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by Peicheng Wang, Wanzhi Jiang, Tianyi Lai, Qi Liu, Yingying Shen, Baodong Ye, and Dijiong Wu

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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[1,2,3], Baodong Ye[1,2,3], Dijiong Wu[1,2,3,4]

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

Corresponding authors:

Dr. Dijiong Wu (Ph.D., M.D.), Department of Hematology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), Hangzhou (310006), Zhejiang, P.R. China; E-mail: wudijiong@zcmu.edu.cn, Tel: +86-0571-86620325

Dr. Baodong Ye (Ph.D., M.D.), Department of Hematology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), Hangzhou (310006), Zhejiang, P.R. China; E-mail: 13588453501@163.com Tel: +86-0571-86620325

* *Equal contribution*

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Abstract

Aplastic anemia (AA) is a disease characterized by hematopoiesis failure, 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 an unignorable role in the onset and progression of acquired AA. These variants, associated with inherited bone marrow failure syndromes (IBMFS) and inborn errors of immunity (IEI), 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, detailed the clinical significance of germline variants in diagnosing and clinical management of this condition. More works are encouraged to better understand the role of immunogenic pathogenic variants and whether somatic mutation participate as secondary “hit” in the development of bone marrow failure.

Keywords: aplastic anemia; germline variants; inherited bone marrow failure syndromes; immunosuppressive therapy; hematopoietic stem cell transplantation

1. Background

Aplastic anemia (AA) is the most common syndrome of bone marrow failure, classified into inherited and acquired form based on clinical manifestation and genomic background. Inherited AA, often referred to as inherited bone marrow failure syndromes (IBMFS), is represented by Fanconi anemia (FA) and dyskeratosis congenita (DC), which are clinically rare (1). Acquired AA is characterized by immune-mediated bone marrow injury, i.e., hypoplastic, fatty bone marrow, with a profound reduction in the numbers of hematopoietic stem/progenitor cells that lead to defective hematopoiesis and peripheral pancytopenia (2). Immunosuppressive therapy (IST) with cyclosporine A (CSA), combined with or without anti-thymocyte globulin (ATG), is considered the first-line therapy that achieves hematological remission in approximately 60%–70% of instances in acquired AA cases (3). However, the 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 (HSCs) also play a significant role in the development of the disease (6).

With the advancement and application of next-generation sequencing (NGS) and whole-exome sequencing (WES) (7), HSC defects of in acquired AA patients have been progressively revealed. Somatic variants, including *ASXL1*, *DNMT3A*, *TP53*, and *RUNX1*, have been shown to be associated with worse IST responses 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 ACMG 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 (IEI) 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.

2. Distinguishing IBMFS in the diagnosis of acquired AA

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.

2.1. Features and specific examinations of IBMFS

Inherited AA or IBMFS is a collection of rare disorders resulting from pathogenic germline inheritance, including FA, DC, Diamond-Blackfan anemia (DBA), Shwachman-Diamond syndrome (SDS), severe congenital neutropenia (SCN), congenital amegakaryocytic thrombocytopenia (CAMT), MIRAGE 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 (DC), pulmonary fibrosis (DC), and exocrine pancreatic insufficiency (SDS) (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 FA (18). Additionally, an extremely short telomere length (<1st percentile for age) can be used as a highly sensitive and specific marker for DC detection (19).

2.2. Heterogeneity of clonal hematopoiesis

Clonal hematopoiesis can be viewed as the adaptive response of HSCs to environmental stress (20). The heterogeneity of clones reflects pathogenic mechanisms that is distinct for acquired AA and IBMFS. In acquired AA, HSCs 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, which are considered clinically beneficial; additionally, *DNMT3A*, *ASXL1*, *TP53*, *RUNX1*, and *CSMD1* variants are

associated with a decreased survival rate and progression to myelodysplastic syndromes/acute myeloid leukemia (MDS/AML), which are considered clinically unfavorable (8, 21).

Comparable features have been observed in IBMFS, referred to as somatic compensation. Unlike acquired AA, the pressures confronting HSCs 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 these 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 FA, DC, 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 DC and loss of *EIF6* in SDS. Pathway-independent compensation involves acquiring growth advantages through alternative pathways or mechanisms, exemplified by *TP53* variants in SDS, TBD, and DBA, RAS pathway variants in FA, *RUNX1* variants in FA, and *ASXL1* variants in GATA2-related disorders (24-28). Somatic compensation endows HSCs with proliferative advantages within the hematopoietic microenvironment of IBMFS, leading to two possible outcomes: disease reversal or malignant transformation.

