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Germline mutations and blood malignancy (Review)

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Abstract. Germline mutations are congenital genetic mutations in germ cells that originate from sperm or ovum and are generally incorporated into every cell of the offspring's body. Somatic mutations are acquired genetic mutations that form under the influence of environmental factors during embryo formation and epigenetic development. Generally, only a portion of the cells in the human body have the same somatic mutations. Clinical detection of germline mutations is intended to determine inherited malignancies and identify high-risk families, and detection of somatic mutation is proposed to find targeted drugs, monitor tumor loading for guided therapy, and evaluate prognosis. Large-scale population cohort studies have shown that germline mutations are closely related to the occurrence, development, and prognosis of diseases. Patients with cancer-predisposition germline mutations can be used as sentinels in high-risk families. Traditional histopathology is no longer enough to identify types of cancers. Even within a particular type of tumor, there is great heterogeneity between internal molecules. The Pan-Cancer Research Program as well as other projects seek to use large quantities of data from different types of tumor research databases to carry out integrated analysis in order to establish potential non-tumor-specific tumor markers and targets by increasing the sample size to identify more molecular mechanisms. This review intends to summarize some of the relevant mechanisms underlying germline mutations in blood disorders.

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1. Introduction

Over the past 10 years, the development of basic sequencing technologies, including next-generation sequencing (NGS) and single-cell sequencing, and research projects, such as the Human Genome Project (hg19), the The Cancer Genome Atlas (TCGA) Project, and the TCGA-Pan-Cancer Project, have made great progress. For example, TCGA includes 33 common cancers and over 11,000 tumor samples with data of about 2.5 petabytes. There are three parts including cell-of-origin patterns, oncogenic processes and oncogenic pathway in TCGA Pan-cancer Atlas. In 2016, one study found that 11.8% of male patients diagnosed with metastatic prostate cancer carry pathogenic germline DNA-repair gene mutations. The Gleason score in this group was statistically higher than that in non-metastasis patients and normal individuals (1). In another study of a Chinese cohort, it was also discovered that the mutation rate of the pathogenic germline *BRCA1*, *BRCA2* and *ATM* genes in patients with non-metastatic prostate cancer was 7.55% totally, and that patients experienced metastasis at a rate of 9.68%. The pathogenic germline *ATM* gene mutation was related to the early occurrence of prostate cancer (P=0.011) (2). In 2015, one study found the incidence of germline mutations in 1,120 child tumor patients to be 8.5%, which was significantly different from the 1000 Genomes Project group (including autistic children) with a mutation rate of 0.6% to 1.1%. The most common mutant genes were *TP53, APC, BRCA2, NF1, PMS2, RB1*, and *RUNX1* (3). In 2018, one study reported that the mutagenesis rate of germline mutations in 10,389 adult patients with 33 types of tumors was 8%. *BCRA1/2, ATM, RET, NF1, VHL, SDHB*, and *MET* were found to be the most common mutant genes (4). Mutant gene detection in children and young patients who engaged in hematopoietic stem cell transplantation (HSCT) from 1999 to 2012 owing to aplastic anemia (AA) or myelodysplastic syndrome (MDS) showed that there was a 5.1% (5/98) mutation rate in AA patients and 13.6% (15/110) rate in MDS patients, with

most of the mutations being germline mutations. MDS-related mutant genes were found to include *FANCA, GATA2, MPL, RTEL1, RUNX1, SBDS, TERT, TTNF2,* and *TP53* (5). In 2014, 59 cases in 17 families were identified through detection of mutations in the MDS/acute myeloid leukemia (AML) predisposition genes. Among them, there were five cases of pathological germline mutations in five pedigrees, and often, these familial inheritances progressed to MDS/AML with the occurrence of somatic mutations (6). This frequency rate is very high, even for families with high-risk breast cancer that can be explained with known genes, which warrants increased research attention. Germline genetic mutations are associated with the type of disease, characteristics of invasiveness and mechanism of inheritance. In breast cancer, the *TBX3* gene is associated with high breast cancer risk (an autosomal dominant model, rs2242442, P=0.01, OR=0.76, 95% CI: 0.64-0.92) and decreased invasiveness of the tumor (7). Among the elderly, irrespective of the presence or absence of candidate-initiating genes, clonal hematopoiesis (CH) is common. The whole-genome sequencing of 11,262 people found CH in 1,043 people, thereby elucidating the frequency of CH. The acquired mutations of certain genes, such as *TET2, DNMT3A, ASXL1,* and *PPMID*, were identified to be strongly related to CH; however, in most cases, no initiating genes have been identified (8).

