New progress in the study of germline susceptibility genes of myeloid neoplasms (Review)

LEI BI[1], TIANYUAN MA[2*], XU LI[3*], LAI WEI[3*], ZINUO LIU[3*], BINGYUE FENG[3], BAOXIA DONG[1] and XIEQUN CHEN[1,4]

[1]Department of Hematology, Xijing Hospital, Fourth Military Medical University; [2]Department of Stomatology, Fourth Military Medical University; [3]College of Basic Medicine, Fourth Military Medical University, Xi'an, Shaanxi 710032; [4]Hematology and Oncology Center, Affiliated Hospital of Northwest University and Xian No. 3 Hospital, Xi'an, Shaanxi 710082, P.R. China

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Abstract. In 2016, the World Health Organization incorporated 'myeloid neoplasms with germline predisposition' into its classification of tumors of hematopoietic and lymphoid tissues, revealing the important role of germline mutations in certain myeloid neoplasms, particularly myelodysplastic syndrome and acute myeloid leukemia. The awareness of germline susceptibility has increased, and some patients with myeloid neoplasms present with a preexisting disorder or organ dysfunction. In such cases, mutations in genes including CCAAT enhancer binding protein α (CEBPA), DEAD (Asp-Glu-Ala-Asp) box polypeptide 41 (DDX41), RUNX family transcription factor 1 (RUNX1), GATA binding protein 2 (GATA2), Janus kinase 2 (JAK2) and ETS variant transcription factor 6 (ETV6) have been recognized. Moreover, with the application of advanced technologies and reports of more cases, additional germline mutations associated with myeloid neoplasms have been identified and provide insights into the formation, prognosis and therapy of myeloid neoplasms. The present review discusses the well-known CEBPA, DDX41, RUNX1, GATA2, JAK2 and ETV6 germline mutations, and other mutations including those of lymphocyte adapter protein/SH2B adapter protein 3 and duplications of autophagy related 2B, GSK3B interacting protein αnd RB binding protein 6, ubiquitin ligase, that remain to be confirmed or explored. Recommendations for the management of diseases associated with germline mutations are also provided.

Contents

1. Introduction

Myeloid neoplasms are a group of heterogeneous neoplasms formed by the clonal proliferation of hematopoietic stem cells (HSCs) with multidirectional differentiation potential in the bone marrow, and they include acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPNs). The pathogenesis of myeloid neoplasms is complicated, and germline mutations play a critical role in this process. Patients with these mutations have been observed to have a predisposition to MDS/AML and other myeloid neoplasms. This concept was added to the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia as a new category, indicating the importance of germline mutations in these hematopoietic malignancies (1). This revision is profound, as patients with myeloid neoplasms caused by germline mutations may have different clinical manifestations, responses to clinical management and prognoses from those with *de novo* MDS and AML (2). Patients with MDS/MPNs who have germline mutations also have a higher risk of developing AML, which is also likely to have more malignant clinical features and poor outcomes. Therefore, it is essential to identify the germline mutations in myeloid neoplasms, understand their mechanisms and take early therapeutic measures with long-term follow-ups. Myeloid neoplasms with germline mutations are sporadic, but with the application of whole-genome and targeted sequencing and more familial hematopoietic disorders being reported, a greater number of germline mutations have been identified

Correspondence to: Professor Baoxia Dong or Professor Xiequn Chen, Department of Hematology, Xijing Hospital, Fourth Military Medical University, 127 Chang'le West Road, Xi'an, Shaanxi 710032, P.R. China
E-mail: freshupdbx@hotmail.com
E-mail: xiequnchen@fmmu.edu.cn

*Contributed equally

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and more clearly defined. The present review summarizes basic information about germline mutations, and the features of associated clinical syndromes or diseases in Tables I and II.

2. Germline mutation of myeloid neoplasms

In the 2016 revision of the WHO classification guidelines for myeloid neoplasms and acute leukemia (1), the molecular recognition of germline predisposition to hematopoietic neoplasms was formalized. For example, the primary genetic susceptibility factors used to identify bone marrow malignancies, namely RUNX family transcription factor 1 (RUNX1), CCAAT enhancer binding protein α (CEBPA) and GATA binding protein 2 (GATA2), are hematopoietic transcription factors, which are often associated with the onset of malignancies in young individuals. While idiopathic MDS has a typical onset age of >60 years, it is common for patients with GATA2 deficiency to develop MDS at a younger age, with a median age at onset of 29 years; in these patients, the prevalence of MDS during their lifetime is estimated to be 90%, and GATA2-associated MDS is a high-risk pre-leukemic disease that may rapidly develop into AML (3). The recent identification of DEAD (Asp-Glu-Ala-Asp) box polypeptide 41 (DDX41) germline mutations in familial myeloid malignancies has also led to a shift in the view of susceptibility to hematologic malignancies, indicating an association of germline mutations with age at onset and potential function. Therefore, further research into the myeloid neoplasms associated with germline mutations is recommended.

