) and 500 cells are seeded onto 3ml of mouse methylcellulose media (R&D Systems, HSC007) with APH (50-100ng/ml) or imTOR of Rapamycin (Thermo PHZ1235, diluted with DMSO for 10mM and used as 1:1000, final concentration 10μM) or iTgf-β of SD-208 (CAYMAN 16619, diluted with DMSO for 10mM and used as 1:1000, final concentration 10μM). The media was equally divided into three 3.5cm dishes and incubated at 37°C. Colony numbers were counted after 14 days.

Statistics

Statistical analyses were performed with t-test and one-way ANOVA (if the parameter is over 2) by using prism software (GraphPad).

Results

RS temporarily increases HSC mitochondrial activity and mitophagy *in vivo*

We previously described that FL HSCs experience RS due to their proliferative nature. We also showed that RS responses are increased in Fancd2 KO FL HSCs compared to BM HSCs from adult Fancd2 KO mice (Figure S3A) (5). Here we tested whether RS alters HSC mitochondrial activity and mitophagy. Administration of 5 fluorouracil (5-FU) to WT mice depletes lineage cells and causes subsequent proliferation and RS in adult BM HSCs (15). It was previously described that 5-FU treatment changes Sca-1 expression so that we excluded Sca-1 marker and measured as $CD150^{+}$ CD48- LK Hematpoietic Stem and Progenitor Cells (HSPCs) (15). After 6 days of 5-FU administration, HSPCs exhibited a significant increase in MMP (Figures 1A, S1A–C), which returned to baseline by day 12 when cells no longer proliferate. In parallel to changes in MMP, mitochondrial reactive oxygen species (mtROS) in HSPCs were also elevated at day 6 and returned to baseline by day 12 (Figure 1B).

We next analyzed the mitochondrial morphology in HSPCs. Alterations in mitochondrial morphology and distribution in HSCs have been previously reported in myelodysplastic syndrome (MDS) and leukemia; mitochondria in HSCs from MDS models diffuse and spread out while mitochondria in leukemic stem cells (LSC) exhibit polarity and are concentrated (16, 17). Imaging flow cytometer (IFM) was utilized to calculate mitochondrial area, concentration, and polarity in individual HSPCs. Following the 5-FU challenge, the mitochondrial area decreased and diffused, producing a polar redistribution pattern on day 6 that continued through day 12 (Figure 1C).

Adequate levels of mitophagy and lysosomal activity help maintain HSPCs potential (18–20). When we evaluated mitophagy and lysosomal activity, we found both increased on day 6, and HSPCs lysosomal activity remained high on day 12 (Figures 1D, E). Furthermore, the long-term effect of experimental RS on HSPCs was analyzed. HSPCs from mice 1 month after 5-FU injection (1Mo HSC) continued to exhibit a significantly larger mitochondrial area (Figure 1H), but comparable MMP and mtROS compared to control HSPCs (Figures 1F, G). Together, these data indicate that *in vivo* RS drastically alters MMP, mtROS, mitophagy and mitochondrial distribution in adult HSPCs.

RS increases mitochondrial activity and mitophagy in cultured HSC

We next investigated changes in mitochondrial activity in cultured HSCs subjected to aphidicolin (APH) which induces RS by inhibiting DNA polymerase (21). After 7 days of culture APH-treated HSCs exhibited a significant decline in number compared to control HSCs (Figure 2A). APH-treated HSCs exhibited significantly fewer cell divisions compared to control HSCs (Figure S1D). APH-treated HSCs did not exhibit an increase in apoptosis as shown by Annexin-V staining (Figure S1E). Hematopoietic progenitor colony formation of HSCs was significantly increased with APH-treatment (Figure S1F). MMP of APH-treated HSCs transiently increased on day 2 but normalized by day 7 (Figure 2B). Both on day 2 and on day 7, APH-treated HSCs exhibited unaltered mtROS level (Figure 2C). Mitophagy activity was also significantly upregulated in APH-treated HSCs at day 2 but comparable at day 7 (Figure 2D). These data indicate that BM HSCs under experimental RS in an ex vivo culture upregulate MMP and mitophagy.

Fancd2 KO BM HSC exhibits decreased mitophagy

Next, we focused on whether Fancd2 deficiency affected mitochondrial metabolism in quiescent adult BM HSCs, which do not exhibit spontaneous RS (5). While there was variation, Fancd2 KO BM HSCs exhibited comparable MMP to Fancd2 WT BM HSCs and HSPCs (Figures 3A, S2A, B). mtROS was slightly upregulated in Fancd2 KO BM HSCs but was not altered in HSPCs (Figures 3B, S2C). Mitochondria distribution was diffused and not polar in Fancd2 BM KO HSCs (Figure 3C). These data indicate mitochondrial activity in FANCD2-deficient BM HSCs was comparable to WT cells. We further observed that mitophagy activity significantly decreased in Fancd2 KO BM HSCs (Figures 3D, S2E), whereas lysosome activity did not change in Fancd2 KO BM HSCs (Figures 3E, S2F). Consistent with existing reports (10), these data illustrate that FANCD2-deficiency significantly decreases mitophagy in quiescent adult BM HSCs.

FANCD2-deficiency increases metabolic activity and mitophagy in FL HSC

Fetal HSCs show increased mitochondrial metabolisms compared to adult BM HSCs (9). Here, we focused on how FANCD2-deficiency changes mitochondrial metabolism in the rapidly proliferating FL HSCs. Unlike Fancd2 KO BM, FL HSCs showed an increased RS response, illustrated by characteristic gains in ssDNA (Figure 4A) (5). We first measured MMP and found it to be significantly higher in Fancd2 KO FL HSCs and HSPCs compared to WT and Fancd2 heterozygous (HET) (Figures 4B, S3A–G), while mtROS levels were

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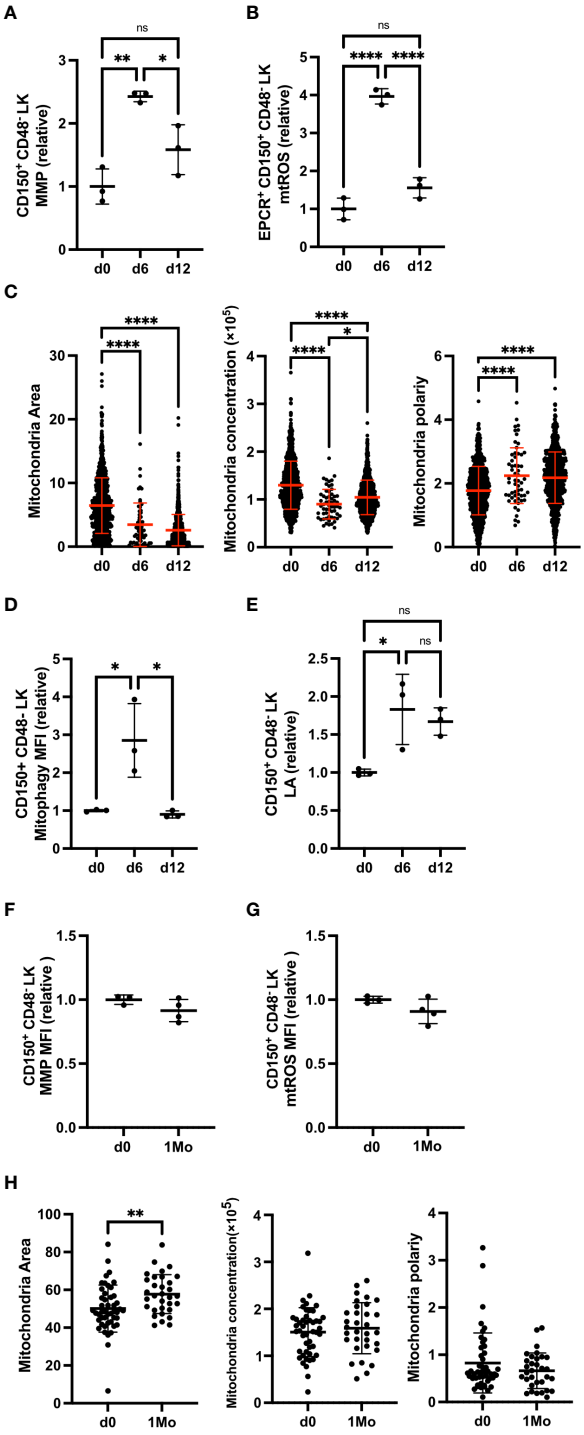

FIGURE 1
5-FU-induced RS increases MMP and mitophagy. **(A, B)** Relative mean fluorescence intensity (MFI) of MMP (TMRE) **(A)** and mtROS (MSR) **(B)** in HSPCs (EPCR+ CD150+ CD48- c-Kit+ Lin-) at untreated (d0), day 6 (d6) and day 12 (d12) after 5-FU injection (d0 n=3 mice, d6 n=3 mice, d12 n=3 mice). **(C)** Mitochondrial area, mitochondrial concentration, and mitochondrial polarity of HSPCs from mitoDendra mouse, at d0, d6 and d12 after 5-FU injection (d0 n=931 cells, d6 n=58 cells, d12 n=867 cells). Data are analyzed by IFM. **(D, E)** Relative MFI of mitophagy **(D)** and lysosome acidification (LA, LTR) **(E)** in HSPCs at d0, d6 and d12 after 5-FU injection (d0 n=3 mice, d6 n=3 mice, d12 n=3 mice). **(F, G)** Relative MFI of MMP **(F)** and mtROS **(G)** in HSPCs at 1 month (1Mo) after 5-FU injection (d0 n=3 mice, d6 n=3 mice, d12 n=3 mice). **(H)** Mitochondrial area, mitochondrial concentration and mitochondrial polarity in HSPCs at 1 month (1Mo) after 5-FU injection (d0 n=47 cells, 1Mo n=32 cells). Data are analized by IFM. All relative data are divided with average value of d0 and show mean ± SD. *P<0.05, **P<0.01, ****P<0.0001, ns, not significant.

unchanged in Fancd2 KO FL HSCs (Figure 4C). Further, Fancd2 KO FL HSPCs exhibited a significant increase in mitochondrial area, while the distribution of mitochondria was concentrated and polarized, indicating an overall increase in mitochondrial activity (Figure 4D). We also measured mitophagy and lysosome acidification in FL HSC. In contrast to adult BM HSCs, mitophagy and lysosome acidification were all upregulated in Fancd2 KO FL HSCs and HSPCs (Figures 4E, F, S3H, I).

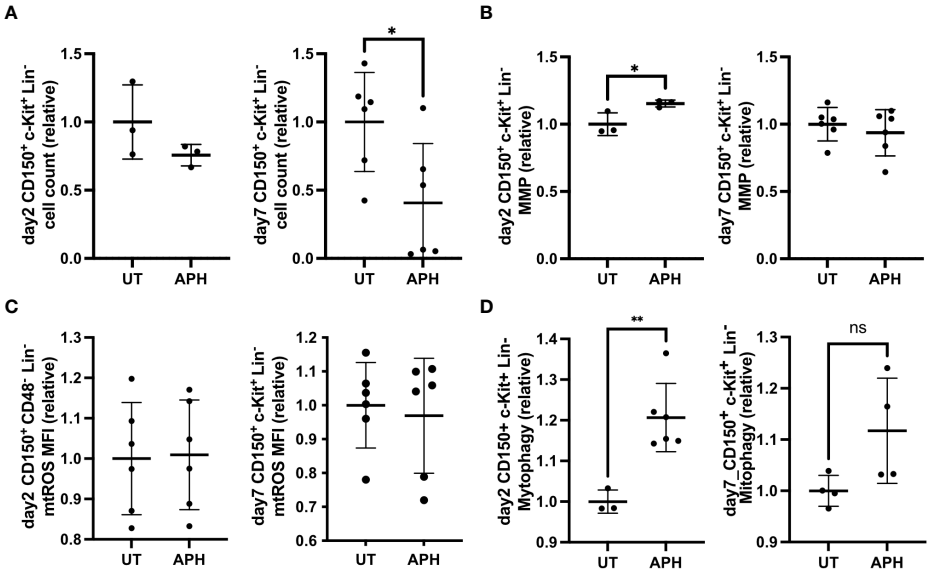

FIGURE 2
APH-induced RS increases MMP and mitophagy. **(A-D)** Relative cell count **(A)**, MFI of MMP **(B)**, MFI of mtROS **(C)** and MFI of mitophagy **(D)** of cultured HSCs (CD150+ c-Kit+ Lin-) at day 2 (left panel) and day 7 (right panel), with or without APH (UT) (day 2 UT n=3-6 wells, APH n=3-6 wells, day 7 UT n=6 wells, APH n=6 wells). All relative data are divided with average of UT and show mean ± SD. *P<0.05, **P<0.01, ns, not significant.

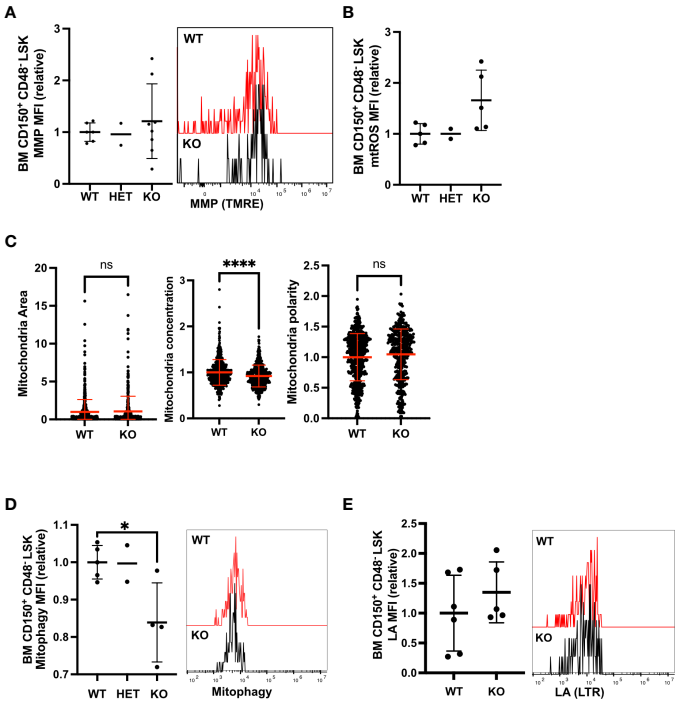

FIGURE 3
Mitophagy activity was decreased in Fancd2-deficient BM HSC. **(A)** Relative MFI (left panel) and representative flow cytometric plots (right panel) showing MMP in adult (8-12 weeks) HSCs (Fancd2 WT n=6 mice, HET n=2 mice, KO n=8 mice, left panel). **(B)** Relative MFI of mtROS in adult HSCs (Fancd2 WT n=5 mice, HET n=2 mice, KO n=5 mice). **(C)** Mitochondrial area, mitochondrial concentration and mitochondrial polarity in HSCs. HSCs were stained with anti-Tomm20 and analyzed by IFM (Fancd2 WT n=559 cells, KO n=431 cells). **(D)** Relative MFI (left panel) and representative flow cytometric plots (right panel) of mitophagy in BM HSCs (Fancd2 WT n=5 mice, HET n=2 mice, KO n=4 mice). **(E)** Relative MFI (left panel) and representative FACS plots (right panel) of LA in BM HSCs (Fancd2 WT n=6 mice, KO n=5 mice, left panel). All relative data are divided with average of controls (WT or HET) and show mean ± SD. *P<0.05, ****P<0.0001, ns, not significant.

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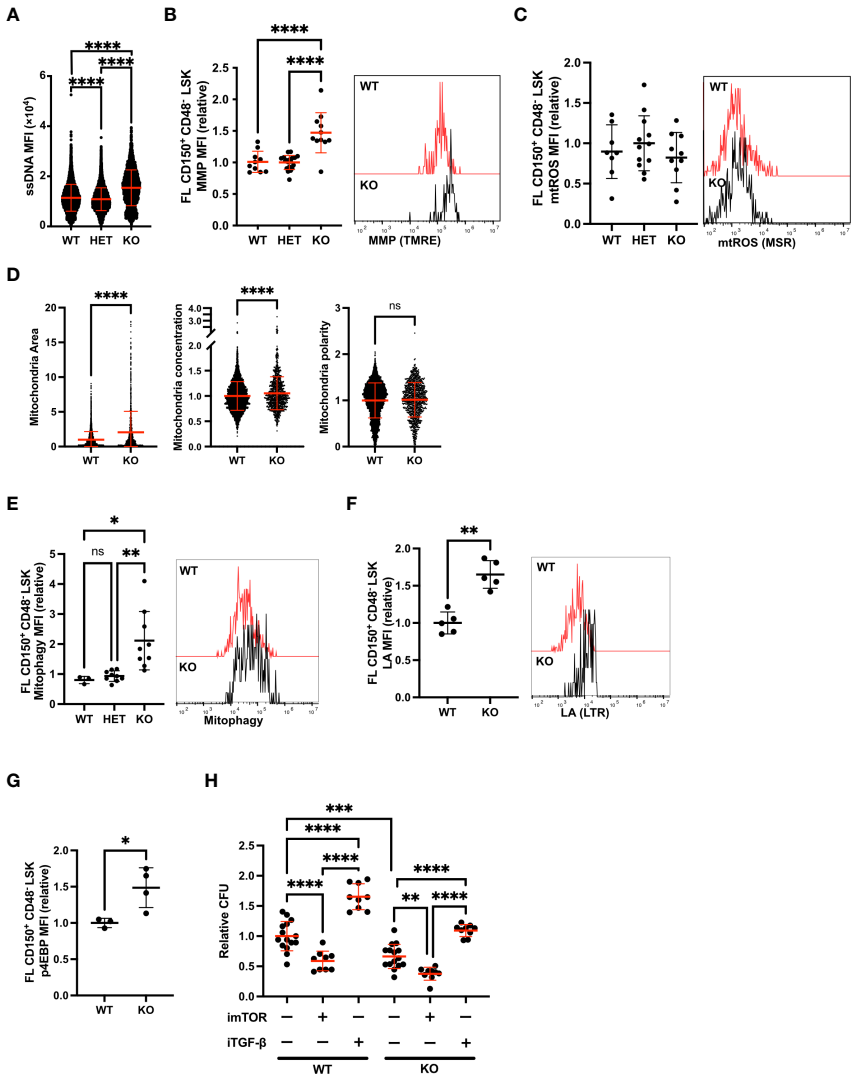

FIGURE 4
Fancd2 deficiency increases MMP and mitophagy in FL HSC. **(A)** MFI of AF-488 ssDNA in HSPCs (LSK) at E14.5 (Fancd2 WT n=7003 cells, Het n=12954 cells, KO n=2673 cells). IF of ssDNA was performed and data are analysed by IFM. **(B)** Relative MFI (left panel) and representative flow cytometric plots (right panel) of MMP in FL HSCs at E13.5 (CD150+ CD48- c-Kit+ Sca-1 Lin- (LSK)) (Fancd2 WT n=10 mice, HET n=19 mice, KO n=11 mice, left panel). **(C)** MFI (left panel) and representative flow cytometric plots (right panel) of mtROS in FL HSCs at E13.5 (Fancd2 WT n=8 mice, HET n=12 mice, KO n=10 mice, left panel). **(D)** Mitochondrial area, mitochondrial concentration and mitochondrial polarity in HSPCs (LSK) at E14.5 (Fancd2 WT n=4446 cells, KO n=987 cells). IF of Tomm20 was performed and data are analysed by IFM. **(E)** Relative MFI (left panel) and representative flow cytometric plots (right panel) of mitophagy in FL HSCs at E13.5 (Fancd2 WT n=3 mice, HET n9 mice, KO n=9 mice, left panel) **(F)** Relative MFI (left panel) and representative flow cytometric plots (right panel) of LA in FL HSCs at E13.5 (Fancd2 WT n=5 mice, HET n=4 mice, KO n=4 mice). **(G)** MFI of p4EBP in E13.5 FL HSCs (Fancd2 WT n=3 mice, KO n=4 mice). **(H)** CFU assay of HSCs with Rapamycin (imTOR) or SD-208 (iTgf-β) or untreatment (UT). Some independent experiments were performed and data are shown as relative numbers to the average of each WT UT (Fancd2 WT UT n=15 imTOR n=9 dishes, iTgf-β n=9 dishes, Fancd2 KO UT n=15 imTOR n=9 dishes, iTgf-β n=9 ishes). All relative data are divided with average of controls (WT or HET) and show mean ± SD. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, ns, not significant.

mTOR signaling is closely associated with mitochondrial metabolism and lysosome biogenesis in HSCs (22). We thus analyzed changes in phosphorylated 4EBP (p4EBP), a direct substrate of mTOR and OPP incorporation as a measure of global protein synthesis in Fancd2 KO FL HSCs. Both measures of metabolic activity were significantly increased (Figures 4G, S3J). These data indicate that FANCD2 deficiency stimulates mitochondrial activity during fetal development in concert with an increase in mTOR signaling. Additionally, we performed CFU with treatment of mTOR inhibitor (imTOR) of Rapamycin or Tgf-β inhibitor (i Tgf-β) which rescued the RS of Fancd2 KO FL HSPCs (5). Result showed CFU number was significantly decreased in imTOR treated Fancd2-KO HSC compared to untreated Fancd2-KO HSC indicating upregulated metabolism protect from RS (Figure 4H). Collectively, our data indicate the RS response results increasing metabolism including mTOR pathway, mitochondrial activity and mitophagy and it sustains HSC function in FANCD2-deficient FL HSCs. These results mirror experimental observations following experimental RS in WT BM HSCs (Figure S3K).

Discussion

In this study, we extended our observations on the unique fetal HSC phenotype in FA. We showed that fetal RS, in the context of FA

results in a concurrent increase in mitochondrial metabolism and mitophagy. In contrast, adult quiescent BM HSCs show lower levels of mitochondrial metabolism and decreased mitophagy in Fancd2 KO. These observations provide an *in vivo* link of RS with mitochondrial metabolism and mitophagy. In 5-FU-treated mice, prominent mitochondrial changes were observed at day 6 after administration, the phase when HSCs are subjected to proliferation (15). Both FL HSCs and BM HSCs respond to RS by increasing mitochondrial metabolism and mitophagy, which suggests an inherent HSC response to RS. However, the change in mtROS and morphology in FANCD2-deficient FL HSCs and 5-FU BM HSCs differed. FL HSCs exhibit higher mtROS levels compared to BM HSC (9). mtROS level closely reflects mitochondrial quality and investigating how fetal and adult HSCs differ in mitochondrial quality control processes would be addressed in the future.

We previously described the role of RS in decreasing FA FL HSCs (5). Metformin, an inhibitor of mitochondrial complex I, was reported to ameliorate adult FANCD2-deficient HSC decline in number and potential (13) suggesting a link between FA and fetal mitochondrial metabolism. However, low placental penetration of metformin prevented its use to investigate the effect on FL HSCs (data not shown).

Previous reports showed defective mitophagy as a non-canonical phenotype of FA cells and illustrate the role of several FA proteins in mitophagy (10, 11). Our results revealed a progressive decrease in mitophagy through development. While a mechanism has not been established, the investigation should be done in the future.

The direct correlation between RS, mitochondrial metabolism and mitophagy is difficult to explain. Our data indicate that mTOR signaling and mTOR-related lysosomal biogenesis may sense RS and increase overall metabolic activity including c-Myc (23) and ribosome biogenesis (24), though further investigation is needed. In conclusion, this manuscript provides evidence for the involvement of RS in the metabolic regulation of HSCs by FANCD2 deficiency and highlights the unique characteristics of the FL HSCs compared to adult BM HSCs.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by TWMU animal experiment committee.

Author contributions

MM-K and NO performed experiments. MM-K, KF, SA, PK, AN-I wrote manuscript, MG, AI, KS and AN-I funded research. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1108430/full#supplementary-material

SUPPLEMENTARY FIGURE 1
APH-treated HSC of phenotypes in ex vivo. **(A-C)** Representative flow cytometric plots **(A)** and relative frequency to total live cells **(B)** and total analyzed cell count **(C)** of CD150+ CD48- c-Kit+ Lin- HSC in d0, d6, d12 after 5-FU injection (d0 n=3 mice, d6 n=3 mice, d12 n=3 mice). **(D, E)** Relative MFI of cell trace violet (CTV, C34557 Thermo Fisher) **(D)** and relative percentage of Annexin-V single positive (SP) cells **(E)** in BM HSCs (CD150+ c-Kit+ Lin-) ex vivo cultured for 7 days with (APH) or without APH (UT) (UT n=6 wells, APH n=6 wells). **(F)** CFU assay of BM HSCs with or without APH. 3 independent experiments were performed and data are shown as relative numbers to the average of each UT (UT n=9 dishes, APH n=9 dishes). All data show mean ± SD.

SUPPLEMENTARY FIGURE 2
Metabolism data for Fancd2-deficient BM HSPCs. **(A, B)** Relative MFI of MMP in BM HSPCs **(A)** and BM total live cells **(B)** (Fancd2 WT n=5 mice, HET n=3 mice, KO n=8 mice). **(C-E)** Relative MFI of mtROS in HSPCs **(C)** (Fancd2 WT n=5 mice, HET n=2 mice, KO n=5 mice) and relative MFI of mitophagy **(D)** (Fancd2 WT n=5 mice, HET n=2 mice, KO n=5 mice), MFI of lysosome acidification **(E)** (Fancd2 WT n=6 mice, KO n=5 mice). All data show mean ± SD.

SUPPLEMENTARY FIGURE 3
Metabolism data for Fancd2-deficient FL HSPCs. **(A)** Scheme for FA deficient FL HSC. **(B-D)** Representative flow cytometric plots **(B)** and relative frequency to total live cells **(C)** and total analyzed cell count **(D)** of CD150+ CD48- c-Kit+ Sca-1+ Lin- WT and Fancd2 KO E13.5 FL HSC. **(E)** Relative frequency of HSPCs to total live cell. **(F, G)** Relative MFI of MMP in E13.5 FL HSPCs **(F)** and total live cells (G, A-G, Fancd2 WT n=10 mice, HET n=19 mice, KO n=11 mice). **(H, I)** Relative MFI of mitophagy (H, Fancd2 WT n=3 mice, HET n=8 mice, KO n=9 mice, BM HSC n=3 mice: mom of pups, Fancd2 HET) and MFI of LA **(I)** (Fancd2 WT n=5 mice, HET n=4 mice, KO n=5 mice) in E13.5 FL HSCs and BM HSCs. **(J)** MFI of OPP in E13.5 FL HSCs and BM HSCs (Fancd2 WT n=5 mice, HET n=6 mice, KO n=5 mice, BM HSC n=2 mice: mom of pups, Fancd2 HET). **(K)** Scheme for RS increases mitochondrial metabolism and mitophagy in FANCD2 deficient FL HSC. How mTOR signal senses RS should be described in the future. All data show mean ± SD. *P<0.05, **P<0.01, ****P<0.0001.