Owing to the few tests and studies of germline mutations, which have predominantly focused on somatic mutant pathogenesis, the majority of studies concerning germline mutation are only at the statistical and bioinformatics level, Additionally, only a few genes related to disease mechanisms are included in the World Health Organization (WHO) classification (9). Germline mutations are the driving force underlying the genome and genetic disease evolution. The mutation rate in families increases with parental age, but the number of extra-age mutations increases more than twice across different families (8). Meta-analysis of 6,570 mutations showed that germline methylation affects the mutation rate, whereby, the mutation rate of single-cell divisions increases during embryo formation and primordial pelvis formation, and decreases significantly during sperm formation in adolescents (8). According to current theories, germline mutations in genes increase the susceptibility to tumors, while somatic mutations are the secondary reason for the occurrence of tumors. However, even for the same tumor type in different patients, it is still unclear whether the tumor has been caused by either only germline or somatic mutations or both. Somatic mutations are dynamic processes, which lead to the occurrence and development of diseases. The mechanisms underlying the initiation, maintenance, and progression of these mutations have yet not been clearly studied, which could be a research focus of future research.

2. Studies on relevant mechanisms underlying blood disorder-related germline mutations: Germline *CEBPA* mutations

The CCAAT enhancer binding protein alpha (*CEBPA*) gene is located on chromosome 19, and there are two types of this gene according to the WHO 2016 Classification (9). The *CEBPA* biallelic mutation has a germline mutation at the 3' site and a somatic mutation at the 5' site, or two somatic mutations at the 5' site. In 2008, one study first reported that patients with the same *CEBPA* germline mutation appeared in the first proband's family, and somatic mutations at other sites led to the disease (10). Only biallelic *CEBPA* mutations indicate a robust prognosis (11). Usually, germline mutations occur at the N-terminal and somatic mutations occur at the C-terminal. Somatic mutations are unstable over the course of disease, and different mutant sites and mechanisms underlying mutations are detected as the disease progresses, which can be used to monitor tumor burden (12). Taskesen *et al* reported that the frequency of somatic mutations in AML was 7% (13). Pabst *et al* showed that 11.1% of AML patients carry *CEBPA* germline mutations (10) and all are located at the N-terminal. The frequency of other gene co-mutations with biallelic *CEBPA* mutations is low, and the prognosis is independent of *NPM1* and/or *FLT3-ITD* mutations (13). Patients with biallelic mutations were found to have prolonged overall survival (OS), event-free survival (EFS), and relapse-free survival (RFS) than patients with single mutations, and researchers have proposed that AML patients with biallelic *CEBPA* mutations should be separated from AML patients with *CEBPA* mutations (12). Compared to the incidence of 7-9% for somatic *CEBPA* mutations in sporadic cases, 83% of patients with *CEBPA* germline mutations had somatic mutations at the C-terminal (14), which suggest that patients with *CEBPA* germline mutations should consider hematopoietic stem-cell transplantation (HSCT) early at the onset of the disease to replace mutant stem cells, improve the bone marrow hematopoietic microenvironment, and avoid the risk of C-terminal secondary somatic mutations as well as the relapse of leukemia (14). Ram *et al* reported that not all patients with germline mutations have a family history of cancer and germline mutations may not entirely be an issue (15). Therefore, patients without previous family histories should also be monitored. In a large-scale study of C-terminal mutant families, the complete penetrance rate was only 46% (16). Another study showed that GATA binding protein 2 (*GATA2)* gene mutation at the ZF1 site attenuated the activity of transcription factors that enhance *CEBPA* regulation, indicating that *CEBPA* double mutations downregulate the expression of certain target genes during the process of malignant tumor transformation (17).