2.3. 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 (PPV) and specificity (29-31). PNH is characterized by the HSC *PIGA* clone, where *PIGA* variants impede glycosylphosphatidylinositol-anchored proteins (GPI-AP) synthesis, 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 GPI-AP renders inactivation of immune response-related cell surface proteins (33), which is a common immune escape mechanism observed in 20%–60% of acquired AA patients (34, 35). The detection of PNH clones (>1%) in acquired AA patients is considered to exclude patients from IBMFS and has been validated in clinical and research settings (11, 13, 36, 37). Similarly, the loss of *HLA* class I allelic genes endows HSCs with the capacity to establish clones with cellular immunity in 20% of patients, involving the mechanisms of *HLA* variants and 6p UPD/ 6p CN-LOH (31). UPD (uniparental disomy) or CN-LOH (copy number-neutral loss of heterozygosity) describes the phenomenon of heterozygous loss where one parental chromosome region is replaced by the other (38, 39), occurring frequently in the *HLA* class I region of chromosome 6p for 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% PPV 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 application of IST results in a significant reduction in the clonal expansion of CD8+ CD28− T cells, considered robust evidence for the 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).

3. Contribution of germline variants to acquired AA

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 IEI, the genes involved maintain the stability, repair, and renewal of HSCs under normal conditions (**Figure 1**, **Figure 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.

3.1. DNA repair deficiencies accelerate bone marrow failure

DNA damage repair has a crucial role in maintaining the response of HSCs to both external and internal stimuli, as well as their self-renewal process (51). The FA pathway is one of the important mechanisms involved in DNA damage repair (**Figure 2**). In acquired AA and hematologic malignancies patients, the prevalence of common heterozygous *FANC* variants is 8.38% (52). Whereas in Chinese acquired AA patients below the age of 40, the prevalence of non-pathogenic heterozygous *FANC* variants can reach 45.9%, with likely benign and uncertain significance variants accounting for 97.56% of all events (7). To date, 22 *FANC* genes have been identified, and their homozygous states are closely associated with FA, 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 FA (53). The risk of developing cancer in heterozygous carriers is comparable to that of the general population, and they are generally considered non-pathogenic (54, 55). However, in a study integrating AA and hematological malignancies, heterozygous carriers of *FANC* variants showed susceptibility to both conditions, suggesting that in certain disease contexts, heterozygosity may be insufficient to maintain the proper function (52). The underlying mechanism may involve the diminished ability of heterozygous variants for DNA repair, 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 acquired AA cases with *FANC* heterozygous variants, followed by *BRCA2* and *FANCD2*, which have a pattern of variant frequencies similar to that in FA. The proportions of *FANCG* and *FANCC* variants are relatively small, possibly due to the limited sample size. The enrichment of rare variants in *FANCN* and *SLX4*, which are less commonly observed in FA patients, may indicate susceptibility in acquired AA or could be attributed to sample size, necessitating further comprehensive investigations (7, 18, 52).

3.2. Excessive immune responses aggravate HSCs 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 (FHL), a childhood-onset fatal disorder 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 (APCs), leading to the activation and proliferation of cytotoxic T cells (50, 59). Accompanied by the secretion of IFN-γ, they ultimately resulting in the destruction of HSCs (60). Recently, two acquired AA patients were found to have heterozygous variants in Myb-like SWIRM and MPN domains 1 (*MYSM1*), a regulator of hematopoiesis and immune cell development, known to cause IBMFS in homozygous state (61, 62). These patients had the 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 (WGS) of patients with AA/PNH have revealed that 65% (37/57) carried heterozygous germline variants associated with IEI. Among the identified 60 variants, 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.