CEBPA. The CEBPA gene is located on chromosome 19q13.1, and encodes a transcription factor that is essential for granulocytic differentiation and cellular growth arrest (4,5). CEBPA consists of three domains, which comprise two N-terminal transactivating domains, and a basic region with leucine-zipper (bZIP) for specific DNA sequence binding and dimerization, respectively, at the C-terminal end (6). The bZIP domain of the transcription factor is able to recognize the CCAAT motif in the promoters of target genes (4). Alternative in-frame non-AUG (GUG) and AUG start codons result in protein isoforms with different lengths, namely p42 and p30. CEBPA-p42, as the full-length isoform, has been widely studied, and its function has been shown to be associated with the proliferation and differentiation of myeloid progenitors (7). A study by Zhang *et al* (8) found that the loss of CEBPA-p42 in mice disrupted the normal development of terminally differentiated granulocytes and macrophages, and increased the self-renewal of HSCs. The truncated protein CEBPA-p30 is a negative factor responsible for blocking differentiation in AML, and mutations in CEBPA are critical in the disruption of myeloid differentiation in AMLs (9).

Smith *et al* (6) reported the first case of CEBPA mutation in 2004. The case was a member of a family in which three members were affected by AML. The researchers identified a 212delC mutation in CEBPA by analyzing DNA extracted from peripheral blood samples. Two siblings possessed a normal karyotype and were diagnosed with a rare disease, namely M2 with eosinophilia, and their father was diagnosed with M1. The researchers screened for the 212delC mutation in five healthy family members and found the wild-type sequence, indicating that the germline CEBPA mutation truly contributed to the development of AML. The characteristics of these three familial cases were consistent with French-American-British subtype M1 or M2 classification.

Sellick *et al* (10) reported further cases with a germline mutation in CEPBA that caused AML, supporting the previous conclusion. The authors screened five members of a family, three of whom were diagnosed with AML. The affected patients were found to harbor an out-of-frame germline 217insC mutation in the N-terminal area of CEBPA, leading to reduced expression of the 42-kDa isoform and enhanced production of the 30-kDa isoform. Corresponding results were observed in two subsequent studies (11,12). It is worthy of note that the locations and types of mutations found by Sellick *et al* (10) are almost identical to the mutations in the aforementioned family with AML, indicating that these mutation of CEBPA may serve to initiate the pathogenesis of AML as a dependent factor.

In subsequent years, other germline CEBPA mutations have been reported, including 350-351insCTAC, 291delC, 465-466insT and 217-218insC (11-13). All of these mutations are located in the N-terminal region. Yan *et al* (14) also reported that a patient with AML that may have developed from MDS carried a germline CEBPA mutation: C.134dupC. The germline CEBPA mutation was an initial event in this case, and the patient presented with myelodysplasticity, indicating that this mutation may participate in the transition from MDS to AML.

It has become increasingly clear that familial AML with germline CEBPA mutation involves the inheritance of a single copy of mutated CEBPA encoding a granulocyte differentiation factor (4). The AML is associated with biallelic CEBPA mutations, typically with the germline mutation at the 5'-end of the gene and a somatic mutation at the 3'-end of the allele acquired when progression to AML occurs (13).

DDX41. DDX41 is a receptor belonging to the DEAD/H-box helicase family, encoded by a gene comprising 17 exons on chromosome 5 (5q35.3) (15). DEAD-box proteins such as DDX41 have a core consisting of two major domains involved in nucleotide binding, with sites for RNA binding and ATP hydrolysis. The functions of the N- and C-terminal regions are not specific to particular proteins. In terms of RNA metabolism, DDX41 takes part in pre-mRNA splicing, mRNA export, transcriptional and translational regulation, ribosome biogenesis and RNA decay (16).

In multiple families with MDS/AML, DDX41 mutations have been identified as germline and acquired somatic mutations, and most of the germline mutations are frameshift mutations. DDX41 mutations can affect the development of tumors, as the loss of DDX41 has been shown to result in a loss of tumor-suppressive function (17,18). Somatic mutations have also been detected in the majority of tumors with germline mutations (19). Clinically, DDX41 mutation leads to the development of high-risk MDS. Notably, the functions of DDX41 contribute to various biological processes, including mRNA splicing, innate immunity and rRNA processing (15).

In 2015, Polprasert *et al* (17) first described adult cases of MDS/AML caused by germline mutations (p.I396T, p.F183I, p.Q52fs and p.M155I) in the DDX41 gene. The authors identified a higher incidence of DDX41 mutations and deletions in

patients with advanced MDS (19%) compared with low-risk MDS (6%). They also observed that patients with DDX41 mutations or deletions had poorer overall survival, In addition, they noted that the occurrence of somatic DDX41 mutation is closely associated with the existence of germline DDX41 mutations, with ~50% of patients with germline DDX41 mutations also acquiring somatic mutations (p.A225D, p.E247K, p.P321L and split-donor site mutations), compared with 0.8% of patients with wild-type DDX41.

Lewinsohn *et al* (18) also provided evidence that DDX41 mutations are an important cause of MDS/AML induction, suggesting that DDX41 is an effective tumor suppressor. The authors screened 289 families with hematological malignancies by whole-exon sequencing, and detected heterozygous germline DDX41 mutations in nine families. Three of these families carried a p.D140gfs*2 repeat mutation, one family carried a germline c.1574G>A p.R525H mutation, and five carried new mutations that had not been reported before. In addition, the average age of the germline DDX41 mutation carriers at the onset of MDS or AML was 57 years, which is younger than the previously reported age of 67 years. Further germline DDX41 mutations have been reported in subsequent studies (19,20), including c.711G>T p.L237F, c.712C>A p.P238T, c.155dupA, c.1586_1587delCA and c.719delTinsCG.