3. Germline *RUNX1* mutations

Runt-related transcription factor 1 (*RUNX1*), expressed by the *RUNX1* gene located on chromosome 21, is a member of the RUNX transcription factor family, containing 138 amino acids with Runx homologous functional areas. *RUNX1* has been categorized by the WHO 2016 Classification (9). Germline *RUNX1* mutations are mainly associated with familial thrombopenia/AML. Ripperger *et al* reported that the father of a 13-year-old female proband was also diagnosed with AML and carried a similar *RUNX1* germline mutation (c.520>T, p. Arg174X). Both had abnormal karyotypes accompanied by different somatic mutations (18). It is possible to use these rare familial platelet disorder with predisposition to myeloid malignancy (FPD/MM)-related malignant tumors as a multiple-step model of MDS/AML. It was reported that an 18-year-old male with a negative parental gene test had a spontaneous

RUNX1 germline mutation and a secondary somatic nonsense mutation of exon 8 of the *RUNX1* gene (c.837G>A), which resulted in the premature stop of transcription in the exons of DNA binding the inhibition region with progression from congenital thrombopenia to acute granulocytic monocytic leukemia (19). In the absence of coexistence of other somatic mutations, gene germline co-mutations with somatic mutations produced a stronger dominant negative effect mutation in only one way (19). Churpek *et al* performed gene-panel tests on 264 cases, revealing that 67% of young cases (<50 years old) and asymptomatic *RUNX1* carriers had hematopoietic clone distortion (6). In an *in vitro* study, Antony-Debré *et al* induced pluripotent stem cells from two patients with *RUNX1* germline mutations, one of which was a monoallelic deletion of RUNX domain with only the presence of thrombopenia and the other mutation site was R174Q, which was a dominant negative-phase mutation with a nearly complete functional deletion, associated with thrombopenia and leukemia (20). A semi-deletion was found to lead to defects in erythrogenesis, megakaryocytic hematopoiesis and pre-platelet formation, as well as complete loss of activity causing the amplification of the granulocyte-monocyte chamber and the increase of genomic instability (20). The protein volume from *RUNX1* gene expression can explain the different phenotypes expressed by different *RUNX1* site mutations. In most cases, haploid dose deficiency only leads to thrombocytopenia, but a greater degree of gene deletion tends to occur in leukemia. Dowdy *et al* emphasized the pivotal role of *RUNX1* in hematopoietic regulation. In their study, a full-length *RUNX1* protein expressed by the *RUNX1* gene in mice was established with a germline point mutation at HTY350-352AAA (21). The mutant mice could bypass the lethal embryo and live to adulthood without pathogenic changes in hematopoietic stem cells, but there were multi-line hematopoietic differentiation changes in adulthood with hematopoietic directional progenitor cell growth control defects. Downregulation of B line lymphocytes and myeloid lines along with delaying megakaryocytic and erythroid development and maturation were present (21). These results showed that the germline *RUNX1* gene also plays a regulatory role in embryogenesis, final hematopoiesis, and differentiation of multiple hematopoietic lines.