References

1. Garaycoechea JI, Patel KJ. Why does the bone marrow fail in fanconi anemia? *Blood* (2014) 123:26–34. doi: 10.1182/blood

2. Kamimae-Lanning AN, Goloviznina NA, Kurre P. Fetal origins of hematopoietic failure in a murine model of fanconi anemia. *Blood* (2013) 121:2008–12. doi: 10.1182/blood-2012-06-439679

3. Yoon Y, Storm KJ, Kamimae-Lanning AN, Goloviznina NA, Kurre P. Endogenous DNA damage leads to p53-independent deficits in replicative fitness in fetal murine Fancd2–/–Hematopoietic stem and progenitor cells. *Stem Cell Rep* (2016) 7:840–53. doi: 10.1016/j.stemcr.2016.09.005

4. Ceccaldi R, Parmar K, Mouly E, Delord M, Kim JM, Regairaz M, et al. Bone marrow failure in fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. *Cell Stem Cell* (2012) 11:36–49. doi: 10.1016/j.stem.2012.05.013

5. Mochizuki-Kashio M, Yoon Y, Menna T, Grompe M, Kurre P. FANCD2 alleviates physiologic replication stress in fetal liver HSC. *Bio Rxiv* (2020). doi: 10.1101/2020.09.30.320796

6. Nakamura-Ishizu A, Ito K, Suda T. Hematopoietic stem cell metabolism during development and aging. *Dev Cell* (2020) 54:239–55. doi: 10.1016/j.devcel.2020.06.029

7. Filippi M-D, Ghaffari S. Mitochondria in the maintenance of hematopoietic stem cells: New perspectives and opportunities. *Blood* (2019) 133:1943–52. doi: 10.1182/blood-2018-10-808873

8. Takihara YJ, Nakamura-Ishizu A, Tan DQ, Fukuda M, Matsumura T, Endoh M, et al. High mitochondrial mass is associated with reconstitution capacity and quiescence of hematopoietic stem cells. *Blood Adv* (2019) 3:2323–7. doi: 10.1182/bloodadvances.2019032169

9. Manesia JK, Xu Z, Broekaert D, Boon R, van Vliet A, Eelen G, et al. Highly proliferative primitive fetal liver hematopoietic stem cells are fueled by oxidative metabolic pathways. *Stem Cell Res* (2015) 15:715–21. doi: 10.1016/j.scr.2015.11.001

10. Sumpter R, Sirasanagandla S, Fernández ÁF, Wei Y, Dong X, Franco L, et al. Fanconi anemia proteins function in mitophagy and immunity. *Cell* (2016) 165:867–81. doi: 10.1016/j.cell.2016.04.006

11. Shyamsunder P, Esner M, Barvalia M, Wu YJ, Loja T, Boon HB, et al. Impaired mitophagy in fanconi anemia is dependent on mitochondrial fission. *Oncotarget* (2016) 7:58065–74. doi: 10.18632/oncotarget.11161

12. Fernandes P, Miotto B, Saint-Ruf C, Said M, Barra V, Nähse V, et al. FANCD2 modulates the mitochondrial stress response to prevent common fragile site instability. *Commun Biol* (2021) 4(1):127. doi: 10.1038/s42003-021-01647-8

13. Zhang Q-S, Tang W, Deater M, Phan N, Marcogliese AN, Li H, et al. Metformin improves defective hematopoiesis and delays tumor formation in fanconi anemia mice. *Blood* (2016) 128:2274–784. doi: 10.1182/blood

14. Houghtaling S, Timmers C, Noll M, Finegold MJ, Jones SN, Stephen Meyn M, et al. Epithelial cancer in fanconi anemia complementation group D2 (Fancd2) knockout mice. *Genes Dev* (2003) 17:2021–35. doi: 10.1101/gad.1103403

15. Umemoto T, Hashimoto M, Matsumura T, Nakamura-Ishizu A, Suda T. Ca2+–mitochondria axis drives cell division in hematopoietic stem cells. *J Exp Med* (2018) 215:2097–113. doi: 10.1084/jem.20180421

16. Pei S, Minhajuddin M, Adane B, Khan N, Stevens BM, Mack SC, et al. AMPK/FIS1-mediated mitophagy is required for self-renewal of human AML stem cells. *Cell Stem Cell* (2018) 23:86–100.e6. doi: 10.1016/j.stem.2018.05.021

17. Aoyagi Y, Hayashi Y, Harada Y, Choi K, Matsunuma N, Sadato D, et al. Mitochondrial fragmentation triggers ineffective hematopoiesis in myelodysplastic syndromes. *Cancer Discov* (2022) 12:250–69. doi: 10.1158/2159-8290.CD-21-0032

18. Mortensen M, Soilleux EJ, Djordjevic G, Tripp R, Lutteropp M, Sadighi-Akha E, et al. The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. *J Exp Med* (2011) 208:455–467. doi: 10.1084/jem.20101145

19. Liang R, Arif T, Kalmykova S, Kasianov A, Lin M, Menon V, et al. Restraining lysosomal activity preserves hematopoietic stem cell quiescence and potency. *Cell Stem Cell* (2020) 26:359–376.e7. doi: 10.1016/j.stem.2020.01.013

20. Ho TT, Warr MR, Adelman ER, Lansinger OM, Flach J, Verovskaya Ev, et al. Autophagy maintains the metabolism and function of young and old stem cells. *Nature* (2017). doi: 10.1038/nature21388

21. Vesela E, Chroma K, Turi Z, Mistrik M. Common chemical inductors of replication stress: Focus on cell-based studies. *Biomolecules* (2017) 7:19. doi: 10.3390/biom7010019

22. Mochizuki-Kashio M, Shiozaki H, Suda T, Nakamura-Ishizu A. Mitochondria turnover and lysosomal function in hematopoietic stem cell metabolism. *Int J Mol Sci* (2021) 22:4627. doi: 10.3390/ijms22094627

23. Rodríguez A, Zhang K, Färkkilä A, Filiatrault J, Yang C, Velázquez M, et al. MYC promotes bone marrow stem cell dysfunction in fanconi anemia. *Cell Stem Cell* (2021) 28:33–47.e8. doi: 10.1016/j.stem.2020.09.004

24. Gueiderikh A, Maczkowiak-Chartois F, Rosselli F. A new frontier in fanconi anemia: From DNA repair to ribosome biogenesis. *Blood Rev* (2022) 52. doi: 10.1016/j.blre.2021.100904

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Emerging bone marrow failure syndromes- new pieces to an unsolved puzzle

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Inherited bone marrow failure (BMF) syndromes are genetically diverse — more than 100 genes have been associated with those syndromes and the list is rapidly expanding. Risk assessment and genetic counseling of patients with recently discovered BMF syndromes is inherently difficult as disease mechanisms, penetrance, genotype-phenotype associations, phenotypic heterogeneity, risk of hematologic malignancies and clonal markers of disease progression are unknown or unclear. This review aims to shed light on recently described BMF syndromes with sparse concise data and with an emphasis on those associated with germline variants in *ADH5/ALDH2*, *DNAJC21*, *ERCC6L2* and *MECOM*. This will provide important data that may help to individualize and improve care for these patients.

KEYWORDS

bone marrow failure, ADH5, ALDH2, DNAJC21, ERCC6L2, MECOM, early onset myeloid malignancies

Introduction

Bone marrow failure (BMF) syndromes are defined by decreased production of one or more hematopoietic lineages, which leads to diminished or absent hematopoietic precursors in the bone marrow and subsequent cytopenia in the peripheral blood. BMF can be distinguished into an acquired form and an inherited form. The acquired form, which is likely caused by an autoimmune reaction (1), may be successfully treated with immunosuppressant regimens. Inherited BMF syndromes include a broad spectrum of heterogenous diseases such as Fanconi anemia, telomere biology disorders, Shwachman-Diamond syndrome, Diamond-Blackfan anemia, congenital cytopenia, immunodeficiency and others (2). In excess of 100 genes have been associated with inherited BMF to date (3–8). The first inherited BMF syndrome, Fanconi anemia, was described in 1927 by the Swiss pediatrician Guido Fanconi, who reported a family with three boys with physical birth defects and a condition resembling pernicious anemia (9). The first causative gene, *FANCC*, was successfully cloned in 1992 (10). A number of genes have emerged as new *bona fide* genes associated with the development of BMF in the past ten years: In 2014, variants in *ERCC6L2* were shown to cause autosomal recessive BMF and predisposition to myeloid malignancies (11). *MECOM* as causative gene for inherited BMF has been described in

2015 (12), but its association with a predisposition to hematologic malignancies was only reported three years later (13, 14). Homozygous/compound heterozygous variants in *DNAJC21* were linked to a Shwachman-Diamond-like BMF with additional telomeropathy-like features in 2016 (15). In 2020, a digenic ADH5/ALD2H2 deficiency causing severe BMF, early-onset myelodysplastic syndrome (MDS), short stature and intellectual disability was connected to the inability to detoxify formaldehyde (16).

Particularly for the recently described syndromes, data on disease mechanism, penetrance, overall risk of developing hematologic malignancies, and molecular or cytogenetic factors indicating a risk of worsening cytopenia, development of bone marrow dysplasia or leukemogenesis is sparse. This is an important and incomplete pillar for counseling patients and providing them with the most complete and up-to-date information specific to their underlying condition (17). Penetrance, risk of hematologic malignancy and phenotypic heterogeneity may influence the decision towards early (preventive) hematopoietic stem cell transplantation (HSCT) versus 'watch and wait' and a more specific follow-up program tailored to the early detection of clonal evolution and disease progression. Amino acid hotspots, genotype-phenotype correlations and disease mechanisms based on reported variants are crucial to determine the strength and validity of the underlying genetic diagnosis and the expected/predicted phenotype and course of disease. This review is therefore based on the recently described syndromes with germline variants in *ERCC6L2, MECOM, DNAJC21*, and *ADH5/ALDH2* that lack concise reviews at this point in time.

ERCC6L2 acts as crucial non-homologous end joining factor

In 2014, whole-exome sequencing (WES) of three children and young adults (ages nine to nineteen years old) with BMF and neurological abnormalities (microcephaly, developmental delay) and a history of consanguinity revealed homozygous *ERCC6L2* variants in two index patients (11). *ERCC6L2* belongs to the Snf2-like *ERCC6* family, which also includes *ERCC6* and *ERCC6L*. Functional studies revealed that the molecular mechanism of ERCC6L2 deficiency is an impaired nucleotide excision repair mechanism and an increased amount of reactive oxygen species *via* a defect in the mitochondrial function of ERCC6L2 (11). The short ERCC6L2 isoform contains an N-terminal TUDOR and a C-terminal DEAD/DEAH ATP-helicase domain. Zhang et al. (18) later reported an alternative *ERCC6L2* transcript translating a new protein, Hebo (helicase mutated in BMF), which differs from the ERCC6L2 protein by an 850-amino acid sequence and an additional HEBO domain. Hebo is ubiquitously expressed and is recruited to sites of DNA damage (18). A subsequent study by Tummala et al. postulated the underlying mechanism as primary transcription deficiency rather than a DNA repair defect based on patients being defective in the repair of transcription-associated DNA damage leading to genomic instability (19). Liu et al. described that ERCC6L2 clusters with core subunit non-homologous end joining (NHEJ) genes. ERCC6L2-deficient cells were depleted upon treatment with γ-irradiation, zeocin and etoposide inducing double-strand breaks, lending itself to a similar, but less severe phenotype than that observed in cells lacking the NHEJ ligase LIG4. They could also demonstrate that *Ercc6l2*−/− mice were viable and ERCC6L2 deficiency resulted in an approximately 50% reduction in orientation-specific class switch recombination of antibody genes (20). A CRISPR-Cas9 screen against genotoxic agents also identified ERCC6L2 as a canonical NHEJ pathway factor (21). SFPQ, a member of the SFPQ-NONO complex that has recently been attributed a putative function in NHEJ, has been described as novel interaction partner of ERCC6L2 (22). Somatic *ERCC6L2* variants have been described in a variety of hematologic and solid malignancies, most commonly in patients with uterine corpus endometrial carcinoma. Upon treatment with radiotherapy, these patients showed a strikingly longer disease-free and overall survival than patients with wild-type ERCC6L2, indicating that ERCC6L2 loss may be clinically relevant (22). The most recent study described an impaired clonogenic capacity and erythroid differentiation in *ERCC6L2*-silenced HSPCs and a probable impact on mesenchymal stromal cells and their differentiation potential (23).

Consanguinity has been described in at least 8 of the 24 families (33%) reported to date (Table 1). The disease is caused by loss-of-function (LOF) variants and all but two variants, D272N and S658N (NM_020207.7) (7, 19), are truncating variants affecting both isoforms or just the long isoform with its HEBO domain (Tables 1, S1 and Figure 1). Two variants, R644* and I475fs, have been found in more than one family and are present in the heterozygous state in gnomAD (https://gnomad.broadinstitute.org) with significant allele frequencies in the European (Non-Finnish) and Finnish subpopulation, respectively (Table 1 and Figure 1). One copy number variant (CNV), a homozygous intragenic deletion of exon 11, has been reported as the causative allele in a patient with BMF (Figure 1) (24). The association with neurological abnormalities such as microcephaly, congenital mirror movements and developmental delay of various degrees was discovered in three studies (18, 24, 25) and may be part of the phenotype or could be an independent effect of the underlying consanguinity in these cases. Of note, ataxia, microcephaly, and developmental delay have also been described in diseases associated with variants in other NHEJ factors such as *ATM*, *MRE11*, *NBS1*, *NHEJ1*, *PRKDC*, *RAD50*, and *XRCC4* (26).

There is no known genotype-phenotype association and the phenotype ranges from mild cytopenia to severe BMF in childhood and/or development of MDS/acute myeloid leukemia (AML) (Table 1). The overall penetrance is high with an estimate of 94% with two asymptomatic homozygotes still being very young (Table 1). Cytopenia and/or overt BMF develop early at an average age of 14 years and were reported in 24 out of 36 patients (66%, range 2 to 47 years (n=24), Table 1) (7, 11, 18, 19, 23, 25, 27, 28). The development of hematologic malignancies (MDS/AML) has been described in approximately 31% of *ERCC6L2* germline-mutated patients at an average age of 35 years (range 2–59 years (n=12), Table 1) (7, 19, 24, 28–30). Importantly, only one in four showed signs of cytopenia or BMF beforehand, which is in line with reports that cytopenia can be subtle, intermittent and go unnoticed.

TABLE 1 Overview of emerging bone marrow failure syndromes including genetic and phenotypic features.

Gene	OMIM	inheritance/ consanguinity/ *de novo* occurence	mechanism of disease	amino acid hotspots	penetrance	phenotype	heterogeneity	genotype-phenotype association	age of onset	acquired somatic variants/disease progression	risk of heme malignancy, type	HSCT	PMID
ADH5/ALDH2 (NM_000671.4)	619151	digenic, AR for *ADH5*	LOF (missense and truncating SNVs), either heterozygous or homozygous for *ALDH2*2*	W322* described in all but one family; A278P described in seven out of 13 families	complete	cytopenia/BMF and/ or MDS/AML [100%] short stature [100%] intellectual disability [100%] microcephaly [67%] abnormal skin pigmentation [58%] *(retinal degeneration, facial dysmorphia, skeletal, and endocrine abnormalities)*	high penetrance, severe BMF and MDS/AML often requiring HSCT	individuals with homozygous *ALDH2*2* indicative of more severe (neurological) phenotype	MDS/ AML: age 7 years (range 0 to 18, n=9)	73% with gain of the long arm of chromosome 1, other recurrent cytogenetic alterations included monosomy 7, trisomy 8 and 21p alterations	80%, MDS/ AML	75%, after MDS/ AML diagnosis	33147438 33355142 34458631
DNAJC21 (NM_001012339.3)	617052	AR, consanguinity in 57%	LOF (missense and truncating SNVs and CNVs)	K34E described in seven individuals from four families; R173* described in two families	complete	cytopenia/BMF and/ or AML [100%] growth delay and/or short stature [95%] develomental delay, intellectual disability and/or neurological abnormalities [68%] skeletal abnormalities [63%] skin abnormalities [63%] microcephaly [42%] facial dysmorphia [37%] dental abnormalities [32%] osteopenia/ osteoporosis [32%] (high) myopia, astigmatism and other visual field defects [32%]	although penetrance is high, spontaneous, intermittent or prolonged improvement of cytopenia has been reported	unknown	cytopenia/ BMF: age 2 years (range 0 to 15, n=19) AML: ages 12 and 15 years (n=2)	complex karyotype, deletions of 17p13 and 20q and a derivative chromosome 15 with translocation t(1;15) described in four patients without hematologic malignancy	11%, AML^	21%, mostly due to severe BMF	35298850 27346687 35464845 30755392 29146883 28062395 29700810

(Continued)

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TABLE 1 Continued

Gene	OMIM	inheritance/ consanguinity/ *de novo* occurence	mechanism of disease	amino acid hotspots	penetrance	phenotype	heterogeneity	genotype-phenotype association	age of onset	acquired somatic variants/disease progression	risk of heme malignancy, type	HSCT	PMID
						retinal (rod-cone) dystrophy and other retinal abnormalities [32%] pancreas lipomatosis/ exocrine pancreatic dysfunction [26%]							
ERCC6L2 (NM_020207.7)	615715	AR, consanguinity in 33%	LOF (truncating SNVs and CNVs), two missense variants (D272N and S658N)	R644* and F486fs (Finnish founder) described in more than one family	high (94%), two asymptomatic homozygotes	cytopenia, BMF [66%] MDS, AML (particularly acute erythroid leukemia) [31%] *(neurological abnormalities such as microcephaly, congenital mirror movements and intellectual disability)*	may vary from subtle intermittent cytopenia to severe BMF and/or early onset MDS/ AML	unknown	cytopenia/ BMF: age 14 years (range 2 to 47, n=24) MDS/ AML: age 35 years (range 2 to 59, n=12)	complex or monosomal karyotype with loss of chromosomes 5, 7 or 17 or isolated monosomy 7, *TP53* variants (often multi-hit)	33%, MDS/ AML	28%, either due to severe BMF or after the onset of MDS/ AML	29633571 24507776 30936069 28815563 29987015 33209984 29146883 35969835 33510405 36156210
MECOM (NM_004991.4)	616738	AD, *de novo* in 16%	LOF (missense and truncating SNVs and CNVs)	R938W, P948A and variants affecting the splice sites between exons 7 and 8 have been described in more than one family	high (96%) for any related features (RUS/ other skeletal abnormalities, deafness, cytopenias/ BMF)	cytopenia, BMF [80%] RUS [54%] brachy-, campto-, and clinodactyly and/or other finger abnormalities [38%] sensorineural hearing impairment/ congenital deafness [20%] cardiac abnormalities [18%] prematurity, hydrops fetalis or polyhydramnios	high penetrance, spontaneous, intermittent or prolonged improvement of cytopenias has been reported	distinct genotype-phenotype association- all but one variant associated with the co-presentation of RUS and hematologic disease cluster in the region spanning zinc fingers 8 and 9 (specific variants R969C/ H/L, I971T, and Q965E have solely been associated with RUS without any other features)	cytopenia/ BMF: at birth/in infancy (range 0 to 42, n=43) MDS or MDS/ MPN-U: ages 37, 42 and 73 years (n=3)	unclear, translocation t(1;14)(q44;q32) described in one patient with MDS/MPN-U	5%, MDS/ MPN^	50%, due to severe BMF	35020829 30536840 32064714 29200407 29519864 29439187 36082647 29097497 29540340 26581901 29146883 22972950 26554871 29496554

(Continued)

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TABLE 1 Continued

Gene	OMIM	inheritance/ consanguinity/ *de novo* occurence	mechanism of disease	amino acid hotspots	penetrance	phenotype	heterogeneity	genotype-phenotype association	age of onset	acquired somatic variants/disease progression	risk of heme malignancy, type	HSCT	PMID
						[13%] micro- or macrocephaly, structural brain abnormalities or intellectual disability/ cognitive impairment [11%] patellar hypoplasia [10%] metatasus adductus, hallux valgus and other toe abnormalities [9%] clubfoot [7%] renal abnormalities [7%] MDS or MDS/ MPN-U [5%] (*hip dysplasia, cleft palate, early-onset ischemic insults, facial dysmorphia, precocious puberty/ gynecomastia in infancy, paralysis of the larynx/ laryngomalacia)*							35484980 35150448

AD, autosomal dominant, AML- acute myeloid leukemia; AR, autosomal recessive; BMF, bone marrow failure; CNV, copy number variant; HSCT, hematopoietic stem cell transplantation; LOF, loss-of-function; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; RUS, radioulnar synostosis; SNV, single-nucleotide variant; MPN-U, myeloproliferative neoplasm- unclassified.
Phenotypic features in parentheses and Italics are not clearly associated with the underlying BMF syndrome (yet).
^Only few patients reported.

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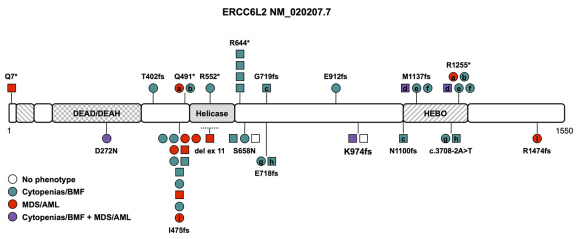

FIGURE 1
Schematic of the *ERCC6L2* transcript (NM_020207.7) and its protein domains with the location of all reported germline variants. Circles represent females and squares represent males. Symbols on the same horizontal level are individuals from the same family. Letters within the symbols indicate individuals with compound heterozygous genotype, while symbols without letters indicate homozygosity for the variant. The color fill depicts different phenotypes, no color fill designates healthy individuals carrying homozygous/compound heterozygous causative variants. The dotted line shows the location of the sole described copy number variant. AML, acute myeloid leukemia; BMF, bone marrow failure; HEBO, helicase mutated in bone marrow failure; MDS, myelodysplastic syndrome.

Close to all patients with MDS fell into high-risk groups with a complex karyotype or isolated monosomy 7 and co-occurring (often multi-hit) *TP53* alterations (Table 1) (7, 19, 24, 28–30). Several patients with acute erythroid leukemia have been reported, either progressing from MDS or as isolated disease, leading to the assumption that this AML subtype seems to be much more prevalent in *ERCC6L2* germline-mutated patients (Table 1) (28). Acute erythroid leukemia, defined by excess of maturation-arrested primitive erythroblasts, is a rare subtype of AML, occurring in about 3% of all AML patients (31). It is characterized by a significantly higher frequency of *TP53* variants (36%), especially bi-allelic/multi-hit *TP53* alterations with relatively lower somatic mutational burden compared to other AML subtypes (31, 32). While acute erythroid leukemia by itself does not seem to carry an additional prognostic impact as independent risk factor (33), its frequent association with complex karyotypes and multi-hit *TP53* alterations does confer to a dismal outcome in at least the subset of cases with these abnormalities (34). Cytogenetic abnormalities in the twelve *ERCC6L2* patients with AML and MDS presented often as a complex or monosomal karyotype with loss of chromosomes 5, 7 or 17 or isolated monosomy 7. In addition, multi-hit *TP53* alterations were reported in seven out of twelve patients with MDS/AML. An assessment of the allelic state of these *TP53* alterations was not performed (Table 1) (7, 19, 24, 28–30). The prognosis of MDS/AML in patients with *ERCC6L2* germline variants is poor, especially when progression to acute erythroid leukemia is noted, with no known survivors of this AML subtype so far (28). HSCT was performed in at least ten individuals (28%), either because of severe, transfusion-dependent BMF or after MDS/AML development (Table 1). Given the high frequency of monosomy 7, complex/monosomal karyotypes and *TP53* variants, which may be associated with disease progression and development of MDS/AML, HSCT should be considered early, especially when these aberrations are discovered in the context of clonal evolution and bone marrow dysplasia.

MECOM deficiency serves as an example of a genotype-phenotype association

Heterozygous variants in *HOXA11* are known to cause radioulnar synostosis (RUS) (a congenital proximal fusion of the radius and ulna) with amegakaryocytic thrombocytopenia (RUSAT) (35). However, families with RUSAT without *HOXA11* variants were reported (36), suggesting that additional candidate genes/loci exist. Consequently, Niihori et al. (12) performed WES on three individuals with RUSAT without an identified variant in *HOXA11* and detected heterozygous missense variants in *MECOM* in all three patients. This was the first time RUSAT has been linked to variants in *MECOM*.

The MDS1-EVI1 complex locus (MECOM) gives rise to several transcripts through alternative splicing of the N-terminus that encode at least three different isoforms: full length EVI1-145 kDa, EVI1-Δ324, which lacks zinc fingers 6 and 7, and MDS1-EVI1. MDS1-EVI1 comprises an N-terminal so-called PRDF1-RIZ homology domain, two C2H2 zinc finger DNA binding domains, one at the N-terminus including seven zinc fingers, and the other at the C-terminus including three zinc fingers, a proline-rich repressor domain and a small aspartate/glutamate-rich acidic region located in the C-terminal region (Figure 2) (37). MECOM acts as crucial transcription factor in hematopoiesis, playing an important role in the formation and self-renewal of long-term hematopoietic stem and progenitor cells (HSPCs) (38) as well as myeloid differentiation through interaction with transcription factors including GATA1 (39), SPI1 (40), RUNX1 (41) and others (42). The inversion or

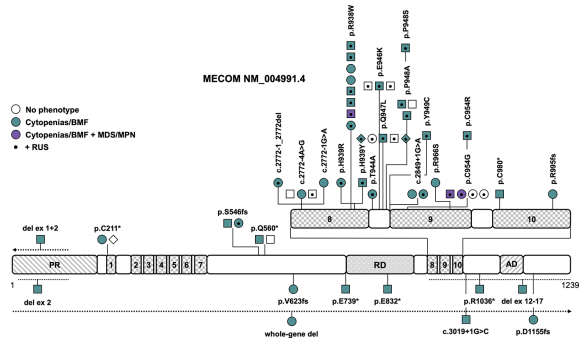

FIGURE 2
Schematic of the *MECOM* transcript (NM_004991.4) and its protein domains with the location of all reported germline variants. Checkered domains with the numbers 1 to 10 stand for zinc fingers 1 to 10. Circles represent females, squares represent males and diamonds represent unknown gender. Symbols on the same horizontal level are individuals from the same family. The color fill depicts different phenotypes, no color fill indicates healthy individuals carrying a causative variant. A dot in the middle of the symbol designates the presence of RUS in these individuals. The dotted lines show the location of the described copy number variants, the arrow denotes that the copy number variant extends in this direction. AD, acidic domain; BMF, bone marrow failure; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; PR, PRDF1-RIZ domain; RD, repression domain; RUS, radioulnar synostosis.

translocation of chromosome 3 drives inv(3)/t(3;3) AML *via* structural rearrangement of an enhancer that upregulates transcription of *EVI1*. It is associated with poor overall survival in AML patients and HSCT is usually mandated whenever possible (43). Furthermore, overexpression of *EVI1* has been reported in 6 to 11% of AML patients without 3q aberrations (44).