3.3. SAMD9/SAMD9L exaggerate HSCs defect

Sterile alpha motif domain-containing protein 9/ 9-like (*SAMD9/9L*) encodes two proteins involved in anti-tumor 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 multi-system disorder characterized by a predisposition to MDS and loss of chromosome 7 (16). Meanwhile, WES also revealed that *SAMD9L* was the cause of ataxia-pancytopenia syndrome (65). Both syndromes share similarities in their excessive antiproliferative 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 HSCs proliferation and self-renewal, promote inflammation, and exacerbate bone marrow failure (69, 70). Intense survival pressure drives HSCs 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 SAA 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%), distinguishing it from what is observed in SAMD9/9L-related disorders. The most common variant types observed are missense, nonsense, and frameshift. Carriers of these variants tend to be younger and exhibit lower levels of neutrophils, reticulocytes, and platelet counts (47). However, our understanding of these variants and their pathogenic mechanisms remains incomplete. The regulation of HSCs and the microenvironment by *SAMD9/9L* is highly complex. Loss of function variants are also linked to a predisposition to MDS and abnormal expression of specific inflammatory pathway (71). *SAMD9/9L* variants in adult acquired AA can be considered to contribute to mild functional disruptions, including dysregulation of HSCs proliferation, immune-inflammatory responses, ultimately leading to T-cell destruction and bone marrow failure.

3.4. Other variants

With the application of WES, numerous reports on other germline variants continue to emerge. Heterozygous variants in ribosome maturation protein (*SBDS*) account for

approximately 5% of acquired AA cases (49). Biallelic variants in *SBDS* are found in over 90% of SDS patients and are a major genetic factor associated with SDS (72). SDS is a genetic syndrome characterized by an elevated risk of bone marrow failure, exocrine pancreatic insufficiency, skeletal defects, and hematological malignancies (73). Acquired AA patients with heterozygous *SBDS* variants do not have such manifestations but share pancytopenia and telomere shortening with those having SDS (49). The telomere shortening may be attributed to the SBDS dysfunction in interacting with shelterin, a complex that protects telomeres and initiates telomerase recruitment (74).

Conserved telomere maintenance component 1 (*CTC1*) is a component of 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 DC, where 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 cases (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 towards 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, have been identified in three acquired AA patients (77, 78). GOF variants in the signal transducer and activator of the transcription 1 (*STAT1*) have been reported in two cases of AA, which lead to increased T helper type 1 (TH1) cells and the overexpression of IFN-γ (79, 80).Other concomitant variants have provided further information, but their pathogenicity yet to be clarified, including the heterozygous variants of *RTEL1*, *DDX41, ZRSR2, NFKB1* and *ETV6* (81-85).

4. Clinical significance of germline variants in acquired AA

4.1. Germline variants co-existence with PNH clones

PNH is a hallmark of acquired bone marrow failure, characterized by a clone of *PIGA* somatic cell variants (86). The PNH clone (>1%) detected in AA patients is considered an exclusion of the patient's IBMFS (11, 36, 37). However, evidence suggests that patients with acquired AA who carry germline variants can also have clones of PNH. In cases of *CTC1*-Tier-1 carriers, three of four (75%) were found to have large PNH clones (clone size > 50%) (48). The presence of PNH clones was frequently observed in adult patients with SAA 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 IEI 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.

4.2. IST response diversity

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 acquired AA cases with heterozygous germline variants in *CTC1* demonstrated a response to IST (87). A patient carrying a heterozygous variant of *MYSM1* showed a response to ATG + CSA (61). The heterozygous variants in *SMAD9/9L* was found to have minimal impact on the efficacy of IST, with approximately 85% of patients carrying these variants showing a response to IST + eltrombopag (EPAG) (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 CSA-dependent (50). In a group of eight AA patients with the *FANC* heterozygous variants, the efficacy of IST (ATG+CSA) was only 25%, whereas a response rate of up to 100% was obtained in the control group (six patients) (7).

4.3. Choices of HSCT and prognosis

HSCT is considered the ultimate and effective approach for SAA cases 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 (homozygous or compound heterozygous is required for recessive inheritance) 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) showed no significant difference in post-transplant survival compared to non-carriers (11). The study also identified graft-versus-host disease (GVHD) as the primary cause of mortality post-transplantation in these carriers, distinct from the organ failure observed in patients with P/LP variants. Specifically, in acquired AA patients with *FANC* heterozygous germline variants of likely benign and uncertain significance, they underwent a consistent treatment approach with non-carriers, and the final hematological 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 FA (7, 90).

