REVIEW ARTICLE

Germline variants in acquired aplastic anemia: current knowledge and future perspectives

Peicheng Wang,[1,2*] Wanzhi Jiang,[1,2*] Tianyi Lai,[1,2*] Qi Liu,[1,2] Yingying Shen,[1-3] Baodong Ye[1-3] and Dijiong Wu[1-4]

[1]Department of Hematology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), Hangzhou; [2]The First School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou; [3]National Traditional Chinese Medicine Clinical Research Base (Hematology), Hangzhou and [4]Department of Oncology and Hematology, Wenzhou Hospital of Integrated Traditional Chinese and Western Medicine affiliated to Zhejiang Chinese Medicine University, Wenzhou, Zhejiang, China

PW, WJ and TL contributed equally as first authors.

Correspondence: D. Wu
wudijiong@zcmu.edu.cn
B. Ye
13588453501@163.com

Received: September 16, 2023.
Accepted: April 9, 2024.
Early view: July 11, 2024.

https://doi.org/10.3324/haematol.2023.284312

Abstract

Aplastic anemia (AA) is a disease characterized by failure of hematopoiesis, bone marrow aplasia, and pancytopenia. It can be inherited or acquired. Although acquired AA is believed to be immune-mediated and random, new evidence suggests an underlying genetic predisposition. Besides confirmed genomic mutations that contribute to inherited AA (such as pathogenic mutations of *TERT* and *TERC*), germline variants, often in heterozygous states, also play a not negligible role in the onset and progression of acquired AA. These variants, associated with inherited bone marrow failure syndromes and inborn errors of immunity, contribute to the disease, possibly through mechanisms including gene homeostasis, DNA repair, and immune injury. This article explores the nuanced association between acquired AA and germline variants, detailing the clinical significance of germline variants in diagnosing and managing this condition. More work is encouraged to better understand the role of immunogenic pathogenic variants and whether somatic mutations participate as secondary “hits” in the development of bone marrow failure.

Introduction

Aplastic anemia (AA) is the most common bone marrow failure syndrome and is classified into inherited and acquired forms based on clinical manifestations and genomic background. Inherited AA, often referred to as inherited bone marrow failure syndrome (IBMFS), is represented by Fanconi anemia and dyskeratosis congenita, both of which are clinically rare.[1] Acquired AA is characterized by immune-mediated bone marrow injury, manifested as a hypoplastic, fatty bone marrow, with a profound reduction in the numbers of hematopoietic stem/progenitor cells that leads to defective hematopoiesis and peripheral pancytopenia.[2] Immunosuppressive therapy (IST) with cyclosporine A, combined or not with antithymocyte globulin, is considered the first-line therapy that achieves hematologic remission in approximately 60%–70% of cases of acquired AA.[3] However, immune system involvement alone cannot fully explain the pathogenesis and progression of acquired AA. The specific triggering factors and antigen targets responsible for the hyperactivity of T cells in acquired AA remain unclear.[4,5] Notably, abnormalities in the bone marrow microenvironment and hematopoietic stem cells (HSC) also play a significant role in the development of the disease.[6]

With the advancement and application of next-generation sequencing and whole-exome sequencing,[7] HSC defects in acquired AA patients have been progressively revealed. Somatic variants, including those involving *ASXL1*, *DNMT3A*, *TP53*, and *RUNX1*, have been shown to be associated with worse responses to IST, but are still insufficient to explain IST failure in nearly 40% of cases.[8] Approximately 5%–30% of young patients diagnosed with AA carry IBMFS-associated germline variants, potentially leading to adverse outcomes.[9-11] These variants can be categorized according to American College of Medical Genetics and Genomics guidelines as pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), and benign (B).[12] Dr. Neal S. Young’s research indicates that concomitant variants, excluding P/LP variants, are considered acquired AA variants,[13] which should include recessive heterozygotes and VUS or B/LB variants in dominant genes. Despite lacking typical symptoms due to their mild pathogenicity,

individuals carrying germline variants associated with IBMFS and inborn errors of immunity eventually experience immune-mediated bone marrow destruction as deleterious factors accumulate. Our review aims to explore this phenomenon: clinically non-pathogenic variants (B/LB, VUS, or recessive heterozygotes) hold significant implications for acquired AA, as their enrichment is not accidental.

Distinguishing inherited bone marrow failure syndromes in the diagnosis of acquired aplastic anemia

Before defining novel subgroups in acquired AA, it is essential to retrospectively review the characteristics of both acquired AA and IBMFS using their unique features.

Features and specific examinations of inherited bone marrow failure syndrome

Inherited AA or IBMFS is a collection of rare disorders resulting from the inheritance of pathogenic germline variants and includes Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, Shwachman-Diamond syndrome, severe congenital neutropenia, congenital amegakaryocytic thrombocytopenia, MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) syndrome, and GATA2 deficiency.[14-16] These diseases often have distinctive manifestations that differentiate them from acquired AA, such as developmental abnormalities, physical deformities, mucocutaneous triad (dyskeratosis congenita), pulmonary fibrosis (dyskeratosis congenita), and exocrine pancreatic insufficiency (Shwachman-Diamond syndrome).[17] Thorough investigation into family history and specific testing is crucial. A family history displaying abnormalities heightens suspicion of IBMFS. Positive results in chromosomal breakage tests serve as the diagnostic standard for identifying Fanconi anemia.[18] An extremely short telomere length (<1st percentile for age) can be used as a highly sensitive and specific marker for the detection of dyskeratosis congenita.[19]

Heterogeneity of clonal hematopoiesis

Clonal hematopoiesis can be viewed as the adaptive response of HSC to environmental stress.[20] The heterogeneity of clones reflects pathogenic mechanisms that are distinct for acquired AA and IBMFS. In acquired AA, HSC tend to have variants with immune escape and proliferation in overcoming the immune response and the toxic microenvironment. This grants them a competitive advantage in clone formation, which is observed in around 50%-70% of patients.[21-23] Somatic variants, including *PIGA*, *BCOR*, and *BCORL1*, are characterized by an improved response to IST and overall survival, and are considered clinically beneficial. On the other hand, *DNMT3A*, *ASXL1*, *TP53*, *RUNX1*, and *CSMD1* variants are associated with a lower survival rate and progression to myelodysplastic syndromes/acute myeloid leukemia, and are considered clinically unfavorable.[8,21] Comparable features have been observed in IBMFS, and are referred to as somatic compensation. Unlike in acquired AA, the pressures confronting HSC in IBMFS stem primarily from their inherent genetic defects. Consequently, the significance of these clones in IBMFS is centered around compensating for or restoring the original function caused by the intrinsic deficiencies. Somatic variants can be categorized into three types based on the specific compensation mechanisms:[24] gene-specific, pathway-specific, and pathway-independent. Gene-specific compensation is observed in variants that fully or partially restore original gene function. It usually occurs in diseases such as Fanconi anemia, dyskeratosis congenita, and SAMD9/9L-related disorders. Pathway-specific compensation maintains pathway integrity through compensatory variants in other genes, such as the acquired promoter variants of telomerase reverse transcriptase (*TERT*) in dyskeratosis congenita and loss of *EIF6* in Shwachman-Diamond syndrome. Pathway-independent compensation involves acquiring growth advantages through alternative pathways or mechanisms, exemplified by *TP53* variants in Shwachman-Diamond syndrome, telomere biology disorders, and Diamond-Blackfan anemia, RAS pathway variants in Fanconi anemia, *RUNX1* variants in Fanconi anemia, and *ASXL1* variants in GATA2-related disorders.[24-28] Somatic compensation endows HSC with proliferative advantages within the hematopoietic microenvironment of IBMFS, leading to two possible outcomes: disease reversal or malignant transformation.