Patients with DDX41 mutations who develop MDS/AML usually present with leukopenia with or without other cytopenias and macrocytosis, in addition to hypocellular bone marrow with prominent erythroid dysplasia, and a normal karyotype, often leading to erythroleukemia (18). The prognosis of these patients is generally poor. On the basis of findings in a limited number of patients, cases with DDX41 mutation may respond to lenalidomide (15). However, more data are required to verify the efficacy of this treatment.

RUNX1. RUNX1 is located on chromosome band 21q22, and is associated with the development of normal hematopoiesis. It is a key regulator of hematopoietic and bone marrow differentiation. The protein is a member of a family of transcription factors with a homologous region called the runt homology domain (RHD). The RHD directs the binding of RUNX1 to the DNA sequence of the target gene and mediates the interaction between RUNX1 and core binding factor-β. Mutations in RUNX1 are associated with leukemia and MDS, and patients with these mutations often have a favorable outcome (21).

In 1999, Osato *et al* (22) first revealed three types of mutations within the Runt domain of the RUNX1 gene, namely silent mutations, missense mutations and nonsense or frameshift mutations. These mutations can affect the function of RUNX1, leading to leukemia. Since then, additional RUNX1 mutants have been identified in patients with MDS/AML, indicating that germline RUNX1 mutation is one of the main pathogenic mechanisms of MDS and AML. RUNX1 mutations can inhibit the differentiation of HSCs and the onset and development of MDS/AML (22). Ismael *et al* (23) found RUNX1 mutations, including V90-K117del, Val117fsX124 and T300fsX311, in three of five pediatric patients diagnosed with MDS/MPN-unclassified.

Owen *et al* (24) conducted a study of familial platelet disease with propensity to myeloid malignancies (FPD/AML), an autosomal dominant syndrome characterized by platelet abnormalities and susceptibility to MDS/AML that is caused by the genetic mutation of RUNX1. The authors identified germline RUNX1 mutations in five families with a history of MDS/AML, including 1007_1013del, G336fsX563, 83insG and A28fsX109, and found a 35% incidence of MDS/AML in carriers of RUNX1 mutations. In another study of FPD/AML, Cavalcante de Andrade Silva *et al* (25) examined two brothers who had been diagnosed with hematological malignancies and their families. The study revealed a microdeletion encompassing exons 1-2 of RUNX1 in six family members.

Harada and Harada (26) proposed a new category of myelodysplastic neoplasms, comprising MDS refractory anemia with excess blasts and AML with myelodysplasia-related changes, and sought to elucidate the relationship between RUNX1 mutations and secondary MDS and AML. In their analysis, 20% of patients with this new category of disease were found to have RUNX1 mutations. The study concluded that RUNX1 mutations are likely to be an initial factor in the development of AML, in addition to other genetic abnormalities.

Different families with germline RUNX1 mutations exhibit varying risks of developing MDS and AML (11-100%), and the median age of patients with such mutations at the onset of MDS/AML is 33 years, which is younger than that of sporadic MDS/AML (2,27).

GATA2. The GATA2 gene, located on chromosome 3, encodes a zinc finger transcription factor that contains two zinc fingers and a nuclear localization signal. This protein is vital in the development and proliferation of hematopoietic and endocrine cell lineages (28). GATA2 is crucial for the production and maintenance of HSCs in embryonic and adult hematopoietic processes and plays a regulatory role by combining with downstream targets, including transcription factor PU.1 (SPI1), LIM domain only 2, T-cell acute lymphocytic leukemia protein 1, Friend leukemia integration 1 transcription factor and RUNX1 (3). Hematopoietic cells are susceptible to changes in GATA2 levels (3). Mutations in the exons and intron 5 of this gene have been identified to cause several hematopoietic diseases, including MDS, AML and chronic myelomonocytic leukemia (CMML). In humans, germline mutations are an important cause of GATA2 deficiency.

While MDS primarily affects the elderly, it is often associated with an underlying genetic predisposition in children and young adults. An *et al* (29) found that germline GATA2 mutations accounted for 8.5% of primary MDS cases in a cohort of children in China. Wang *et al* (30) investigated the prevalence of GATA2 mutations in pediatric hematological diseases related to MDS and AML. Using Sanger sequencing to analyze all exons and intron 5 of GATA2 in children with MDS from three families with recorded pathogenic mutations, they detected GATA2 germline mutations in all three families (c.892dupT p.C298LfsX86, c.1168_1170delAAG p.K390del, c.802G>T p.G268X). In the follow-up sequencing of target genes in six familial MDS patients, GATA2 germline mutation was also found. In two consecutive prospective studies conducted in Germany over 15 years, Wlodarski *et al* (31) investigated 426 child and adolescent patients with MDS and a further 82 patients with secondary MDS. It was found that GATA2 germline mutations accounted for 15% of advanced

primary MDS cases and 7% of all cases. However, in children with MDS secondary to treatment or acquired aplastic anemia, these mutations were not present. Carriers of GATA2 mutations were older than patients with wild-type GATA1 when diagnosed and were more likely to have monosomy 7 and advanced disease. A further 108 patients with primary MDS were then subjected to a stratified analysis. Among these patients, a total of 57 cases with GATA2 mutations were identified, with 44 different germline mutations, 31 of which were new. It was observed that GATA2 mutations had a prevalence of 37% in patients of all age groups with monosomy 7, and peaked at puberty when they were present in 72% of adolescents with monosomy 7. The study identified GATA2 mutation as the most common germline defect, with monosomy 7 predisposing adolescents to infantile MDS and a high prevalence of GATA2 mutations.