4. Germline *GATA2* mutations

Clinical manifestations of congenital *GATA2* deficiency are non-tuberculosis mycobacterial infections, opportunistic infections, severe monocyte, natural killer (NK) cell, and B cell defects, and progress from hypoplastic MDS to myeloid leukemia. Compared with non-progressive MDS, the germline *GATA2* mutation in progressive MDS had a higher carrying rate (15%, 13:85 vs. 4% 15:341, P<0.01), older age (12.3 vs. 10.3 years, P<0.001) and a larger proportion of chromatid 7 (70 vs. 11%, P<0.01), and 71% of cases had no blood disorder family history (22). Most of the studies on the mechanisms underlying *GATA2* germline mutations have revealed a single mutation site, such as germline point mutation p.Arg396Gln, leading to the loss of *GATA2* function, which affects the binding ability of the DNA region and inability to maintain the undifferentiated characteristics of hematopoietic stem cells or mature progenitor cells (23). Wild-type *GATA2* can regulate its transcription through the special domain of 2.4 kb from IS TSS (a transcription structure region), but the mutant type cannot regulate transcription correctly. A functional study of the three most common germline *GATA2* mutations (gT354M, gR396Q, and gR398W), showed that the intensity of binding and the ability to activate mutant DNA decreased (24). These results may be based on the complete amino acid deletion of the structural integrity of the ZF2 domain of DNA-bound arginine or zinc finger proteins. Mutants of T354M or C373R bind more closely to hematopoietic differentiation factor PU.1 and interfere with differentiation, and some mutations will make the cells progress toward granulocytic diseases. The normal physiological activities of genes are closely regulated in the processes of replication, transcription, and translation. With this, cell type-specific enhancers strictly regulate the physiological activity of *GATA2*. The 9.5 bp enhancer of the *GATA2* gene produces a variety of conserved *cis* elements, and the germline mutation of the *cis* element is pathogenic. Soukup *et al* established a mouse model with a germline *GATA2* enhancer mutation (25). The multi-motif results in embryonic lethality, but the single-nucleotide ET motif mutation can bypass embryo lethality and live to adulthood while also maintaining normal steady-state hematopoiesis. However, the mutation of the ET motif makes hematopoietic stem cells and progenitor cells lose their effects under different stresses. Overall, the results showed that single-nucleotide mutations have disease tendencies while retaining development activities, inactivating the regeneration ability of enhancers, destroying the hematopoietic regulation mechanism, leading to hematopoietic failure (25). Abnormal clone hematopoietic formation can be observed in both MDS patients with symptomatic germline *GATA2* mutations and patients with peripheral cytopenia but no obvious morphological evidence of dysplasia in bone marrow (26). The hypocellular state may be a pre-MDS stage. A total of 14/28 (29%) of patients with germline *GATA2* deletions had heterogeneous and somatic *ASXL1* mutations, of which 4/5 had proliferating chronic myelomonocytic leukemia (27). *GATA2* with the *ASXL1* mutation is related to an unfavorable prognosis. However, whether the two are synergistic cannot be identified. The ASXL transcriptional regulator 1 (*ASXL1*) mutation transforms into myeloid disease in patients with germline *GATA2* mutations, especially in terms of progressing to chronic myelomonocyticleukemia (CMML). *GATA2* germline mutation patients with *ASXL1* somatic mutation are younger, almost all of them are female (13/14), and each has a high risk of progressing to CMML (27). Early allogenic stem cell transplantation can benefit patients before they progress to AML or CMML. The status of *GATA2* does not affect the prognosis of biallelic *CEBPA/FLT-ITD* negative patients (28).

5. *DDX41/ETV6/ANKRD26* mutations

In the 2016 edition of the WHO Classification, myeloid neoplasms with germline predisposition was considered a major sectional change, specifically including genes *DDX41, ANKRD26, ETV6* and *GATA2* (9). DEAD-box helicase 41 (*DDX41)* is composed of 17 exons and is located on chromosome 5 (5q35.3). The majority of germline mutations that occur in *DDX41* are frameshift mutations that lead to loss of function

in *DDX41* acting as a tumor suppressor (29). Quesada *et al* found only *TP53* (n=11, 32%), ASXL transcriptional regulator 1 (*ASXL1*) (n=8, 24%), and Janus kinase 2 (*JAK2*) (n=4, 12%) were recurrent with *DDX41* gene mutations in myeloid neoplasm disease (30). Germline mutations in ankyrin repeat domain 26 (*ANKRD26*) and ETS variant transcription factor 6 (*ETV6*) have been reported with inherited thrombocytopenia (IT) and platelet disorders with germline predisposition to myeloid neoplasia (31). These patients are characterized by early onset or are vulnerable to attack by other somatic mutant genes with such a genetic background. Many cases have been initially misdiagnosed as Immune thrombocytopenia purpura (ITP) or sporadic MDS. Progression to MDS/AML may be associated with bi- or pancytopenia, multi-lineage dysplasia, acquisition of cytogenetic abnormalities, or somatic mutations, indicating clonal progression and/or bi-allelic mutations (31). Perez Botero *et al* first reported a case of chronic myelomonocytic leukemia-1 in a patient with a germline *ANKRD6* mutation diagnosed with thrombocytopenia 2 (THC2), a non-syndromic, autosomal dominant thrombocytopenia (32). Targeted NGS of *ETV6* performed in germline DNA samples from 4,405 children with acute lymphoblastic leukemia (ALL) determined 31 rare *ETV6* variants that were potentially related to ALL predisposition. In children harboring these mutations, ALL had distinct clinical features, such as older age at diagnosis, which indicated a unique mechanism of leukemia pathogenesis related to these *ETV6* variants. The authors estimated that approximately 1% of patients with childhood ALL potentially carry highly penetrant *ETV6* variants (33).