Value of immune mechanisms

Somatic variants, such as *ASXL1*, *RUNX1*, and *TP53*, lack specificity in bone marrow failure diseases, as well as the variable penetrance of IBMFS, posing a challenge for differentiation. The immune escape mechanisms of paroxysmal nocturnal hemoglobinuria (PNH) and *HLA* class I allelic gene loss (Figure 1), along with the immune characteristics of TCR-Vβ oligoclonal expansion, collectively serve as markers for acquired AA, providing diagnostic references with high positive predictive value and specificity.[29-31] PNH is characterized by the HSC *PIGA* clone, where *PIGA* variants impede the synthesis of glycosylphosphatidylinositol-anchored proteins, resulting in the loss of CD55 and CD59 expression. This deficiency triggers complement activation, leading to hemolysis and thrombosis.[21,32] Meanwhile, the deficiency of glycosylphosphatidylinositol-anchored proteins renders inactive immune response-related cell surface proteins,[33] which is a common immune escape mechanism observed in 20%–60% of patients with acquired AA.[34,35] The detection of PNH clones (>1%) in patients with acquired AA is considered to exclude patients from having IBMFS and has been validated in clinical and research settings.[11,13,36,37] Similarly, the loss of *HLA* class I allelic genes endows HSC

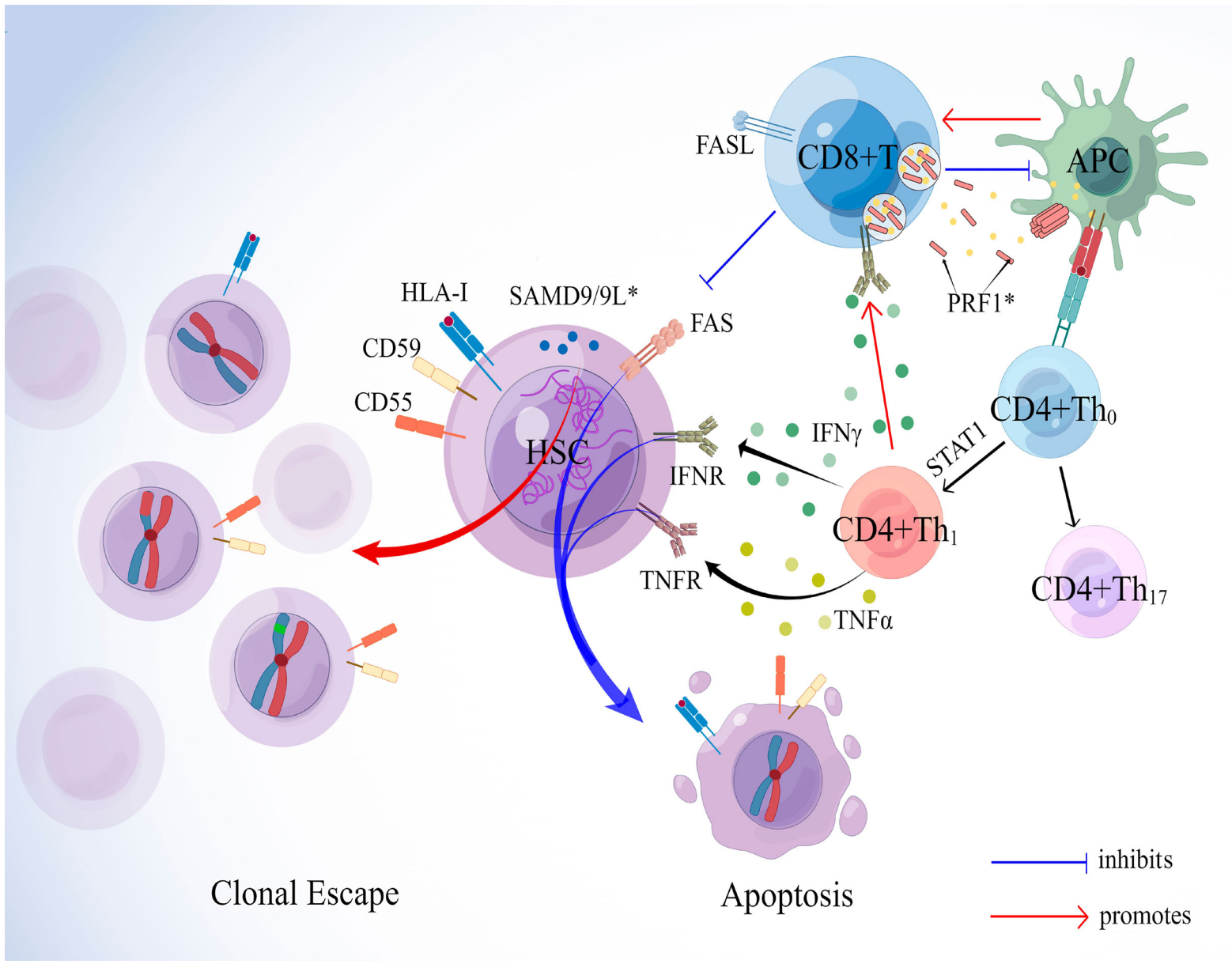

Figure 1. Immune destruction and involvement of germline variants. Antigen-presenting cells (APC) process antigens, presenting them to T cells. $CD4^+$Th0 cells differentiate into Th1 and Th17 cells, maintaining a relative balance. Among them, STAT1 promotes the differentiation of Th0 to Th1 cells, leading to the secretion of cytokines interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) by $CD4^+$ Th1 cells, which bind to their respective receptors (IFNR and TNFR), promoting apoptosis of hematopoietic stem cells (HSC). Additionally, increased IFNγ stimulates $CD8^+$ T-cell proliferation and promotes HSC apoptosis through FASL-FAS signaling. $CD8^+$ T cells also release granzyme and perforin encoded by *PRF1*, leading to the elimination of APC and negative regulation of immune responses. Furthermore, SAMD9/9L is associated with antitumor and anti-infection responses, although the specific mechanism of action remains unclear. The formidable survival pressure prompts HSC to employ mechanisms such as *PIGA*, 6p uniparental disomy, and *HLA-I* mutations to escape from immune responses. *Genes affected by germline variants involved in acquired aplastic anemia.

with the capacity to establish clones with cellular immunity in 20% of patients, involving the mechanisms of *HLA* variants and 6p copy number-neutral loss of heterozygosity (CN-LOH) (also known as 6p uniparental disomy).[31] CN-LOH describes the phenomenon of heterozygous loss where one parental chromosome region is replaced by the other,[38,39] and occurs frequently in the *HLA* class I region of chromosome 6p in acquired AA.[21] *HLA* variants cause acquired loss of partial *HLA* class I genes. Both losses are concentrated at sites such as *HLA-B*14:02*, *HLA-A*02:01*, and *HLA-B*40:02*, outlining the classic immune escape landscape in acquired AA.[21,31] In a previous study, 6p CN-LOH showed almost 100% positive predictive value for acquired AA, highlighting its significant diagnostic value.[29] Limited usage of TCR-Vβ indicates the oligoclonal expansion of $CD8^+CD28^-$ T cells,[40-42] suggesting immune dysregulation at the T-cell level under chronic antigen stimulation,[43] which is a common occurrence in acquired AA. Successful use of IST results in a significant reduction in the clonal expansion of $CD8^+CD28^-$ T cells, considered robust evidence for an immune-mediated mechanism.[44,45] Recent evidence indicates that oligoclonal patterns are also present in effector memory $CD8^+CD28^-CD57^+$ T cells. Notably, effector memory T cells pose a persistent threat and play a crucial role in the recognition and recurrence of acquired AA.[30,46]

Contribution of germline variants to acquired aplastic anemia

In patients with acquired AA, genetic reports of individuals characterized by more severe phenotypes or unfavorable

treatment responses often show common occurrences of germline heterozygous recessive variants, as well as VUS or B/LB variants in dominant genes.[7,47] Related to IBMFS and inborn errors of immunity, the genes involved maintain the stability, repair, and renewal of HSC under normal conditions (Figures 1 and 2). When mutated, impaired or exaggerated functions may contribute to the pathogenesis and development of acquired AA (Table 1).[7,47-50] Exploring the prevalence and mechanisms of germline variants in acquired AA will contribute to a more comprehensive understanding of the etiology and risk factors of the disease.