Sibling donor transplantation is considered the preferred treatment for acquired AA over unrelated donor transplantation (91, 92). However, it's important to note that family members or unrelated individuals carrying germline variants may still serve as donors for HSCT, introducing the possibility of transplanting imperfect HSCs to patients. Previous findings in other hematological 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 GVHD (93). However, the impact of donor germline variants on HSCT in AA remains inadequately explored. One case reported the death of a patient due to graft failure, who received HSCT from a histocompatible sibling with an unrecognized mutation of telomerase RNA component (*TERC*) (94). Another case highlighted a 43-year-old male patient who experienced remission for four years after receiving a transplant from a sibling carrying heterozygous variants in *FANCJ* Arg814Cys and *TINF2* Leu429Val (95). These suggest that strategy for donor selection requires urgent attention. Sibling transplantation has been performed for decades for the treatment of autosomal recessive genetic diseases such as FA. In

China, haploidentical HSCT was conducted more frequently than unrelated donor transplantation in acquired AA (96, 97), which suggested a higher incidence of transmitting germline variants. The occurrence of GVHD (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 HSCT selection criteria.

5. Outlook and future directions

5.1. Considerations of 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 in other systems or isolated cases. Recently, WGS identified that 65% of AA/PNH patients carry germline variants associated with IEI (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 experience an inability to tolerate CSA + EPAG 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 involve 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.

5.2. Enhanced identification and comprehensive understanding

In acquired AA, many non-pathogenic variants remain associated with the condition. Current research reveals the correlation between acquired AA and variants in genes related to IBMFS and IEI, though 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 like *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 to 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 their acquired AA 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 be associated with poor response to IST, but the underlying mechanisms remain unclear (7).

5.3. Novel therapeutic approaches

Currently, there is a limited understanding of germline variants in acquired AA, and the treatment framework primarily adhere to the general protocols established for acquired AA. When IST regimen fails to achieve satisfactory efficacy, considering other drugs with different mechanisms is necessary. Androgens have shown promising response rates and safety in conditions such as DC, FA and acquire AA (102, 103). Treatment with androgens increases *TERT* expression and improved telomere maintenance (104), potentially beneficial to patients with short telomeres and acquired AA variants. However, the use of androgen for SDS is unconventional. When considering androgen therapy for heterozygous *SBDS* patients, careful evaluation of potential benefits is necessary. As suggested by some cases involving

TINF2 variants (105), augmenting telomerase activity alone may not effectively address recruitment failure. Acquired AA patients with *FANC* heterozygous variants appear to inherit somewhat 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), which 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 EPAG. Itacitinib, a selective JAK1 inhibitor, has shown preliminary efficacy in acute GVHD treatment (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 non-mutated acquired AA patients, suggesting a potential therapeutic approach targeting STAT1 in AA (79, 80). However, the effectiveness of itacitinib beyond *STAT1* (GOF) AA patients require further validation. More research and data are required to refine the management of patients with acquired AA.

6. 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. Moreover, an urgent need exists 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 promote the development of precision medicine.

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Table 1. Incidence and Functional Mechanisms of germline Variants in Acquired Aplastic Anemia

Classification	Gene involved	Carrying ratio	Function	Genotype	References
Repair Disorders	FANC	45.9% (age <40)	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)	Involved in anti-tumor and antiviral responses	Heterozygous (pathogenicity unclear)	47
Others	CTC1	2.3% (AA/PNH)	Component of CST complex, regulates telomeres	Recessive heterozygous	48
	SBDS	5%	Involved in ribosome biogenesis	Recessive heterozygous	49

FA, Fanconi anemia.

Table 2. Treatment and Efficacy of 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)	33.3% (N=9)
		IST (CSA+ATG)	25% (N=8)

ITS, immunosuppressive therapy; CSA, cyclosporine A; ATG, anti-thymocyte globulin; EPAG, eltrombopag.