Later reports broadened the phenotype caused by germline *MECOM* variants, including BMF without RUS (7, 13, 45–50), predisposition to myeloid malignancies (14, 51), abnormalities of other organ systems (7, 13, 14, 45–47, 49–55), and RUS without any other phenotypic features (56). Causative variants include LOF variants that are scattered across the entire gene and missense variants that are solely located in zinc fingers 8 and 9 (Figure 2 and Tables 1, S1). Confirmed *de novo* variants have been reported in 16% of patients (7, 48–51). The variants R938W and P948A (NM_004991.4) as well as variants affecting the splice sites between exons 7 and 8 have been described in more than one family (Figure 2 and Table 1). Interestingly, there seems to be a rather distinct genotype-phenotype association — all but one variant associated with the co-presentation of RUS and hematologic disease cluster in the region spanning zinc fingers 8 and 9, which includes mostly missense, but also canonical and non-canonical splice variants (Figure 2 and Table 1). Specific missense variants in zinc fingers 8 and 9 (namely R969C/H/L, I971T, and Q965E) have been described in 21 individuals from 6 families with RUS and finger malformations without hematological abnormalities (56). Little is known about this association of RUS with missense variants in zinc fingers 8 and 9. It was shown that the *Evi1* expression pattern is temporally and spatially restricted in mouse embryos with a transient expression in the emerging limb buds (57). *Junbo* mice with an *Evi1* variant affecting zinc finger 9 had extra digits on their forelimbs, suggesting that the C-terminal zinc finger domain may be relevant in digit development (58). Both the *MECOM* missense variants H939R and R969C have displayed attenuated suppression of TGFB1 (12, 56), which has been previously shown to play a role in digit formation during mouse development (59). LOF variants in *MECOM* seem to cause BMF but not RUS. CNVs have been described in four patients and were all confirmed or presumed *de novo* (Figure 2) (46, 48–50). One 751kb 3q26 microdeletion encompassing the entire *MECOM* gene and the pseudogene *EGFEM1P* was reported in one patient with BMF without other phenotypic features (48). Intragenic deletions of exons 1 + 2 (49), exon 2 (50) and exons 12-17 (46), affecting zinc finger and acidic domains, were reported in three patients with BMF at birth and a range of congenital skeletal and/or heart abnormalities. The intragenic deletion of exons 1 and 2 extended to other genes and also included the telomere biology gene *TERC*, so that specific phenotypic features cannot be attributed to either gene in this case (49). Overall penetrance of any related features (RUS/other skeletal abnormalities, deafness, cytopenia/BMF) is high at an estimated 96% (Table 1). Cytopenia/BMF was present in 80% of patients with an average age of onset at birth/in infancy (n=43) (7, 12–14, 45–55, 60). Although cytopenia can be severe and present at birth, even leading to intrauterine death, and may require early HSCT, three cases (5%) with spontaneous resolution or improvement of cytopenia/BMF have been reported (13, 45, 51). RUS was the most frequent non-hematopoietic feature in 54% of patients (7, 12–14, 51–55, 60), followed by brachy-, campto-, and clinodactyly and/or other finger abnormalities in 38% of patients (7, 12, 13, 45, 46, 51–53). Other frequent abnormalities included sensorineural hearing impairment/congenital deafness in 20% (12–14, 51), cardiac abnormalities such as atrial/ventricular septal defects, tetralogy of Fallot, aortic coarctation, pulmonary stenoses/atresias, pulmonary venous return anomaly, patent ductus arteriosus and myocardial atrophy in 18% (7, 13, 47, 49–51, 54) and prematurity, hydrops fetalis or polyhydramnios in 13% of patients (12, 47, 50, 53–55). Less frequent phenotypic features are micro- or macrocephaly, structural brain abnormalities or developmental delay/cognitive impairment in 11% (12, 13, 46, 51, 52), patellar hypoplasia (13, 14), metatarsus adductus, hallux valgus and other toe abnormalities (13, 14) in 9% and clubfoot (7, 51, 52) and renal abnormalities (7, 13, 55) in 7% of patients each (Table 1). Other features such as hip dysplasia (13, 52, 53), cleft palate (12, 13, 49), early-onset ischemic insults (13, 14, 51), facial dysmorphia (7, 49, 51), precocious puberty/gynecomastia in infancy (13), and paralysis of the larynx/laryngomalacia (45, 49) have been described in only two to three individuals and consequently the association with germline *MECOM* variants may not be entirely clear or proven in these cases. Three patients (5%) were reported to develop hematologic malignancies, specifically MDS with refractory cytopenia with multilineage dysplasia at 37 years (51), MDS with excess blasts-2 at 73 years with interstitial deletion of the long arm of chromosome 9 (14) and MDS/myeloproliferative disease-unclassifiable at 42 years with a translocation t(1;14)(q44;q32) (Table 1) (14). All patients reportedly had a history of thrombocytopenia or BMF with earlier onset (14, 51). HSCT has been performed in 27 out of 55 patients (50%) because of severe BMF.

DNAJC21 deficiency- a new Shwachman-Diamond syndrome-like disorder with telomeropathy aspects

DNAJC21 is ubiquitously expressed and encodes a protein with 531 amino acids, containing a highly conserved N-terminal DnaJ molecular chaperone homology domain, a central coiled coil region as well as two zinc fingers (Figure 3). The first studies in yeast showed that it functions together with the cytoplasmic zinc finger protein Znf622 to stimulate the ATPase activity of the Hsp70 chaperone protein Hspa8, thereby initiating the removal/recycling process of Arx1, a ribosome maturation factor (61, 62). In 2016, Tummala et al. screened a cohort of 28 unrelated individuals with BMF and syndromal features by WES and identified 3 individuals with homozygous variants in *DNAJC21*. By targeted re-sequencing of *DNAJC21* in patients with similar phenotype, a fourth patient with a homozygous *DNAJC21* variant was found (15). Functional studies on patient-derived lymphoblastoid cell lines implicated involvement of DNAJC21 in rRNA biogenesis and 60S ribosome maturation — thereby resembling the function of SBDS — leading to decreased interaction with HSPA8, ZNF622 and PA2G4 and

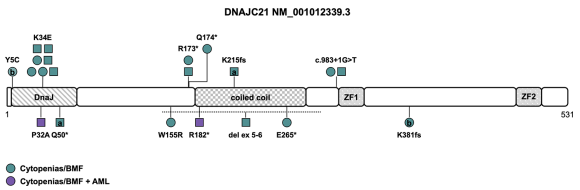

FIGURE 3
Schematic of the *DNAJC21* transcript (NM_001012339.3) and its protein domains with the location of all reported germline variants. Circles represent females and squares represent males. Symbols on the same horizontal level are individuals from the same family. Letters within the symbols indicate individuals with compound heterozygous genotype, while symbols without letters indicate homozygosity for the variant. The color fill depicts different phenotypes. The dotted line shows the location of the sole described copy number variant. AML, acute myeloid leukemia; BMF, bone marrow failure; ZF, zinc finger.

increased cell death in patients with DNAJC21 deficiency (15). So far, 19 patients from 14 different families have been described in the literature (7, 15, 63–67), in 8 families (57%) a history of consanguinity was reported (Table 1). There is no confirmed case of a *de novo* variant in *DNAJC21* reported to date.

Causative variants include missense variants, particularly within the N-terminal DnaJ-domain affecting the amino acids 5, 32 and 34 (Y5C, K34E, P32A, NM_001012339.3), with two of those located in the universally conserved HPD motif (H33-P34-D35), which is essential for stimulation of ATPase activity. The K34E variant is the most common variant described in seven individuals from four families (Figure 3 and Tables 1, S1) (64, 65), reversing the surface charge of a key amino acid adjacent to the HPD motif and also likely disrupting the interaction with HSPA8 (68). The P32A variant potentially alters the fold of the HPD motif, disrupting the interaction with HSPA8 and stimulation of its ATPase activity (68). Truncating variants encompassing nonsense, frameshift and canonical splice site variants (Table S1) are predicted to undergo nonsense mediated decay, leading to significant reduction of DNAJC21 protein expression (7, 15, 65–67). While most patients were homozygous for a causative *DNAJC21* variant, two individuals were found to be compound heterozygous (7, 66). Besides single-nucleotide variants, one individual with a homozygous intragenic deletion of exons 5 and 6 was reported as well (Figure 3 and Table 1) (65).

While some features such as exocrine pancreatic dysfunction are consistent with a (classic) Shwachman-Diamond phenotype (as has been described in patients with bi-allelic inactivation of *SBDS* and less likely of *EFL1* or heterozygous variants in *SRP45* (69)), other features such as skin hypopigmentation, dental and retinal abnormalities seem to resemble characteristics of telomeropathies (64). There is no known genotype-phenotype association. The penetrance of a hematologic phenotype in the sense of single- or multiple lineage cytopenia/BMF seems to be complete (Table 1). The average age of onset for BMF is two years (range 0 to 15 years, n=19). Spontaneous, intermittent or prolonged improvement of cytopenia was reported in at least four patients (64–66), while four patients (21%) needed a HSCT (Table 1). AML developed in two patients (11%) at the age of twelve and fifteen years, respectively (15, 67). Somatic cytogenetic or molecular alterations were unknown or not reported. However, one case of acute megakaryoblastic leukemia was described (15). Other cytogenetic abnormalities, including a complex karyotype (7), a derivative chromosome 15 with translocation t(1;15) (65), a deletion of 17p13 (64) and a deletion of 20q (64) have been reported in patients with BMF without hematologic malignancy and consequently their significance in disease progression and development of MDS/AML is unclear. Growth delay and/or short stature as the most frequent non-hematopoietic feature has been described in all but one patient (95%). Other frequent abnormalities included developmental delay/intellectual disability and/or neurological abnormalities (68%), skeletal abnormalities (particularly hip dysplasia, thoracic deformities, genu valgum and metaphyseal dysplasia) and skin abnormalities (mainly hypopigmentation and palmoplantar cutis laxa) at 63% each, as well as microcephaly (42%), facial dysmorphia (37%), dental abnormalities (32%), and osteopenia/osteoporosis (32%). Pancreas lipomatosis was reported in five cases (26%), with four out of the five patients suffering from exocrine pancreatic dysfunction with preserved endocrine function. Intriguingly, while (high) myopia, astigmatism and other visual field defects were described in six patients (32%), rare features of retinal (rod-cone) dystrophy and other retinal abnormalities were also identified in six patients (32%), albeit one patient developed symptoms after HSCT (65) and another patient carried a homozygous variant in *PCARE*, known to cause an autosomal recessive form of retinitis pigmentosa (Table 1) (65). This suggests retinal abnormalities may be part of the developing phenotype. Due to the low number of reported patients, specific phenotypic features need to be defined and refined over time.

Sixteen Italian patients with Shwachman-Diamond syndrome and bi-allelic *SBDS* variants were screened for additional variants in *DNAJC21*, *EFL1*, and *SRP45*. One of the two germline-mutated *SBDS* patients with compound heterozygous *SBDS* variants and an additional heterozygous *DNAJC21* variant was reported to suffer from a more severe hematologic phenotype, in particular severe neutropenia (70). Both *DNAJC21* variants identified in this study (70), E276K and V342M, are reasonably rare in 0,32% and 0,0016% of the gnomAD population but ensemble *in-silico* predictions are contradicting or in favor of no significant impact on the protein structure.

Digenic ADH5/ALDH2 deficiency causes BMF through defective formaldehyde detoxification

Formaldehyde is a ubiquitous endogenous and environmental metabolite, which has been classified as a group I human carcinogen by the International Agency for Research on Cancer as it may cause nasopharyngeal cancer, lung cancer and is associated with

development of AML (71). Specifically, it was shown that formaldehyde exposure induces chromosomal aneuploidy, in particular aberrations of chromosomes 5, 7, and 8, which are frequently seen in AML (72, 73). It was also reported to induce hematopoietic toxicity to both mature and stem/progenitor cells in the bone marrow of mice exposed to formaldehyde by affecting myeloid progenitor growth and survival through oxidative damage apoptosis and dysregulation of colony stimulating factor receptors (74).

ADH5, located in the cytoplasm, is the most widely expressed alcohol dehydrogenase and the main formaldehyde-detoxifying enzyme (75). It contains a catalytic and zinc-binding domain of the alcohol dehydrogenase (Figure 4). ALDH2, a mitochondrial enzyme oxidizing acetaldehyde to acetate, is important in ethanol metabolism, and deficiency of this enzyme is very common in humans, leading to facial flushing, nausea, headaches, cardiac palpitations, and overall discomfort in response to drinking alcohol (76). The *ALDH2*2* allele, defined by the c.1510G>A (NM_000690.4) variant (rs671), encoding an E504K amino acid substitution, reduces enzyme activity to less than 50% in heterozygotes and less than 4% in homozygotes in a dominant-negative manner (76). It is common in the East Asian population with an allele frequency of about 25% in the gnomAD (https://gnomad.broadinstitute.org/) population. ALDH2, active mainly in detoxifying acetaldehyde, also takes part in formaldehyde detoxification (75).

Dingler et al. described the first seven children/young adults with homozygous or compound heterozygous *ADH5* variants and the heterozygous *ALDH2*2* allele associated with BMF and predisposition to MDS/AML that is solely driven by formaldehyde accumulation (16). A subsequent study by Oka et al. reported seven individuals from five different families with BMF and development of MDS/AML at young age (77). Of those, four individuals were heterozygous and three homozygous for the *ALDH2*2* allele. The three homozygotes were reported to harbor more severe phenotypes including neurological deterioration and early death (77). Because of its association with short stature and intellectual disability, it was subsequently also called AMeD syndrome (for Anemia, Mental retardation and Dwarfism) (10). One male patient with a history of anemia since the age of 8 years who developed MDS at 18 years old was diagnosed with ADH5/ALDH2 deficiency and sensorineural hearing loss based on concurrent compound heterozygous variants in *ADGRV1* (78).

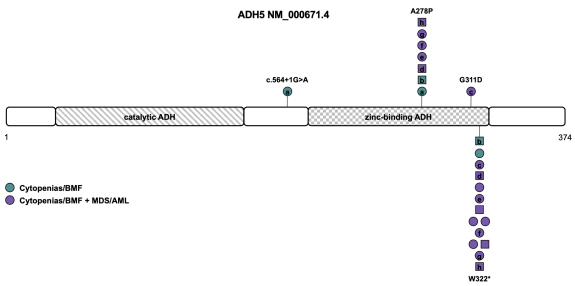

FIGURE 4
Schematic of the *ADH5* transcript (NM_000671.4) and its protein domains with the location of all reported germline variants. Circles represent females and squares represent males. Symbols on the same horizontal level are individuals from the same family. Letters within the symbols indicate individuals with compound heterozygous genotype, while symbols without letters indicate homozygosity for the variant. The color fill depicts different phenotypes. ADH, alcohol dehydrogenase; AML, acute myeloid leukemia; BMF, bone marrow failure; MDS, myelodysplastic syndrome.

Of the 15 individuals from 13 families described so far, 8 were compound heterozygous and seven homozygous for variants in *ADH5*. Consanguinity or occurrence of *de novo* variants was not reported (Table 1). Causative variants include LOF variants c.564+1G>A (NM_000671.4), resulting in retention of intron 5 (L188fs), and the recurrent variant W322*, that has been described in all but one individual (Table S1). Missense variants were located within the zinc-binding domain of ADH5, namely the G311D variant and the recurrent A278P variant found in seven patients (Tables 1, S1 and Figure 4) (16, 77, 78). Penetrance is complete with all patients diagnosed with either BMF or early-onset MDS/AML and nine out of twelve patients (75%) undergoing HSCT (Table 1). Based on the published data, MDS/AML was diagnosed in 12 out of 15 patients (80%) at an average age of 7 years (range 0 to 18 years (n=9), Table 1, Figure 4). In eight out of eleven patients (73%) with reported cytogenetic information, a gain of the long arm of chromosome 1, frequently seen in Fanconi anemia (79, 80) was described. Other recurrent cytogenetic alterations included monosomy 7, trisomy 8 and 21p alterations (Table 1) (16, 77, 78). All patients with reported additional phenotypical data were short of stature and displayed mild to moderate intellectual disability. Microcephaly was described in 67% and abnormal skin pigmentation was found in 58% of patients (Table 1). Although the phenotype mimics Fanconi anemia, radial ray defects have not been detected so far and chromosomal breakage tests are negative. Other features such as retinal degeneration, facial dysmorphia, skeletal, and endocrine abnormalities have only been described in single individuals so that the clinical association remains unclear to date (16, 77, 78).

Using a CRISPR-Cas9 functional screen, *ADH5* was (together with *ESD* and the *FANC* family genes) described as a top candidate gene dramatically increasing cellular formaldehyde sensitivity when disrupted (81). Concordantly, *Adh5*$^{-/-}$ *Aldh2*$^{-/-}$ double-deficient mice recapitulated some of the hematopoietic phenotypes seen in these patients such as reduced proliferation of HSPCs and loss of differentiation (16, 77). Another group reported that *Adh5*$^{-/-}$ deficient mice with wildtype Aldh2 are born and develop normally, while double-deficient mice showed significantly lower body weight, which mimics the short stature seen in humans (82).

Formaldehyde also triggers a cellular redox imbalance that can lead to reactive oxygen species accumulation and cytotoxicity, which may cause BMF development even in the presence of functional DNA repair mechanisms by overwhelming the DNA-repair capacity in HSPCs (83). Using patient-derived lymphoblasts, fibroblasts, induced pluripotent stem cells (iPSCs), and CRISPR/Cas9-engineered cell lines, Mu et al. were able to demonstrate that patient-derived iPSCs were sensitive to exogenous treatment with formaldehyde, which

induced drastically defective cell expansion when stimulated into hematopoietic differentiation and increased levels of DNA damage. This phenotype was attenuated upon expression of *ADH5* and less so by addition of an ALDH2 activator (84). Therapies aiming to lower endogenous formaldehyde could be a promising treatment strategy for ADH5/ALDH2 deficiency. C1, a new small molecule acting as agonist of ALDH2, was well tolerated and able to partially reverse the HSPC expansion/differentiation defect in iPSCs *in vitro* (84). The combination of a formaldehyde scavenger such as metformin and glutathione precursors (for instance N-acetyl-L-cysteine) (83) may also benefit patients with Fanconi anemia.

In whose transcriptional reprogramming during differentiation of HSPCs may lead to acute accumulation of endogenous DNA damage, most likely arising from formaldehyde, an obligate by-product during transcriptional regulation (85). Further studies are needed to determine if aldehydes are the major cause of pathology in Fanconi anemia patients, who have functional ALDH2 and ADH5 to mediate aldehyde metabolism.

Discussion

There may be a confounding bias for all described syndromes by the short period of clinical observations since these syndromes have been discovered. The likelihood of developing hematologic malignancies and the penetrance of these diseases may be estimated as too low since many patients are still children or young adults and others already underwent HSCT to treat severe early-onset BMF.

Suspicion of an inherited BMF syndrome should arise when patients are diagnosed with BMF in infancy/early-childhood (7) and/or MDS at young age (below 40 years old) (29, 30). A positive family history and other organ manifestations also point towards an inherited rather than acquired BMF syndrome (2, 7, 17). Germline BMF panel-based next-generation sequencing (NGS) is a reasonable first-tier option (86). WES or whole-genome sequencing (WGS) should be considered when suspicion of an inherited BMF syndrome is high and initial panel-based results are negative. WES covers all coding genes, however, if genes have not been described as candidate genes at the time of the analysis, the genetic variant causing the phenotype may be missed. Regular re-analysis of WES, as was done in one patient with BMF and bi-allelic *DNAJC21* variants (66) should be included (87). Even if this initially may only increase the number of variants of unknown significance (88), these could be upgraded over time when new information such as observation in multiple probands, segregation with disease, or functional impact of the variant becomes available. Intragenic and whole-gene CNVs were described in *DNAJC21* (65), *ERCC6L2* (24), and *MECOM* (46, 48–50), so that high-density microarrays or bioinformatic analysis of panel-based NGS/WES data have to be incorporated (89). Non-canonical, deeply intronic or exonic synonymous splice variants may require additional RNA sequencing to unravel the effects on splicing and prove pathogenicity of these unusual but not infrequent variants (89–91).

Crucially, unrecognized inherited BMF syndromes may lead to use of related donors carrying the same variants (29), as well as excessive death upon HSCT, which can be reduced using tailored conditioning regimens (92). Adapted non-myeloablative conditioning protocols have been used successfully as conditioning regimens in telomere biology disorders and Fanconi anemia (93, 94). A small case series of six patients with germline *MECOM* variants reported that reduced-intensity conditioning was an effective treatment and reduced toxicity-related morbidities (95). However, comprehensive HSCT data from patients with germline *ERCC6L2, MECOM, DNAJC21*, and *ADH5/ALDH2* variants are lacking to date, including donor choice, conditioning regimens and non-relapse morbidity and mortality.

A clear genotype-phenotype correlation has so far only been established for *MECOM* variants, where the co-presentation of RUS and hematologic disease appears to be caused by variants spanning zinc fingers 8 and 9. Although there is indication that homozygosity for the *ALDH2*2* allele may lead to a more severe (neurological) phenotype in patients with ADH5/ALDH2 deficiency (77), the number of individuals is too low to draw comprehensive conclusions at this time. The discovery of additional mechanisms of disease and amino acid hotspots may help predict the individual, variant-based risk of hematologic malignancies, severe BMF and other phenotypic features.

Some data suggest clonal genetic markers of disease progression such as (multi-hit) *TP53* variants in patients with *ERCC6L2* germline variants may be indicative of disease progression (24, 28–30) and given the poor prognosis of MDS/acute erythroid leukemia in these patients, early HSCT should be performed. Similar data have been reported for *TP53* variants in other inherited BMF syndromes (96, 97), although a clear link to disease progression in patients with somatic *TP53* variants in the absence of other high-risk molecular or cytogenetic markers is unclear (98). Early pre-emptive HSCT comes also with the potential of HSCT-related mortality and morbidity so that more valid early markers of disease progression are needed (99).

In summary, this review provides new insights into four distinct and recently described BMF syndromes and will thereby improve the clinical management for these patients. New data will over time further refine these syndromes and add more pieces to the yet unsolved puzzle of inherited BMF syndromes.

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SF conceptualized and designed the study and wrote the manuscript.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1128533/full#supplementary-material

References

1. Giudice V, Selleri C. Aplastic anemia: Pathophysiology. *Semin Hematol* (2022) 59 (1):13–20. doi: 10.1053/j.seminhematol.2021.12.002

2. Savage SA, Dufour C. Classical inherited bone marrow failure syndromes with high risk for myelodysplastic syndrome and acute myelogenous leukemia. *Semin Hematol* (2017) 54(2):105–14. doi: 10.1053/j.seminhematol.2017.04.004

3. Pecci A, Balduini CL. Inherited thrombocytopenias: an updated guide for clinicians. *Blood Rev* (2021) 48:100784. doi: 10.1016/j.blre.2020.100784

4. Sieff C. *Diamond-blackfan anemia*. Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW and Amemiya A, editors. GeneReviews® [Internet] Seattle (WA): University of Washington, Seattle (1993).

5. Revy P, Kannengiesser C, Bertuch AA. Genetics of human telomere biology disorders. *Nat Rev Genet* (2023) 24(2):86–108. doi: 10.1038/s41576-022-00527-z

6. Mehta PA, Ebens C. *Fanconi anemia*. Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, et al, editors. GeneReviews® [Internet] Seattle (WA): University of Washington, Seattle (1993). Available at: http://www.ncbi.nlm.nih.gov/pubmed/20301575. GeneReviews®.

7. Bluteau O, Sebert M, Leblanc T, Peffault de Latour R, Quentin S, Lainey E, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood* (2018) 131(7):717–32. doi: 10.1182/blood-2017-09-806489

8. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* (2020) 40(1):24–64. doi: 10.1007/s10875-019-00737-x

9. Lobitz S, Velleuer E. Guido Fanconi (1892-1979): a jack of all trades. *Nat Rev Cancer* (2006) 6(11):893–8. doi: 10.1038/nrc2009

10. Strathdee CA, Gavish H, Shannon WR, Buchwald M. Cloning of cDNAs for fanconi's anaemia by functional complementation. *Nature* (1992) 356(6372):763-7. doi: 10.1038/356763a0

11. Tummala H, Kirwan M, Walne AJ, Hossain U, Jackson N, Pondarre C, et al. ERCC6L2 mutations link a distinct bone-Marrow-Failure syndrome to DNA repair and mitochondrial function. *Am J Hum Genet* (2014) 94(2):246–56. doi: 10.1016/j.ajhg.2014.01.007

12. Niihori T, Ouchi-Uchiyama M, Sasahara Y, Kaneko T, Hashii Y, Irie M, et al. Mutations in MECOM, encoding oncoprotein EVI1, cause radioulnar synostosis with amegakaryocytic thrombocytopenia. *Am J Hum Genet* (2015) 97(6):848–54. doi: 10.1016/j.ajhg.2015.10.010

13. Germeshausen M, Ancliff P, Estrada J, Metzler M, Ponstingl E, Rütschle H, et al. MECOM-associated syndrome: a heterogeneous inherited bone marrow failure syndrome with amegakaryocytic thrombocytopenia. *Blood Adv* (2018) 2(6):586–96. doi: 10.1182/bloodadvances.2018016501

14. Ripperger T, Hofmann W, Koch JC, Shirneshan K, Haase D, Wulf G, et al. MDS1 and EVI1 complex locus (MECOM): a novel candidate gene for hereditary hematological malignancies. *Haematologica* (2018) 103(2):e55–8. doi: 10.3324/haematol.2017.178723

15. Tummala H, Walne AJ, Williams M, Bockett N, Collopy L, Cardoso S, et al. DNAJC21 mutations link a cancer-prone bone marrow failure syndrome to corruption in 60S ribosome subunit maturation. *Am J Hum Genet* (2016) 99(1):115–24. doi: 10.1016/j.ajhg.2016.05.002

16. Dingler FA, Wang M, Mu A, Millington CL, Oberbeck N, Watcham S, et al. Two aldehyde clearance systems are essential to prevent lethal formaldehyde accumulation in mice and humans. *Mol Cell* (2020) 80(6):996–1012.e9. doi: 10.1016/j.molcel.2020.10.012

17. Wegman-Ostrosky T, Savage SA. The genomics of inherited bone marrow failure: from mechanism to the clinic. *Br J Haematol* (2017) 177(4):526–42. doi: 10.1111/bjh.14535

18. Zhang S, Pondarre C, Pennarun G, Labussiere-Wallet H, Vera G, France B, et al. A nonsense mutation in the DNA repair factor hebo causes mild bone marrow failure and microcephaly. *J Exp Med* (2016) 213(6):1011–28. doi: 10.1084/jem.20151183

19. Tummala H, Dokal AD, Walne A, Ellison A, Cardoso S, Amirthasigamanipillai S, et al. Genome instability is a consequence of transcription deficiency in patients with bone marrow failure harboring biallelic ERCC6L2 variants. *Proc Natl Acad Sci* (2018) 115(30):7777–82. doi: 10.1073/pnas.1803275115

20. Liu X, Liu T, Shang Y, Dai P, Zhang W, Lee BJ, et al. ERCC6L2 promotes DNA orientation-specific recombination in mammalian cells. *Cell Res* (2020) 30(9):732–44. doi: 10.1038/s41422-020-0328-3

21. Olivieri M, Cho T, Álvarez-Quilón A, Li K, Schellenberg MJ, Zimmermann M, et al. A genetic map of the response to DNA damage in human cells. *Cell* (2020) 182 (2):481–96. doi: 10.1016/j.cell.2020.05.040

22. Francica P, Mutlu M, Blomen VA, Oliveira C, Nowicka Z, Trenner A, et al. Functional radiogenetic profiling implicates ERCC6L2 in non-homologous end joining. *Cell Rep* (2020) 32(8):108068. doi: 10.1016/j.celrep.2020.108068

23. Armes H, Bewicke-Copley F, Rio-Machin A, di Bella D, Philippe C, Wozniak A, et al. Germline ERCC excision repair 6 like 2 (ERCC6L2) mutations lead to impaired erythropoiesis and reshaping of the bone marrow microenvironment. *Br J Haematol* (2022) 199(5):754–64. doi: 10.1111/bjh.18466

24. Thams S, Islam M, Lindefeldt M, Nordgren A, Granberg T, Tesi B, et al. Heterozygous variants in DCC. *Neurol Genet* (2020) 6(6):e526. doi: 10.1212/NXG.0000000000000526

25. Shabanova I, Cohen E, Cada M, Vincent A, Cohn RD, Dror Y. ERCC6L2-associated inherited bone marrow failure syndrome. *Mol Genet Genomic Med* (2018) 6 (3):463–8. doi: 10.1002/mgg3.388

26. Slatter MA, Gennery AR. Update on DNA-double strand break repair defects in combined primary immunodeficiency. *Curr Allergy Asthma Rep* (2020) 20(10):57. doi: 10.1007/s11882-020-00955-z

27. Järviaho T, Halt K, Hirvikoski P, Moilanen J, Möttönen M, Niinimäki R. Bone marrow failure syndrome caused by homozygous frameshift mutation in the ERCC6L2 gene. *Clin Genet* (2018) 93(2):392–5. doi: 10.1111/cge.13125

28. Douglas SPM, Siipola P, Kovanen PE, Pyörälä M, Kakko S, Savolainen ER, et al. ERCC6L2 defines a novel entity within inherited acute myeloid leukemia. *Blood* (2019) 133(25):2724–8. doi: 10.1182/blood-2019-01-896233