6. Germline mutations in ALL

Germline mutations also include lymphoid neoplasms that are not only specific to myeloid tumors. Many gene mutations have been reported to be associated with hematological malignancies, especially in children with ALL, such as mutations in *TP53,* protein tyrosine phosphatase non-receptor type 11 (*PTPN11*) (34), ATRX chromatin remodeler (*ATRX*) (35), IKAROS family zinc finger *1* (*IKZF1*) (36), ETS-related gene (*ERG*), homeobox D4 (*HOXD4*), and SH2B adaptor protein 3 (*SH2B3).* Here, we describe certain disease-related mechanisms and associated novel research progress. In a cohort study, somatic mutations affecting the RAS pathway were found in relapse ALL patients, along with epigenetic and developmental alterations. However, germline mutations also influence DNA-repair pathways (37). Germline heterozygous *IKZF1* gene mutations cause dysgammaglobulinemia; hematologic abnormalities, including B-cell defects; and autoimmune diseases (36). The germline *ERG* mutation is required for definitive hematopoiesis, adult hematopoietic stem cell function, and the maintenance of normal peripheral blood platelet numbers (38). The germline *PTPN11* gene (encoding a Shp2 protein) has also been recognized as the cause of such a deadly disease owing to the occurrence of germline mutations at the interface of the PTP and SH2 domains (39). Functional analysis of the murine Hoxd4 homolog uncovered that mutant HOXD4 protein had lower transcriptional activity than wild-type protein *in vitro*, resulting in a partial loss of function, which might be involved in childhood ALL (40). Research has also determined that loss of *SH2B3* increases Janus kinase, a signal transducer and activator of transcription signaling, promoting lymphoid cell proliferation and accelerating leukemia development in a mouse model of *NOTCH1*-induced ALL (41). Li-Fraumeni syndrome (LFS) is characterized as an autosomal dominant cancer predisposition disorder caused by germline *TP53* gene mutations. Germline *TP53* variants in childhood hypodiploid ALL suggest another manifestation of LFS. Qian *et al* identified 49 unique non-silent, rare *TP53* coding variants in 77 (2.0%) of 3,801 patients sequenced, of which 22 variants were classified as pathogenic, suggesting gene loss of function. *TP53* pathogenic variants were significantly over-represented in ALL compared with non-ALL controls, characterized by older ALL diagnosis, more likely to have hypodiploid ALL, inferior EFS, short OS, and higher risk of second malignant neoplasms (42). For B-ALL patients with a germline *TP53* c.818G>A (p.R273H) mutation, Chimeric antigen receptor (CAR) T-cell therapy may be an alternate choice as traditional chemotherapy and allogenic stem cell transplantation (SCT) are not effective strategies for those patients with adverse outcomes (43).