McReynolds *et al* (3) conducted a clinical, hematological and genetic evaluation of 25 patients with GATA2 mutations, including missense (11/24, 46%), transcoding (2/24, 8%), acquisition (5/24, 21%) and regulatory (6/24, 25%) mutations. Hematological analyses revealed that 48% of the patients met the criteria for the diagnosis of MDS or CMML. The study also suggested that abnormal clonal hematopoiesis is frequent in MDS patients with symptomatic GATA2 mutation, indicating the importance of the close monitoring of disease progression in early MDS.

Bödör *et al* (32) investigated familial cases of MDS/AML with GATA2 germline mutations and observed a GATA2 p.Thr354Met mutation in five individuals from one family pedigree. Furthermore, high-risk MDS syndrome with monosomy 7 was noted in two first cousins with somatic ASXL1 c.1934dupG p.Gly646TrpfsX12. These findings confirm that individuals with germline GATA2 mutations are prone to familial MDS/AML, and the occurrence of monosomy 7 and ASXL1 mutation may be prevalent secondary genetic abnormalities.

Ding *et al* (28) sequenced the whole genome and exons of a high-risk MDS family comprising an affected father and son, and healthy daughter. On the basis of sequencing results, the affected family members were diagnosed with monocytopenia and mycobacterial infection (MonoMAC) syndrome with a heritable germline GATA2 mutation (R396Q) as a risk factor. Mutations in stromal antigen 2 and ryanodine receptor 2 were also detected in bone marrow samples of the father and son. However, the mutations occurred at different locations, suggesting that these mutations were independently acquired.

Pasquet *et al* (33) described the preliminary identification of a GATA2 mutation (R396Q) in a mother and her three children by exon sequencing; the mutation was associated with a history of chronic mild neutropenia that developed into AML or MDS. Ten patients with severe chronic neutropenia from six different families were subsequently identified as having six distinct and previously unreported GATA2 mutations (R204X, E224X, R330X, A372T, M388V and complete deletion of the GATA2 locus). The frequent occurrence of MDS and AML in these patients with chronic neutropenia suggests that it is important to screen for GATA2 mutations in chronic neutropenia. The suggestion that GATA2 mutation contributes to the initiation and progression of MDS/AML was supported by Hahn *et al* (34), who identified a compound in-cis GATA2 germline mutation in a pedigree with MDS/AML and thrombocytopenia.

GATA2-associated disorders include familial MDS/AML, chronic myeloid leukemia, MonoMAC syndrome and dendritic cell, monocyte, B and NK lymphoid deficiency (30).

Janus kinase 2 (JAK2). The JAK2 gene is located on chromosome 9p. It encodes a non-receptor tyrosine kinase associated with a variety of tumors, particularly hematologic neoplasms. This enzyme is involved in numerous important biological processes, including cell proliferation, differentiation, apoptosis and immune regulation, via participation in the JAK-STAT pathway. The well-known mutation JAK2-V617F is strongly associated with MPNs, including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) (35).

PMF carries a risk of developing into secondary AML, and mutations of JAK2 have been shown to initiate this progression. Engle *et al* (36) performed an in-depth sequencing analysis of 649 validated somatic single-nucleotide variants in a single patient at different disease stages. The results indicated that a clonal group comprising JAK2 and U2 small nuclear RNA auxiliary factor 1 represented the founding clone, and included mutations present at high frequencies in all three disease stages.

The majority of mutations in ET are somatic; however, Yoshimitsu *et al* (37) reported the first case of ET caused by JAK2-T875N mutation in a patient with a family history of thrombocytosis and cerebral infarction, which may have been associated with germline mutations. Marty *et al* (38) identified two families with germline mutations that led to ET. One family had the JAK2 R867Q mutation, and the other presented with two JAK2 mutations, S755R and R938Q. These unusual mutations exhibited lower sensitivity to JAK2 and HSP90 inhibitors than the JAK2 V617F mutation. Another germline mutation, JAK2 R564Q, was reported by Etheridge *et al* (39). The authors found that although JAK2 R564Q shows similar levels of increased kinase activity to the JAK2 V617F mutation, the growth-promoting effects of JAK2 R564Q are much milder than those of JAK2 V617F. Although these mutations are of the same gene, their differing drug sensitivities and disease action mechanisms suggests that they affect different functions of the same gene. These distinctions may be associated with the site of the mutation.

ETS variant transcription factor 6 (ETV6). The ETV6 gene is located on chromosome 12. It encodes an ETS family transcription factor with three functional domains necessary for hematopoiesis and the vascular development. Mutations of this gene indicate a predisposition to MDS and acute leukemia, including AML and B-cell acute lymphoblastic leukemia (B-ALL). Patients with ETV6 mutations appear to have decreased platelet numbers with normal-sized platelets and a mild-to-moderate bleeding tendency (40). Several studies have described germline ETV6 variations as a susceptibility factor for hematologic malignancies.