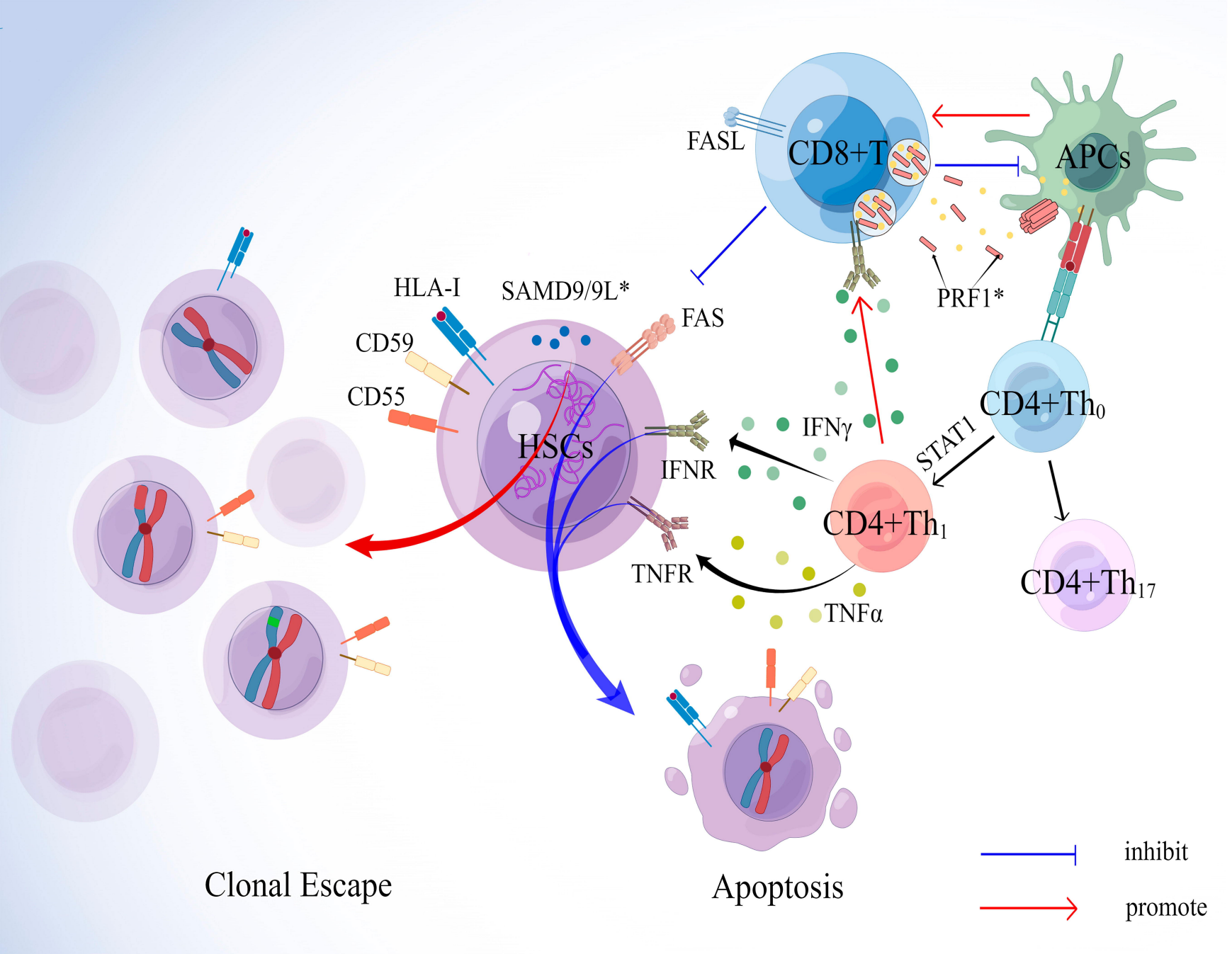
Figure legends

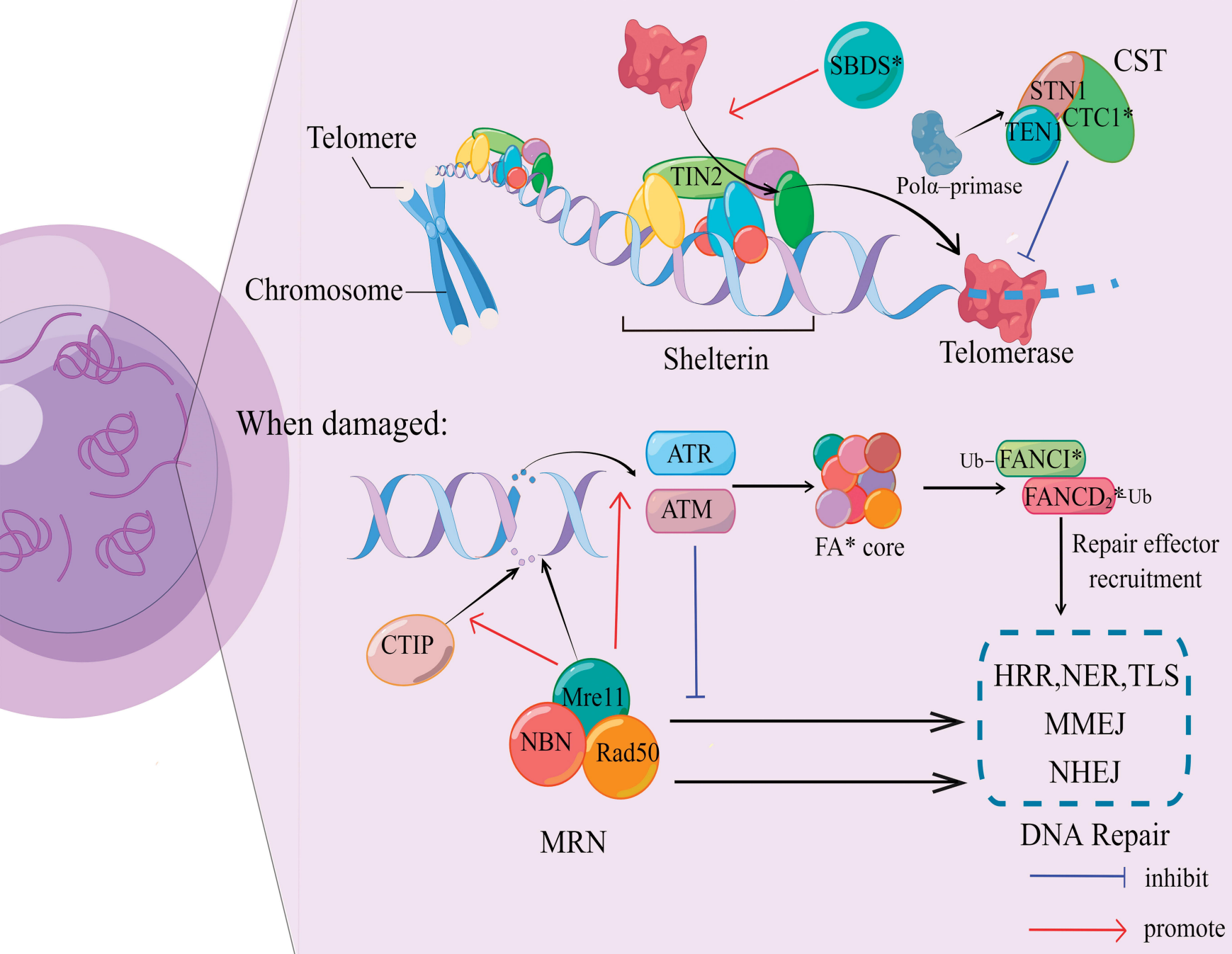
Figure 1. Immune destruction and germline variants involvement.

Antigen-presenting cells (APCs) process antigens, presenting them to T cells. $CD4^+$Th0 cells differentiate into Th1 and Th17, maintaining relative balance. Among them, STAT1 promotes Th0 differentiation toward Th1, leading to the secretion of cytokines IFNγ and TNFα by $CD4^+$Th1 cells, which bind to IFNR and TNFR, promoting HSC apoptosis. 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 APCs and negative regulation of immune responses. Furthermore, SAMD9/9L is associated with anti-tumor and anti-infection responses, but its specific mechanism remains unclear. The formidable survival pressure prompts HSCs to employ mechanisms such as PIGA, 6p UPD/CN-LOH, and HLA-I mutations to escape from immune responses. The * denotes genes affected by germline variants involved in acquired aplastic anemia. The figure was drawn by Figdraw (www.figdraw.com).

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 (MMEJ) and non-homologous end joining (NHEJ). ATR and ATM also facilitate the formation of the FA core and are crucial for FANCD2-FANCI monoubiquitylation, ultimately promoting homologous recombinational repair (HRR), nucleotide excision repair (NER), and translesion synthesis (TLS) DNA repair pathways. The * denotes genes affected by germline

variants, in acquired aplastic anemia. The figure was drawn by Figdraw (www.figdraw.com).


FASL
CD8+T
APCs
PRF1*
HLA-I
SAMD9/9L*
FAS
CD59
CD55
HSCs
IFNγ
IFNR
STAT1
CD4+Th0
CD4+Th1
TNFR
TNFα
CD4+Th17
Clonal Escape
Apoptosis
inhibit
promote


SBDS*
CST
STN1
CTC1*
TEN1
Telomere
TIN2
Polα–primase
Chromosome
Shelterin
Telomerase
When damaged:
ATR
ATM
FA* core
Ub–FANCI*
FANCD2*–Ub
Repair effector recruitment
CTIP
Mre11
NBN
Rad50
MRN
HRR,NER,TLS
MMEJ
NHEJ
DNA Repair
inhibit
promote