7. Other germline mutations

Hamadou *et al* reported a germline ASXL transcriptional regulator 1 (*ASXL1*) deletion in p.Arg402Gln for the first time in a case of non-Hodgkin lymphoma, whereby bioinformatics analysis predicted potentially harmful effects (44). Seiter *et al* performed NGS using samples of AML, which was diagnosed as MDS transformed by both a 46-year-old son and his 75-year-old father, and found c.2957A>G in both blood and non-blood tissues. Mutations were no longer detected in peripheral blood after stem cell transplantation (45). Furthermore, it was reported that the germline deletion mutation of 8 bp of intron 3 of the telomerase reverse transcriptase (*TERT*) gene tended to have CH abnormity ($P=7.4x10^{-12}$, OR=1.37) (46). A Chinese family with AML exhibited autosomal dominant inheritance, and 11 patients of four generations of this family carried the transglutaminase 6 (*TGM6*) gene deletion mutation (c.1550T>G, p.L517W) (47). The amino acid alterations affecting the activation of *TGM6* in a highly conserved region may be involved in the development of disease. Pauli *et al* reported a 6-year-old boy with Noonan syndrome (NS) suffering from B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with the germline *PTPN11* mutation (34). Moreover, Hahn *et al* sequenced the exons of 144 patients with MDS and those with other hematological malignancies, demonstrating that the carrying rate of five genes (*TET2*, *MET*, *GATA2*, *ASXL1*, *NOTCH1*) was 1.5- to 6.0-fold higher in patients than in the control group (ExAC database or control group), such as *NOTCH1* R912W, with a 6.5-fold higher carrying rate than that in controls (48). AML patients with somatic mutations, compared with the normal population before the occurrence of disease, had a higher frequency of mutations and VAF, while the mutations of specific genes were more diverse (49). This indicated that there are differences and specificities in terms of whether the disease occurs or not in cases of both somatic and germline mutations. Although inheritance of AML is rare, the results obtained for these families could help describe the potential pathogenesis of more common and sporadic cases. To a large extent, tumor progression is influenced by genetic polymorphisms carried by germline cells. There was an association between the diversity in

germline gene mutations and somatic events (including specific tissue tumorigenesis and oncogene formation) (50). In the same disease category, different gene mutations can represent different disease subtypes and highlight various clinical characteristics. Bluteau *et al* sequenced the whole exons of skin fibers of 179 patients with non-hereditary bone marrow failure syndrome from 2002 to 2016 (51) and found that the new germline mutation of sterile alpha motif domain containing 9 like (*SAMD9L*) was present at a rate of 5.6% (10/179), followed by telomerase RNA component (*TERC*) at 5.0% (9/179) and *GATA2* at 3.9% (7/179). Unlike clearly diagnosed hereditary bone marrow failure syndrome, different germline mutations and inexplicitly genetic bone marrow failure in patients represent different disease types such as MDS1 and EVI1 complex locus protein EVI1 (*MECOM*), which is a subgroup of severe aplastic anemia requiring urgent HSCT. In these cases, thorough and broader genetic testing is necessary, including that of regulatory regions, introns, and other splicing regions. According to the classification of diseases based on cytogenetics and molecular biology, the diagnosis and targeted treatment of hematological diseases will be carried beyond the traditional classification of diseases, and individualized treatment along with precision medicine will achieve further progress. We have summarized all the mutant sites in this article and their related references in Table I.

8. Cancer-predisposing syndrome

Cancer-predisposing syndrome (CPS) is the occurrence of certain human malignant tumors with familial aggregation, wherein multiple members of a family have the same tumor or several tumors. The syndrome is characterized by early onset, a high degree of malignancy, and multiple recurrences. Multiple CPS have been identified, such as LFS, neurofibromatosis type 1, APC-related adenomatous polyposis, Beckwith-Wiedemann syndrome, multiple endocrine neoplasia 1, ataxia telangiectasia, *RUNX1* deficiency, Fanconi anemia, Bloom syndrome, and PTEN hamartoma tumor syndrome (52). To date, BMF syndrome, telomere biology disorders, neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders, and Down syndrome have been included in the WHO 2016 revision of myeloid neoplasms and the acute leukemia classification (9). Variable and incomplete penetrance could not only explain why inherited diseases are occasionally transmitted through unaffected parents, but also why clinically healthy individuals can carry potentially pathogenic variants without expressing features of the disease. New sequencing techniques and karyotype analysis are more helpful in the diagnoses of these diseases, which have already been reported to be linked to one or multiple genetic mutations. As shown in Table I, specific one-gene mutations correspond to relative disease phenotypes. However, many genetic mutations have not been reported to specifically be associated with blood malignancy diseases, or these diseases are caused due to the involvement of multiple genes. This includes mutations of the Fanconic anemia (*FANC*) *genes*, such as *FANCA*, FANCD and *FANCW* in Fanconi anemia, *TP53* in LFS, and Cbl proto-oncogene (*CBL*) in CNL; however, how these mutations impact tumorigenesis needs to be determined. For children, in particular, early onset malignancies with a positive family history should be listed separately for diagnosis and treatment. Epigenetic mechanisms and alterations in DNA-repair genes warrant further investigation and may be promising treatment targets.