Melazzini *et al* (41) described ETV6 mutations (P214L, R369W, W380R and N385VFs) leading to ALL or PV. The average platelet counts of these patients were mild to low. The patients were diagnosed at a younger age than those

without such mutations, with the exception of PV. Similar observations were also made in another studies, in which the ETV6 mutations included R359X, L349P, L358M, R399C and R369Q (40,42-45). Among the carriers of these ETV6 mutations, patients with L349P, N385VFs, P214L and R399C presented with features of MDS. Thrombocytopenia was also observed as a common feature in these patients, but some of the germline mutations were associated with a low platelet count without hematopoietic malignancies. To identify whether ETV6 mutations contribute to childhood leukemia, Topka *et al* (44) screened a cohort of 588 patients with leukemia. Nineteen distinct ETV6 variants were identified, including two rare germline variants (V37M and R181H).

Lymphocyte adapter protein (LNK)/SH2B adapter protein 3 (SH2B3). The LNK gene, which is also known as SH2B3 and insulin-dependent diabetes mellitus 20, is located on chromosome 12, and encodes a lymphocyte adaptor protein. This gene is a negative regulator of multiple cytokine signaling molecules and tyrosine kinase receptors; notably, the lymphocyte adaptor protein αcts as a negative regulator of the mutant protein in MPNs with JAK2-V617F mutations (46).

Mutations of LNK/SH2B3 are often present in exon 2, and disturb the normal homeostasis process of HSCs (47-49). Although the majority of LNK variants in MPNs are somatic, germline mutations are also present in sporadic cases, indicating that germline predisposition, including single-nucleotide polymorphisms and haplotypes, may strongly affect the occurrence of MPNs, particularly during leukemic transformation and in idiopathic erythrocytosis (50).

Luque Paz *et al* (50) reported the case of an 80-year-old man who presented with chronic thrombocytosis. Using next-generation sequencing (NGS), an LNK mutation (c.639C>A p.Ser213Arg) in the PH domain was detected, a mutation that was also reported by Spolverini *et al* (51).

A germline LNK-E208Q mutation has also been reported (46,52,53). An LNK-E208Q mutation in the PH domain was detected in five members of a family, two of whom were diagnosed with MPNs. However, this mutation showed low capacity for the promotion of autonomous progenitor cell proliferation, which may explain why the other three family members with this variant did not develop MPN (52).

Oh *et al* (46) studied 33 samples from patients with JAK2 V617F-negative MPNs and identified two novel mutations in exon 2 of LNK (603_607delGCGCT and 613C>G). These two mutations were identified from DNA in the skin, so it was deduced that both variants were germline mutations. Germline LNK mutations were also detected in a further study of 341 patients with hematopoietic malignancies, including a 1-bp deletion that led to a frameshift and premature stop codon (Q72fs) in a patient with CMML, and a missense mutation (S186I) in four patients, one with PMF and three with CMML (54).

Autophagy related 2B (ATG2B) and GSK3B interacting protein (GSKIP). Both ATG2B and GSKIP are located on chromosome 14q32. They play a synergistic role in megakaryopoiesis (55). Hematopoietic malignancies caused by ATG2B and GSKIP may be associated with a duplication in 14q32. Plo *et al* (56) identified a 700-kb tandem duplication at the 14q32.13-q32.2 locus in four families from the West Indies. More than 30 members of these families presented with AML, MDS, CMML or MPN. This locus contains five protein-coding genes: TCL1 family AKT coactivator A, GSKIP, ATG2B, and bradykinin receptors B1 and B2. The researchers found that simultaneous downregulation of ATG2B and GSKIP in this locus led to the reduction of the spontaneous growth of megakaryocytic progenitors, and thereby revealed a novel predisposition locus. However, the opposite conclusion was proposed by Babushok *et al* (55), in a study of a North American family with an autosomal dominant predisposition to myeloid neoplasms. A duplication of chromosome 14q32 without duplication of GSKIP and ATG2B was detected. Presumably, the duplication in this region may affect the expression of GSKIP and ATG2B. However, whether the duplication of ATG2B and GSKIP is necessary for myeloid neoplasms remains to be determined.

RB binding protein 6, ubiquitin ligase (RBBP6). The human RBBP6 gene is located on chromosome 16p12.2, and influences cell proliferation and apoptosis by interacting with p53 and pRb. The RBBP6 gene encodes three proteins: Isoforms 1 and 2 bind the tumor-suppressive proteins p53 and pRb via a RING domain (57,58). Although isoform 3 has no named domain, it may be a cell cycle regulator involved in mitosis and apoptosis (58). Notably, mutations on RBBP6 isoform 3 have shown to be a predisposing factor in several cancers, including lung cancer, breast cancer and MPNs (57,59). Harutyunyan *et al* (60) detected germline RBBP6 mutations in ~5% of familial cases of MPN (3/67) and ~0.6% of sporadic cases (3/490). In familial MPN, they found five mutations on this gene, namely E1654G, R1451T, R1569H, S1444E and A1673V, suggesting that RBBP6 mutations are strong candidates for familial predisposition to MPN.