29. Feurstein SK, Trottier AM, Estrada-Merly N, Pozsgai MJ, McNeely KE, Drazer MW, et al. Germline predisposition variants occur in myelodysplastic syndrome patients of all ages. *Blood* (2022) 140(24):2533–48. doi: 10.1182/blood.2022015790

30. Feurstein S, Churpek JE, Walsh T, Keel S, Hakkarainen M, Schroeder T, et al. Germline variants drive myelodysplastic syndrome in young adults. *Leukemia* (2021) 35(8):2439–44. doi: 10.1038/s41375-021-01137-0

31. Rose D, Haferlach T, Schnittger S, Perglerová K, Kern W, Haferlach C. Subtype-specific patterns of molecular mutations in acute myeloid leukemia. *Leukemia* (2017) 31(1):11–7. doi: 10.1038/leu.2016.163

32. Montalban-Bravo G, Benton CB, Wang SA, Ravandi F, Kadia T, Cortes J, et al. More than 1 TP53 abnormality is a dominant characteristic of pure erythroid leukemia. *Blood* (2017) 129(18):2584–7. doi: 10.1182/blood-2016-11-749903

33. Santos FPS, Faderl S, Garcia-Manero G, Koller C, Beran M, O'Brien S, et al. Adult acute erythroleukemia: an analysis of 91 patients treated at a single institution. *Leukemia* (2009) 23(12):2275–80. doi: 10.1038/leu.2009.181

34. Boddu P, Benton CB, Wang W, Borthakur G, Khoury JD, Pemmaraju N. Erythroleukemia-historical perspectives and recent advances in diagnosis and management. *Blood Rev* (2018) 32(2):96–105. doi: 10.1016/j.blre.2017.09.002

35. Thompson AA, Nguyen LT. Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with HOXA11 mutation. *Nat Genet* (2000) 26(4):397–8. doi: 10.1038/82511

36. Castillo-Caro P, Dhanraj S, Haut P, Robertson K, Dror Y, Sharathkumar AA. Proximal radio-ulnar synostosis with bone marrow failure syndrome in an infant without a HOXA11 mutation. *J Pediatr Hematol Oncol* (2010) 32(6):479–85. doi: 10.1097/MPH.0b013e3181e5129d

37. Maicas M, Vázquez I, Alis R, Marcotegui N, Urquiza L, Cortés-Lavaud X, et al. The MDS and EVI1 complex locus (MECOM) isoforms regulate their own transcription and have different roles in the transformation of hematopoietic stem and progenitor cells. *Biochim Biophys Acta (BBA) - Gene Regul Mech* (2017) 1860 (6):721–9. doi: 10.1016/j.bbagrm.2017.03.007

38. Kataoka K, Sato T, Yoshimi A, Goyama S, Tsuruta T, Kobayashi H, et al. Evi1 is essential for hematopoietic stem cell self-renewal, and its expression marks hematopoietic cells with long-term multilineage repopulating activity. *J Exp Med* (2011) 208(12):2403–16. doi: 10.1084/jem.20110447

39. Laricchia-Robbio L, Fazzina R, Li D, Rinaldi CR, Sinha KK, Chakraborty S, et al. Point mutations in two EVI1 zn fingers abolish EVI1-GATA1 interaction and allow erythroid differentiation of murine bone marrow cells. *Mol Cell Biol* (2006) 26 (20):7658–66. doi: 10.1128/MCB.00363-06

40. Laricchia-Robbio L, Premanand K, Rinaldi CR, Nucifora G. EVI1 impairs myelopoiesis by deregulation of PU.1 function. *Cancer Res* (2009) 69(4):1633–42. doi: 10.1158/0008-5472.CAN-08-2562

41. Senyuk V, Sinha KK, Li D, Rinaldi CR, Yanamandra S, Nucifora G. Repression of RUNX1 activity by EVI1: A new role of EVI1 in leukemogenesis. *Cancer Res* (2007) 67(12):5658–66. doi: 10.1158/0008-5472.CAN-06-3962

42. Voit RA, Tao L, Yu F, Cato LD, Cohen B, Fleming TJ, et al. A genetic disorder reveals a hematopoietic stem cell regulatory network co-opted in leukemia. *Nat Immunol* (2023) 24(1):69–83. doi: 10.1038/s41590-022-01370-4

43. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* (2022) 140(12):1345–77. doi: 10.1182/blood.2022016867

44. Hinai AA, Valk PJM. Review: Aberrant EVI1 expression in acute myeloid leukaemia. *Br J Haematol* (2016) 172(6):870–8. doi: 10.1111/bjh.13898

45. Weizmann D, Pincez T, Roussy M, Vaillancourt N, Champagne J, Laverdière C. New MECOM variant in a child with severe neonatal cytopenias spontaneously resolving. *Pediatr Blood Cancer* (2020) 67(5). doi: 10.1002/pbc.28215

46. van der Veken LT, Maiburg MC, Groenendaal F, van Gijn ME, Bloem AC, Erpelinck C, et al. Lethal neonatal bone marrow failure syndrome with multiple congenital abnormalities, including limb defects, due to a constitutional deletion of 3′ MECOM. *Haematologica* (2018) 103(4):e173–6. doi: 10.3324/haematol.2017.185033

47. Deliloğlu B, Tüfekçi Ö, Tüzün F, Aykut A, Ceylan Eİ, Durmaz A, et al. A novel mecom gene mutation associated with amegakaryocytic thrombocytopenia in a premature infant. *Turk J Pediatr* (2022) 64(4):736. doi: 10.24953/turkjped.2021.4855

48. Nielsen M, Vermont CL, Aten E, Ruivenkamp CAL, van Herrewegen F, Santen GWE, et al. Deletion of the 3q26 region including the EVI1 and MDS1 genes in a neonate with congenital thrombocytopenia and subsequent aplastic anaemia. *J Med Genet* (2012) 49(9):598–600. doi: 10.1136/jmedgenet-2012-100990

49. Bouman A, Knegt L, Gröschel S, Erpelinck C, Sanders M, Delwel R, et al. Congenital thrombocytopenia in a neonate with an interstitial microdeletion of 3q26.2q26.31. *Am J Med Genet A* (2016) 170A(2):504–9. doi: 10.1002/ajmg.a.37451

50. Kjeldsen E, Veigaard C, Aggerholm A, Hasle H. Congenital hypoplastic bone marrow failure associated with a *de novo* partial deletion of the MECOM gene at 3q26.2. *Gene* (2018) 656:86–94. doi: 10.1016/j.gene.2018.02.061

51. Walne A, Tummala H, Ellison A, Cardoso S, Sidhu J, Sciuccati G, et al. Expanding the phenotypic and genetic spectrum of radioulnar synostosis associated hematological disease. *Haematologica* (2018) 103(7):e284–7. doi: 10.3324/haematol.2017.183855

52. Niihori T, Tanoshima R, Sasahara Y, Sato A, Irie M, Saito-Nanjo Y, et al. Phenotypic heterogeneity in individuals with MECOM variants in 2 families. *Blood Adv* (2022) 6(18):5257–61. doi: 10.1182/bloodadvances.2020003812

53. Lord S v, Jimenez JE, Kroeger ZA, Patrick CS, Sanchez-Pena I, Ziga E, et al. A MECOM variant in an African American child with radioulnar synostosis and thrombocytopenia. *Clin Dysmorphol* (2018) 27(1):9–11. doi: 10.1097/MCD.0000000000000200

54. Coste T, Vincent-Delorme C, Stichelbout M, Devisme L, Gelot A, Deryabin I, et al. COL4A1/COL4A2 and inherited platelet disorder gene variants in fetuses showing intracranial hemorrhage. *Prenat Diagn* (2022) 42(5):601–10. doi: 10.1002/pd.6113

55. Al-Abboh H, Zahra A, Adekile A. A novel MECOM variant associated with congenital amegakaryocytic thrombocytopenia and radioulnar synostosis. *Pediatr Blood Cancer* (2022) 69(12):e29761. doi: 10.1002/pbc.29761

56. Shen F, Yang Y, Zheng Y, Li P, Luo Z, Fu Y, et al. MECOM-related disorder: Radioulnar synostosis without hematological aberration due to unique variants. *Genet Med* (2022) 24(5):1139–47. doi: 10.1016/j.gim.2022.01.021

57. Wieser R. The oncogene and developmental regulator EVI1: expression, biochemical properties, and biological functions. *Gene* (2007) 396(2):346–57. doi: 10.1016/j.gene.2007.04.012

58. Parkinson N, Hardisty-Hughes RE, Tateossian H, Tsai HT, Brooker D, Morse S, et al. Mutation at the Evi1 locus in junbo mice causes susceptibility to otitis media. *PLoS Genet* (2006) 2(10):e149. doi: 10.1371/journal.pgen.0020149

59. Sader F, Roy S. Tgf-β superfamily and limb regeneration: Tgf-β to start and bmp to end. *Dev Dyn* (2022) 251(6):973–87. doi: 10.1002/dvdy.379

60. Loganathan A, Munirathnam D, Ravikumar T. A novel mutation in the MECOM gene causing radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT-2) in an infant. *Pediatr Blood Cancer* (2018) 66(4): e27574. doi: 10.1002/pbc.27574

61. Meyer AE, Hoover LA, Craig EA. The cytosolic J-protein, Jjj1, and Rei1 function in the removal of the pre-60 s subunit factor Arx1. *J Biol Chem* (2010) 285(2):961–8. doi: 10.1074/jbc.M109.038349

62. Meyer AE, Hung NJ, Yang P, Johnson AW, Craig EA. The specialized cytosolic J-protein, Jjj1, functions in 60S ribosomal subunit biogenesis. *Proc Natl Acad Sci U.S.A.* (2007) 104(5):1558–63. doi: 10.1073/pnas.0610704104

63. Alsavaf MB, Verboon JM, Dogan ME, Azizoglu ZB, Okus FZ, Ozcan A, et al. A novel missense mutation outside the DNAJ domain of DNAJC21 is associated with shwachman–diamond syndrome. *Br J Haematol* (2022) 197(6):e88–93. doi: 10.1111/bjh.18112

64. D'Amours G, Lopes F, Gauthier J, Saillour V, Nassif C, Wynn R, et al. Refining the phenotype associated with biallelic DNAJC21 mutations. *Clin Genet* (2018) 94 (2):252–8. doi: 10.1111/cge.13370

65. Dhanraj S, Matveev A, Li H, Lauhasurayotin S, Jardine L, Cada M, et al. Biallelic mutations in DNAJC21 cause shwachman-diamond syndrome. *Blood* (2017) 129 (11):1557–62. doi: 10.1182/blood-2016-08-735431

66. Chirita-Emandi A, Petrescu CAM, Zimbru CG, Stoica F, Marian C, Ciubotaru A, et al. Case report: Novel biallelic variants in DNAJC21 causing an inherited bone marrow failure spectrum phenotype: An odyssey to diagnosis. *Front Genet* (2022) 13:870233/full. doi: 10.3389/fgene.2022.870233/full

67. Ji J, Shen L, Bootwalla M, Quindipan C, Tatarinova T, Maglinte DT, et al. A semiautomated whole-exome sequencing workflow leads to increased diagnostic yield and identification of novel candidate variants. *Mol Case Stud* (2019) 5(2):a003756. doi: 10.1101/mcs.a003756

68. Warren AJ. Molecular basis of the human ribosomopathy shwachman-diamond syndrome. *Adv Biol Regul* (2018) 67:109–27. doi: 10.1016/j.jbior.2017.09.002

69. Nelson A, Myers K. *Shwachman-diamond syndrome* Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW and Amemiya A, editors. GeneReviews® [Internet] Seattle (WA): University of Washington, Seattle (1993). Available at: http://www.ncbi.nlm.nih.gov/pubmed/20301722.

70. Morini J, Nacci L, Babini G, Cesaro S, Valli R, Ottolenghi A, et al. Whole exome sequencing discloses heterozygous variants in the DNAJC21 and EFL1 genes but not in SRP54 in 6 out of 16 patients with shwachman-diamond syndrome carrying biallelic SBDS mutations. *Br J Haematol* (2019) 185(3):627–30. doi: 10.1111/bjh.15594

71. Protano C, Buomprisco G, Cammalleri V, Pocino RN, Marotta D, Simonazzi S, et al. The carcinogenic effects of formaldehyde occupational exposure: A systematic review. *Cancers (Basel)* (2021) 14(1):165. doi: 10.3390/cancers14010165

72. Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, et al. Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. *Cancer Epidemiol Biomarkers Prev* (2010) 19(1):80–8. doi: 10.1158/1055-9965.EPI-09-0762

73. Lan Q, Smith MT, Tang X, Guo W, Vermeulen R, Ji Z, et al. Chromosome-wide aneuploidy study of cultured circulating myeloid progenitor cells from workers occupationally exposed to formaldehyde. *Carcinogenesis* (2015) 36(1):160–7. doi: 10.1093/carcin/bgu229

74. Wei C, Wen H, Yuan L, McHale CM, Li H, Wang K, et al. Formaldehyde induces toxicity in mouse bone marrow and hematopoietic stem/progenitor cells and enhances benzene-induced adverse effects. *Arch Toxicol* (2017) 91(2):921–33. doi: 10.1007/s00204-016-1760-5

75. Edenberg HJ, McClintick JN. Alcohol dehydrogenases, aldehyde dehydrogenases, and alcohol use disorders: A critical review. *Alcohol Clin Exp Res* (2018) 42(12):2281–97. doi: 10.1111/acer.13904

76. Matsumura Y, Stiles KM, Reid J, Frenk EZ, Cronin S, Pagovich OE, et al. Gene therapy correction of aldehyde dehydrogenase 2 deficiency. *Mol Ther Methods Clin Dev* (2019) 15:72–82. doi: 10.1016/j.omtm.2019.08.004

77. Oka Y, Hamada M, Nakazawa Y, Muramatsu H, Okuno Y, Higasa K, et al. Digenic mutations in ALDH2 and ADH5 impair formaldehyde clearance and cause a multisystem disorder, AMeD syndrome. *Sci Adv* (2020) 6(51). doi: 10.1126/sciadv.abd7197

78. Kinoshita S, Ando M, Ando J, Ishii M, Furukawa Y, Tomita O, et al. Trigenic ADH5/ALDH2/ADGRV1 mutations in myelodysplasia with usher syndrome. *Heliyon* (2021) 7(8):e07804. doi: 10.1016/j.heliyon.2021.e07804

79. Sebert M, Gachet S, Leblanc T, Rousseau A, Bluteau O, Kim R, et al. Clonal hematopoiesis driven by chromosome 1q/MDM4 trisomy defines a canonical route toward leukemia in fanconi anemia. *Cell Stem Cell* (2023) 30(2):153–70. doi: 10.1016/j.stem.2023.01.006

80. Merfort LW, Lisboa M de O, Cavalli LR, Bonfim CMS. Cytogenetics in fanconi anemia: The importance of follow-up and the search for new biomarkers of genomic instability. *Int J Mol Sci* (2022) 23(22):14119. doi: 10.3390/ijms232214119

81. Zhao Y, Wei L, Tagmount A, Loguinov A, Sobh A, Hubbard A, et al. Applying genome-wide CRISPR to identify known and novel genes and pathways that modulate formaldehyde toxicity. *Chemosphere* (2021) 269:128701. doi: 10.1016/j.chemosphere.2020.128701

82. Nakamura J, Holley DW, Kawamoto T, Bultman SJ. The failure of two major formaldehyde catabolism enzymes (ADH5 and ALDH2) leads to partial synthetic lethality in C57BL/6 mice. *Genes Environ* (2020) 42(1):21. doi: 10.1186/s41021-020-00160-4

83. Umansky C, Morellato AE, Rieckher M, Scheidegger MA, Martinefski MR, Fernández GA, et al. Endogenous formaldehyde scavenges cellular glutathione resulting in redox disruption and cytotoxicity. *Nat Commun* (2022) 13(1):745. doi: 10.1038/s41467-022-28242-7

84. Mu A, Hira A, Niwa A, Osawa M, Yoshida K, Mori M, et al. Analysis of disease model iPSCs derived from patients with a novel fanconi anemia–like IBMFS ADH5/ALDH2 deficiency. *Blood* (2021) 137(15):2021–32. doi: 10.1182/blood.2020009111

85. Shen X, Wang R, Kim MJ, Hu Q, Hsu CC, Yao J, et al. A surge of DNA damage links transcriptional reprogramming and hematopoietic deficit in fanconi anemia. *Mol Cell* (2020) 80(6):1013–24. doi: 10.1016/j.molcel.2020.11.040

86. Gálvez E, Vallespín E, Arias-Salgado EG, Sánchez-Valdepeñas C, Giménez Y, Navarro S, et al. Next-generation sequencing in bone marrow failure syndromes and isolated cytopenias: Experience of the Spanish network on bone marrow failure syndromes. *Hemasphere* (2021) 5(4):e539. doi: 10.1097/HS9.0000000000000539

87. Robertson AJ, Tan NB, Spurdle AB, Metke-Jimenez A, Sullivan C, Waddell N. Re-analysis of genomic data: An overview of the mechanisms and complexities of clinical adoption. *Genet Med* (2022) 24(4):798–810. 10.1016/j.gim.2021.12.011

88. Mørup SB, Nazaryan-Petersen L, Gabrielaite M, Reekie J, Marquart H v, Hartling HJ, et al. Added value of reanalysis of whole exome- and whole genome sequencing data from patients suspected of primary immune deficiency using an extended gene panel and structural variation calling. *Front Immunol* (2022) 13:906328/full. doi: 10.3389/fimmu.2022.906328/full

89. Feurstein S, Drazer M, Godley LA. Germline predisposition to hematopoietic malignancies. *Hum Mol Genet* (2021) 30(R2):R225–35. doi: 10.1093/hmg/ddab141/6294510

90. Blombery P, Fox L, Ryland GL, Thompson ER, Lickiss J, McBean M, et al. Utility of clinical comprehensive genomic characterization for diagnostic categorization in patients presenting with hypocellular bone marrow failure syndromes. *Haematologica* (2020) 106(1):64–73. doi: 10.3324/haematol.2019.237693

91. Wlodarski MW. The arrival of personalized genomics in bone marrow failure. *Haematologica* (2021) 106(1):11–3. doi: 10.3324/haematol.2020.265124

92. McReynolds LJ, Rafati M, Wang Y, Ballew BJ, Kim J, Williams V v, et al. Genetic testing in severe aplastic anemia is required for optimal hematopoietic cell transplant outcomes. *Blood* (2022) 140(8):909–21. doi: 10.1182/blood.2022016508

93. Fink O, Even-Or E, Avni B, Grisariu S, Zaidman I, Schejter YD, et al. Two decades of stem cell transplantation in patients with fanconi anemia: Analysis of factors affecting transplant outcomes. *Clin Transplant* (2023) 37(1). doi: 10.1111/ctr.14835

94. Tummala H, Walne A, Dokal I. The biology and management of dyskeratosis congenita and related disorders of telomeres. *Expert Rev Hematol* (2022) 15(8):685–96. doi: 10.1080/17474086.2022.2108784

95. Irie M, Niihori T, Nakano T, Suzuki T, Katayama S, Moriya K, et al. Reduced-intensity conditioning is effective for allogeneic hematopoietic stem cell transplantation in infants with MECOM-associated syndrome. *Int J Hematol* (2022). doi: 10.1007/s12185-022-03505-7

96. Toki T, Yoshida K, Wang R, Nakamura S, Maekawa T, Goi K, et al. *De novo* mutations activating germline TP53 in an inherited bone-Marrow-Failure syndrome. *Am J Hum Genet* (2018) 103(3):440–7. doi: 10.1016/j.ajhg.2018.07.020

97. Kennedy AL, Myers KC, Bowman J, Gibson CJ, Camarda ND, Furutani E, et al. Distinct genetic pathways define pre-malignant versus compensatory clonal hematopoiesis in shwachman-diamond syndrome. *Nat Commun* (2021) 12(1):1334. doi: 10.1038/s41467-021-21588-4

98. Choijilsuren HB, Park Y, Jung M. Mechanisms of somatic transformation in inherited bone marrow failure syndromes. *Hematol Am Soc Hematol Educ Program* (2021) 2021(1):390–8. doi: 10.1182/hematology.2021000271

99. Pierri F, Faraci M, Giardino S, Dufour C. Hematopoietic stem cell transplantation for classical inherited bone marrow failure syndromes: an update. *Expert Rev Hematol* (2021) 14(10):911–25. doi: 10.1080/17474086.2021.1977119

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Hematopoietic cell transplantation and gene therapy for Diamond-Blackfan anemia: state of the art and science

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Diamond-Blackfan anemia (DBA) is one of the most common inherited causes of bone marrow failure in children. DBA typically presents with isolated erythroid hypoplasia and anemia in infants. Congenital anomalies are seen in 50% of the patients. Over time, many patients experience panhematopoietic defects resulting in immunodeficiency and multilineage hematopoietic cytopenias. Additionally, DBA is associated with increased risk of myelodysplastic syndrome, acute myeloid leukemia and solid organ cancers. As a prototypical ribosomopathy, DBA is caused by heterozygous loss-of-function mutations or deletions in over 20 ribosomal protein genes, with *RPS19* being involved in 25% of patients. Corticosteroids are the only effective initial pharmacotherapy offered to transfusion-dependent patients aged 1 year or older. However, despite good initial response, only ~20-30% remain steroid-responsive while the majority of the remaining patients will require life-long red blood cell transfusions. Despite continuous chelation, iron overload and related toxicities pose a significant morbidity problem. Allogeneic hematopoietic cell transplantation (HCT) performed to completely replace the dysfunctional hematopoietic stem and progenitor cells is a curative option associated with potentially uncontrollable risks. Advances in HLA-typing, conditioning regimens, infection management, and graft-versus-host-disease prophylaxis have led to improved transplant outcomes in DBA patients, though survival is suboptimal for adolescents and adults with long transfusion-history and patients lacking well-matched donors. Additionally, many patients lack a suitable donor. To address this gap and to mitigate the risk of graft-versus-host disease, several groups are working towards developing autologous genetic therapies to provide another curative option for DBA patients across the whole age spectrum. In this review, we summarize the results of HCT studies and review advances and potential future directions in hematopoietic stem cell-based therapies for DBA.

KEYWORDS

Diamond-Blackfan anemia, DBA, anemia, HCT, HSCT, gene therapy, RPS19, ribosomopathy

Introduction

Diamond-Blackfan anemia (DBA) is an intriguing and enigmatic disease that has held the attention of clinicians and scientists alike for close to a century. First described as congenital hypoplastic anemia in 1936 (1, 2), it is characterized by macrocytic anemia, reticulocytopenia, erythroblastopenia, and constitutional anomalies including craniofacial, skeletal, and cardiac abnormalities in about 50% of the patients (3). DBA is an ultra-rare disease, with an incidence of 2-8 per million live births per year (3, 4). Approximately 90% of the patients are diagnosed in the first year of life with 35% presenting in the first 4 months of life with progressive anemia (3, 5). The phenotypic presentation is diverse and can widely vary in between family members with the same mutation (5). Despite profound improvements in our understanding of the molecular mechanisms of this disorder, no newer therapies have met clinical approval since original report of corticosteroids as an effective option in 1951 (6).

Pathophysiology and genetics

DBA is a ribosomopathy caused by loss-of-function mutations or deletions involving ribosomal protein genes (5). Out of the 83 ribosomal protein (RP) genes, 23 RP genes have so far been reported to be associated with DBA, where the clinical features are secondary to impaired ribosomal biogenesis (3). *RPS19* mutations account for 25% of cases. Very rarely, mutations in non-RP genes such as *EPO* and *GATA1* have been reported cause a DBA-like disease characterized by erythroblastopenia, without any impairment ribosomal biogenesis (5). Several studies have partially elucidated the mechanisms of DBA-associated anemia. DBA bone marrow shows increased apoptosis and impaired maturation of erythroid progenitors (7–10). Potential explanations for these erythroid defects include reduced translation of the essential erythroid transcription factor *GATA1* (11), build-up of cytotoxic free heme due to reduced translation of globin proteins (12), and activation of TP53 via several potential mechanisms (9, 13, 14). In addition to a specific erythroid failure seen in younger patients, bone marrow hypocellularity, progressive multilineage cytopenias and immunodeficiency are noted in older patients with DBA, suggesting impairment of hematopoietic stem and progenitor cells (HSPCs) (15–17). Mechanisms of DBA HSPCs dysfunction are not clear, although TP53 activation appears to play a critical role (18). Additionally, systematic review of published case reports suggested an increased risk of cancer in DBA, which was subsequently confirmed by pioneering studies lead by the DBA Registry of North America (DBAR) (19–21). These prospective studies established DBA as a cancer predisposition syndrome with increased risk of MDS/AML and solid tumors such as colon carcinoma and osteosarcoma (20, 22, 23). While the exact mechanisms underlying this risk of malignancy are unknown, acquired somatic mutations leading to clonal hematopoiesis have been reported in DBA (24). For a more thorough review of the DBA pathophysiology, the readers are referred to recent review articles by Da Costa et al. (3, 5, 25)

Current treatment options

The three major therapeutic options for anemia in DBA are packed red blood cell (pRBC) transfusions, corticosteroids (prednisone), and allogeneic hematopoietic cell transplantation (HCT) (Figure 1A) (27). Majority of patients are diagnosed during their infancy and initially supported with pRBC transfusions during the first year of life after which corticosteroid treatment is initiated. Oral corticosteroids have been the mainstay of DBA treatment for well over half a century. Oral prednisone is started at a dose of 2 mg/kg/day (or 80 mg/day in adults) for a 2-week period and then tapered over several weeks to months to a maximum maintenance dose of 0.3 mg/kg/day. Although 60-80% of the patients respond to corticosteroids initially, at least half of them will either lose responsiveness or develop steroid intolerance over time (27). Patients who require a maintenance dose > 0.3 mg/kg/day or those that do not respond to prednisone are supported with chronic pRBC transfusions. A second trial of prednisone may be attempted at least 12 months after the first attempt as anecdotally some children may subsequently become responders (27). Prompt evaluation and treatment for iron overloading are critical in transfusion-dependent patients to avoid secondary organ damage and to improve outcomes for a potential HCT.

Investigational medical therapies

Advances in the understanding of the pathobiology underlying DBA has led to newer medical therapies which are currently being investigated. L-leucine has been shown to improve translational efficiency and activate mTOR pathway resulting in improvement of anemia in animal and cell models of DBA and was recently demonstrated to be safe with modest efficacy in patients with DBA (28–30). Recent studies have suggested that RP deficiency can lead to reduced globin chain production in erythroid cells, resulting in accumulation of free heme that is cytotoxic to erythroid progenitors and precursors (12, 31). Based on this hypothesis, eltrombopag is predicted to improve erythropoiesis in DBA by chelating intracellular iron. Preliminary studies were affected by eltrombopag-induced thrombocytosis and showed low response rate (32). Alternate approaches to inhibit heme accumulation, such as glycine transporter 1 inhibitors (33), are currently being explored (34). Trifluoperazine, which was shown to improve erythroid defect in experimental DBA models by reducing TP53 activity via calmodulin inhibition, is being evaluated for clinical use (14).