9. Laboratory detection and clinical study of germline mutations

In order to avoid the contamination of normal DNA with tumor cell DNA, bone marrow and peripheral blood are not considered to be suitable specimen sources when detecting germline mutations in leukemia patients. Skin biopsies are regarded as the 'gold standard', while hair and nails are relatively robust sources followed by oral epithelial cells and saliva; the quality and quantity of the DNA obtained from hair and nail samples are inadequate (53). Therefore, we propose that the peripheral blood of healthy relatives of probands can be used as a relatively uncontaminated and abundant source of DNA for detection of the same germline mutations as those in patients diagnosed with hematological diseases for the first time, enabling the detection of concomitant secondary somatic mutations in patients. Drazer *et al* believed that the use of non-hematopoietic stem cells for genetic testing, such as dermal tissue fibroblasts, could be beneficial in avoiding misdiagnosis of gene mutations in the laboratory, owing to the lower frequency of allele mutations in blood corpuscles (54). In addition, it is important to identify somatic or germline mutations by sequencing of the secondary tissue or parental genes. Family history or physical examination sometimes cannot fully predict the existence of germline mutations. In particular, for those diseases caused by multiple genetic mutations, genes that are damaged or seem to have disappeared after chemotherapy are suggested to have harbored somatic mutations, while the remaining genes are suggested to have had germline mutations. The prognostic impact of the presence of gene mutations before and after complete remission also needs to be analyzed. Even when considering the same gene, the phylogenetics may occur in a reciprocal fashion (50). NGS of the tumor tissue can also be used to identify patients with high-risk hereditary hematologic malignancy syndrome. Drazer *et al* reported that 74/360 (21%) healthy individuals carried mutations in genes that were pathogenic or potentially pathogenic, and eventually 25 pathological mutations with VAF >40% were identified, of which 6/25 (24%) were germline-derived pathogenic mutations. However, even if clear family history was available, germline mutant testing did not necessarily lead to valid results (54). Király *et al* tested the members of three families (including eight genes: *RUNX1*, *CEBPA*, *GATA2*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT*, *SRP72*) who showed a significant link with myeloid malignancies in nine patients and reported that, to date, quite a few disease-related genes have not been investigated for their association with morbidity and that there were technical limitations in performing such studies. Whole-exome sequencing or whole-genome sequencing may be necessary for these families (55).

10. Discussion

A number of solid tumors show a definite familial inheritance pattern with gene mutations. The onset of hereditary syndromes in childhood is the main clinical manifestation.

Table I. Germline mutations discussed in this review.