DNA repair deficiencies accelerate bone marrow failure

DNA damage repair has a crucial role in maintaining the response of HSC to both external and internal stimuli, as well as their self-renewal process.[51] The Fanconi anemia pathway is one of the important mechanisms involved in DNA damage repair (Figure 2). The prevalence of common heterozygous *FANC* variants is 8.38% in patients with acquired AA and hematologic malignancies.[52] In Chinese patients with acquired AA below the age of 40, the prevalence of non-pathogenic heterozygous *FANC* variants was 45.9%, with LB variants and VUS accounting for 97.56% of all cases.[7] To date, 22 *FANC* genes have been identified, and their homozygous states are closely associated with Fanconi anemia, cancer susceptibility, and hereditary breast tumors.[18] Previous studies have suggested that *FANC* heterozygous carriers typically do not have the congenital anomalies and chromosome breakage observed in Fanconi anemia.[53] The risk of developing cancer in heterozygous carriers is comparable to that in the general population, and these states are generally considered non-pathogenic.[54,55] However, in a study integrating AA and hematologic malignancies, heterozygous carriers of *FANC* variants showed susceptibility to both conditions, suggesting that in certain disease contexts, heterozygosity may be insufficient to maintain proper function.[52] The underlying mechanism may involve the diminished ability of heterozygous variants to repair DNA, leading to unresolved DNA damage that activates P53 and results in HSC exhaustion. Alternatively, without P53 inhibition, accumulated mutations may trigger immune destruction or evolve into hematologic malignancies.[52,56] *FANCA* variants are the most common among cases of acquired AA with *FANC* heterozygous variants, followed by *BRCA2* and *FANCD2*, which have a pattern of variant frequencies similar to that in Fanconi anemia. The proportions of *FANCG* and *FANCC* variants are relatively small, possibly due to limited sample sizes. The enrichment of rare variants in *FANCN* and *SLX4*, which are less commonly observed in patients with Fanconi anemia, may indicate susceptibility in acquired AA or could be attributed to sample size, necessitating further comprehensive investigations.[7,18,52]

Excessive immune responses aggravate hematopoietic stem cell damage

Some patients with acquired AA carry immune-related germline variants, exacerbating the immune response in disease progression. Heterozygous recessive variants of perforin (*PRF1*) have been identified in acquired AA, accounting for approximately 6% of cases.[50] Homozygous *PRF1* variants are associated with familial hemophagocytic lymphohistiocytosis, a fatal disorder of childhood onset characterized by functional perforin deficiency.[57] In a previous study, the p. A91V variant was most frequently observed, and four out of the five patients had increased hemophagocytes in the bone marrow, without other typical clinical features of hemophagocytic syndrome.[50] The p. A91V variant was confirmed to induce sustained inflammation, chronic antigen presentation, and release of inflammatory mediators.[58] Low perforin caused by *PRF1* variants impairs the elimination of antigen-presenting cells, leading to the activation and proliferation of cytotoxic T cells.[50,59] Accompanied by the secretion of interferon-γ, this ultimately results in the destruction of HSC.[60] Recently, two patients with acquired AA were found to have heterozygous variants in Myb-like SWIRM and MPN domains 1 (*MYSM1*), a regulator

Table 1. Incidence and functional mechanisms of germline variants in acquired aplastic anemia.

Classification	Gene involved	Carrier ratio, %	Function	Genotype	References
Repair disorders	*FANC*	45.9 (age <40 years)	Constitutes the FA pathway for DNA repair	Recessive heterozygous	7
Immune damage	*PRF1*	6	Encodes perforin, involved in cell lysis and immune regulation	Recessive heterozygous	50
	MYSM1	Unknown	Deubiquitinase catalysis, hematopoietic and immune regulation	Recessive heterozygous	61
SAMD9/9L disorders	*SAMD9/9L*	20 (age >18 years)	Involved in antitumor and antiviral responses	Heterozygous (pathogenicity unclear)	47
Others	*CTC1*	2.3 (AA/PNH)	Component of the CST complex, regulates telomeres	Recessive heterozygous	48
	SBDS	5	Involved in ribosome biogenesis	Recessive heterozygous	49

FA: Fanconi anemia; AA: aplastic anemia; PNH: paroxysmal nocturnal hemoglobinuria.