Transplant indications and outcomes

Allogeneic HCT remains the only option for hematopoietic cure in patients with DBA. The first HCT for DBA was performed in 1976, proving that hematological cure is possible (35). Numerous case reports since then confirmed the feasibility of HCT in DBA. The current accepted recommendations for HCT in DBA include

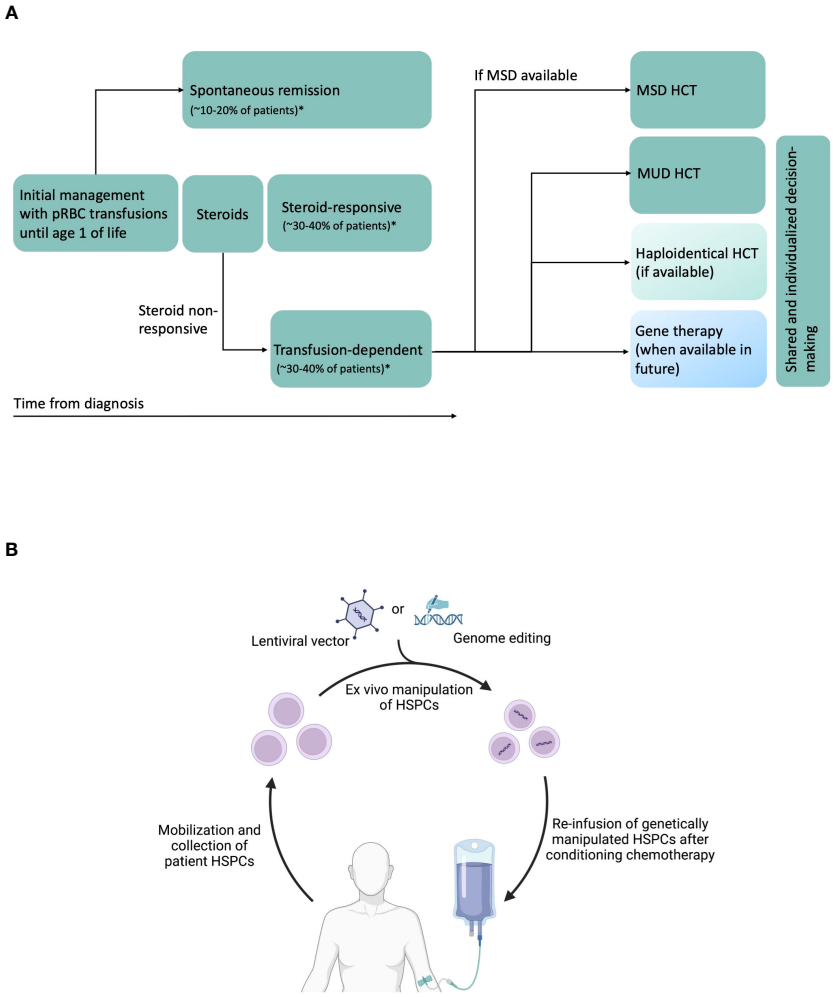

FIGURE 1
(A) Schematic of DBA natural history, and current and potential future therapies. Transfusion-dependent patients are eligible for HCT. Current standards are to perform MSD (preferred) or MUD HCT. Haploidentical HCT can be performed in the absence of MSD or MUDs, preferably on a clinical trial. In future, gene therapy could be another potential therapeutic option. In patients without MSD, treatment decisions will have to be individualized, shared between physicians, patients and patient family members, and consider availability of newer approaches such as haploidentical HCT and gene therapy in the future. *Data from DBA Registry of North America (26) and unpublished data from German DBA registry. **(B)** Schematic of future gene therapy approaches using autologous HSPCs. $CD34^+$ HSPCs are mobilized from bone marrow using agents such as G-CSF and plerixafor, collected by apheresis, purified, modified ex vivo with lentiviral vectors or undergo genome editing using novel agents such as CRISPR/Cas9, base editors or prime editors, and infused back into the patient after administration of conditioning chemotherapy. Created with BioRender.com.

transfusion dependence, non-responsiveness to steroids, requiring > 0.3 mg/kg/day of steroids, unacceptable adverse effects from steroid therapy, clinically relevant cytopenias such as neutropenia and transfusion-dependent thrombocytopenia, and evolution to hematologic malignancy (36).

The earliest published data from DBAR showed 87% overall survival (OS) after HCT from HLA-matched related donors (MRD) (37). However, in this early report OS after alternative donor HCTs was only 14%. Multiple retrospective studies, across the globe, have since confirmed the good outcomes after MRD HCT (Table 1) (38, 41–44, 46). Outcomes for HLA-matched unrelated donor (MUD) HCTs have shown improvement over the last two decades, mainly due to advances in supportive care, high-resolution HLA typing and better donor-recipient matching. More recent data from the Franco-German DBA registries with 70 DBA patients showed comparable OS between MRD and MUD HCT recipients (91% vs 92%). However, in patients that underwent HCT after year 2000, recipients of MUD HCT had slightly lower chronic graft-versus-host disease (cGVHD)-free survival (GFS) compared to MRD HCT recipients (87% vs. 100%, p=0.06) (47). Similar observations about comparable OS after MRD HCT and MUD HCT were made by the European Society for Blood and Marrow Transplantation (EBMT) and Italian Association of Pediatric Hematology and Oncology Registry (Table 1), although GFS was not reported (41, 46). Overall, these data show that 1) MRD HCT outcomes continue to be excellent and 2) OS after MUD HCT is comparable to those after MRD HCT. In the following sections, we will discuss more specific factors affecting outcomes after HCT in DBA.

TABLE 1 Summary of results from key DBA HCT studies since year 2000.

Study, year, and country	MRD, OS	Alternative Donor, OS	Conditioning regimen (number of patients)	Graft failure (number of patients)	aGVHD (II-IV)	cGVHD
Vlachos et al, 2001, USA (37)	N=8; 87.5%	N=12; 14.1%	Bu/Cy – 12 TT/Cy -3	1	ND	ND
Roy et al, 2005, USA (38)	N=41; 76%	N=20; 39%	BU/Cy – 44 Cy/Rad - 13	6	28%	26%
Lipton, 2006, USA (26)	N=21; 72.7%	N=15; 19.1%	ND	1	ND	ND
Mugishima et al, 2007, Japan (39)	N=8; 100%	N=11; 81.8%	Bu/Cy – 8 Cy/Rad - 11	2	25%	6.3%
Vlachos et al, 2010, USA (27)	N=ND 90% (<9 years old) 70% (>9years old)	N=ND 23.1% (prior 2000) 85.7% (since 2000)	ND	ND	ND	ND
Bizzetto et al, 2011, EBMT (40)	N=13 (UCBT); 100%	N=8 (UCBT); 37.5%	ND	0	ND	ND
Fagioli et al, 2014, Italy (41)	N=16; OS 80.4%	N=14; OS 69.9%	Bu/TT/Flu-15 Bu/Cy – 4 Treo/TT/Flu-4	1	41%	21%
Strahm et al, 2018, France and Germany (42)	N=45; 91%	N=25; 92%	Bu/Cy – 35 Treo/TT/Flu – 13	1	24%	11%
Junior et al, 2020, Brazil (43)	N=25; 80%	N=19; 55%	Bu/Cy – 25 Bu/Flu - 15	8	25%	20%
Behfar et al, 2019, Turkey (44)	N=9; 77.8%	N=1; 100%	Bu/Cy – 10	1	60%	10%
Koyamaishi et al, 2021, Japan (45)	N=5; ND	N=22; ND	Bu/Cy – 12 Mel/Flu – 9	0	48.1%	33.3%
Miano et al, 2021, EBMT (46)	N=58; >80%	N=37; >80%	Bu/Cy – 47 Bu/Flu – 16 Treo/Flu – 15	5	30%	15%

N, number of patients; MSD, matched sibling donor; MMD, mismatched donor; OS, overall survival; aGVHD, acute graft versus host disease; cGVHD, chronic graft versus host disease; UCBT, umbilical cord blood transplant; Bu, Busulfan; Cy, Cyclophosphamide; Flu, Fludarabine; Treo, Treosulfan; TT, Thiotepa; Rad, radiation; ND, not documented.

Pre-transplant factors

One of the major questions facing DBA patients and caregivers is to decide on when to undergo and how to prepare for allogeneic HCT. Several studies suggest better outcomes in younger patients (age < 10 years) compared to older patients (41–43, 46). While some of the newer data show improvements in OS for older patients, the incidence of cGVHD and transplant-related mortality (TRM) rates remain high. This is likely due to a higher transfusion exposure leading to iron-related organ toxicity, potentially development of donor-specific antibodies, and increased interval between diagnosis and transplant – all of which could affect organ function. Additionally, there is some evidence to suggest DBA is a pro-inflammatory condition (9, 48, 49), which could contribute to the end-organ damage and poor TRM in older patients. Therefore, HCT is recommended before age 10 years in the most recent EBMT guidelines (36). Iron overloading also increases the risk of sinusoidal obstruction syndrome (SOS) or hepatic veno-occlusive disease (VOD), especially after busulfan-based myeloablative conditioning (MAC) regimens (45). Therefore, DBA patients require early aggressive and effective chelation which remains a challenge across the world due to lack of experience, restricted access to the drugs, and lack of comprehensive surveillance. For example, it is evident that ferritin is not a reliable biomarker of iron load in DBA patients, which often results in a delay in the initiation of chelation and under-chelation (50–52). Instead, iron content determination by MRI is a widely available and reliable method for iron measurement which utilizes the specific characteristic of iron that shortens T1, T2, and T2* relaxation times. MRI liver and heart are therefore routinely performed and are instrumental in assessing hemosiderosis and guide chelation management in DBA. Imaging-based quantification of other iron-loading organs in DBA such as pancreas or pituitary gland are not available in clinical settings. In some DBA patients, higher non-transferrin bound iron (NTBI), a highly toxic form of reactive iron in blood, is reported even with normal ferritin and liver iron content, suggesting a more complex

mechanism of iron overloading and toxicity (50–52). Furthermore, iron overload-mediated damage is often irreversible and not completely corrected by chelation (53). Therefore, iron chelation should be promptly and effectively started earlier in the course of the disease to reduce cumulative iron exposure to reduce organ damage, and not just before an upcoming HCT (52, 53). Deferoxamine, deferasirox and deferiprone are the commonly used iron chelators in DBA. While singe-agent therapy can be used in infants and younger children, combination therapy, such as deferasirox during the day and deferoxamine during night, is often required in older transfusion-dependent patients for effective chelation. Deferiprone is typically reserved for patients with severe cardiac iron loading and/or heart dysfunction. EBMT recommends to reduce liver iron content (LIC) to ≤ 2 mg/g dry weight before HCT (36). However, based on our experience and the international standard among DBA experts, LIC values should be optimally lowered before HSCT to be as close as possible to 3mg Fe/g and not exceed 7mg/g, although due to rarity of the disease and lack of randomized trials, there is no evidence for lowest acceptable LIC values before HCT in DBA. In children with increased LIC levels, our practice has been to perform elastography to assess liver stiffness, which is reliably associated with fibrosis (54).

Other pre-transplant factors to consider are congenital anomalies and organ dysfunction such as cardiac and renal defects, endocrine evaluation, and allo-sensitization and donor-specific antibodies due to multiple prior transfusions (36).

Conditioning regimen

The current standard is to use MAC regimen for DBA (36). Some expert panels favor treosulfan over busulfan due to reduced toxicities with treosulfan and it remains to be answered in future studies whether treosulfan has clear benefit over busulfan (36, 47). An on-going Blood and Marrow Transplant Clinical Trials Network consortium study in the US is investigating the role of treosulfan based-conditioning for bone marrow failure diseases, including DBA (ClinicalTrials.gov Identifier: NCT04965597). A single-center study from US in patients with BMF disorders transplanted with treosulfan-based preparative regimen (n=23, including 4 patients with DBA) showed a 2-year OS and event-free survival (EFS) of 96%, with a 1-year GVHD-free, EFS of 87% (55).

Reduced-intensity conditioning (RIC) regimens have shown favorable results in several bone marrow failure disorders such as Fanconi anemia and dyskeratosis congenita (56, 57). Limited reports demonstrate good outcomes with reduced-intensity conditioning in DBA, particularly in younger children (< 5 years of age) (45, 58). In a retrospective study of 27 patients with DBA, MAC and RIC regimens had comparable outcomes (OS: 100% vs. 92.9%) (45). Majority of the patients in this study received a melphalan-based RIC therapy (45). This is consistent with laboratory observations where wildtype hematopoietic stem/progenitor cells had a competitive advantage over *RPS19* haploinsufficient cells in mouse and human cell models of DBA, providing rationale for use of RIC regimens (18, 59). Moreover, in the context of poor outcomes in older patients with DBA, use of RIC therapy could potentially be an attractive option for this patient population (27).

Novel non-genotoxic antibody-based conditioning agents have been shown to be effective in promoting engraftment without prominent adverse effects in preclinical models. These involve monoclonal antibodies targeting CD45 or CD117, either naked or conjugated with cytotoxic payloads (60, 61). Preliminary data using CD117-targeting antibody in patients with Fanconi anemia are promising (62). Future prospective studies could explore the role of RIC and antibody-based conditioning in DBA.

Donor selection

MRDs remain first choice for DBA HCT (27, 36). Importantly, related donors need to be screened for DBA mutations before graft donation. If patient does not have a known DBA mutation, selection of potential related donors relies on clinical and laboratory parameters (i.e., complete blood count, HbF percentage, erythrocyte adenosine deaminase (eADA) level, and potentially bone marrow exam). A previous case report of a persistent erythroid failure in a DBA patient following HCT from a sibling donor with undiagnosed DBA highlights the importance of rigorous screening for DBA in any potential related donors (63).

Outcomes for MUD HCTs have improved tremendously over the last two decades, and OS after MUD HCTs is comparable to MRD HCTs, as described in several studies from Europe (41, 42, 46). However, MUD HCTs are associated with higher cGVHD, especially in children over 10 years of age (42). Therefore, it is key to use shared decision-making after discussion of risk-benefit from MUD HCT for older DBA patients (36).

Unrelated cord blood (UCB) HCTs are associated with inferior outcomes, primarily due to graft failure and higher TRM (39–42, 46). Advances in haploidentical HCT in general have improved accessibility and expanded the pool of donors, but the available evidence for DBA patients remains limited to case-reports (64–66).

Stem cell source

Bone marrow is preferred over PBSCs whenever possible for non-malignant diseases (36). Additionally, unlike UCB, sibling CB HCTs from sibling donors has shown very good outcomes (42). In one of the larger retrospective DBA studies, 7 sibling CB HCTs were described with 100% OS and one patient with limited cGVHD (42).

GVHD

Improvements in high-resolution HLA typing has reduced GVHD rates in the recent cohorts. Strahm et al. reported a cumulative incidence of 7% (95% CI, 3-17) for aGVHD (Grade III-IV) and 11% (95% CI, 5-22) for cGVHD (Table 1) (42). They also noted that none of the patients who received MRD HCT after year 1999 developed cGVHD. Data from the Italian Registry (n=30,

out of which 26 received their HCTs after year 1999), showed slightly higher numbers at 24% for III-IV aGVHD and 21% for cGVHD (n=5, extensive cGVHD in 3 patients) (41).

Retrospective analysis by EBMT showed that iron overloading was associated with extensive cGVHD (24% vs 0%, $P = 0.04$) (46). Other factors that appear to impact cGVHD were occurrence of aGVHD, age of patient (increased risk in older patients), year of transplant (reduced risk in HCTs after year 2000), and donor type (reduced risk with MRD HCTs) (42). Most of the studies have reported calcineurin inhibitor (CNI) with methotrexate (MTX) as the most commonly used GVHD prophylaxis regimen (41, 46). Mycophenolate mofetil (MMF) was used instead of MTX in some cases. A recent report of abatacept added to CNI/MMF combination for unrelated HCTs in bone marrow failure patients looks promising (67). Advances in GVHD prophylaxis have the potential to improve cGVHD-free survival (cGFS) for MUD HCTs in DBA.

Chimerism

The minimum percentage of donor chimerism to correct erythroid failure in DBA is not known. Also, while partial chimerism could correct anemia, it does not completely eliminate the risk of future MDS/AML. In the Franco-German HCT cohort, 5 patients (10%) were reported to have mixed chimerism without transfusion requirements but the exact chimerism was unavailable (42). In another small study, one patient with 23.5% donor-chimerism was transfusion-dependent, whereas the two others with 76.7% and 54.6% donor-chimerism remained transfusion-free (45). These limited data suggest that a donor chimerism of at least 50% or more is needed to correct erythroid failure after HCT in DBA.

Long-term monitoring after HCT

While several reports have demonstrated improved outcomes after allogeneic HCT for DBA recently, there is limited data on long-term follow-up of these patients. In addition to the standard long-term follow-up for patients undergoing HCT, DBA patients require additional disease-specific late effects screening (36, 68–70). DBA patients will require follow-up related to prior treatment with corticosteroids, iron overloading and iron chelation agents. This includes vision screening and cataract exams, iron load monitoring using ferritin and/or T2* MRI (preferable) and appropriate management of iron load, evaluation of end-organ damage caused by iron overloading, and hearing screen which can be an adverse effect of certain iron chelators. Patients who are iron-overloaded post-HCT will require routine phlebotomy. DBA patients have several other non-hematological issues, such as craniofacial defects, cardiac, renal, and genitourinary abnormalities which will necessitate multidisciplinary care (27). DBA patients are at slightly increased risk of solid tumors such as osteosarcoma and colorectal cancers, which could be increased by HCT (36). Lastly, DBA patients are at risk for delayed puberty and reduced fertility, which can be further accentuated by exposure to myeloablative chemotherapy used in conventional HCTs (71–73). Therefore, counselling and fertility preservation needs to be offered to all DBA patients considering HCT.

Gene therapy – progress and challenges

Once *RPS19* was identified as the first DBA gene (followed by discovery of >20 other DBA genes), gene complementation emerged as a potential therapeutic option (74). Although major advances have been made in the last few decades, several preclinical safety, efficacy and regulatory challenges remain before these transformative therapies are ready for clinical use. Autologous HCT of genetically-manipulated HSPCs could overcome several disadvantages of allogeneic HCT, namely a) lack of suitable donors for many patients b) immune toxicities such GVHD c) risk of graft rejection, and d) risk of donor-related hematopoiesis. In general, autologous gene therapy for hematological diseases is carried out by isolating patient HSPCs, genetically correcting them ex vivo, followed by conditioning chemotherapy and infusion of corrected HSPCs (Figure 1B). Genetic correction of HSPCs can be achieved using lentiviral vector to express wild type (healthy) copy of the affected gene or using CRISPR/Cas9 or related nucleases for targeted genetic correction of the mutation in the endogenous locus.

Early preclinical work on DBA gene therapies in laboratory models, primarily used retroviral vectors and subsequently used self-inactivating (SIN) lentiviral vectors (75–78). The third-generation SIN lentiviral vectors incorporate several safety features such as deletion of viral enhancer/promoter sequences within the U3 region of 3' Long Tandem Repeat (LTR), removal of all viral protein genes from the vector plasmid and deletion of viral *Tat* sequence (79). These changes improve safety by reducing the risks of insertional gene activation and replication-competent lentiviral vector generation (79). Preclinical DBA gene therapy studies have primarily focused on *RPS19*, the most common DBA gene (5). One of the major challenges has been limited access to DBA $CD34^+$ HSPCs for clinical development, as typically millions of $CD34^+$ cells are required for these studies. We recently developed a CRISPR/Cas9-based approach to model *RPS19*-mutated DBA using $CD34^+$ HSPCs from healthy donors (18). Utilizing this model, we demonstrated the efficacy of an *RPS19*-expressing SIN lentiviral vector (17, 18). Recently, reduced translation of the erythroid transcription factor GATA1 was suggested to cause the characteristic erythroid defect of DBA, regardless of the mutated RP gene (11). This raises the possibility of forced erythroid-specific expression of *GATA1* as a DBA gene-agnostic therapeutic strategy. Preliminary data using this approach are promising (80).

One of the disadvantages of lentiviral vectors is the inability to control gene dosage in transduced HSPCs. While this is not an issue for RPS19 protein, whose levels are tightly regulated (75), RPL5 and RPL11 overexpression can potentially lead to TP53 stabilization by inhibiting MDM2, a negative regulator of TP53 (81). Therefore, overexpression of RPL5 or RPL11 can potentially lead to TP53-dependent cell death. Hence, targeted approaches such as knock-in

of wildtype cDNA cassette following CRISPR/Cas9 editing, precise nucleotide base editing and prime editing need to be explored as future therapeutic options. These novel and promising technologies have shown effectiveness in preclinical setting for other inherited hematopoietic disorders (82–84). Gene correction using knock-in approaches have been shown to be effective in preclinical models of diseases such as chronic granulomatous disease and sickle cell anemia (85, 86). Base editing involves Cas9-nuclease-derived proteins that can precisely convert an adenine to guanine or cytosine to thymine (87). Base editing has been shown to be effective in correcting preclinical models of monogenic diseases such as sickle cell disease (83, 88). However, the diversity of pathogenic mutations that cause DBA also means a single base editor-guide RNA combination can only be used for patients with identical mutations, which poses a unique set of challenges from a preclinical, regulatory, ethical and health economics standpoint. An alternative approach could be prime editing to correct a several adjacent mutations within a short segment of DBA using a single prime editing guide RNA (89). This, for instance, could be a viable approach particularly for *RPS19* between codons 52 and 62, which is a hotspot for several DBA mutations (90). However, it has to be noted that prime editors are less advanced than base editors in terms of preclinical development. Although there are rapid preclinical advances, several practical challenges remain. These include feasibility of mobilization of adequate HSPCs from patients, optimization of lentiviral transduction or genome editing of DBA HSPCs, thoroughly evaluating safety of gene therapy approaches, and regulatory and financial challenges in developing clinical trials for ultra-rare diseases.

In sum, various approaches are being explored towards development of hematological curative therapy for DBA. These approaches could offer a potentially safe and effective therapeutic option for transfusion-dependent DBA patients, especially for those without an MRD (Figure 1A). While MRD HCTs have excellent outcome, there is potential clinical equipoise between MUD HCTs and a future gene therapy approach, given the slightly higher rates of cGVHD following MUD HCTs. Furthermore, gene therapy approaches could ultimately prove to be more cost-effective in the long run compared to MUD HCT due to economies of scale. Therefore, the long-term efficacy and safety will have to be determined through carefully designed clinical trials. In particular, it remains to be seen how the risk of MDS/AML is altered following autologous HCT with gene-corrected HSPCs.

Conclusion and future directions

Overall, outcomes after allogeneic HCT have remarkably improved over the last two decades. This has made HCT a safe and viable option for many patients. However, several unanswered questions and unaddressed challenges remain. Outcomes after HCT in adolescent children is improving but remains suboptimal while there is limited data on HCT outcomes in adult patients with DBA. Even less is known about outcomes in DBA patients who develop MDS/AML or aplastic anemia. Other challenging questions include the roles of reduced-intensity conditioning regimen, antibody-based conditioning approaches and haploidentical HCT in DBA. The risk of solid tumors post-HCT is unknown and long-term follow-up studies are needed. Renewed interest in gene therapy has raised hopes for a safer and effective curative option for select DBA patients. Concurrent advances in our understanding of DBA mechanisms have raised interest and hope in other therapeutic agents such as L-leucine (30) and trifluoperazine (14). Progress in medical therapies including iron chelation have the potential to improve life expectancy and quality of life for DBA patients that are unable to undergo HCT. Therefore, outcomes of patients that undergo HCT and gene therapy will have to be continually evaluated and compared with more current outcome data of non-transplanted patients to determine the risk-benefit of these transformative therapies. The critical need for such continual assessment of natural history and outcomes in a rare disease population highlights the important role played by disease registries, patient advocacy groups and global alliances, and the need for synergy and interaction between the various stakeholders.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Josephs HW. Anaemia of infancy and early childhood. *Medicine* (1936) 15 (3):307–451. doi: 10.1097/00005792-193615030-00001

2. Diamond LK. Hypoplastic anemia. *Am J Dis Child.* (1938) 56:464.

3. Da Costa L, Narla A, Mohandas N. An update on the pathogenesis and diagnosis of Diamond-Blackfan anemia. *F1000Res* (2018) 7. doi: 10.12688/f1000research.15542.1

4. Engidaye G, Melku M, Enawgaw B. Diamond Blackfan anemia: genetics, pathogenesis, diagnosis and treatment. *Ejifcc* (2019) 30(1):67–81.