Mutant genes [symbol (full name)]	Mutant sites		Disease hematologic phenotype	Other phenotypes	EXAC
	c.	p.			
ASXL1	c.1205G>A	R402Q	NHL	Bohring-Optiz	NR
(additional sex combs like 1)	c.2957A>G	N986S	MDS trans AML	syndrome	NR
TERT	c.1280826_128083	NR	Predisposition to	Thyroid cancer	NR
(telomerase reverse transcriptase)					
TGM6 (transglutaminase)	3delAGCCCACC		CH		
PTPN11	c.1550T4G	L517W	AML	Sanger-Brown's ataxia	NR
(protein tyrosine phosphatase,	c.922A> G	N308D	BCP-ALL	Noonan syndrome	NR
non-receptor type 11)					
TET2	NR	Y867H	MDS	NR	0.00693
(tet methylcytosine dioxygenase 1)					
		P1723S			0.005895
		L1721W			0.00008987
		H1778R			NR
		Q1084P			0.002635
		V1718L			0.002964
NOTCH1 (notch 1)	NR	R912W	MDS	Esophageal, stomach, cervical and colorectal cancer	NR
RUNX1	c.520>T,	R174X	AML	NR	NR
(runt-related transcription factor 1)					
	c.837G>A	NR			NR
	c.952T>G,	S318A			0.0007742
	c.1098-1103 delCGGCAT,	I366-367del			0.00002871
	c.620G>A,	R207Q			0.0002065
	c.155T>A,	NR			NR
	c.554_560 delAAGTCGC	M52K			NR
CEBPA [CCAAT/enhancer binding protein (C/EBP), alpha]	c.584_589dup	NR	AML		NR
GATA2 (GATA binding protein 2)		T354M	AML	Cytopenia, bone	NR
		R396Q		marrowfailure, severe	NR
		R398W		immunodeficiency	NR
		A164T			0.2056
		A161A			0.009627
MET (met proto-oncogene)		E168D	MDS	NR	0.000008321
		R988C			0.002853
		T1010I			0.00001662
TP53 (tumor suppressor protein 53)	c.818G>A	R273H	ALL	Li-Fraumeni syndrome	NR
HOXD4 (homeobox D4)	c.242A4T	E81V	ALL	Bone disease	NR

ExAC, The Exome Aggregation Consortium (http://exac.broadinstitute.org/. ExAC data is available in the gnomAD browser or can be downloaded from gs://gnomad-public/legacy). NR, not reported or not found in the EXaC database; NHL, non-Hodgkin lymphoma; AML, acute myeliod leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CH, clonal hematopoiesis; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; g., genomic sequence; c., coding DNA sequence; p., protein.

Germline mutations in adults do not impact their growth and development but do affect hematopoietic generation and differentiation. Furthermore, the detection of these mutations can aid in the identification of certain special disease types, guide risk prognosis stratification, and inspire appropriate treatment plans that can further lead to improving the quality

of life of patients. During the genetic screening of potential related donors for HSCT, it is very important to avoid using a mutation-positive related donor to prevent donor-derived MDS/AML or other hematologic malignancy. Xiao *et al* reported that a 36-year-old male [donor with the same *CEBPA* germline mutation (584-589dup)] underwent transplantation and relapsed after 15 months. No somatic mutation was detected in the donor, and complete chimerism was detected in the recipient after recurrence when considering donor-derived leukemia (56). Galera *et al* reported three families with *GATA2* germline mutations undergoing transplantations. Of them, a 21-year-old male (46, XY, -7,13) with an NRAS somatic mutation received cells with the same *GATA2* germline mutation from his 64-year-old mother. He was diagnosed with donor-derived MDS three years later (57). Donors with germline mutations may have no clinical manifestation, but in the recipient, the same germline mutation re-enters the same recipient environment, which may trigger a donor-derived somatic mutation and lead to the recurrence of the disease or new emergence of the donor-derived disease. Early molecular recognition of these mutations can provide molecular and genetic support to ensure the success of transplantation and avoid disease recurrence.

Second, in addition to the medical value of determining germline mutations for affected patients, attention should be paid to the physical and mental health of the affected family members. Clinical symptom monitoring, genetic screening, disease diagnosis, and counseling should be carried out for these affected family members, as well as long-term pre-disease status monitoring. In addition, diseases are also influenced by epigenetics and penetrance rates, so more attention should be paid to the psychology of family members, ensuring there is minimal cause of anxiety, panic, excessive examination or even excessive treatment, as well as ensuring there is minimal wastage of social resources.

Finally, with the development of detection technology and increase in false-positive rates brought about by the improvement in specificity, clinicians should make accurate judgments of the needs of affected family members and the type of genetic tests that should be performed, formulate more normative guidelines, and conserve medical and social resources.

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Authors' contributions

JD and YG conceptualized the study. JD and XW collected the reference. JD and YG wrote, reviewed, and edited the manuscript. All authors read and approved the final version of the manuscript.

Ethics statement and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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