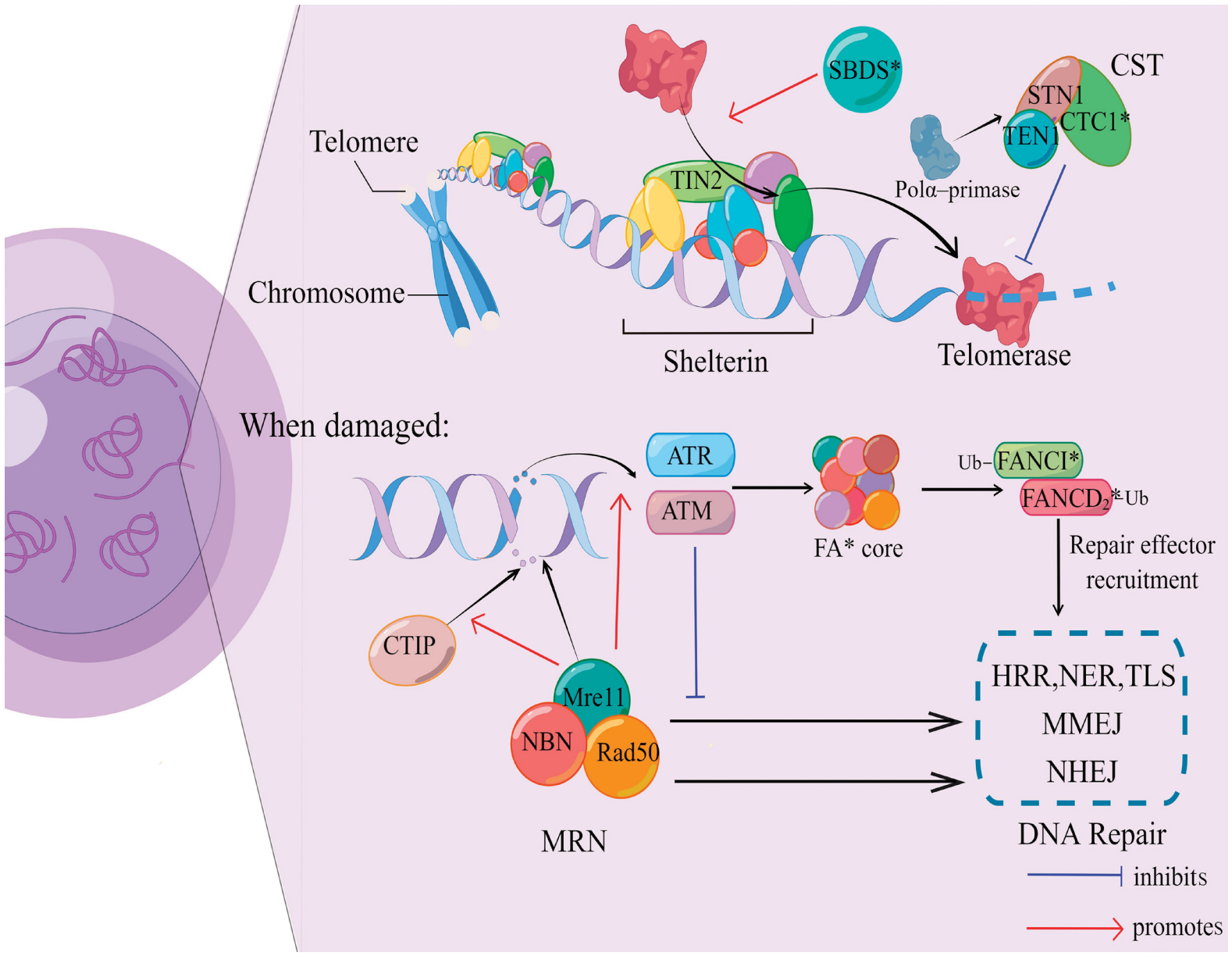

Figure 2. Germline variants involved in genome stability and DNA repair. Telomeres are repeated DNA segments located at the ends of chromosomes, protected by shelterin proteins and extended by telomerase. The recruitment of telomerase by shelterin proteins is a crucial step for telomere elongation, a process in which SBDS also participates. The CST complex (CTC1-STN1-TEN1) has a dual role: recruiting Polα-primase for C-strand synthesis and inhibiting telomere extension, providing intricate regulation of telomeres. In response to DNA damage, the MRN complex serves as a sensor, signaler, and effector to mediate DNA repair. It promotes the recruitment of CTIP, ATR, and ATM, facilitating DNA repair pathways such as microhomology-mediated end joining and non-homologous end joining. ATR and ATM also facilitate the formation of the Fanconi anemia core and are crucial for FANCD2-FANCI monoubiquitylation, ultimately promoting homologous recombinational repair, nucleotide-excision repair, and translesion synthesis DNA repair pathways. *Genes affected by germline variants, in acquired aplastic anemia. Ub: ubiquitylation; HRR: homologous recombinational repair; NER: nucleotide-excision repair; TLS: translesion synthesis; MMEJ: microhomology-mediated end joining; NHEJ: non-homologous end joining.

of hematopoiesis and immune cell development, known to cause IBMFS in a homozygous state.[61,62] These patients had loss of *HLA-A*02:06*, and one also had PNH. Tatsuya *et al*. suggested that *MYSM1* heterozygous defects lead to an excessive immune response to exogenous antigens, increasing susceptibility to acquired AA.[61] The association between acquired AA and immune-related variants appears to be non-incidental. Whole-genome sequencing of patients with AA/PNH revealed that 65% (37/57) carried heterozygous germline variants associated with inborn errors of immunity. Among the 60 variants identified, VUS were predominant, involving 37 autosomal recessive variants and 23 autosomal dominant variants, offering a new perspective on acquired AA.[63] In conclusion, heterozygous recessive variants or VUS in dominant genes may lead to aberrant responses or incomplete reactions to antigens, ultimately resulting in the development of acquired AA due to the failure of feedback regulatory mechanisms, as detailed in the preceding discussion.

SAMD9/SAMD9L exaggerate defects of hematopoietic stem cells

Sterile alpha motif domain-containing protein 9/9-like (*SAMD9/9L*) encode two proteins involved in antitumor and antiviral responses; however, their precise functions and regulation remain elusive.[64] In 2016, *SAMD9* was first identified as the causative gene for MIRAGE syndrome, a multisystem disorder characterized by a predisposition to myelodysplastic syndromes and loss of chromosome 7.[16] Meanwhile, whole-exome sequencing also revealed that

SAMD9L was the cause of ataxia-pancytopenia syndrome.[65] Both syndromes share similarities in their excessive anti-proliferative effects due to gain-of-function (GOF) variants, leading to the manifestation of cytopenia or even pancytopenia.[66,67] Pediatric cohorts with *SAMD9/9L* (GOF) variants tend to develop hematologic malignancies.[37,67,68] *SAMD9L* (GOF) variants were found to impair HSC proliferation and self-renewal, promote inflammation, and exacerbate bone marrow failure.[69,70] Intense survival pressure drives HSC to counteract the variant damage through somatic compensation or CN-LOH,[69] resulting in transient early-life AA episodes.[37] Recent studies have revealed that up to 20% of adult patients with severe AA harbor *SAMD9/9L* (non-GOF) heterozygous germline variants.[47] These variants frequently co-occur with typical features of acquired AA, including 6p CN-LOH, PNH clones, and variants in *HLA* and *PIGA* genes. Notably, the probability of chromosome 7 abnormalities in these patients was relatively low, 2/40 cases (5%), differently from what is observed in SAMD9/9L-related disorders. The most common types of variants observed are missense, nonsense, and frameshift. Carriers of these variants tend to be younger and exhibit lower levels of neutrophils, reticulocytes, and platelets.[47] However, our understanding of these variants and their pathogenic mechanisms remains incomplete. The regulation of HSC and the microenvironment by *SAMD9/9L* is highly complex. Loss-of-function variants are also linked to a predisposition to myelodysplastic syndromes and abnormal expression of specific inflammatory pathways.[71] *SAMD9/9L* variants in adult acquired AA can be considered to contribute to mild functional disruptions, including dysregulation of HSC proliferation and immune-inflammatory responses, ultimately leading to T-cell destruction and bone marrow failure.

Other variants

With the application of whole-exome sequencing, numerous reports on other germline variants continue to emerge. Heterozygous variants in ribosome maturation protein (*SBDS*) account for approximately 5% of cases of acquired AA.[49] Biallelic variants in *SBDS* are found in over 90% of patients with Shwachman-Diamond syndrome and are a major genetic factor associated with this disorder.[72] Shwachman-Diamond syndrome is a genetic syndrome characterized by an elevated risk of bone marrow failure, exocrine pancreatic insufficiency, skeletal defects, and hematologic malignancies.[73] Patients with acquired AA with heterozygous *SBDS* variants do not have such manifestations but share pancytopenia and telomere shortening with those having Shwachman-Diamond syndrome.[49] The telomere shortening may be attributed to the dysfunction in SBDS in interacting with shelterin, a complex that protects telomeres and initiates telomerase recruitment.[74]

Conserved telomere maintenance component 1 (*CTC1*) is a part of the CST complex.[75] Approximately 2.3% of patients with acquired AA and PNH harbor heterozygous variants in *CTC1* and do not have any signs and family history of IBMFS.[48] Homozygous *CTC1* variants are common in Coats plus syndrome and dyskeratosis congenita, in which these variants impair the function of the CST complex, leading to telomere dysfunction and genomic instability.[75,76] Of four cases of *CTC1*-Tier-1 carriers, three (75%) were found to have large PNH clones (clone size >50%), indicating the widespread occurrence of immune escape. While these heterozygous *CTC1* variants do not significantly affect telomere length, they still exhibit a tendency to acquired bone marrow failure and PNH, needing further exploration.

Moreover, additional cases have offered insights for future investigation. A B/LB variant of TRF1-interacting nuclear factor 2 (*TINF2*), Ser245Tyr, associated with late-onset recurrent AA, has been identified in three patients with acquired AA.[77,78] GOF variants in the signal transducer and activator of the transcription 1 (*STAT1*) have been reported in two cases of AA and led to increased T helper type 1 cells and the overexpression of interferon-γ.[79,80] Other concomitant variants, including heterozygous variants of *RTEL1*, *DDX41*, *ZRSR2*, *NFKB1*, and *ETV6*, have provided further information, but their pathogenicity has yet to be clarified.[81-85]

Clinical significance of germline variants in acquired aplastic anemia

Co-existence of germline variants and paroxysmal nocturnal hemoglobinuria clones

PNH, characterized by a clone of *PIGA* somatic cell variants, is a hallmark of acquired bone marrow failure.[86] The PNH clone (>1%) detected in AA patients is considered to exclude IBMFS.[11,36,37] However, evidence suggests that patients with acquired AA who carry germline variants can also have clones of PNH. In four cases of *CTC1*-Tier-1 carriers, three (75%) were found to have large PNH clones (clone size >50%).[48] The presence of PNH clones was frequently observed in adult patients with severe AA carrying *SAMD9/9L* variants.[47] In one AA patient carrying the *MYSM1* variant, the PNH clone accounted for 30%.[61] Moreover, some patients with variants in inborn errors of immunity show a tendency to develop PNH clones.[63] In conclusion, the co-existence of PNH and germline variants indicates their distinction from IBMFS. Excluding germline variants by the presence of PNH clones may not be entirely conclusive.