Other mutations. In recent decades, with the application of advanced biotechnology approaches, an increasing number of germline mutations have been discovered. For example, Narumi *et al* (61) revealed the loss of chromosome 7 carrying sterile α motif domain-containing 9 germline mutations in two patients with MDS by exome sequencing and other methods. Noris *et al* (62) found that mutations in 5'-untranslated region of ankyrin repeat domain 26 (ANKRD26) on chromosome 10p12 may lead to a 30-fold increase in the incidence of MDS/AML. Takaoka *et al* (63) reported a new germline helicase-like transcription factor mutation (E259K) in familial MDS. They demonstrated that this mutation may lead to the accumulation of DNA double-strand breaks and the weakening of PCNA polyubiquitination. Both telomerase RNA component (TERC) and telomerase reverse transcriptase (TERT) take part in the assembly of telomerase, which protects chromosomes and stabilizes the genome (64,65). Mutations in TERT or TERC may lead to multiple diseases as presented in Tables I and II. As regard to signal recognition particle 72 (SRP72), limited information is available; however, mutation in this gene is associated with hematological diseases (64,66). Although the existing research on these susceptible genes is relatively superficial, it provides evidence on the pathogenesis of myeloid neoplasms and suggests potential strategies for further diagnosis and treatment.

Table I. Summary of germline mutations.

Gene	Location	Main product	Main construction	Functions
CEBPA	Chromosome 19q13.1	A transcription factor (4)	N-terminal transactivation domains, C-terminal basic regions, leucine-zipper regions (6)	Controlling granulocytic differentiation and cellular growth arrest (4,5)
DDX41	Chromosome 5q35.3	An RNA helicase protein (20)	N-terminal domain, DEAD-box domain, helicase C domain, C-terminal domain (15)	Tumor suppressor with unknown mechanism (19)
RUNX1	Chromosome 21q22	A transcription factor (24)	Runt homology domain, transactivation domain, inhibitory domain (21)	Regulating definitive hematopoiesis and myeloid differentiation (24)
GATA2	Chromosome 3	A hematopoietic transcription factor (30)	Two zinc finger domains, a transactivation domain (33)	Regulating the production and maintenance of hematopoietic stem cells in the embryo and during adult definitive hematopoiesis (3)
JAK2	Chromosome 9p	Janus kinase 2 (38)	FERM domain (a 4-point, ezrin, radixin, moesindomain), Src homology 2-like domain, JAK homology 2 pseudokinase domain, JAK homology 1 active tyrosine kinase domain (38)	Participating in activation of the thrombopoietin/thrombopoietin receptor axis
ETV6	Chromosome 12	A transcription factor (41)	N-terminal pointed domain, central regulatory domain, ETS (DNA-binding domain) (45)	Necessary for hematopoiesis, particularly thrombopoiesis, and development of the vascular network (41)
LNK/SH2B3/ IDDM20	Chromosome 12	Lymphocyte adaptor protein/SH2B adaptor protein 3 (50)	Pleckstrin homology domain, Src homology 2 domain (50)	Negatively regulating cytokine-initiated cell signaling with important roles in the homeostasis of HSCs and lymphoid progenitors (47-49)
ATG2B and GSKIP duplication	Chromosome 14q32	Autophagy-related protein 2 homolog B and GSK3B interacting protein (56)	Belongs to the genomic duplication at chromosome 14 (56)	Synergistic roles in megakaryopoiesis (55)
RBBP6	Chromosome 16p12.2	Retinoblastoma binding protein 6 (58)	Domain with no name, CCHC zinc finger, RING finger domain, Rb-binding domain, proline-rich domain, serine-arginine rich domain, nuclear localization sequence	Influencing cell proliferation and apoptosis by interacting with p53 and pRb (58)
SRP72	Chromosome 4q11	Signal recognition particle 72-kDa gene	Protein-binding domain, RNA-binding domain (79)	Halting the translation of nascent secretory or extracellular proteins and directing them to the endoplasmic reticulum (70)

Table I. Continued.

Gene	Location	Main product	Main construction	Functions
TERC	Chromosome 3q26	An RNA template	Transcriptase, telomerase enzyme, RNA template and other associated proteins (64,65)	Constitutes a ribonucleoprotein enzyme complex (telomerase), which protects chromosomes and stabilizes the genome as a whole, and telomere preservation (64,65)
TERT	Chromosome 5p15.33	A telomerase enzyme	Reverse transcriptase, telomerase enzyme, RNA template and other associated proteins (64,65)	Constitutes a ribonucleoprotein enzyme complex (telomerase), which protects chromosomes and stabilizes the genome as a whole, and telomere preservation (64,65)

CEBPA, CCAAT enhancer binding protein α; DDX41, DEAD (Asp-Glu-Ala-Asp) box polypeptide 41; RUNX1, RUNX family transcription factor 1; GATA2, GATA binding protein 2; JAK2, Janus kinase 2; ETV6, ETS variant transcription factor 6; LNK, lymphocyte adapter protein; SH2B3, SH2B adapter protein 3; IDDM20, insulin-dependent diabetes mellitus 20; ATG2B, autophagy related 2B; GSKIP, GSK3B interacting protein; RBBP6, RB binding protein 6, ubiquitin ligase; SRP72, signal recognition particle 72; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase; HSCs, hematopoietic stem cells.

3. Clinical significance

Pathogenicity and tumor mutation burden. Pathogenicity may result from the accumulation of various genetic mutations rather than known mutations. In patients with germline mutations, other mutated genes have also been detected (12,17,30). These secondary mutations increase the mutational load, leading to changes in the original disease course and a rapid switch to malignancy (5,30,67). As they accumulate, the pathogenic mechanism appears more complicated. Thus, the detection of gene mutations and mutational burden helps in the analysis and explanation of the pathogenesis and progress of disease from the genetic perspective, and can further improve the level of accurate diagnosis and treatment.