5. Da Costa L, Leblanc T, Mohandas N. Diamond-Blackfan anemia. *Blood* (2020) 136(11):1262–73. doi: 10.1182/blood.2019000947

6. Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol* (2008) 142(6):859–76. doi: 10.1111/j.1365-2141.2008.07269.x

7. Ohene-Abuakwa Y, Orfali KA, Marius C, Ball SE. Two-phase culture in Diamond Blackfan anemia: localization of erythroid defect. *Blood* (2005) 105(2):838–46. doi: 10.1182/blood-2004-03-1016

8. Iskander D, Psaila B, Gerrard G, Chaidos A, En Foong H, Harrington Y, et al. Elucidation of the EP defect in Diamond-Blackfan anemia by characterization and prospective isolation of human EPs. *Blood* (2015) 125(16):2553–7. doi: 10.1182/blood-2014-10-608042

9. Iskander D, Wang G, Heuston EF, Christodoulidou C, Psaila B, Ponnusamy K, et al. Single-cell profiling of human bone marrow progenitors reveals mechanisms of failing erythropoiesis in Diamond-Blackfan anemia. *Sci Transl Med* (2021) 13(610): eabf0113. doi: 10.1126/scitranslmed.abf0113

10. Perdahl EB, Naprstek BL, Wallace WC, Lipton JM. Erythroid failure in Diamond-Blackfan anemia is characterized by apoptosis. *Blood* (1994) 83(3):645–50. doi: 10.1182/blood.V83.3.645.645

11. Ludwig LS, Gazda HT, Eng JC, Eichhorn SW, Thiru P, Ghazvinian R, et al. Altered translation of GATA1 in Diamond-Blackfan anemia. *Nat Med* (2014) 20 (7):748–53. doi: 10.1038/nm.3557

12. Yang Z, Keel SB, Shimamura A, Liu L, Gerds AT, Li HY, et al. Delayed globin synthesis leads to excess heme and the macrocytic anemia of Diamond Blackfan anemia and del (5q) myelodysplastic syndrome. *Sci Trans Med* (2016) 8(338):338ra67–ra67. doi: 10.1126/scitranslmed.aaf3006

13. Dutt S, Narla A, Lin K, Mullally A, Abayasekara N, Megerdichian C, et al. Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. *Blood* (2011) 117(9):2567–76. doi: 10.1182/blood-2010-07-295238

14. Taylor AM, Macari ER, Chan IT, Blair MC, Doulatov S, Vo LT, et al. Calmodulin inhibitors improve erythropoiesis in Diamond-Blackfan anemia. *Sci Transl Med* (2020) 12(566). doi: 10.1126/scitranslmed.abb5831

15. Giri N, Kang E, Tisdale JF, Follman D, Rivera M, Schwartz GN, et al. Clinical and laboratory evidence for a trilineage haematopoietic defect in patients with refractory Diamond-Blackfan anaemia. *Br J Haematol* (2000) 108:167–75. doi: 10.1046/j.1365-2141.2000.01796.x

16. Iskander D, Roberts I, Rees C, Szydlo R, Alikian M, Neale M, et al. Impaired cellular and humoral immunity is a feature of Diamond-Blackfan anaemia; experience of 107 unselected cases in the United Kingdom. *Br J Haematol* (2019) 186(2):321–6. doi: 10.1111/bjh.15915

17. Bhoopalan SV, Yen J, Mayuranathan T, Yao Y, Mayberry K, Zhou S, et al. A novel RPS19-edited hematopoietic stem cell model of Diamond-Blackfan anemia for development of lentiviral vector gene therapy. *Blood* (2021) 138(Supplement 1):859. doi: 10.1182/blood-2021-147557

18. Bhoopalan SV, Yen JS, Mayuranathan T, Mayberry KD, Yao Y, Lillo Osuna MA, et al. An RPS19-edited model for Diamond-Blackfan anemia reveals TP53-dependent impairment of hematopoietic stem cell activity. *JCI Insight* (2023) 8(1):e161810. doi: 10.1172/jci.insight.161810

19. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev* (2010) 24(3):101–22. doi: 10.1016/j.blre.2010.03.002

20. Savage SA, Dufour C. Classical inherited bone marrow failure syndromes with high risk for myelodysplastic syndrome and acute myelogenous leukemia. *Semin Hematol* (2017) 54(2):105–14. doi: 10.1053/j.seminhematol.2017.04.004

21. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Blood* (2012) 119(16):3815–9. doi: 10.1182/blood-2011-08-375972

22. Lipton JM, Federman N, Khabbaze Y, Schwartz CL, Hilliard LM, Clark JI, et al. Osteogenic sarcoma associated with Diamond-Blackfan anemia: a report from the Diamond-Blackfan Anemia Registry. *J Pediatr Hematol Oncol* (2001) 23(1):39–44. doi: 10.1097/00043426-200101000-00009

23. Lipton JM, Molmenti CLS, Desai P, Lipton A, Ellis SR, Vlachos A. Early onset colorectal cancer: an emerging cancer risk in patients with Diamond Blackfan anemia. *Genes (Basel).* (2021) 13(1). doi: 10.3390/genes13010056

24. Nash M, Vlachos A, Wlodarski MW, Lipton JM. Clonal hematopoiesis in patients with Diamond Blackfan anemia. *Blood* (2020) 136(Supplement 1):25–6. doi: 10.1182/blood-2020-139652

25. Da Costa L, O'Donohue MF, van Dooijeweert B, Albrecht K, Unal S, Ramenghi U, et al. Molecular approaches to diagnose Diamond-Blackfan anemia: The EuroDBA experience. *Eur J Med Genet* (2018) 61(11):664–73. doi: 10.1016/j.ejmg.2017.10.017

26. Lipton JM, Atsidaftos E, Zyskind I, Vlachos A. Improving clinical care and elucidating the pathophysiology of Diamond Blackfan anemia: an update from the Diamond Blackfan Anemia Registry. *Pediatr Blood Cancer.* (2006) 46(5):558–64. doi: 10.1002/pbc.20642

27. Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. *Blood* (2010) 116 (19):3715–23. doi: 10.1182/blood-2010-02-251090

28. Vlachos A, Atsidaftos E, Lababidi ML, Muir E, Rogers ZR, Alhushki W, et al. L-leucine improves anemia and growth in patients with transfusion-dependent Diamond-Blackfan anemia: Results from a multicenter pilot phase I/II study from the Diamond-Blackfan Anemia Registry. *Pediatr Blood Cancer.* (2020) 67(12):e28748. doi: 10.1002/pbc.28748

29. Payne EM, Virgilio M, Narla A, Sun H, Levine M, Paw BH, et al. L-Leucine improves the anemia and developmental defects associated with Diamond-Blackfan anemia and del(5q) MDS by activating the mTOR pathway. *Blood* (2012) 120 (11):2214–24. doi: 10.1182/blood-2011-10-382986

30. Jaako P, Debnath S, Olsson K, Bryder D, Flygare J, Karlsson S. Dietary L-leucine improves the anemia in a mouse model for Diamond-Blackfan anemia. *Blood* (2012) 120(11):2225–8. doi: 10.1182/blood-2012-05-431437

31. Doty RT, Fan X, Young DJ, Liang JA, Singh K, Pakbaz Z, et al. Studies in a mosaic DBA patient and chimeric mice reveal erythroid cell-extrinsic contributions to erythropoiesis. *Blood* (2022) 139(23):3439–49. doi: 10.1182/blood.2021013507

32. Duncan BB, Lotter J, Superata J, Barranta ME, Darden I, Venugopal S, et al. Treatment of refractory Diamond-Blackfan anemia with eltrombopag. *Blood* (2022) 140(Supplement 1):5824–5. doi: 10.1182/blood-2022-160297

33. Taher AT, Viprakasit V, Cappellini MD, Kraus D, Cech P, Volz D, et al. Haematological effects of oral administration of bitopertin, a glycine transport inhibitor, in patients with non-transfusion-dependent β-thalassaemia. *Br J Haematol* (2021) 194(2):474–7. doi: 10.1111/bjh.17479

34. Machado TR, Young DJ. Study of the selective glyT1 inhibitor bitopertin for steroid-refractory Diamond-Blackfan anemia. Available at: www.clinicaltrials.gov (Accessed 6 Aug 2023).

35. August CS, King E, Githens JH, McIntosh K, Humbert JR, Greensheer A, et al. Establishment of erythropoiesis following bone marrow transplantation in a patient with congenital hypoplastic anemia (Diamond-Blackfan syndrome). *Blood* (1976) 48 (4):491–8. doi: 10.1182/blood.V48.4.491.491

36. Diaz-de-Heredia C, Bresters D, Faulkner L, Yesilipek A, Strahm B, Miano M, et al. Recommendations on hematopoietic stem cell transplantation for patients with Diamond-Blackfan anemia. On behalf of the Pediatric Diseases and Severe Aplastic Anemia Working Parties of the EBMT. *Bone Marrow Transplant.* (2021) 56(12):2956–63. doi: 10.1038/s41409-021-01449-w

37. Vlachos A, Federman N, Reyes-Haley C, Abramson J, Lipton JM. Hematopoietic stem cell transplantation for Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Bone Marrow Transplant.* (2001) 27(4):381–6. doi: 10.1038/sj.bmt.1702784

38. Roy V, Pérez WS, Eapen M, Marsh JCW, Pasquini M, Pasquini R, et al. Bone marrow transplantation for Diamond-Blackfan anemia. *Biol Blood Marrow Transplantation.* (2005) 11(8):600–8. doi: 10.1016/j.bbmt.2005.05.005

39. Mugishima H, Ohga S, Ohara A, Kojima S, Fujisawa K, Tsukimoto I. Hematopoietic stem cell transplantation for Diamond-Blackfan anemia: a report from the Aplastic Anemia Committee of the Japanese Society of Pediatric Hematology. *Pediatr Transplant.* (2007) 11(6):601–7. doi: 10.1111/j.1399-3046.2006.00616.x

40. Bizzetto R, Bonfim C, Rocha V, Socié G, Locatelli F, Chan K, et al. Outcomes after related and unrelated umbilical cord blood transplantation for hereditary bone marrow failure syndromes other than Fanconi anemia. *Haematologica* (2011) 96 (1):134–41. doi: 10.3324/haematol.2010.027839

41. Fagioli F, Quarello P, Zecca M, Lanino E, Corti P, Favre C, et al. Haematopoietic stem cell transplantation for Diamond Blackfan anaemia: a report from the Italian Association of Paediatric Haematology and Oncology Registry. *Br J Haematol* (2014) 165(5):673–81. doi: 10.1111/bjh.12787

42. Strahm B, Loewecke F, Niemeyer CM, Albert M, Ansari M, Bader P, et al. Favorable outcomes of hematopoietic stem cell transplantation in children and adolescents with Diamond-Blackfan anemia. *Blood Adv* (2020) 4(8):1760–9. doi: 10.1182/bloodadvances.2019001210

43. Darrigo Junior LG, Loth G, Kuwahara C, Vieira A, Colturato V, Rodrigues AL, et al. Hematopoietic cell transplantation for Diamond Blackfan anemia: A report from the Pediatric Group of the Brazilian Bone Marrow Transplantation Society. *Eur J Haematology.* (2020) 105(4):426–33. doi: 10.1111/ejh.13463

44. Behfar M, Koochakzadeh L, Yazdanian N, Salajegheh P, Rostami T, Khodayari-Namini N, et al. Outcome of allogeneic Hematopoietic Stem Cell Transplantation on Diamond-Blackfan anemia using busulfan-based myeloablative regimen. *Turk J Pediatr* (2019) 61(3):407–12. doi: 10.24953/turkjped.2019.03.013

45. Koyamaishi S, Kamio T, Kobayashi A, Sato T, Kudo K, Sasaki S, et al. Reduced-intensity conditioning is effective for hematopoietic stem cell transplantation in young pediatric patients with Diamond-Blackfan anemia. *Bone Marrow Transplant.* (2021) 56 (5):1013–20. doi: 10.1038/s41409-020-01056-1

46. Miano M, Eikema DJ, de la Fuente J, Bosman P, Ghavamzadeh A, Smiers F, et al. Stem cell transplantation for Diamond-Blackfan anemia. A retrospective study on behalf of the severe aplastic anemia working party of the European blood and marrow transplantation group (EBMT). *Transplant Cell Ther* (2021) 27(3):274.e1–.e5. doi: 10.1016/j.jtct.2020.12.024

47. De Latour RP, Peters C, Gibson B, Strahm B, Lankester A, De Heredia C, et al. Recommendations on hematopoietic stem cell transplantation for inherited bone marrow failure syndromes. *Bone marrow transplantation.* (2015) 50(9):1168. doi: 10.1038/bmt.2015.117

48. Kapralova K, Jahoda O, Koralkova P, Gursky J, Lanikova L, Pospisilova D, et al. Oxidative DNA damage, inflammatory signature, and altered erythrocytes properties in Diamond-Blackfan anemia. *Int J Mol Sci* (2020) 21(24). doi: 10.3390/ijms21249652

49. Matsui K, Giri N, Alter BP, Pinto LA. Cytokine production by bone marrow mononuclear cells in inherited bone marrow failure syndromes. *Br J Haematol* (2013) 163(1):81–92. doi: 10.1111/bjh.12475

50. Porter J, Walter PB, Neumayr LD, Evans P, Weyhmiller MG, Harmatz P, et al. Inflammatory and vitamin bio-markers of iron trafficking and distribution in transfusional overload: insights from comparing Diamond Blackfan anemia with sickle cell disease and thalassemia (MCSIO PILOT study). *Blood* (2013) 122 (21):1014. doi: 10.1182/blood.V122.21.1014.1014

51. Porter JB, Walter PB, Neumayr LD, Evans P, Bansal S, Garbowski M, et al. Mechanisms of plasma non-transferrin bound iron generation: insights from comparing transfused diamond blackfan anaemia with sickle cell and thalassaemia patients. *Br J Haematol* (2014) 167(5):692–6. doi: 10.1111/bjh.13081

52. Roggero S, Quarello P, Vinciguerra T, Longo F, Piga A, Ramenghi U. Severe iron overload in Blackfan-Diamond anemia: A case-control study. *Am J Hematol* (2009) 84 (11):729–32. doi: 10.1002/ajh.21541

53. John Porter VV, Kattamis A, Cappellini MD, Cohen A, Porter J, Taher A, et al. Iron overload and chelation. In: Cappellini MD, Cohen A, Porter J, et al, editors. *Guidelines for the Management of Transfusion Dependent Thalassaemia, 3rd ed*. Nicosia (CY: Thalassaemia International Federation (2014).

54. Sande JA, Verjee S, Vinayak S, Amersi F, Ghesani M. Ultrasound shear wave elastography and liver fibrosis: A Prospective Multicenter Study. *World J Hepatol* (2017) 9(1):38–47. doi: 10.4254/wjh.v9.i1.38

55. Burroughs LM, Shimamura A, Talano JA, Domm JA, Baker KK, Delaney C, et al. Allogeneic hematopoietic cell transplantation using treosulfan-based conditioning for treatment of marrow failure disorders. *Biol Blood Marrow Transplant.* (2017) 23 (10):1669–77. doi: 10.1016/j.bbmt.2017.06.002

56. Bhoopalan SV, Wlodarski M, Reiss U, Triplett B, Sharma A. Reduced-intensity conditioning-based hematopoietic cell transplantation for dyskeratosis congenita: Single-center experience and literature review. *Pediatr Blood Cancer.* (2021) 68(10): e29177. doi: 10.1002/pbc.29177

57. MacMillan ML, DeFor TE, Young JA, Dusenbery KE, Blazar BR, Slungaard A, et al. Alternative donor hematopoietic cell transplantation for Fanconi anemia. *Blood* (2015) 125(24):3798–804. doi: 10.1182/blood-2015-02-626002

58. Crazzolara R, Kropshofer G, Haas OA, Matthes-Martin S, Kager L. Reduced-intensity conditioning and stem cell transplantation in infants with Diamond Blackfan anemia. *Haematologica* (2017) 102(3):e73–e5. doi: 10.3324/haematol.2016.157149

59. Liu Y, Dahl M, Debnath S, Rothe M, Smith EM, Grahn THM, et al. Successful gene therapy of Diamond-Blackfan anemia in a mouse model and human CD34(+) cord blood hematopoietic stem cells using a clinically applicable lentiviral vector. *Haematologica* (2022) 107(2):446–56. doi: 10.3324/haematol.2020.269142

60. Czechowicz A, Palchaudhuri R, Scheck A, Hu Y, Hoggatt J, Saez B, et al. Selective hematopoietic stem cell ablation using CD117-antibody-drug-conjugates enables safe and effective transplantation with immunity preservation. *Nat Commun* (2019) 10 (1):617. doi: 10.1038/s41467-018-08201-x

61. Gao C, Schroeder JA, Xue F, Jing W, Cai Y, Scheck A, et al. Nongenotoxic antibody-drug conjugate conditioning enables safe and effective platelet gene therapy of hemophilia A mice. *Blood Adv* (2019) 3(18):2700–11. doi: 10.1182/bloodadvances.2019000516

62. Agarwal R. Depleted donor stem cell transplant in children and adults with Fanconi anemia after being conditioned with a regimen containing JSP191 antibody. (2023). Available at: www.clinicaltrials.gov (Accessed 6 Aug 2023).

63. Wynn RF, Grainger JD, Carr TF, Eden OB, Stevens RF, Will AM. Failure of allogeneic bone marrow transplantation to correct Diamond-Blackfan anaemia despite haemopoietic stem cell engraftment. *Bone Marrow Transplant.* (1999) 24(7):803–5. doi: 10.1038/sj.bmt.1701982

64. Bonfim C, Ribeiro L, Nichele S, Loth G, Bitencourt M, Koliski A, et al. Haploidentical bone marrow transplantation with post-transplant cyclophosphamide for children and adolescents with Fanconi anemia. *Biol Blood Marrow Transplant.* (2017) 23(2):310–7. doi: 10.1016/j.bbmt.2016.11.006

65. Zecca M, Strocchio L, Pagliara D, Comoli P, Bertaina A, Giorgiani G, et al. HLA-haploidentical T cell-depleted allogeneic hematopoietic stem cell transplantation in children with Fanconi anemia. *Biol Blood Marrow Transplant.* (2014) 20(4):571–6. doi: 10.1016/j.bbmt.2014.01.015

66. Ramzan MB, Bairwa RC, Khan T, Katewa S. Successful T cell replete haploidentical peripheral blood hematopoietic stem cell transplantation in a young girl with Diamond-Blackfan anemia. *Pediatr Neonatal Biol* (2018) 3(1):000113. doi: 10.23880/PNBOA-16000113

67. Stenger EO, Watkins B, Rogowski K, Chiang K-Y, Haight AE, Leung K, et al. Abatacept GVHD prophylaxis in unrelated hematopoietic cell transplantation for pediatric bone marrow failure. *Blood Adv* (2023) 7(10):2196–205. doi: 10.1182/bloodadvances.2022008545

68. Bonfim C. Special pre- and posttransplant considerations in inherited bone marrow failure and hematopoietic malignancy predisposition syndromes. *Hematol Am Soc Hematol Educ Program.* (2020) 2020(1):107–14. doi: 10.1182/hematology.2020000095

69. Dietz A C, Savage S A, Adrianna Vlachos A, Mehta P, Bresters D, Tolar J, et al. Late effects screening guidelines after hematopoietic cell transplantation for inherited bone marrow failure syndromes: consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after ped. *Biol Blood Marrow Transplantation.* (2017) 23(9):1422–8. doi: 10.1016/j.bbmt.2017.05.022

70. Dietz AC, Mehta PA, Vlachos A, Savage SA, Bresters D, Tolar J, et al. Current Knowledge and Priorities for Future Research in Late Effects after Hematopoietic Cell Transplantation for Inherited Bone Marrow Failure Syndromes: Consensus Statement from the Second Pediatric Blood and Marrow Transplant Consortium International Conference on Late Effects after Pediatric Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant.* (2017) 23(5):726–35. doi: 10.1016/j.bbmt.2017.01.075

71. Tufano AM, Osorio DS, Atsidaftos E, Sison CP, Lipton JM, Vlachos A. Deleterious consequences of Diamond Blackfan anemia on reproductive health and pregnancy outcomes: A report from the Diamond Blackfan anemia registry (DBAR). *Blood* (2014) 124(21):4399. doi: 10.1182/blood.V124.21.4399.4399

72. Gansner JM, Achebe MM, Gray KJ, Yefidoff-Freedman R, Labovitis E, Parnes A, et al. Pregnancy outcomes in inherited bone marrow failure syndromes. *Blood* (2017) 130(14):1671–4. doi: 10.1182/blood-2017-08-798462

73. Giri N, Stratton P, Savage SA, Alter BP. Pregnancies in patients with inherited bone marrow failure syndromes in the NCI cohort. *Blood* (2017) 130(14):1674–6. doi: 10.1182/blood-2017-08-802991

74. Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, et al. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. *Nat Genet* (1999) 21(2):169–75. doi: 10.1038/5951

75. Flygare J, Olsson K, Richter J, Karlsson S. Gene therapy of Diamond Blackfan anemia CD34(+) cells leads to improved erythroid development and engraftment following transplantation. *Exp Hematol* (2008) 36(11):1428–35. doi: 10.1016/j.exphem.2008.06.012

76. Jaako P, Debnath S, Olsson K, Modlich U, Rothe M, Schambach A, et al. Gene therapy cures the anemia and lethal bone marrow failure in a mouse model of RPS19-deficient Diamond-Blackfan anemia. *Haematologica* (2014) 99(12):1792–8. doi: 10.3324/haematol.2014.111195

77. Debnath S, Jaako P, Siva K, Rothe M, Chen J, Dahl M, et al. Lentiviral vectors with cellular promoters correct anemia and lethal bone marrow failure in a mouse model for Diamond-Blackfan anemia. *Mol Ther* (2017) 25(8):1805–14. doi: 10.1016/j.ymthe.2017.04.002

78. Hamaguchi I, Flygare J, Nishiura H, Brun AC, Ooka A, Kiefer T, et al. Proliferation deficiency of multipotent hematopoietic progenitors in ribosomal protein S19 (RPS19)-deficient diamond-Blackfan anemia improves following RPS19 gene transfer. *Mol Ther* (2003) 7(5 Pt 1):613–22. doi: 10.1016/S1525-0016(03)00091-1

79. Milone MC, O'Doherty U. Clinical use of lentiviral vectors. *Leukemia* (2018) 32 (7):1529–41. doi: 10.1038/s41375-018-0106-0

80. Voit RA, Liao X, Cohen B, Armant M, Kamal E, Huang M-M, et al. Regulated expression of GATA1 as a gene therapy cure for Diamond-Blackfan anemia. *Blood* (2022) 140(Supplement 1):986–7. doi: 10.1182/blood-2022-165848

81. Aspesi A, Monteleone V, Betti M, Actis C, Morleo G, Sculco M, et al. Lymphoblastoid cell lines from Diamond Blackfan anaemia patients exhibit a full ribosomal stress phenotype that is rescued by gene therapy. *Sci Rep* (2017) 7(1):12010. doi: 10.1038/s41598-017-12307-5

82. Bhoopalan SV, Yen JS, Levine RM, Sharma A. Editing human hematopoietic stem cells: advances and challenges. *Cytotherapy* (2023) 25(3):261–9. doi: 10.1016/j.jcyt.2022.08.003

83. Newby GA, Yen JS, Woodard KJ, Mayuranathan T, Lazzarotto CR, Li Y, et al. Base editing of haematopoietic stem cells rescues sickle cell disease in mice. *Nature* (2021) 595(7866):295–302. doi: 10.1038/s41586-021-03609-w

84. Everette KA, Newby GA, Levine RM, Mayberry K, Jang Y, Mayuranathan T, et al. Ex vivo prime editing of patient haematopoietic stem cells rescues sickle-cell disease phenotypes after engraftment in mice. *Nat BioMed Eng* (2023) 7(5):616–28. doi: 10.1038/s41551-023-01026-0

85. Sweeney CL, Pavel-Dinu M, Choi U, Brault J, Liu T, Koontz S, et al. Correction of X-CGD patient HSPCs by targeted CYBB cDNA insertion using CRISPR/Cas9 with 53BP1 inhibition for enhanced homology-directed repair. *Gene Ther* (2021) 28(6):373–90. doi: 10.1038/s41434-021-00251-z

86. Dever DP, Bak RO, Reinisch A, Camarena J, Washington G, Nicolas CE, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature* (2016) 539(7629):384–9. doi: 10.1038/nature20134

87. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat Rev Genet* (2018) 19(12):770–88. doi: 10.1038/s41576-018-0059-1

88. Ravi NS, Wienert B, Wyman SK, Bell HW, George A, Mahalingam G, et al. Identification of novel HPFH-like mutations by CRISPR base editing that elevate the expression of fetal hemoglobin. *eLife* (2022) 11:e65421. doi: 10.7554/eLife.65421

89. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* (2019) 576(7785):149–57. doi: 10.1038/s41586-019-1711-4

90. Willig TN, Draptchinskaia N, Dianzani I, Ball S, Niemeyer C, Ramenghi U, et al. Mutations in ribosomal protein S19 gene and diamond blackfan anemia: wide variations in phenotypic expression. *Blood* (1999) 94(12):4294–306.

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Germline landscape of *RPA1, RPA2 and RPA3* variants in pediatric malignancies: identification of *RPA1* as a novel cancer predisposition candidate gene

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Replication Protein A (RPA) is single-strand DNA binding protein that plays a key role in the replication and repair of DNA. RPA is a heterotrimer made of 3 subunits – RPA1, RPA2, and RPA3. Germline pathogenic variants affecting *RPA1* were recently described in patients with Telomere Biology Disorders (TBD), also known as dyskeratosis congenita or short telomere syndrome. Premature telomere shortening is a hallmark of TBD and results in bone marrow failure and predisposition to hematologic malignancies. Building on the finding that somatic mutations in RPA subunit genes occur in ~1% of cancers, we hypothesized that germline RPA alterations might be enriched in human cancers. Because germline *RPA1* mutations are linked to early onset TBD with predisposition to myelodysplastic syndromes, we interrogated pediatric cancer cohorts to define the prevalence and spectrum of rare/novel and putative damaging germline *RPA1*, *RPA2*, and *RPA3* variants. In this study of 5,993 children with cancer, 75 (1.25%) harbored heterozygous rare (non-cancer population allele frequency (AF) < 0.1%) variants in the RPA heterotrimer genes, of which 51 cases (0.85%) had ultra-rare (AF < 0.005%) or novel variants. Compared with Genome Aggregation Database (gnomAD) non-cancer controls, there was significant enrichment of ultra-rare and novel *RPA1*, but not *RPA2* or *RPA3*, germline variants in our cohort (adjusted p-value < 0.05). Taken together, these findings suggest that germline putative damaging variants affecting *RPA1* are found in excess in children with cancer, warranting further investigation into the functional role of these variants in oncogenesis.

KEYWORDS

RPA1, RPA2, RPA3, germline mutation, cancer

Introduction

Maintenance of genome integrity requires efficient DNA repair. The perturbation of processes engaged in repair of DNA damage by somatic mutations is a well-known mechanism for oncogenesis. Germline biallelic inactivation of genes governing DNA repair leads to classic cancer predisposition syndromes such as Fanconi anemia, ataxia telangiectasia and Bloom syndrome, among others (1–4). Monoallelic mutations impacting some of these genes can also increase the risk for cancer (5–9). We recently discovered that germline heterozygous mutations in the Replication Protein A1 (*RPA1*) gene cause Telomere Biology Disorder (TBD), a hereditary condition classically associated with pathological shortening of telomeres resulting in bone marrow failure (BMF), pulmonary and liver fibrosis, mucocutaneous fragility, and predisposition to solid tumors, myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) (10).

The RPA1 protein is the largest subunit of Replication Protein A (RPA), a heterotrimeric complex consisting of RPA1 (RPA70), RPA2 (RPA32) and RPA3 (RPA14). As a complex, RPA tightly binds single-strand DNA (ssDNA) to protect it from nucleases while maintaining DNA accessible to essential DNA-DNA and DNA-protein interactions. Consistent with the ubiquitous and ongoing formation of ssDNA, RPA is present and required across almost all cellular processes during replication, recombination, and repair of DNA. In fact, RPA is involved in all ssDNA repair pathways (nucleotide excision, base excision, mismatch) and double strand DNA repair mechanisms (homologous recombination, non-homologous end joining) (11–13). RPA participates in such diverse pathways through its ability to dynamically bind ssDNA while facilitating DNA repair and cell cycle protein interactions (11).

The essential role of RPA in DNA repair might lend RPA to be mutated in cancers. By mining the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (14), we found that somatic mutations in *RPA1*, *RPA2*, *RPA3* are found in 1.4%, 0.5%, and 0.9% of human cancers, respectively. In our previously published cohort of 4 patients with TBD, one patient who carried a germline *RPA1* p.V227A mutation developed advanced MDS requiring hematopoietic stem cell transplantation. All 3 *RPA1* germline mutations (p.V227A, p.E240K, p.T270A) identified in the 4 cases were missense and 2 out of 3 exerted a gain-of-function effect, resulting in increased binding to single strand and telomeric DNA (10). Besides these descriptions associating germline *RPA1* variants with bone marrow failure or hematologic malignancies, the *RPA2* or *RPA3* genes have not been linked to any human diseases thus far. Moreover, the landscape of germline variants in RPA heterotrimer in malignancies has not been systematically assessed. To address this knowledge gap, we investigated the occurrence of novel and rare germline variants in *RPA1*, *RPA2* and *RPA3* genes, in a cohort of 5,993 children with cancers. We found that ultra-rare and novel germline variants in the *RPA1* gene were significantly more common among pediatric cancer patients than non-cancer controls. Furthermore, we examined a separate cohort of 41 young adults with AML and identified potentially deleterious *RPA1* germline variants in 3 cases. Our studies indicate that the *RPA1* gene may be a novel risk factor for malignancies.

Methods

Data sources

For this study, we used publicly available whole exome sequencing datasets previously collected across studies at St. Jude Children's Research Hospital or through dbGaP. Specifically, we used the Pediatric Cancer Genome Project (PCGP) (15), real-time clinical genomics (RTCG/G4K) (16), St. Jude Lifetime Cohort (SJLIFE) (17), and TARGET datasets (TARGET URL is https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000218). In sum, we interrogated 5,993 germline samples across 24 cancer types including hematologic, non-central nervous system (CNS) solid tumors, and CNS tumors. Cancers were stratified into hematologic (n = 3,452; 58%), solid (n = 1,974; 33%) or CNS (n = 1,068; 18%) cancers and further subclassified as follows: i) hematologic malignancies: B-cell (B) acute lymphoid leukemia (B-ALL), T-cell ALL (T-ALL), acute myeloid leukemia (AML), Hodgkin's lymphoma, and non-Hodgkin's lymphoma; ii) solid tumors: germ cell tumor (GCT), melanoma (MEL), neuroblastoma (NBL), nasopharyngeal carcinoma (NPC), papillary thyroid carcinoma (PTC), sarcomas (Ewing's (EWS), osteosarcoma (OS), rhabdomyosarcoma (RMS), synovial), Wilms tumor (WT); and iii) CNS tumors: ependymoma (EP), low grade glioma (LGG), medulloblastoma (MB), and high grade glioma (HGG). An external cohort was queried, which consisted of 41 patients with AML from the German Study Alliance Leukemia that met following criteria: age below 35, blast-free remission after chemotherapy, karyotype aberrations (n = 12 with < 3, n = 29 with ≥ 3 aberrations detected in diagnostic karyotype or FISH analysis), and samples of peripheral blood or bone marrow at remission (18). The current study was approved by the Institutional Review Board at St. Jude Children's Research Hospital.