Diversity of response to immunosuppressive therapy

Patients with germline variants are generally considered to have a poor response to IST.[11] However, specific outcomes may vary due to gene variations and individual differences (Table 2). For example, four out of five cases of acquired AA with heterozygous germline variants in *CTC1* demonstrated a response to IST.[87] A patient carrying a heterozygous variant of *MYSM1* showed a response to antithymocyte globulin

Table 2. Treatment and its efficacy in acquired aplastic anemia with germline variants.

Gene	Genotype	Treatment	Efficacy/therapeutic response
MYSM1	Recessive heterozygous	IST (CSA+ATG)/Danazol	100% (N=2)
CTC1	Recessive heterozygous	IST	80% (N=5)
SMAD9/9L	Heterozygous (pathogenicity unclear)	IST+EPAG	85% (N=40)
SBDS	Recessive heterozygous	IST	25% (N=4), transient response
PRF1	Recessive heterozygous	IST	40% (N=5), CSA-dependent
FANC	Recessive heterozygous	IST (CSA) IST (CSA+ATG)	33.3% (N=9) 25% (N=8)

IST: immunosuppressive therapy; CSA: cyclosporine A; ATG: anti-thymocyte globulin; EPAG: eltrombopag.

plus cyclosporine A.[61] Heterozygous variants in *SMAD9/9L* were found to have a minimal impact on the efficacy of IST, with approximately 85% of patients carrying these variants showing a response to IST plus eltrombopag.[47] However, no favorable outcomes were achieved with the use of IST in patients carrying other germline variants. In another trial, three out of four AA patients with *SBDS* heterozygous variants did not respond to IST, and the remaining patient showed only a transient response.[49] Additionally, three out of five patients with *PRF1* heterozygous variants did not respond to IST; the remaining two were dependent on cyclosporine A.[50] In a group of eight AA patients with *FANC* heterozygous variants, the efficacy of IST (antithymocyte globulin plus cyclosporine A) was only 25%, whereas a response rate of up to 100% was obtained in the control group (6 patients).[7]

Choice of hematopoietic stem cell transplantation and prognosis

Hematopoietic stem cell transplantation (HSCT) is considered the ultimate and effective approach for cases of severe AA failing to respond to IST and other treatment options.[88] The impact of recessive germline variants on transplantation in bone marrow failure has long been unknown. An earlier study provided overarching insights: P/LP variants (a homozygous or compound heterozygous state is required when inheritance is recessive) led to poorer post-transplant survival. On the other hand, carriers with single recessive P/LP variants or VUS (defined as acquired AA in our review) did not show a significant difference in post-transplant survival compared to non-carriers.[11] The study also identified graft-*versus*-host disease as the primary cause of mortality after transplantation in these carriers, distinct from the organ failure observed in patients with P/LP variants. Specifically, in patients with acquired AA with *FANC* heterozygous germline LB variants or VUS who underwent a treatment approach consistent with that of non-carriers, the final hematologic response showed no difference.[7] Similar conclusions were drawn for carriers with heterozygous *FANC* P/LP variants in another study.[89] Both indicated that *FANC* carriers do not require reduced-intensity conditioning regimens, highlighting their heterogeneity from patients with Fanconi anemia.[7,90] Sibling donor transplantation is considered the preferred treatment for acquired AA over unrelated donor transplantation.[91,92] However, it is important to note that family members or unrelated individuals carrying germline variants could be donors for HSCT, introducing the possibility of transplanting imperfect HSC to patients. Previous findings in other hematologic diseases have indicated that donor-derived pathogenic germline mutations could increase the risks of malignancies and engraftment failure. Certain variants, such as *DDX41*, have been linked to a higher incidence of acute graft-*versus*-host disease.[93] However, the impact of donor germline variants on HSCT in AA remains inadequately explored. There is one reported case of a patient who died of graft failure after having undergone HSCT from a histocompatible sibling with an unrecognized mutation of telomerase RNA component (*TERC*).[94] Another report described the case of a 43-year-old male patient who experienced remission for 4 years after receiving a transplant from a sibling carrying heterozygous variants in *FANCJ* Arg814Cys and *TINF2* Leu429Val.[95] These cases suggest that the strategy for donor selection requires urgent attention. Sibling transplantation has been performed for decades for the treatment of autosomal recessive genetic diseases such as Fanconi anemia. In China, haploidentical HSCT was conducted more frequently than unrelated donor transplantation in acquired AA,[96,97] which suggests a higher incidence of transmission of germline variants. The occurrence of graft-*versus*-host disease[97] may be associated with acquired AA variants, whether originating from patients or donors. Nevertheless, the impact of these variants on HSCT engraftment, survival, and the long-term risks have not been comprehensively evaluated.[11] Further studies are needed to answer these questions and refine the selection criteria for HSCT.

Outlook and future directions

Considerations on immunogenic pathogenic variants

In the current conceptual framework, the diagnosis of acquired AA primarily emphasizes the exclusion of pathogenic variants associated with IBMFS,[98] with less attention given

to other systems or isolated cases. Recently, whole-genome sequencing showed that 65% of AA/PNH patients carry germline variants associated with inborn errors of immunity.[63] In acquired AA, *PIGA* is considered a beneficial variant, often indicating a more favorable response to IST.[8,21] However, P/LP immunogenic germline variants are also frequently associated with PNH, exhibiting higher autoimmune activity and poorer response to IST.[63] For instance, patients with *NFKB1* haploinsufficiency do not tolerate cyclosporine A plus eltrombopag treatment.[84] The association of complement germline variations with PNH[99] further underscores the importance of identifying relevant variants in AA/PNH. The next consideration should be whether pathogenic immunogenic variants should be incorporated into acquired bone marrow failure. This may require a multifaceted evaluation, considering distribution frequency, efficacy, and pathogenicity, to provide a more precise definition of acquired AA.

Enhanced identification and comprehensive understanding

Many non-pathogenic variants remain associated with acquired AA. Current research is revealing the correlation between acquired AA and variants in genes related to IBMFS and inborn errors of immunity, although more exploration is still needed to identify others. For instance, the identification of variants corresponding to *FZR1* deficiency, proven to induce AA, holds significant meaning in this context.[100] Recently, machine-learning models have made strides in predicting acquired *versus* inherited AA with an accuracy rate of 89%. However, specific identification of germline variants, such as *SAMD9/9L*, and other heterozygous recessive variants remains limited.[13] Carriers of these variants exhibit distinct clinical features such as younger age, lower counts of neutrophils, reticulocytes, platelets, and $CD34^+$ cells, suggesting the potential for using machine-learning algorithms for discrimination.[7,47] Applying machine-learning models in this area will bring very interesting results.

The mechanism through which germline variants cause acquired AA is not yet fully understood. Some germline variants may contribute to acquired AA through a co-occurring heterozygous mode, such as *FANC* and telomere biology disorders, *SAMD9/9L*, etc.[11,47,101] This also provides another perspective, suggesting the cumulative effect of non-pathogenic factors. As previously discussed, somatic compensation is a distinctive phenomenon in IBMFS. It is worth exploring whether similar mechanisms exist in the subset of acquired AA with germline variants. The pathogenesis of the acquired AA in these cases may be linked to specific clonal populations, aligning with the concept of a genetic "second hit". Nonetheless, the relationship between germline variants in acquired AA and the response to conventional treatment has not been fully established. *FANC* heterozygous carriers were found to have a poor response to IST, but the underlying mechanisms remain unclear.[7]