Germline mutations in clinical prediction. Various germline mutations have been identified to be associated with hematological malignancies, but heterogeneity is evident in penetrance, the age of disease onset, clinical manifestations and prognoses. Certain mutations often lead to specific types of hematological tumors appearing in specific clinical syndromes. Mutations at different loci of the same gene may also be a cause of heterogeneity. For example, hereditary MDS is a heterogeneous disease that develops from congenital bone marrow failure syndrome. It seems appropriate that the risk management approach for MDS should differ according to the associated genetic mutations and their diagnoses, including those with current risk such as Fanconi anemia, short-term risk such as RUNX1 mutations, and long-term risk such as DDX41 mutations (68). The following characteristics suggest that genetic susceptibility should be considered in patients with MDS: Physical deformities and dysplasia, recurrent hemopenia, macrocyte and bone marrow failure, repeated infections since childhood, rare types of infections such as *Mycobacterium avium* infection, multiple concurrent tumors, severe side effects of chemotherapy or radiotherapy, or multiple family members with a family history of malignancy (69,70).

Germline mutations may act as biomarkers for the prediction of drug efficacy. At the 25th European Hematology Association Congress, it was disclosed that patients with DDX41 germline mutations had improved overall survival (HR 0.63, P=0.009) compared with those with wild-type DDX41 when treated with azacytidine. This observation may help physicians to predict the therapeutic response (71).

Germline mutation in treatments. Due to the heterogeneity of myeloid neoplasms, there are no uniform recommendations for treatment. However, HSC transplantation (HSCT) is considered to be an essential treatment for this type of neoplasm. Timely HSCT can avoid the occurrence of primary infections, organ dysfunction and malignancy. Nevertheless, as germline mutations have familial aggregation, family members with germline mutations also have a high risk of acquiring diseases, and the use of HSCT donations from relatives can lead to recurrence (72). Therefore, donors must be screened for potential germline mutations to eliminate the potential risk of donor-derived recurrence. Moreover, genetic consulting and testing is also recommended for other family members (73). Abnormal bone marrow is a good predictor of prognosis, and

Table II. Germline mutation-associated syndromes or diseases in myeloid neoplasms.

Genes	Associated syndromes or diseases	Clinical features
CEBPA	Familial AML, particularly FAB M1, M1Eo, M2 and M2Eo (11-13), MDS (11-13)	Early age of AML onset (11,12); favorable outcomes in patients with AML (11-13)
DDX41	Familial MDS/AML, CML, non-Hodgkin lymphoma, Hodgkin lymphoma (18)	Late age of MDS/AML onset (17,18); inferior overall survival (17); improved response to lenalidomide (17)
RUNX1	Familial platelet disease/AML, MDS/AML, MPNs (26)	Outcomes for MDS/AML are heterogeneous and effective treatment options are limited (24)
GATA2	Familial MDS/AML, CML, MonoMAC syndrome, DCML deficiency, Emberger syndrome (lymphoedema, monosomy 7 and MDS) (30)	Early age of MDS onset with a high risk of AML (29); heterogeneous manifestations in multiple systems; GATA2 mutational status does not negatively affect the outcome of MDS or HSCT (31)
JAK2	MPNs, including PV, ET and PMF (35), secondary AML from PMF (36)	JAK2 V617F is a canonical mutation in MPN (37); different mutations of JAK2 vary in drug sensitivity
ETV6	ETV6-RT inherited thrombocytopenia predisposes to childhood ALL (41), ALL (45), red cell macrocytosis (45)	Thrombocytopenia; early leukemic transformation is a major risk (41); children with ALL with germline ETV6 variants are significantly older (42)
LNK/SH2B3/ IDDM20	Pediatric B-ALL (48), familial MPNs, including PV, ET and PMF (46,52,53)	High SH2B3 levels are associated with longer event-free survival and overall survival in pediatric patients with B-ALL (48)
ATG2B and GSKIP duplication	Familial MPNs (56)	Unclear whether the duplications of the GSKIP and ATG2B genes are necessary for familial myeloid neoplasms (55,56)
RBBP6	Familial MPNs	Clinical features and survival of familial MPNs are similar to those of sporadic MPN (59)
SRP72	Familial BMF syndrome with elevated risk of MDS/AML (64,66)	Little known due to limited cases (64,66)
TERC	Dyskeratosis congenita, BMF syndrome with elevated risk of AA, MDS and AML (65,66,80), AA (81-83), AML (82,84), MDS (82-84), paroxysmal nocturnal hemoglobulinemia (83)	TERC mutations disturb telomerase functions, leading to dyskeratosis congenita, BMF and MPNs and a high risk of AA/MDS/AML; highly variable clinical phenotypes from mild to severe (64,85); patients with AA and TERC mutations have an inadequate response to immunosuppression (86) and exhibit worse survival (65)
TERT	Dyskeratosis congenita, BMF syndrome with elevated risk of AA, MDS and AML (65,66,80), MPNs including PV, ET, PMF (87-89), AML (90,91), AA (92), MDS evolving into AML (86)	TERT mutations disturb telomerase functions, leading to dyskeratosis congenita, BMF and MPNs and a high risk of AA/MDS/AML; highly variable clinical pheno typesfrom mild to severe (64,85); patients with AA and TERT mutations have an inadequate response to immunosuppression (86) and exhibit worse survival (65); in AML, the highest median TERT levels are in M1 and M7, and higher TERT levels indicate a significantly lower overall survival in M1 (90)