Variant calling and filtering

Variant calling and genotyping were performed using Genome Analysis Toolkit's (GATK) best practices workflow with modifications as described previously (19). We retained high quality variants that passed filtering using following criteria: allelic balance > 0.2, genotype quality > 20, variant allelic frequency (VAF) for heterozygous variants between 20-80%, minimum of 10 alternate reads supporting single nucleotide variants (SNVs) and 7 alternate reads supporting InDels, and missingness < 25% of samples. We performed variant annotation using ANNOtate VARiation (ANNOVAR) and variant effect predictor (VEP) tools (20). We also annotated all the variants using InterVar (21) automated clinical interpretation based on the American College of Medical Genetics and Genomics (ACMG) guidelines (22).

We retained coding variants in the RPA heterotrimer (*RPA1*, *RPA2*, and *RPA3* genes) with genome aggregation database (gnomAD) non-cancer cohort allelic frequency (AF) of < 0.5% (23) of the following classes: missense, frameshift insertions and deletions, stop gain, and splice site. We further filtered to retain missense variants with a computed Combined Annotation-Dependent Depletion (CADD) (24) Phred score > 15.

Computational analysis of RPA mutations

We performed local coordinate minimization followed by global side-chain optimization with the Atomic Multipole Optimized Energetics for Biomolecular Applications (AMOEBA) polarizable force field (25) on 5 high resolution structures of RPA fragments collectively comprising 7 modular domain of RPA heterotrimer. These included X-ray structures of the DNA binding domains A and B, DBD-A and DBD-B (PDB: 1JMC) (26), and the RPA trimerization core composed of DBD-C, D and E (PDB: 1L1O) (27) and NMR structures of the DBD-F (5N8A) (28) and the wing helix domain (PDB: 1DPU) (29). Prior to minimization, the ssDNA was removed from the 1JMC structure and bound peptides were removed from the 2 NMR structures. We then used our optimized structures to predict protein stability differences $\Delta\Delta G_{Fold}$ (DDG untrained (DDGun)) (30). DDGun estimates the $\Delta\Delta G_{Fold}$ of missense variants from a linear regression of sequence and biochemical features determined from the protein structure. Destabilizing $\Delta\Delta G_{Fold}$ values indicate a decrease in the ratio of folded to unfolded protein due to the mutation (we define negative $\Delta\Delta G_{Fold}$ values as stabilizing and positive $\Delta\Delta G_{Fold}$ values as destabilizing). We established $\Delta\Delta G_{Fold}$ cut-offs for mutations highly likely to impact protein folding. Our cut-offs were determined based on a $\Delta\Delta G_{Fold}$ that affects the ratio of folded to unfolded protein 12-fold (~1.5 kcal/mol) for both stabilizing and destabilizing mutations.

Statistical analysis

We performed rare-variant burden tests for *RPA1*, *RPA2*, *RPA3* variants using 5,993 cases from all pediatric cancers in our cohorts (pan-cancer) and within each sub-class of cancers, namely, hematologic (n = 3,452), solid (n = 1,974), and central nervous system CNS (n = 1,068) malignancies. For the control set, we retrieved all variants across *RPA1, RPA2*, and *RPA3* from gnomAD v2 non-cancer subset containing 134,187 individuals with no reported malignancy (23). All variants from control dataset were processed through the same variant annotation and filtering workflow as our cancer cohort (AF < 0.5%). Enrichment tests for cases with and without germline ultra-rare (AF < 0.005%) plus novel (AF 0%) and rare (AF < 0.1%) variants in the 3 genes were performed using both two- and one-sided Fisher exact tests using the statistical package R (v4.3) described in previous studies (31, 32). We used Bonferroni correction to adjust for multiple testing with a significance cutoff of adjusted p-value of < 0.05.

Results

Variants identified among the RPA heterotrimer genes

Within the pan-cancer cohort, we identified 80 cases with 55 germline heterozygous *RPA1*, *RPA2* or *RPA3* variants meeting criteria of AF < 0.5% in gnomAD non-cancer cohort and CADD score > 15 for candidate variant selection (Figure 1A). Specifically, 40 *RPA1*, 7 *RPA2* and 8 *RPA3* unique heterozygous germline variants were identified in 63, 7 and 10 cases, respectively (Figure 1B). All variants were classified as variant of uncertain significance (VUS) according to the ACMG criteria (Tables 1–3). Majority of the variants (92% of *RPA1*, 71% of *RPA2* and all *RPA3* variants) had CADD scores > 20, indicating a higher probability of a deleterious effect (Tables 1–3). In addition, looking at variant burden in population, we found that 98% (54/55) of the identified RPA heterotrimer variants had AF < 0.1% (this includes rare, very-rare, ultra-rare, and novel variants, Figure 1B). All *RPA1*, *RPA2* and *RPA3* variants are mutually exclusive and no cases with compound heterozygous or homozygous variants were identified.

RPA1 germline variants and cancers

RPA1 (616 amino acids, 70kDa) is the largest of the 3 subunits of the RPA heterotrimer. We discovered 1.05% (63/5993) of the cohort to harbor heterozygous germline *RPA1* variants (Figure 1C; Tables 1–3), which was statistically not significant compared to gnomAD non-cancer controls for all cancers and cancer subtypes (Table 4). RPA1 has 4 modular oligosaccharide binding-fold domains commonly referred to as functional DNA binding domains (DBD): F, A, B and C spanning the N- to C- terminal regions of the protein. *RPA1* variants were found across all 4 DBDs as follows: 6 in DBD-F, 15 in DBD-A, 10 in DBD-B, and 26 in DBD-C (Figure 1C). Of note, 6 cases were found to have *RPA1* variants in the linker regions between 2 DBDs. All *RPA1* variants were missense (Figure 1C) except for p.L53lfs*53 within DBD-F, which was found in 1 case. Three recurrently mutated amino acids were discovered in RPA1 domains DBD-A (p.V286, 9 cases), DBD-B (p.R389, 5 cases), and DBD-C (p.G437, 5 cases). We next focused specifically on novel and ultra-rare *RPA1* variants (33), present in 14 and 21 cases, respectively (Figure 1B; Tables 1–3). Notably, we found significant enrichment of *RPA1* novel and ultra-rare variants in our cohort (adjusted p-value < 0.05, Table 4).

Prediction of variant structural effect was performed by calculating protein stability change scores ($\Delta\Delta G_{Fold}$) with a $\Delta\Delta G_{Fold}$ that affects the ratio of folded to unfolded protein 12-fold (~1.5 kcal/mol) for both stabilizing and destabilizing mutations. Significant scores (>1.5 kcal/mol) were demonstrated for 4 variants (p.M46T in DBD-F, p. R234G in DBD-A, p.W361L in DBD-B, p.V594G in DBD-C) which were novel or ultra-rare (Tables 1–3). RPA1 p.M46T is likely to destabilize folding of DBD-F resulting in the loss of multiple important protein-protein interactions (11). W361 is a key DNA binding residue in DBD-B and human cells

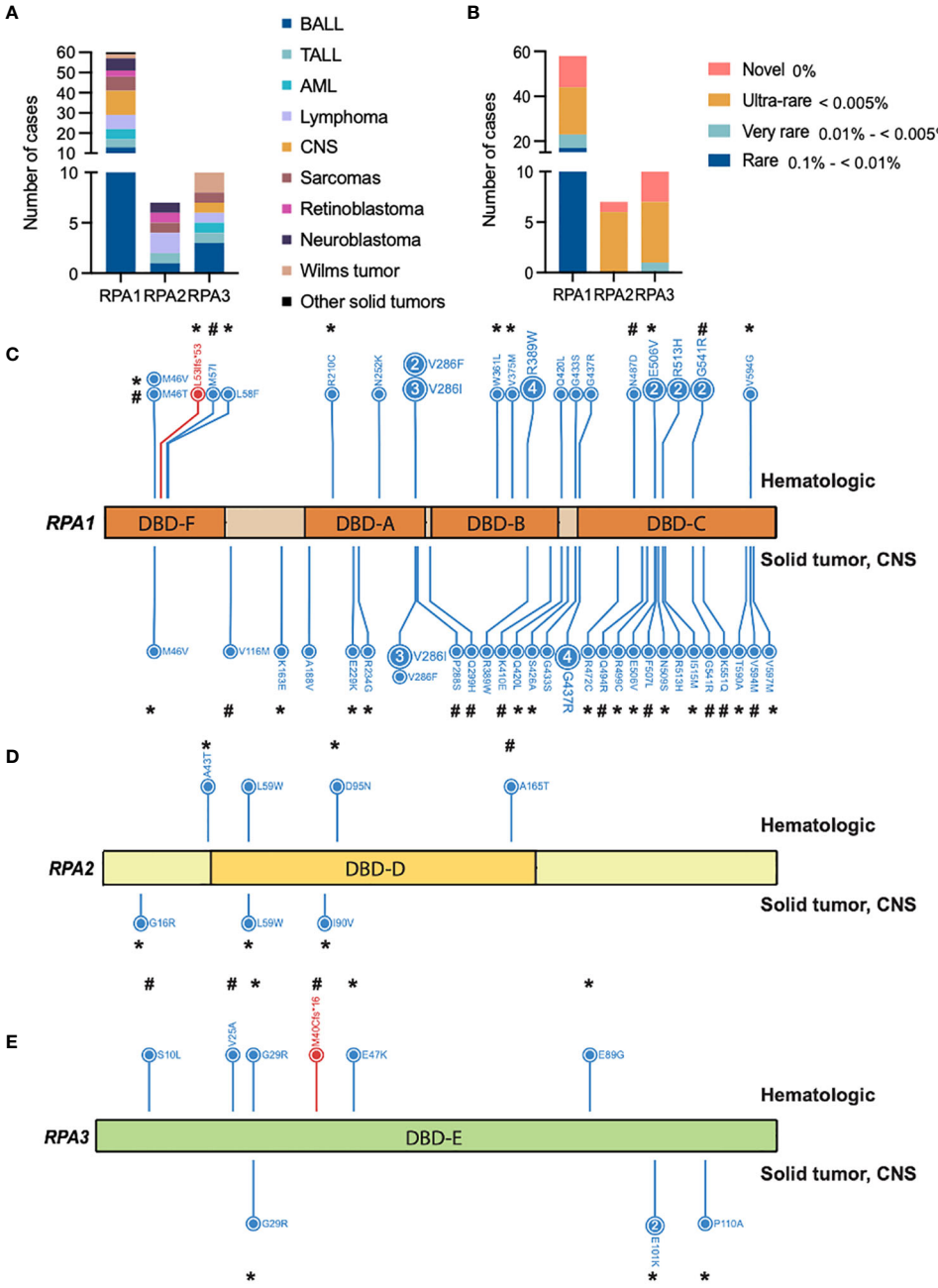

FIGURE 1
Germline heterozygous variants in the RPA heterotrimer in pediatric cancers. **(A)** Number of pediatric cancer cases with either RPA1, RPA2 or RPA3 heterozygous germline variants. B-ALL (B cell acute lymphoid leukemia), T-ALL (T cell acute lymphoid leukemia), AML (acute myeloid leukemia). Unique cancers are identified by different colors represented in the legend. **(B)** Number of cancer cases with either novel (pink), ultra-rare (gold), very rare (light teal), or rare (blue) germline variants in RPA1, RPA2 or RPA3 according to gnomAD allelic frequency. Schematic of human RPA1 (DNA binding domain (DBD- F, A, B, C)) **(C)**, RPA2 (DBD-D) **(D)** and RPA3 (DBD-E) **(E)** proteins with germline variants denoted. Blue and red lettering represents missense and frameshift variants, respectively. Numbers within circles represent the number of cases that harbored that variant while lack of numbering denotes one case per variant. Variants found in hematologic cases are represented on top and solid (intra and extra cranial) malignancies are denoted at the bottom of each protein map. * = ultra-rare variant allelic frequency (< 0.005%), # = novel variants.

with W361A support normal replication but are deficient in DNA repair (12, 34), suggesting that p.W361L may destabilize DBD-B folding resulting in hypomorphic RPA.

We next assessed which types of malignancies were present in patients with *RPA1* variants (Figure 1A). We found comparable frequency of cases with *RPA1* variants across solid tumors (n = 22, 1.1%), CNS cancers (n = 12, 1.1%) and hematological malignancies (n = 29, 0.8%). Among solid tumor cases with *RPA1* variants, 31.8% (7/22) presented with sarcomas and 27.3% (6/22) were diagnosed with neuroblastoma. Notably, the 7 sarcoma cases carried 6 unique *RPA1* variants (n = 2 novel and n = 1 ultra-rare) and only one was noted to have a concomitant germline mutation (Table 2). All 6

TABLE 1 Germline heterozygous variants found in RPA1, RPA2 and RPA3 in pediatric hematological malignancies.

Genomic position	SJID	Diagnosis	Age	RPA1 Domain	Heterozygous RPA1 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
1747265	SJBALL032225	BALL	9.18	F	c.A136G: p.M46V	AMR	0.0013%	0.0029%	22.2	0.28	VUS	none reported	IKZF1 del	unavailable
1747266	SJALL041240	BALL	4.13	F	c.T137C: p.M46T	NFE	novel	novel	25.2	0.456	VUS	none reported	NA	1.9
1747283	SJALL041360	BALL	14.08	F	c.155_156del: p.L53Ifs*53	NFE	0.0008%	0.0020%	NA	NA	VUS	none reported	NA	unavailable
1747879	SJHL042034	HL	17.4	F	c.G171A:p.M57I	NFE	novel	novel	28	0.492	VUS	none reported	NA	-0.2
1747882	SJCBF147	AML	17.68	F	c.G174C:p.L58F	NFE	0.0009%	0.0010%	25.2	0.489	VUS	none reported	NRAS	1.2
1780546	SJTALL021675	TALL	22.38	A	c.C628T: p.R210C	Other	0.0038%	novel	35	0.594	VUS	none reported	none reported	0.6
1782352	SJBALL020994	BALL	24.38	A	c.C756G: p.N252K	Other	0.0063%	0.0178%	26.6	0.267	VUS	none reported	RCSD2-ABL2 fusion, IKZF1 deletion, VPREB deletion	0.2
1782605	SJAML030416	AML	17.11	A	c.G856T: p.V286F	Other	0.0097%	0.0149%	33	0.373	VUS	NOTCH2 (p.P6fs*, novel); FANCD2 (p.V427_E15splice, novel)	TP53(p.D281H), ETV6 (p.F417fs), WT1(p.R414fs), PHF6(p.R225*), FLT3 (p.Y597>11aa)	0.6
1782605	SJAML031075	AML (AMKL)	3.13	A	c.G856A: p.V286I	AMR	0.0410%	novel	16.3	0.373	VUS	MLL (p.I882fs)	JAK1:p.L783F;JAK3: p.A573V;GATA1: p.S30_G31fs;STAG2: p.T149fs	0.6
1782605	SJAML032052	AML (AMML)	16.23	A	c.G856A: p.V286I	NFE	0.0410%	0.0286%	16.3	0.373	VUS	none reported	NPM1:p.W288fs; PTPN11: p.E76K	0.6
1782605	SJAML032355	AML (AMML)	17	A	c.G856A: p.V286I	NFE	0.0410%	0.0286%	16.3	0.373	VUS	HIP1:Amplification	ERCC2:p.M1fs;NPM1: p.W288fs;NRAS:p.G12D; PTPN11:p.A72V;TRIM28: p.I302_K304fs; SLC45A3_ELK4:Fusion	0.6
1782605	SJALL016427	BALL	NA	A	c.G856T: p.V286F	NFE	0.0097%	0.0203%	33	0.373	VUS	none reported	none reported	0.6
1782983	SJALL041859	BALL	13.99	B	c.G1082T: p.W361L	NFE	0.0008%	0.0019%	32	0.752	VUS	none reported	NA	2.2
1783867	SJHL041557	HL	NA	B	c.G1123A: p.V375M	NFE	0.0021%	0.0049%	28.6	0.413	VUS	none reported	NA	1.2

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TABLE 1 Continued

Genomic position	SJID	Diagnosis	Age	RPA1 Domain	Heterozygous RPA1 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
1783909	SJTALL022093	TALL	3.02	B	c.C1165T: p.R389W	AMR	0.1763%	0.0028%	35	0.319	VUS	none reported	none reported	-0.9
1783909	SJBALL001702	BALL	NA	B	c.C1165T: p.R389W	NFE	0.1763%	0.0736%	35	0.319	VUS	none reported	none reported	-0.9
1783909	SJALL015269	TALL	6.3	B	c.C1165T: p.R389W	NFE	0.1763%	0.0736%	35	0.319	VUS	none reported	none reported	-0.9
1783909	SJBALL032592	BALL	12.78	B	c.C1165T: p.R389W	NFE	0.1763%	0.0736%	35	0.319	VUS	ATM (p.W2769*, 0.0008%)	TACC3-FGFR3: focal amplification	-0.9
1787123	SJNHL042753	NHL	7.24	B	c.A1259T: p.Q420L	NFE	0.0019%	0.0042%	22	0.16	VUS	none reported	NA	unavailable
1787161	SJNHL042070	NHL	14.35	coding, non DBD	c.G1297A: p.G433S	AFR	0.0157%	0.0508%	23.5	0.218	VUS	none reported	NA	unavailable
1787173	SJHL042469	HL	11.31	C	c.G1309A: p.G437R	AFR	0.0134%	0.1397%	24.4	0.286	VUS	none reported	NA	unavailable
1792053	SJALL018944	BALL	19.48	C	c.A1459G: p.N487D	NFE	novel	novel	28.6	0.564	VUS	none reported	NA	0.2
1792111	SJPHALL020033	BALL	3.28	C	c.A1517T: p.E506V	AMR	0.0034%	novel	32	0.387	VUS	BRIP1 (p.P47A, 0.03%), TAL1 (p.E1_splice, novel)	none reported	-0.6
1792111	SJALL015640	TALL	5.23	C	c.A1517T: p.E506V	AFR	0.0034%	0.0381%	32	0.387	VUS	none reported	none reported	-0.6
1792132	SJERG020054	BALL	NA	C	c.G1538A: p.R513H	NFE	0.0164%	0.0331%	35	0.535	VUS	none reported	IKZF1 del, CDKN2A del, ETV6 del	0.4
1792132	SJHL019322	HL	15.53	C	c.G1538A: p.R513H	NFE	0.0164%	0.0331%	35	0.535	VUS	none reported	NA	0.4
1795196	SJALL041325	BALL	11.21	C	c.G1621A: p.G541R	NFE	novel	novel	26.1	0.591	VUS	none reported	NA	0.1
1795196	SJNHL018781	NHL	14.7	C	c.G1621A: p.G541R	NFE	novel	novel	26.1	0.591	VUS	CTC1 (p.R224*, 0.00008%)	NA	0.1
1800399	SJALL018992	BALL	7.78	C	c.T1781G: p.V594G	NFE	0.0011%	0.0013%	26.5	0.569	VUS	RAD51D (p.G258fs*,novel)	NA	3.3

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TABLE 1 Continued

Genomic position	SJID	Diagnosis	Age	RPA2 Domain	Heterozygous RPA2 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
28233784	SJHYPO109	BALL	2.11	coding, non DBD	c.G127A:p.A43T	NFE	0.0032%	0.0065%	17.1	0.039	VUS	none reported	none reported	unavailable
28233735	SJTALL022654	TALL	11.66	D	c.T176G: p.L59W	Other	0.0022%	0.0299%	22.6	0.131	VUS	TSC1 (p.E876_E21splice, novel)	none reported	0.1
28233489	SJHL041547	HL	14.62	D	c.G283A: p.D95N	NFE	0.0004%	0.0010%	34	0.409	VUS	POLG (p.R1096C, 0.001%)	NA	-0.2
28223548	SJHL041567	HL	16.45	D	c.G493A: p.A165T	NFE	novel	novel	27.8	0.108	VUS	none reported	NA	0.2
Genomic position	SJID	Diagnosis	Age	RPA3 Domain	Heterozygous RPA3 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
7680021	SJAML042701	AML	0.65	E	c.C29T:p.S10L	NFE	novel	novel	23.2	0.025	VUS	RTEL1 (p.R986*, novel)	NA	-1.4
7679976	SJALL003857	BALL	1.84	E	c.T74C:p.V25A	Other	novel	novel	29.1	0.506	VUS	SH3B2 (p.G470_E8splice, novel)	NA	3.8
7679965	SJALL041268	BALL	4.05	E	c.G85C:p.G29R	NFE	0.0032%	0.0065%	34	0.775	VUS	none reported	NA	0.5
7678756	SJHL041577	HL	11.83	E	c.118delA: p.M40Cfs*16	NFE	novel	novel	NA	NA	VUS	none reported	NA	unavailable
7678736	SJTALL022645	TALL	NA	E	c.G139A:p.E47K	NFE	0.0008%	0.0020%	26.6	0.222	VUS	none reported	none reported	0.3
7677512	SJALL041310	BALL	1.91	E	c.A266G:p.E89G	EAS	0.0004%	0.0057%	27.3	0.33	VUS	none reported	NA	1.9

BALL, B-cell acute lymphoid leukemia; TALL, T-cell acute lymphoid leukemia ALL; AML, acute myeloid leukemia; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; AMR, Admixed/Latino; NFE, Non-Finnish European; AFR, African; EAS, East Asian; VUS, variant of unknown significance; NA, not available; unavailable, lack of structural coverage or accuracy at nucleotide position.

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TABLE 2 Germline heterozygous variants found in RPA1, RPA2 and RPA3 in extra-cranial solid tumors.

Genomic position	SJID	Diagnosis	Age	RPA1 Domain	Heterozygous RPA1 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
1747265	SJNBL017162	NBL	4.69	F	c.A136G:p.M46V	NFE	0.0013%	0.0010%	22.2	0.28	VUS	none reported	none reported	0.1
1756468	SJRHB032408	Sarcoma (RMS)	3.1	coding, non DBD	c.G346A:p.V116M	AMR	novel	novel	23.4	0.151	VUS	CNOT3 (p.P243fs, novel)	BCOR (p.F1385fs)	0.6
1779063	SJWLM019906	WT	4.9	A	c.C563T:p.A188V	NFE	0.0161%	0.0078%	23.7	0.136	VUS	none reported	none reported	-0.4
1780603	SJNBL030203	NBL	4.44	A	c.G685A:p.E229K	AFR	0.0004%	novel	31	0.227	VUS	TP53 (p.A161T, novel)	ALK (p.R1275Q)	0
1782605	SJSTS019601	MEL	9.9	A	c.G856A:p.V286I	NFE	0.0403%	0.0254%	16.32	0.206	VUS	BRIP1 (p.Q685*, 0.006%), MED12 (p.Q2109_Q2115>Q, 0.0009%)	none reported	unavailable
1782605	SJMEL031366	Sarcoma (RMS)	11.42	A	c.G856A:p.V286I	NFE	0.0403%	0.0254%	16.32	0.206	VUS	NA	NA	0.1
1782605	SJRHB000026	Sarcoma (synovial)	9.9	A	c.G856A:p.V286I	NFE	0.0403%	0.0254%	16.32	0.206	VUS	none reported	NA	-0.2
1782611	SJGCT019774	GCT	16.34	A	c.C862T:p.P288S	AFR	novel	novel	31	0.367	VUS	none reported	NA	0.4
1783909	SJRB030058	RB	0.28	B	c.C1165T:p.R389W	NFE	0.1763%	0.0736%	35	0.319	VUS	none reported	none reported	-0.6
1783972	SJOS040162	Sarcoma (OS)	22.78	B	c.A1228G:p.K410E	Other	novel	novel	23.3	0.148	VUS	none reported	none reported	0.1
1787123	SJST032198	PTC	18.13	B	c.A1259T:p.Q420L	NFE	0.0019%	0.0042%	22	0.16	VUS	NTHL1 (p.A237_E5splice, novel)	BRAF (p.V600E)	0.8
1787140	SJEWS019204	Sarcoma (EWS)	16.11	coding, non DBD	c.T1276G:p.S426A	NFE	0.0021%	0.0049%	24.1	0.191	VUS	none reported	NA	-0.9
1787161	SJNBL017202	NBL	1.51	coding, non DBD	c.G1297A:p.G433S	AFR	0.0157%	0.0508%	23.5	0.218	VUS	PALB2 (p.G562_E4splice, novel), NDRG4 (p.M292_E14splice, novel)	none reported	0
1787173	SJSTS042513	Sarcoma (synovial)	8.53	C	c.G1309A:p.G437R	AFR	0.0134%	0.1397%	24.4	0.286	VUS	none reported	NA	unavailable
1787173	SJSTS019626	WT	0.87	C	c.G1309A:p.G437R	AFR	0.0134%	0.1397%	24.4	0.286	VUS	none reported	NA	0.4
1792008	SJRB019561	RB	2.34	C	c.C1414T:p.R472C	AFR	0.0026%	0.0212%	35	0.542	VUS	RB1 (p.L218*,novel)	NA	-0.2
1792111	SJNPC019502	NPC	13.81	C	c.A1517T:p.E506V	AFR	0.0034%	0.0381%	32	0.387	VUS	none reported	NA	0.6
1792120	SJRB017939	RB	0.17	C	c.A1526G:p.N509S	NFE	0.0008%	novel	20.6	0.141	VUS	RB1 (p.R358*, novel)	NA	0.2
1795196	SJNBL018730	NBL	0.11	C	c.G1621A:p.G541R	NFE	novel	novel	26.1	0.591	VUS	MDC1 (p.A710_E6splice, novel)	NA	0.6

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TABLE 2 Continued

Genomic position	SJID	Diagnosis	Age	RPA1 Domain	Heterozygous RPA1 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
1795226	SJNBL017207	NBL	2.42	C	c.A1651C:p.K551Q	NFE	novel	novel	22.7	0.282	VUS	none reported	none reported	unavailable
1800386	SJOS018814	Sarcoma (OS)	11.76	C	c.A1768G:p.T590A	AMR	0.0050%	0.0325%	23.1	0.15	VUS	none reported	NA	-0.2
1800407	SJNBL042729	NBL	0.93	C	c.G1789A:p.V597M	NFE	0.0011%	0.0014%	27.8	0.292	VUS	none reported	NA	unavailable
Genomic position	SJID	Diagnosis	Age	RPA2 Domain	Heterozygous RPA2 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
28233735	SJOS040159	Sarcoma (OS)	19.92	D	c.T176G:p.L59W	Other	0.0022%	0.0299%	22.6	0.131	VUS	none reported	none reported	0.1
28233504	SJRB041658	RB	0.24	D	c.A268G:p.I90V	NFE	0.0004%	0.0010%	18.28	0.077	VUS	RB1 (p.E237*, novel)	NA	unavailable
28240645	SJNBL017483	NBL	3.88	coding, non DBD	c.G46A:p.G16R	AFR	0.0008%	0.0136%	22.6	0.088	VUS	none reported	none reported	unavailable
Genomic position	SJID	Diagnosis	Age	RPA3 Domain	Heterozygous RPA3 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar automated classification	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
7679965	SJLPS014753	Sarcoma (lipo)	22	E	c.G85C:p.G29R	NFE	0.0032%	0.0065%	34	0.775	VUS	NA	NA	0.5
7676696	SJWLM018894	WT	1.05	E	c.G301A:p.E101K	AFR	0.0015%	0.0085%	34	0.325	VUS	WT1 (p.Q238*, novel)	NA	0
7676669	SJWLM043921	WT	1.05	E	c.C328G:p.P110A	NFE	0.0064%	0.0130%	23.1	0.512	VUS	WT1:CNV Del, LIG4 (p.K424fs, novel)	NA	0.3

GCT, germ cell tumor; MEL, melanoma; NBL, neuroblastoma; NPC, nasopharyngeal carcinoma; PTC, papillary thyroid carcinoma; EWS, Ewing's sarcoma; OS, osteosarcoma; RMS, rhabdomyosarcoma; WT, Wilms tumor; AMR, Admixed/Latino; NFE, Non-Finnish European; AFR, African; VUS, variant of unknown significance; NA, not available; unavailable, lack of structural coverage or accuracy at nucleotide position.