Novel therapeutic approaches

Currently, there is a limited understanding of germline variants in acquired AA, and the treatment framework primarily adheres to the general protocols established for acquired AA. When an IST regimen fails to have satisfactory efficacy, other drugs with different mechanisms of action should be considered. Androgens have shown promising response rates and safety in conditions such as dyskeratosis congenita, Fanconi anemia and acquired AA.[102,103] Treatment with androgens increases *TERT* expression and improves telomere maintenance,[104] thus being potentially beneficial to patients with short telomeres and acquired AA variants. However, the use of androgens for Shwachman-Diamond syndrome is unconventional. When considering androgen therapy for heterozygous *SBDS* patients, the potential benefits must be evaluated carefully. Some cases involving *TINF2* variants[105] suggested that augmenting telomerase activity alone may not effectively address recruitment failure. Patients with acquired AA with *FANC* heterozygous variants appear to inherit some characteristics of *FANC* and exhibit a lower response to IST compared to non-variant individuals.[7] The administration of androgens has achieved positive results in the remission of *FANC*,[106] in which it is thought to stabilize telomere and genomes,[103] and may play a role in the remission of *FANC* pathway disorders. Further exploration is required to determine the viability of using androgens in acquired AA patients with heterozygous *FANC* variants, including potential combination with eltrombopag. Itacitinib, a selective JAK1 inhibitor, has shown preliminary efficacy in the treatment of acute graft-*versus*-host disease.[107] Promising results have been observed with itacitinib in a patient with *STAT1* (GOF) variant, and evidence of *STAT1* overexpression has also been found in patients with non-mutated acquired AA, suggesting a potential therapeutic approach targeting STAT1 in AA.[79,80] However, the effectiveness of itacitinib beyond *STAT1* (GOF) AA patients requires further validation. More research and data are required to refine the management of patients with acquired AA.

Conclusion

In acquired AA, individuals carrying certain germline heterozygous recessive variants, along with VUS or B/LB variants in dominant genes, constitute a distinct subgroup. They manifest typical features of acquired AA, with variations in disease severity and treatment response. The variants they carry induce and aggravate AA through various mechanisms such as gene homeostasis, DNA repair, and immune injury, representing risk factors for the development of acquired AA. It is important not to prematurely dismiss the effectiveness of IST, and a comprehensive evaluation of the patient's variant type and condition should be made. When deciding on HSCT, caution should be exercised in considering sibling

and haploidentical donors. There is currently a lack of relevant research to provide specific recommendations for this particular group of patients. There is an urgent need for a more rigorous and authoritative definition of acquired AA, determining whether immune-related pathogenic variants should be encompassed. Exploring further mechanisms in acquired AA carriers may unveil novel mechanisms and treatment approaches, advancing our understanding of AA and promoting the development of precision medicine.

Disclosures

No conflicts of interest to disclose.

Contributions

DW conceived the work. PW, WJ, TL, and DW contributed equally to research, writing, and editing. QL, YS, BY, and DW contributed equally to reviewing and revising the work. All authors contributed to the data analysis and to drafting and critically revising the paper; they agree to be accountable for all aspects of the work.

Acknowledgments

We thank Dr. Didier Blaise for his intellectual advice. We also thank Dr. Charmaine Fay Carcallas Soco and Dr. Daisi Zheng for their advice and assistance with the preparation of the figures. The figures were drawn using Figdraw (www.figdraw.com).

Funding

The present study was supported by the National Natural Science Foundation of China (N. 82174138, 82304937), Zhejiang Scientific Research Fund of Traditional Chinese Medicine (N. 2020ZB085, 2022RC216), Project of Academic Inheritance Studio of Famous and Aged Chinese Medicine Experts in Zhejiang Province (N. GZS2021022), and the Hematological Diseases Program of Chinese Association of Traditional Chinese Medicine (N. 202169-003).

References

1. Schoettler ML, Nathan DG. The pathophysiology of acquired aplastic anemia: current concepts revisited. Hematol Oncol Clin North Am. 2018;32(4):581-594.
2. Medinger M, Drexler B, Lengerke C, Passweg J. Pathogenesis of acquired aplastic anemia and the role of the bone marrow microenvironment. Front Oncol. 2018;8:587.
3. Townsley DM, Winkler T. Nontransplant therapy for bone marrow failure. Hematology Am Soc Hematol Educ Program. 2016;2016(1):83-89.
4. Ahmed M, Dokal I. Understanding aplastic anaemia/bone-marrow failure syndromes. Paediatr Child Health. 2009;19(8):351-357.
5. Risitano AM, Maciejewski JP, Green S, et al. In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing. Lancet. 2004;364(9431):355-364.
6. Li H, Zhou C, Shen Y, et al. Research progress on the hematopoietic microenvironment in aplastic anemia. Eur J Haematol. 2023;111(2):172-180.
7. Shen Y, Liu Q, Li H, et al. Whole-exome sequencing identifies FANC heterozygous germline mutation as an adverse factor for immunosuppressive therapy in Chinese aplastic anemia patients aged 40 or younger: a single-center retrospective study. Ann Hematol. 2023;102(3):503-517.
8. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373(1):35-47.
9. Barade A, Arunachalam AK, Arul D, et al. Germline variants contribute significantly to the pathogenesis of aplastic anemia in India. Blood. 2021;138(Supplement 1):1105.
10. Keel SB, Scott A, Sanchez-Bonilla M, et al. Genetic features of myelodysplastic syndrome and aplastic anemia in pediatric and young adult patients. Haematologica. 2016;101(11):1343-1350.
11. McReynolds LJ, Rafati M, Wang Y, et al. Genetic testing in severe aplastic anemia is required for optimal hematopoietic cell transplant outcomes. Blood. 2022;140(8):909-921.
12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424.
13. Gutierrez-Rodrigues F, Munger E, Ma X, et al. Differential diagnosis of bone marrow failure syndromes guided by machine learning. Blood. 2023;141(17):2100-2113.
14. Wegman-Ostrosky T, Savage SA. The genomics of inherited bone marrow failure: from mechanism to the clinic. Br J Haematol. 2017;177(4):526-542.
15. Dokal I, Vulliamy T. Inherited bone marrow failure syndromes. Haematologica. 2010;95(8):1236-1240.
16. Narumi S, Amano N, Ishii T, et al. SAMD9 mutations cause a novel multisystem disorder, MIRAGE syndrome, and are associated with loss of chromosome 7. Nat Genet. 2016;48(7):792-797.
17. Kim HY, Kim HJ, Kim SH. Genetics and genomics of bone marrow failure syndrome. Blood Res. 2022;57(S1):86-92.
18. Nalepa G, Clapp DW. Fanconi anaemia and cancer: an intricate relationship. Nat Rev Cancer. 2018;18(3):168-185.
19. Fernández García MS, Teruya-Feldstein J. The diagnosis and treatment of dyskeratosis congenita: a review. J Blood Med. 2014;5:157-167.
20. Florez MA, Tran BT, Wathan TK, et al. Clonal hematopoiesis: mutation-specific adaptation to environmental change. Cell Stem Cell. 2022;29(6):882-904.
21. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. Blood. 2016;128(3):337-347.
22. Babushok DV. A brief, but comprehensive, guide to clonal evolution in aplastic anemia. Hematology. 2018;2018(1):457-466.
23. Boddu PC, Kadia TM. Molecular pathogenesis of acquired aplastic anemia. Eur J Haematol. 2019;102(2):103-110.
24. Lundgren S, Keränen M, Wartiovaara-Kautto U, Myllymäki M. Somatic compensation of inherited bone marrow failure. Semin Hematol. 2022;59(3):167-173.