CEBPA, CCAAT enhancer binding protein α; DDX41, DEAD (Asp-Glu-Ala-Asp) box polypeptide 41; RUNX1, RUNX family transcription factor 1; GATA2, GATA binding protein 2; JAK2, Janus kinase 2; ETV6, ETS variant transcription factor 6; LNK, lymphocyte adapter protein; SH2B3, SH2B adapter protein 3; IDDM20, insulin-dependent diabetes mellitus 20; ATG2B, autophagy related 2B; GSKIP, GSK3B interacting protein; RBBP6, RB binding protein 6, ubiquitin ligase; SRP72, signal recognition particle 72; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase; AML, acute myeloid leukemia; FAB, French-American-British classification; M1Eo, M1 with eosinophilia; M2Eo, M2 with eosinophilia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; MPNs, myeloproliferative neoplasms; MonoMAC, monocytopenia and mycobacterial infection; DCML, dendritic cell, monocyte, B and NK lymphoid; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; ALL, acute lymphoblastic leukemia; B-ALL, B-cell ALL; AA, aplastic anemia; BMF, bone marrow failure.

not all mutations indicate the need for HSCT, so monitoring is essential.

Targeted therapy is also a topic of great interest in cancer therapy. Potential targets associated with germline mutations include the mutated gene itself, the products expressed and the pathway involved. Germline mutations result in diseases by influencing the bone marrow microenvironment. It has been reported that leukemias associated with germline mutations cannot be securely managed with HSCT alone; it is also necessary to treat the aberrant microenvironment, as therapy targeting abnormal chemokine production from mutated genes may help reverse the process of leukemia (74).

Gene therapy has potential as an future treatment. The introduction of normal genes into reproductive cells, i.e., sperm and eggs, or preimplantation embryos by germline gene editing can provide favorable outcomes for patients and prevent the passage of genetic disease to future generations (75). The key to this therapy is the choice of genetic vectors and the effective expression of the genes inserted into them. Frequently used vectors of choice include those based on adeno-associated viruses, retroviruses adenoviruses and herpes viruses. Effective expression is essential for the therapeutic level of the protein to be reached. However, at present, this technology is only being researched in mice and human preimplantation embryos so that its safety and efficacy may be studied and for ethical reasons.

Somatic mutation, gene modification and germline mutation. Germline mutations may change the structures of genes, which can lead to the acquisition of somatic mutations in the same genes, with germline mutations acting as an initial trigger (30,67). The somatic mutation is often identified as a second hit and accelerates the disease process by disabling the function of the gene and enhancing the clonal advantages of the germline mutation making the disease more complex. These two types of mutations imply different prognoses. Medullary tumors with DDX41 mutations were reported to have a good prognosis. Moreover, the overall response rate (ORR) to hypomethylating drugs in patients with germline and somatic mutations was 63%, while the ORR of patients with only somatic mutations was 75%. Therefore, it may be concluded that patients who have medullary tumors with DDX41 somatic mutations have a good prognosis and may be considered as an independent population (76). By sequencing samples from the oral mucosa, saliva, fingernails or hair follicles, these individuals may be identified and further clinical measures taken.

Along with genetic mutations, genetic modifications can occur. Variations, which mainly include DNA hypermethylation, histone modifications and changes to non-coding RNAs, play a critical role in the pathogenesis of myeloid neoplasms by inhibiting tumor suppressor genes or increasing the expression of oncogenes (77,78). Drugs targeting DNA methylation and histone deacetylation enzymes are associated with an improved outcome. The use of azacitidine is beneficial for high-risk MDS patients, as it reduces the remission rate, controls the disease and prolongs survival (71). Histone deacetylase inhibitors are an emerging class of drug, as an alternative to hypomethylating agents (HMAs). As MDS is only mildly responsive to HMAs with low CR and PR rate (20-35%) when used as monotherapy (78), genetic modifications may also act as a prognostic biomarker.

4. Summary and prospects

The revision of the myeloid tumor classification guidelines by the WHO in 2016 highlighted the importance of germline susceptibility genes, by creating a category of myeloid tumors with germline susceptibility. In the past few years, the number of susceptibility genes discovered by NGS technology has increased sharply, and includes GATA2, ANKRD26, ETV6, SRP72, DDX41, TERC and TERT. Moreover, most germline mutations are accompanied by somatic mutations, which may be an important reason for secondary genetic abnormalities. The discovery of susceptibility genes reveals the possible pathogenesis of myeloid neoplasms from the perspective of molecular biology. This research avenue provides further directions for clinical diagnosis, drug application and even gene therapy. However, due to differences among samples and the pathological complexity of myeloid neoplasms, genetic predisposition research is relatively scattered and cannot be accurately classified. Nevertheless, as research into this subject deepens, it is likely that scientists will find effective treatments for this intractable disease.

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Availability of data and materials

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Authors' contributions

LB and BD were responsible for conceptualization of the study. LW, ZL and BF curated the data and LB, TM and XL analyzed the data. BD supervised the study and acquired funding. XC contributed to conceptualization, supervised the study and reviewed the manuscript. LB wrote the original draft of the manuscript, and LB and BD reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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