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TABLE 3 Germline heterozygous variants found in RPA1, RPA2 and RPA3 in extra-cranial solid tumors.

Genomic position	SJID	Diagnosis	Age	RPA1 Domain	Heterozygous RPA1 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar auto-mated classifi-cation	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
1778987	SJLGG031132	LGG (Ganglioglioma)	11.23	coding, non DBD	c.A487G:p.K163E	NFE	0.0008%	0.0010%	16.67	0.128	VUS	none reported	BRAF:p.V600E	unavailable
1782296	SJEPD030782	EPD	3.98	A	c.C700G:p.R234G	NFE	0.0008%	0.0019%	28	0.598	VUS	none reported	none reported	1.5
1782605	SJMB030776	MB	3.86	A	c.G856T:p.V286F	AMR	0.0097%	novel	33	0.373	VUS	PBRM1 (p.K128_E4splice, novel)	none reported	-0.4
1782646	SJMB032506	MB	12.8	coding, non DBD	c.G897C:p.Q299H	NFE	novel	novel	22.4	0.081	VUS	C7 (p.R521S, 0.002%); MYH9 (p.F235_E6splice, novel)	none reported	-0.2
1787173	SJHGG117	HGG	2.57	C	c.G1309A:p.G437R	AFR	0.0134%	0.1397%	24.4	0.286	VUS	none reported	none reported	unavailable
1787173	SJHGG030703	HGG (HGNET)	1.49	C	c.G1309A:p.G437R	AFR	0.0134%	0.1397%	24.4	0.286	VUS	none reported	NUTM2B_Deletion	unavailable
1792075	SJHGG067	HGG	5.6	C	:c.A1481G:p.Q494R	EAS	novel	novel	23.5	0.202	VUS	none reported	none reported	-0.1
1792089	SJLGG030365	LGG	1.11	C	c.C1495T:p.R499C	AMR	0.0052%	0.0171%	35	0.435	VUS	SDHA (p.R31*, 0.02%) RUNX1 (p.Q415*, 0.0006%)	KIAA1549_BRAF_Fusion	-0.1
1792113	SJCNS018575	MB	9.19	C	c.T1519C:p.F507L	NFE	novel	novel	28.7	0.179	VUS	NA	NA	0.6
1792132	SJLGG046	LGG	5.1	C	c.G1538A:p.R513H	NFE	0.0164%	0.0331%	35	0.535	VUS	none reported	none reported	0.4
1792139	SJHGG100	HGG	10.99	C	c.C1545G:p.I515M	NFE	0.0008%	novel	17.81	0.074	VUS	none reported	none reported	0.5
1800398	SJST032495	MB	14	C	c.G1780A:p.V594M	NFE	novel	novel	33	0.419	VUS	BRCA1 (p.E1559_E15splice, novel)	NA	0.7
Genomic position	SJID	Diagnosis	Age	RPA3 Domain	Heterozygous RPA1 germline variant	Genetic Ancestry	gnomad non-cancer v.2.1.1 AF	Ancestry specific AF	CADD	REVEL	InterVar auto-mated classifi-cation	Other heterozygous germline variants	Somatic mutations	Stability (ccal/mol)
7676696	SJMB031439	MB	9.37	E	c.G301A:p.E101K	EAS	0.0015%	0.0104%	34	0.325	VUS	ANKRD26 (p.Y1708*), novel	PTCH1(p.Y93fs), AFF4 truncating insertion; CNVs- PTEN, SMARCA2, JAK2	0

EP, ependymoma; LGG, low grade glioma; MB, medulloblastoma; HGG, high grade glioma; AMR, Admixed/Latino; NFE, Non-Finnish European; AFR, African; EAS, East Asian; VUS, variant of unknown significance; NA, not available; unavailable, lack of structural coverage or accuracy at nucleotide position.

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neuroblastoma cases were found to have unique *RPA1* variants (n = 3 novel, n = 2 ultra-rare) of which half were found to have germline variants reported in *PALB2*/*NDRG4*, *MDC1*, or *TP53* genes (Table 2). Three cases of retinoblastoma harbored unique *RPA1* variants (2 ultra-rare) with 2 cases having concomitant germline *RB1* mutation (Table 2). Two cases of Wilms tumor were identified to have germline *RPA1* variants. Among the single cases of solid tumors (germ cell tumor, melanoma, nasopharyngeal carcinoma, and papillary thyroid carcinoma), 2 ultra-rare and 1 novel *RPA1* variants were found.

Among cases with CNS tumors, 4 patients with medulloblastoma harbored novel (n = 3) or ultra-rare (n = 1) *RPA1* variants. Each of these cases also carried other germline mutations (*PBRM1*, *C7* and *MYH9*, *BRCA1*, *ANKRD26*) of which *BRCA1* and *ANKRD26* are cancer predisposition genes (Table 3). Furthermore, 4 cases with high grade glioma harbored 3 *RPA1* variants (n = 1 novel, n = 1 ultra-rare), all clustering within DBD-C domain of RPA1. These patients had no other potentially causative germline variants reported in other predisposition genes. Among the 3 low grade glioma, 3 *RPA1* variants (one ultra-rare) were identified, with one harboring other germline mutations in *SDHA* and *RUNX1*. Lastly, one ultra-rare *RPA1* variant was identified in a case of ependymoma without other germline mutations (Table 3).

From patients with hematologic malignancies, *RPA1* variants were most common in B-ALL (n = 13), followed by lymphoma (n = 7), AML (n = 5), and T-ALL (n = 4) (Table 1). Out of 13 B-ALL cases, 3 and 5 were novel and ultra-rare, respectively. Only 2 cases out of the 13 had heterozygous germline variants in cancer predisposition genes (*RAD51D*, *BRIP1)*. Among lymphomas, we observed 4 Hodgkin's lymphoma (n = 1 novel, n = 1 ultra-rare) and 3 non-Hodgkin's lymphoma (n = 1 novel, n = 1 ultra-rare) with *RPA1* variants. In the AML sub-cohort, we found 3 unique *RPA1* variants in 5 cases, of which 4 were mutated at nucleotide 856 in DBD-A (c.856G>T, c.856G>A coding different amino acids) and 1 ultra-rare variant in DBD-F domain (Table 1). Of the 5 *RPA1*-mutated AML cases, 3 carried other germline variants (MLL, HIP1, NOTCH2 and FANCD2). Four unique *RPA1* variants (n = 2 ultra-rare) were discovered in 4 patients with T-ALL, with one case having additional germline *ATM* variant (Table 1).

Given the occurrence of MDS/AML in one prior patient with germline *RPA1* p.V227A with TBD (10) and 5 AML cases in this study, we queried a cohort of 41 young adults with AML and karyotype aberrations (18) for RPA heterotrimer germline variants. We found 1 ultra-rare (c.460G>A, T154A, AF 0.001%) and 2 rare (c.1397C>G, A466G, AF 0.027%; c.1538G>A, R513H, AF 0.016%) *RPA1* heterozygous variants (Supplemental Table 1).

RPA2 and RPA3 germline variants and cancers

RPA2 is the second largest subunit (270 amino acids, 34kDa) of the RPA heterotrimer. We identified 6 heterozygous germline *RPA2* variants in 7 cases of pediatric malignancies. Five variants are present in DBD-D (Figure 1D) and did not exhibit dysfunctional protein folding scores (Tables 1, 2). All variants were either ultra-rare (n = 5) or novel (n = 1). Four patients (4/3452, 0.1%) had hematological malignancies (n = 1 B-ALL, n = 1 T-ALL, n = 2 Hodgkin's lymphoma) and 3 had solid cancers (n = 1 RBL, n = 1 neuroblastoma, n = 1 sarcoma). Other germline mutations were noted in 3 out of 7 cases (Tables 1, 2).

RPA3, although less than half the size of RPA2 (121 amino acids, 14kDa) had 8 unique germline heterozygous variants (n = 4 ultra-rare, n = 3 novel) in 10 cases of pediatric cancers, including 6 hematologic (B-ALL n = 3, T-ALL n = 1, AML n = 1, Hodgkin's lymphoma n = 1), 3 solid tumors (Wilms tumor n = 2, sarcoma n = 1) and 1 CNS (medulloblastoma) cancers (Figure 1E; Tables 1–3). All were missense except for one frameshift (p.M40Cfs*16). Protein folding scores for 2 out of 7 available *RPA3* variants were greater than 1.5 kcal/mol and were either novel or ultra-rare (Tables 1–3). Half of *RPA3* mutated cases had other germline variants noted (Tables 1–3). The number of cases with *RPA2* or *RPA3* germline variants did not reach statistical significance compared to gnomAD non-cancer controls (Table 4).

Discussion

The RPA heterotrimer is an essential protein for binding ssDNA encountered in cellular transactions to facilitate DNA-DNA and DNA-protein interactions during DNA replication, repair, recombination, RNA transcription, and telomere maintenance. As such, mutations in this genome maintenance protein have been linked to cancer formation in mice (35) and are acquired in up to ~1% of human cancers (14). We recently demonstrated that heterozygous germline *RPA1* mutations *RPA1* c.680T>C p.V227A, c.718G>A p.E240K and c.808A>G p.T270A in DBD-A are associated with TBD, which predisposes to hematologic and solid tumors. In this study, one patient with RPA1-related TBD developed MDS (10). Based on these data, we reasoned that germline defects in RPA1 and possibly also the other 2 components of the RPA heterotrimer (RPA2 and RPA3) might be associated with cancer development. To this end, we investigated comprehensive germline genomic data for the presence of heterozygous variants in *RPA1*, *RPA2* and *RPA3* across a large series of pediatric hematologic, solid and CNS malignancies. We discovered significant enrichment of ultra-rare and novel *RPA1* germline variants in our pediatric cancer cohort compared to non-cancer controls, positioning *RPA1* as a novel candidate predisposition gene. Moreover, in an additional cohort of 41 patients with AML, we identified 3 heterozygous germline *RPA1* variants (c460G>A, p.T154A; c.1397C>G, p.A466G; c.1538G>A, p.R513H) with potential pathogenic effect.

RPA1 harbored the most variants likely due to its larger size compared to *RPA2* and *RPA3*. Although we did not observe a statistically significant enrichment of putative damaging variants in *RPA2* and *RPA3*, some of the identified variants were novel or ultra-rare and could possibly have a deleterious effect. Thus, *RPA2* and *RPA3* could be considered as genes of unknown significance (GUS) yet potentially important in tumor formation. All 3 proteins are required to fold properly to form a functional RPA heterotrimer (13). For this reason, we calculated stabilities of the RPA modular

TABLE 4 Statistical analysis using two- and one-sided Fisher exact tests of ultra-rare plus novel and rare germline heterozygous variants in RPA1, RPA2 and RPA3 across hematologic, extra-cranial solid and CNS tumors.

Ultra-rare or novel variants AF<0.005%												
Gene	Subset	Cancer_AF	Control_AF	Cancer_Alt_Count	Cancer_Total_Count	Control_Alt_Count	Control_Total_Count	p.value (fisher.test-greater)	OR (fisher.test-greater)	FDR_fisher_greater	p.value (Two-sided)	FDR_fisher_twosided
RPA1	PanCancer_UltraRare	0.0029	0.0017	35	11951	466	267908	0.00350858	1.6837	0.028068639	0.00531368	0.042509437
RPA1	HEM_UltraRare	0.0022	0.0017	15	6889	466	267908	0.231331347	1.2518	0.462662694	0.37871645	0.504955267
RPA1	ST_UltraRare	0.0033	0.0017	13	3935	466	267908	0.024524772	1.8993	0.09809909	0.031863803	0.127455213
RPA1	CNS_UltraRare	0.0033	0.0017	7	2129	466	267908	0.083930393	1.8903	0.223814382	0.107377392	0.214754784
RPA2	PanCancer_UltraRare	0.0006	0.0007	7	11979	200	268174	0.785228312	1	0.729135903	0.7835	0.927224074
RPA3	PanCancer_UltraRare	0.0008	0.0006	9	11977	164	268210	0.32195265	1	0.568945016	1.2289	0.853417524
All variants AF<0.5%												
Gene	Subset	Cancer_AF	Control_AF	Cancer_Alt_Count	Cancer_Total_Count	Control_Alt_Count	Control_Total_Count	p.value (fisher.test-greater)	OR (fisher.test-greater)	FDR_fisher_greater	p.value (Two-sided)	FDR_fisher_twosided
RPA1	PanCancer	0.0053	0.0061	63	11923	1614	266760	0.868322713	0.8733	0.982140829	0.33224587	0.504955267
RPA1	HEM	0.0042	0.0061	29	6875	1614	266760	0.982140829	0.6972	0.982140829	0.056909619	0.151758983
RPA1	ST	0.0056	0.0061	22	3926	1614	266760	0.667688785	0.9262	0.890251714	0.835352152	0.954688173
RPA1	CNS	0.0056	0.0061	12	2124	1614	266760	0.631653729	0.9338	0.890251714	1	1
RPA2	PanCancer	0.0006	0.0078	7	11979	2081	266293	1	1	4.73434E-30	0.0748	7.10151E-29
RPA2	HEM	0.0006	0.0078	4	6900	2081	266293	1	1	5.16066E-18	0.0742	3.87049E-17
RPA2	ST	0.0008	0.0078	3	3945	2081	266293	1	1	5.40401E-10	0.0973	2.70201E-09
RPA2	CNS	0	0.0078	0	2136	2081	266293	1	1	1.34364E-07	0	5.03865E-07
RPA3	PanCancer	0.0008	0.0006	10	11976	169	268205	0.237746122	1	0.353635923	1.3252	0.663067356
RPA3	HEM	0.0009	0.0006	6	6898	169	268205	0.276876834	1	0.459624798	1.3804	0.76604133
RPA3	ST	0.0008	0.0006	3	3945	169	268205	0.455502374	1	0.741779259	1.2068	0.927224074
RPA3	CNS	0.0005	0.0006	1	2135	169	268205	0.740267746	1	1	0.7433	1

"PanCancer", all cancers in the cohort; HEM, hematologic; ST, solid tumor; CNS, central nervous system.

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domains harboring mutations to gain insight into the possible effect of identified germline variants on RPA heterotrimer function. Scores greater than 1.5 are highly predictive of protein instability and dysfunction. High protein folding scores were found for 4 unique *RPA1* variants in 4 cases, 3 identified in patients with B-ALL and one in a patient with ependymoma. All were either ultra-rare or novel with CADD scores suggesting high likelihood of pathogenicity. Two *RPA3* variants also harbored high protein folding scores in patients with B-ALL. This suggests that dysfunctional folding of the RPA heterotrimer may lead to genomic instability in these patients.

In our discovery cohort, we identified 5 AML cases with germline *RPA1* variants. One had an ultra-rare *RPA1* p.L58F variant in DBD-F and the remaining 4 had variants affecting nucleotide 856 within DBD-A domain (c.G856A, p.V286I in 3 cases and c.G856T, p.V286F in one case). The resulting amino acid changes do not differ in size or charge from wild-type valine and have a neutral protein folding score of 0.6. However, these mutations may disrupt protein-protein, protein-DNA interactions, or post-translational modifications, which are known mechanisms implicated in pathogenicity of *RPA1* variants in various experimental models (10–13, 35). Additionally, TBD-associated pathogenic *RPA1* variants, p.V227A, p.E240K and p.T270A, have protein folding scores of 1.4, 0.1 and 0.2 (consistent with normal protein folding shown in biochemical assays) yet were shown to exert gain-of-function effect on DNA binding and melting of telomeric G-quadruplexes (10). Three of the 4 AML cases with *RPA1* variants in DBD-A domain had additional germline variants in genes (NOTCH2, FANCD2, MLL, HIP1) which, together with *RPA1* may have an epistatic effect to cause overall genomic instability. Corroborating data from a small cohort of 41 AML patients in which 3 patients carried *RPA1* variants (p.T154A in linker region; p.A466G and p.R513H in DBD-C) deserves further investigation. Beyond *RPA1* in the AML cohort, we also found a novel germline missense variant in *RPA3* in an infant with AML who also harbored a germline truncating variant in the DNA helicase, *RTEL1*, which is associated with TBD (36, 37). More functional studies are needed to determine the pathogenicity of *RPA1* V286I/F alterations and their role in hematologic malignancy.

Among the 13 CNS tumors with variants in RPA heterotrimer genes, 9 cases were high grade neoplasms, including medulloblastoma and high-grade glioma. Interestingly, 3 of the 5 medulloblastoma cases had novel and one very rare germline *RPA1*, as well as one ultra-rare *RPA3* variant. Notably, even though variants in other unrelated genes were also found in 4 of the 5 medulloblastoma cases, none of these genes have been previously associated with medulloblastomas in the literature. Other studies have identified germline defects in DNA repair genes in medulloblastoma (38, 39). It would stand to reason that germline mutations in the RPA heterotrimer, which functions in almost all DNA repair pathways, could potentiate oncogenic transformation. Further investigation should focus on assessing the function of RPA mutant proteins in DNA repair and their contribution to tumor biology.

Our study has several limitations. Although all cases were assessed using a uniform pipeline, the cohort is skewed towards cases with B-ALL (~4-fold higher number of B-ALL compared to solid and CNS cancers). We included all germline and somatic mutations per case that were reported in previously published studies; however, this information was unavailable for a proportion of cases and therefore we cannot make definitive conclusions about *RPA* variants being the sole germline driver in these cancers. Although ultra-rare and novel heterozygous germline variants in *RPA1* were significantly enriched in pediatric cancers, it is difficult to ascertain pathogenicity and clinical relevance without functional follow-up, which falls beyond the scope of this study. It is plausible that variants with high in-silico protein folding energy, ultra-rare and/or novel allelic frequency and high pathogenicity scores may be clinically relevant and should be among the top variants to explore in future studies.

In summary, evasion of DNA repair mechanisms is a common theme among cancers. RPA is an essential protein for DNA replication and repair. Our study describes novel and rare variants with potentially deleterious effect in the *RPA1*, *RPA2* and *RPA3* genes in pediatric malignancies. Moreover, we have identified enrichment of *RPA1* variants in cancer cases compared to non-cancer controls, suggesting that this gene potentially acts as a novel cancer driver. We plan to exploit our findings and perform further functional and biochemical characterization of recurrent cancer associated *RPA1* variants to assess their potential use as targets for future cancer therapies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

RS, KN, and MW: conceptual design of the study and data interpretation. NO and WC: data analysis and statistics. RG, MJS, and MS: computational analysis of RPA mutations and interpretation. MK and FB: conceptual design and interpretation. All authors contributed to manuscript preparation and editing. All authors approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1229507/full#supplementary-material

References

1. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature* (2009) 461:1071–8. doi: 10.1038/nature08467

2. Kennedy RD, D'Andrea AD. DNA repair pathways in clinical practice: Lessons from pediatric cancer susceptibility syndromes. *J Clin Oncol* (2006) 24:3799–808. doi: 10.1200/JCO.2005.05.4171

3. Sharma R, Lewis S, Wlodarski MW. DNA repair syndromes and cancer: Insights into genetics and phenotype patterns. *Front Pediatr* (2020) 8:570084. doi: 10.3389/fped.2020.570084

4. Walsh MF, Chang VY, Kohlmann WK, Scott HS, Cunniff C, Bourdeaut F, et al. Recommendations for childhood cancer screening and surveillance in DNA repair disorders. *Clin Cancer Res* (2017) 23:e23–:e31. doi: 10.1158/1078-0432.CCR-17-0465

5. Croitoru ME, Cleary SP, Di Nicola N, Manno M, Selander T, Aronson M, et al. Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. *J Natl Cancer Inst* (2004) 96:1631–4. doi: 10.1093/jnci/djh288

6. de Voer RM, Hahn MM, Mensenkamp AR, Hoischen A, Gilissen C, Henkes A, et al. Deleterious germline BLM mutations and the risk for early-onset colorectal cancer. *Sci Rep* (2015) 5:14060. doi: 10.1038/srep14060

7. Hall MJ, Bernhisel R, Hughes E, Larson K, Rosenthal ET, Singh NA, et al. Germline pathogenic variants in the ataxia telangiectasia mutated (ATM) gene are associated with high and moderate risks for multiple cancers. *Cancer Prev Res (Phila)* (2021) 14:433–40. doi: 10.1158/1940-6207.CAPR-20-0448

8. Kim J, Light N, Subasri V, Young EL, Wegman-Ostrosky T, Barkauskas DA, et al. Pathogenic germline variants in cancer susceptibility genes in children and young adults with rhabdomyosarcoma. *JCO Precis Oncol* (2021) 5. doi: 10.1200/PO.20.00218

9. Thompson ER, Doyle MA, Ryland GL, Rowley SM, Choong DY, Tothill RW, et al. Exome sequencing identifies rare deleterious mutations in DNA repair genes FANCC and BLM as potential breast cancer susceptibility alleles. *PLoS Genet* (2012) 8:e1002894. doi: 10.1371/journal.pgen.1002894

10. Sharma R, Sahoo SS, Honda M, Granger SL, Goodings C, Sanchez L, et al. Gain-of-function mutations in RPA1 cause a syndrome with short telomeres and somatic genetic rescue. *Blood* (2022) 139:1039–51. doi: 10.1182/blood.2021011980

11. Caldwell CC, Spies M. Dynamic elements of replication protein A at the crossroads of DNA replication, recombination, and repair. *Crit Rev Biochem Mol Biol* (2020) 55:482–507. doi: 10.1080/10409238.2020.1813070

12. Haring SJ, Mason AC, Binz SK, Wold MS. Cellular functions of human RPA1. Multiple roles of domains in replication, repair, and checkpoints. *J Biol Chem* (2008) 283:19095–111. doi: 10.1074/jbc.M800881200

13. Wold MS. Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu Rev Biochem* (1997) 66:61–92. doi: 10.1146/annurev.biochem.66.1.61

14. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: The catalogue of somatic mutations in cancer. *Nucleic Acids Res* (2019) 47:D941–7. doi: 10.1093/nar/gky1015

15. Downing JR, Wilson RK, Zhang J, Mardis ER, Pui CH, Ding L, et al. The pediatric cancer genome project. *Nat Genet* (2012) 44:619–22. doi: 10.1038/ng.2287

16. Newman S, Nakitandwe J, Kesserwan CA, Azzato EM, Wheeler DA, Rusch M, et al. Genomes for kids: The scope of pathogenic mutations in pediatric cancer revealed by comprehensive DNA and RNA sequencing. *Cancer Discov* (2021) 11:3008–27. doi: 10.1158/2159-8290.CD-20-1631

17. Wang Z, Wilson CL, Easton J, Thrasher A, Mulder H, Liu Q, et al. Genetic risk for subsequent neoplasms among long-term survivors of childhood cancer. *J Clin Oncol* (2018) 36:2078–87. doi: 10.1200/JCO.2018.77.8589

18. Kirschner M, Rolles B, Crysandt M, Rollig C, Stolzel F, Kramer M, et al. Impaired overall survival in young patients with acute myeloid leukemia and variants in genes predisposing for myeloid Malignancies. *Hemasphere* (2022) 6:e787. doi: 10.1097/HS9.0000000000000787

19. Chen W, et al. A rare variant analysis framework using public genotype summary counts to prioritize disease-predisposition genes. *Nat Commun* (2022) 13:2592. doi: 10.1038/s41467-022-30248-0

20. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* (2010) 38:e164. doi: 10.1093/nar/gkq603

21. Li Q, Wang K. InterVar: Clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *Am J Hum Genet* (2017) 100:267–80. doi: 10.1016/j.ajhg.2017.01.004

22. Richards CS, Bale S, Bellissimo DB, Das S, Grody WW, Hegde MR, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet Med* (2008) 10:294–300. doi: 10.1097/GIM.0b013e31816b5cae

23. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* (2020) 581:434–43. doi: 10.1038/s41586-020-2308-7

24. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res* (2019) 47:D886–94. doi: 10.1093/nar/gky1016

25. Tollefson MR, Litman JM, Qi G, O'Connell CE, Wipfler MJ, Marini RJ, et al. Structural insights into hearing loss genetics from polarizable protein repacking. *Biophys J* (2019) 117:602–12. doi: 10.1016/j.bpj.2019.06.030

26. Bochkarev A, Pfuetzner RA, Edwards AM, Frappier L. Structure of the single-stranded-DNA-binding domain of replication protein A bound to DNA. *Nature* (1997) 385:176–81. doi: 10.1038/385176a0

27. Bochkareva E, Korolev S, Lees-Miller SP, Bochkarev A. Structure of the RPA trimerization core and its role in the multistep DNA-binding mechanism of RPA. *EMBO J* (2002) 21:1855–63. doi: 10.1093/emboj/21.7.1855

28. Guilliam TA, Brissett NC, Ehlinger A, Keen BA, Kolesar P, Taylor EM, et al. Molecular basis for PrimPol recruitment to replication forks by RPA. *Nat Commun* (2017) 8:15222. doi: 10.1038/ncomms15222

29. Mer G, Bochkarev A, Gupta R, Bochkareva E, Frappier L, Ingles CJ, et al. Structural basis for the recognition of DNA repair proteins UNG2, XPA, and RAD52 by replication factor RPA. *Cell* (2000) 103:449–56. doi: 10.1016/S0092-8674(00)00136-7

30. Montanucci L, Capriotti E, Frank Y, Ben-Tal N, Fariselli P. DDGun: an untrained method for the prediction of protein stability changes upon single and multiple point variations. *BMC Bioinf* (2019) 20:335. doi: 10.1186/s12859-019-2923-1

31. Huang KL, Mashl RJ, Wu Y, Ritter DI, Wang J, Oh C, et al. Pathogenic germline variants in 10,389 adult cancers. *Cell* (2018) 173:355–370 e14. doi: 10.1016/j.cell.2018.03.039

32. Oak N, Cherniack AD, Mashl RJ, Network TA, Hirsch FR, Ding L, et al. Ancestry-specific predisposing germline variants in cancer. *Genome Med* (2020) 12:51. doi: 10.1186/s13073-020-00744-3

33. Rasnic R, Linial N, Linial M. Expanding cancer predisposition genes with ultra-rare cancer-exclusive human variations. *Sci Rep* (2020) 10:13462. doi: 10.1038/s41598-020-70494-0

34. Hass CS, Chen R, Wold MS. Detection of posttranslational modifications of replication protein A. *Methods Mol Biol* (2012) 922:193–204. doi: 10.1007/978-1-62703-032-8_15

35. Wang Y, Putnam CD, Kane MF, Zhang W, Edelmann L, Russell R, et al. Mutation in Rpa1 results in defective DNA double-strand break repair, chromosomal instability and cancer in mice. *Nat Genet* (2005) 37:750–5. doi: 10.1038/ng1587

36. Calado RT, Young NS. Telomere diseases. *N Engl J Med* (2009) 361:2353–65. doi: 10.1056/NEJMra0903373

37. Marsh JCW, Gutierrez-Rodrigues F, Cooper J, Jiang J, Gandhi S, Kajigaya S, et al. Heterozygous RTEL1 variants in bone marrow failure and myeloid neoplasms. *Blood Adv* (2018) 2:36–48. doi: 10.1182/bloodadvances.2017008110

38. Trubicka J, Zemojtel T, Hecht J, Falana K, Piekutowska-Abramczuk D, Ploski R, et al. The germline variants in DNA repair genes in pediatric medulloblastoma: a challenge for current therapeutic strategies. *BMC Cancer* (2017) 17:239. doi: 10.1186/s12885-017-3211-y

39. Carta R, Del Baldo G, Miele E, Po A, Besharat ZM, Nazio F, et al. Cancer predisposition syndromes and medulloblastoma in the molecular era. *Front Oncol* (2020) 10:566822. doi: 10.3389/fonc.2020.566822

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