25. West RR, Calvo KR, Embree LJ, et al. ASXL1 and STAG2 are common mutations in GATA2 deficiency patients with bone marrow disease and myelodysplastic syndrome. Blood Adv. 2022;6(3):793-807.
26. Gregory JJ Jr, Wagner JE, Verlander PC, et al. Somatic mosaicism in Fanconi anemia: evidence of genotypic reversion in lymphohematopoietic stem cells. Proc Natl Acad Sci U S A. 2001;98(5):2532-2537.
27. Choijilsuren HB, Park Y, Jung M. Mechanisms of somatic transformation in inherited bone marrow failure syndromes. Hematology. 2021;2021(1):390-398.
28. Gutierrez-Rodrigues F, Sahoo SS, Wlodarski MW, Young NS. Somatic mosaicism in inherited bone marrow failure syndromes. Best Pract Res Clin Haematol. 2021;34(2):101279.
29. Shah YB, Priore SF, Li Y, et al. The predictive value of PNH clones, 6p CN-LOH, and clonal TCR gene rearrangement for aplastic anemia diagnosis. Blood Adv. 2021;5(16):3216-3226.
30. Giudice V, Feng X, Lin Z, et al. Deep sequencing and flow cytometric characterization of expanded effector memory CD8(+)CD57(+) T cells frequently reveals T-cell receptor Vβ oligoclonality and CDR3 homology in acquired aplastic anemia. Haematologica. 2018;103(5):759-769.
31. Zaimoku Y, Patel BA, Adams SD, et al. HLA associations, somatic loss of HLA expression, and clinical outcomes in immune aplastic anemia. Blood. 2021;138(26):2799-2809.
32. Bessler M, Mason PJ, Hillmen P, et al. Paroxysmal nocturnal haemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. EMBO J. 1994;13(1):110-117.
33. Lee SC, Abdel-Wahab O. The mutational landscape of paroxysmal nocturnal hemoglobinuria revealed: new insights into clonal dominance. J Clin Invest. 2014;124(10):4227-4230.
34. Raza A, Ravandi F, Rastogi A, et al. A prospective multicenter study of paroxysmal nocturnal hemoglobinuria cells in patients with bone marrow failure. Cytometry B Clin Cytom. 2014;86(3):175-182.
35. Griffin M, Kulasekararaj A, Gandhi S, et al. Concurrent treatment of aplastic anemia/paroxysmal nocturnal hemoglobinuria syndrome with immunosuppressive therapy and eculizumab: a UK experience. Haematologica. 2018;103(8):e345-e347.
36. DeZern AE, Symons HJ, Resar LS, et al. Detection of paroxysmal nocturnal hemoglobinuria clones to exclude inherited bone marrow failure syndromes. Eur J Haematol. 2014;92(6):467-470.
37. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. Blood. 2018;131(7):717-732.
38. Benn P. Uniparental disomy: origin, frequency, and clinical significance. Prenat Diagn. 2021;41(5):564-572.
39. Sui Y, Qi L, Wu JK, et al. Genome-wide mapping of spontaneous genetic alterations in diploid yeast cells. Proc Natl Acad Sci U S A. 2020;117(45):28191-28200.
40. Abramova AV, Mikhaylova EA, Fidarova ZT, et al. Oligoclonal expansion and T-cells subpopulations in patients with aplastic anemia at different stages of the disease. Blood. 2018;132(Supplement 1):3864.
41. Risitano AM, Kook H, Zeng W, et al. Oligoclonal and polyclonal CD4 and CD8 lymphocytes in aplastic anemia and paroxysmal nocturnal hemoglobinuria measured by Vβ CDR3 spectratyping and flow cytometry. Blood. 2002;100(1):178-183.
42. Zeng W, Maciejewski JP, Chen G, Young NS. Limited heterogeneity of T cell receptor BV usage in aplastic anemia. J Clin Invest. 2001;108(5):765-773.
43. Strioga M, Pasukoniene V, Characiejus D. CD8+ CD28- and CD8+ CD57+ T cells and their role in health and disease. Immunology. 2011;134(1):17-32.
44. Huang H, Wu DP, Chen G, Fu J. Cyclosporine A effect the CD28 expression on CD8+T cells of patients with aplastic anemia through down regulate FLIP. Blood. 2006;108(11):3767.
45. Zeng W, Nakao S, Takamatsu H, et al. Characterization of T-cell repertoire of the bone marrow in immune-mediated aplastic anemia: evidence for the involvement of antigen-driven T-cell response in cyclosporine-dependent aplastic anemia. Blood. 1999;93(9):3008-3016.
46. Risitano AM. (Auto-)immune signature in aplastic anemia. Haematologica. 2018;103(5):747-749.
47. Hironaka D, Ma X, Patel BA, et al. The role of heterozygous variants in SAMD9/9L and recessive bone marrow failure-related genes in adults with severe aplastic anemia. Blood. 2022;140(Supplement 1):479-480.
48. Shen W, Kerr CM, Przychozen B, et al. Impact of germline CTC1 alterations on telomere length in acquired bone marrow failure. Br J Haematol. 2019;185(5):935-939.
49. Calado RT, Graf SA, Wilkerson KL, et al. Mutations in the SBDS gene in acquired aplastic anemia. Blood. 2007;110(4):1141-1146.
50. Solomou EE, Gibellini F, Stewart B, et al. Perforin gene mutations in patients with acquired aplastic anemia. Blood. 2007;109(12):5234-5237.
51. Li T, Zhou ZW, Ju Z, Wang ZQ. DNA damage response in hematopoietic stem cell ageing. Genomics Proteomics Bioinformatics. 2016;14(3):147-154.
52. Nie D, Zhang J, Wang F, et al. Fanconi anemia gene-associated germline predisposition in aplastic anemia and hematologic malignancies. Front Med. 2022;16(3):459-466.
53. Buchwald M, Moustacchi E. Is Fanconi anemia caused by a defect in the processing of DNA damage? Mutat Res. 1998;408(2):75-90.
54. Berwick M, Satagopan JM, Ben-Porat L, et al. Genetic heterogeneity among Fanconi anemia heterozygotes and risk of cancer. Cancer Res. 2007;67(19):9591-9596.
55. Alter BP, Giri N, McReynolds LJ, Rosenberg PS. Cancer in heterozygote carriers of Fanconi anemia genes. Blood. 2018;132(Supplement 1):3868.
56. Ceccaldi R, Parmar K, Mouly E, 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(1):36-49.
57. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science. 1999;286(5446):1957-1959.
58. Sidore C, Orrù V, Cocco E, et al. PRF1 mutation alters immune system activation, inflammation, and risk of autoimmunity. Mult Scler. 2021;27(9):1332-1340.
59. Terrell CE, Jordan MB. Perforin deficiency impairs a critical immunoregulatory loop involving murine CD8(+) T cells and dendritic cells. Blood. 2013;121(26):5184-5191.
60. Zhang J, Fu R, Wang J, et al. Perforin gene mutations in patients with acquired severe aplastic anemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2011;19(2):431-434.
61. Imi T, Mizumaki H, Hosomichi K, et al. Familial immune-mediated aplastic anaemia in six different families. EJHaem. 2023;4(3):714-718.
62. Liang Y, Bhatt G, Tung LT, et al. Deubiquitinase catalytic activity of MYSM1 is essential in vivo for hematopoiesis and immune cell development. Sci Rep. 2023;13(1):338.
63. Bravo-Perez C, Gurnari C, Guarnera L, et al. Bone marrow failure

and inborn errors of immunity: an immunogenomic crossroad. Blood. 2023;142(Supplement 1):707.
64. Peng S, Meng X, Zhang F, et al. Structure and function of an effector domain in antiviral factors and tumor suppressors SAMD9 and SAMD9L. Proc Natl Acad Sci U S A. 2022;119(4):e2116550119.
65. Gorcenco S, Komulainen-Ebrahim J, Nordborg K, et al. Ataxia-pancytopenia syndrome with SAMD9L mutations. Neurol Genet. 2017;3(5):e183.
66. Davidsson J, Puschmann A, Tedgård U, et al. SAMD9 and SAMD9L in inherited predisposition to ataxia, pancytopenia, and myeloid malignancies. Leukemia. 2018;32(5):1106-1115.
67. Tesi B, Davidsson J, Voss M, et al. Gain-of-function SAMD9L mutations cause a syndrome of cytopenia, immunodeficiency, MDS, and neurological symptoms. Blood. 2017;129(16):2266-2279.
68. Chen DH, Below JE, Shimamura A, et al. Ataxia-pancytopenia syndrome is caused by missense mutations in SAMD9L. Am J Hum Genet. 2016;98(6):1146-1158.
69. Abdelhamed S, Thomas ME 3rd, Westover T, et al. Mutant Samd9l expression impairs hematopoiesis and induces bone marrow failure in mice. J Clin Invest. 2022;132(21):e158869.
70. Thomas ME 3rd, Abdelhamed S, Hiltenbrand R, et al. Pediatric MDS and bone marrow failure-associated germline mutations in SAMD9 and SAMD9L impair multiple pathways in primary hematopoietic cells. Leukemia. 2021;35(11):3232-3244.
71. Congdon RG, Zha J, Qin X, Kunselman L, Olson TS. Impaired efficacy and durability of hematopoietic stem cell engraftment caused by Samd9l-deficiency within bone marrow niches. Blood. 2023;142(Supplement 1):513.
72. Nakhoul H, Ke J, Zhou X, et al. Ribosomopathies: mechanisms of disease. Clin Med Insights Blood Disord. 2014;7:7-16.
73. Dror Y. Shwachman-Diamond syndrome. Pediatr Blood Cancer. 2005;45(7):892-901.
74. Liu Y, Liu F, Cao Y, et al. Shwachman-Diamond syndrome protein SBDS maintains human telomeres by regulating telomerase recruitment. Cell Rep. 2018;22(7):1849-1860.
75. Rice C, Skordalakes E. Structure and function of the telomeric CST complex. Comput Struct Biotechnol J. 2016;14:161-167.
76. Lim CJ, Cech TR. Shaping human telomeres: from shelterin and CST complexes to telomeric chromatin organization. Nat Rev Mol Cell Biol. 2021;22(4):283-298.
77. Vulliamy T, Beswick R, Kirwan MJ, et al. Telomere length measurement can distinguish pathogenic from non-pathogenic variants in the shelterin component, TIN2. Clin Genet. 2012;81(1):76-81.
78. Walne AJ, Vulliamy T, Beswick R, Kirwan M, Dokal I. TINF2 mutations result in very short telomeres: analysis of a large cohort of patients with dyskeratosis congenita and related bone marrow failure syndromes. Blood. 2008;112(9):3594-3600.
79. Rosenberg JM, Peters JM, Hughes T, et al. JAK inhibition in a patient with a STAT1 gain-of-function variant reveals STAT1 dysregulation as a common feature of aplastic anemia. Med. 2022;3(1):42-57.
80. Solimando AG, Desantis V, Palumbo C, et al. STAT1 overexpression triggers aplastic anemia: a pilot study unravelling novel pathogenetic insights in bone marrow failure. Clin Exp Med. 2023;23(6):2687-2694.
81. Marsh JCW, Gutierrez-Rodrigues F, Cooper J, et al. Heterozygous RTEL1 variants in bone marrow failure and myeloid neoplasms. Blood Adv. 2018;2(1):36-48.
82. Zhang Y, Wang F, Chen X, et al. Next-generation sequencing reveals the presence of DDX41 mutations in acute lymphoblastic leukemia and aplastic anemia. EJHaem. 2021;2(3):508-513.
83. Thurlapati A, Boudreaux K, Guntupalli S, Mansour RP, Bhayani S. A case of aplastic anemia and colon cancer with underlying spliceosome mutation: is it an incidental finding or a novel association? Cureus. 2022;14(2):e22632.
84. Sklarz T, Hurwitz SN, Stanley NL, et al. Aplastic anemia in a patient with CVID due to NFKB1 haploinsufficiency. Cold Spring Harb Mol Case Stud. 2020;6(6):a005769.
85. Rampersaud E, Ziegler DS, Iacobucci I, et al. Germline deletion of ETV6 in familial acute lymphoblastic leukemia. Blood Adv. 2019;3(7):1039-1046.
86. Nakao S, Sugimori C, Yamazaki H. Clinical significance of a small population of paroxysmal nocturnal hemoglobinuria-type cells in the management of bone marrow failure. Int J Hematol. 2006;84(2):118-122.
87. Shen W, Hirsch CM, Przychodzen BP, et al. Heterozygous CTC1 variants in acquired bone marrow failure. Blood. 2018;132(Supplement 1):3866.
88. Zhang MX, Wang Q, Wang XQ. Hematopoietic stem-cell transplantation versus immunosuppressive therapy in patients with adult acquired severe aplastic anemia: a cost-effectiveness analysis. Int J Gen Med. 2021;14:3529-3537.
89. McReynolds LJ, Wang Y, Thompson AS, et al. Population frequency of Fanconi pathway gene variants and their association with survival after hematopoietic cell transplantation for severe aplastic anemia. Biol Blood Marrow Transplant. 2020;26(5):817-822.
90. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010;24(3):101-122.
91. Young NS. Aplastic anemia. N Engl J Med. 2018;379(17):1643-1656.
92. Gupta V, Eapen M, Brazauskas R, et al. Impact of age on outcomes after bone marrow transplantation for acquired aplastic anemia using HLA-matched sibling donors. Haematologica. 2010;95(12):2119-2125.
93. Williams LS, Williams KM, Gillis N, et al. Donor-derived malignancy and transplantation morbidity: risks of patient and donor genetics in allogeneic hematopoietic stem cell transplantation. Transplant Cell Ther. 2024;30(3):255-267.
94. Calado RT, Young NS. Telomere diseases. N Engl J Med. 2009;361(24):2353-2365.
95. Yan J, Jin T, Wang L. Hematopoietic stem cell transplantation of aplastic anemia by relative with mutations and normal telomere length: a case report. World J Clin Cases. 2023;11(29):7200-7206.
96. Shen Y, Li Y, Liu Q, et al. Comparison of anti-thymocyte globulin-based immunosuppressive therapy and allogeneic hematopoietic stem cell transplantation in patients with transfusion-dependent non-severe aplastic anaemia: a retrospective study from a single centre. Ann Med. 2023;55(2):2271475.
97. Xu LP, Jin S, Wang SQ, et al. Upfront haploidentical transplant for acquired severe aplastic anemia: registry-based comparison with matched related transplant. J Hematol Oncol. 2017;10(1):25.
98. DeZern AE, Churpek JE. Approach to the diagnosis of aplastic anemia. Blood Adv. 2021;5(12):2660-2671.
99. Prata PH, Galimard J-E, Sicre de Fontbrune F, et al. Rare germline complement factor H variants in patients with

paroxysmal nocturnal hemoglobinuria. Blood. 2023;141(15):1812-1816.
100. Tang X, Wang Z, Wang J, et al. Functions and regulatory mechanisms of resting hematopoietic stem cells: a promising targeted therapeutic strategy. Stem Cell Res Ther. 2023;14(1):73.
101. Shen W, Hirsch CM, Przychodzen BP, et al. Pathogenic germline variants in acquired aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH). Blood. 2018;132(Supplement 1):2583.
102. Argote VEF, Peñafiel COR, Sánchez MHn, et al. Androgen treatment for acquired aplastic anemia in Mexican adults. Blood. 2008;112(11):1046-1046.
103. Bosi A, Barcellini W, Passamonti F, Fattizzo B. Androgen use in bone marrow failures and myeloid neoplasms: mechanisms of action and a systematic review of clinical data. Blood Rev. 2023;62:101132.
104. Bär C, Huber N, Beier F, Blasco MA. Therapeutic effect of androgen therapy in a mouse model of aplastic anemia produced by short telomeres. Haematologica. 2015;100(10):1267-1274.
105. Yamaguchi H, Inokuchi K, Takeuchi J, et al. Identification of TINF2 gene mutations in adult Japanese patients with acquired bone marrow failure syndromes. Br J Haematol. 2010;150(6):725-727.
106. Ribeiro LL, Nichele S, Bitencourt MA, et al. Excelent option therapy of bone marrow failure in Fanconi anemia patients withouth full match donnor. Blood. 2016;128(22):5075.
107. Schroeder MA, Khoury HJ, Jagasia M, et al. A phase 1 trial of itacitinib, a selective JAK1 inhibitor, in patients with acute graft-versus-host disease. Blood Adv. 2020;4(8):1656-1669.