Germline Predisposition to Myeloid Neoplasms in Inherited Bone Marrow Failure Syndromes, Inherited Thrombocytopenias, Myelodysplastic Syndromes and Acute Myeloid Leukemia: Diagnosis and Progression to Malignancy

Rina Kansal*

Versiti Blood Center of Wisconsin, Milwaukee, Wisconsin, USA

Abstract: The diagnosis of any genetic predisposition to any malignancy carries profound significance for the patient and the family, with implications for clinical management that differ from when there is no identifiable heritable cause. The presence of a genetic predisposition to develop hematologic neoplasms is under-recognized. Therefore, such genetic predisposition was added as a separate diagnosis in the diagnostic World Health Organization classification in 2016. Such genetic predisposition may occur in the absence of syndromic or physical signs; even a familial history may be absent in some individuals. Also, currently, surveillance guidelines for individuals who may harbor such a genetic predisposition but have not developed a malignancy are mostly limited to expert opinion. The application of genomic sequencing methods in clinical laboratories has allowed increased recognition of such germline predisposition. Very recently, evidence is beginning to emerge that sheds light on possible steps for progression to a myelodysplastic syndrome or acute myeloid leukemia. This article provides an overview of the clinical aspects of the inherited forms of bone marrow failure syndromes, myelodysplastic syndromes, and acute myeloid leukemia, including for germline mutated *CEBPA, RUNXI, ANKRD26, ETV6, DDX41, GATA2,* and *SAMD9/9L* genes. Considerations for diagnosis are discussed for individuals and families who harbor a genetic or familial predisposition to developing a myeloid malignancy with future perspectives.

Keywords: Biallelic *TP53* mutations, Cancer predisposition, *DDX41*, *ETV6*, Familial AML/MDS, Family history, *GATA2, SAMD9/9L,* Schwachman-Diamond syndrome, WHO classification.

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by a proliferation of myeloid blasts, currently recognized to comprise of numerous diseases with marked genetic and clinical heterogeneity [1], as described in the diagnostic World Health Organization (WHO) classification in 2016 [2]. Myelodysplastic syndromes (MDS) are myeloid neoplasms characterized by cytopenias, ineffective erythropoiesis and a propensity to progress to AML. By current criteria, the diagnosis of MDS requires less than 20% myeloblasts in peripheral blood or bone marrow (BM), with AML as the diagnosis if blasts comprise or exceed 20% in the blood or BM. Both AML and MDS occur most commonly in adults with a median age greater than 65 years, with a median age of 68 years at AML diagnosis, and 23.7% AML diagnosed before the age of 55 years during 2014-2018 [3], and 82% MDS diagnosed at ages $\geq$ 70 years during 2012-2016 in the USA [4]. Until a decade ago, MDS and AML were thought to occur sporadically in the vast majority of cases due to somatically acquired genetic abnormalities that are identifiable in neoplastic cells. An inherited predisposition to AML or MDS is present when a genetic alteration is present in virtually all cells in the body, which occurs due to a germline abnormality that predisposes an individual to, and may lead to, the development of a myeloid malignancy during that individual's lifetime. Therefore, such individuals are considered to harbor a predisposition to develop a myeloid malignancy. Of note, these germline alterations may occur as inherited or *de novo* abnormalities, with the latter occurring only in the patient without inheritance from either parent, but with the capability to transmit to an offspring.

The presence of such inherited predisposition in patients presenting with presumed sporadic AML and MDS has been increasingly recognized in the last decade. The true prevalence of inherited predisposition to MDS and AML among all patients with MDS and AML is currently unknown since that would require examination of all AML and MDS patients for germline genetic abnormalities, which is not feasible in a clinical setting.

In contrast to MDS and AML that occur most commonly in adults but may also occur at any pediatric age, the inherited BM failure (IBMF) syndromes comprise a group of diseases that present most commonly with cytopenias in children but may also be

*Address correspondence to this author at the Versiti Blood Center of Wisconsin, Milwaukee, Wisconsin, USA; E-mail: rinakansal@msn.com

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first diagnosed in adults. These diseases include Fanconi anemia (FA), Diamond-Blackfan syndrome (DBS), Schwachman-Diamond syndrome (SDS), and dyskeratosis congenita (DC), which are defined by the underlying genetic abnormalities in the mechanisms of (1) DNA repair (FA), (2) ribosome synthesis (DBS, and SDS), and (3) telomere maintenance (DC). These constitutional diseases, and severe congenital neutropenia and amegakaryocytic thrombocytopenia, which also present with cytopenias most often in children or infants are known to have an increased predisposition to develop MDS or AML [5-10].

The diagnostic and clinical considerations that are common to all AML and MDS patients with an inherited predisposition to malignancy include (1) diagnosis and clinical management for the proband or the initial patient with AML or MDS, including if the proband is considered for a hematopoietic stem cell transplant (HSCT) from a family member, (2) genetic counseling for the family that may include individuals who may be carriers of the same germline genetic abnormality as the proband, and (3) lifetime surveillance for family members who are carriers of the germline genetic abnormality and may be thus predisposed to developing AML or MDS but have not developed a malignancy, AML or MDS. Therefore, the presence of a germline abnormality that predisposes to a malignancy in the initial patient with AML or MDS carries profound significance for both the patient and the family. The 2016 WHO classification introduced germline predisposition to myeloid neoplasms as a separate entity to encourage this diagnosis, including germline abnormalities in the *RUNX1, CEBPA, ANKRD26, ETV6, DDX41*, and *GATA2* genes [2, 11]. Since the 2016 WHO classification, additional inherited germline abnormalities have been described that predispose to MDS or AML, including in *SAMD9/9L* genes, which are described in a subsequent section.

Accurate diagnosis of such inherited predisposition requires (1) awareness of the clinical and genetic features of the IBMF diseases and for those MDS and AML patients with specific germline genetic abnormalities that predispose to malignancy, to then allow (2) clinical laboratory testing for the presence of a germline abnormality in the patient, with genetic counseling for the family. To eradicate a germline predisposition to developing a myeloid neoplasm, the only currently available treatment is a HSCT. This review describes the diagnostic features and clinical considerations for the inherited forms of BM failure (BMF) syndromes, inherited thrombocytopenias due to germline *RUNXI, ANKRD26*, and *ETV6* alterations, and inherited or familial forms of AML and MDS, including germline alterations in *CEBPA*, *DDX41, GATA2* and *SAMD9/9L* genes, with recent advances relevant for determining when individual carriers or patients with a known disease harboring inherited or known germline abnormalities may progress to MDS or AML.

INHERITED BONE MARROW FAILURE SYNDROMES

The differential diagnosis between acquired and inherited forms of BMF diseases can be challenging, since there is significant overlap in the clinical features (cytopenias) and the BM histopathologic findings (hypocellular marrow) in acquired and inherited BMF. The diagnosis of acquired aplastic anemia first requires exclusion of any inherited disorder. Therefore, genetic testing is often performed in pediatric patients and young adults with BMF [12-14].

Table **1** provides the clinical characteristics of the aforementioned IBMF diseases, including the male: female ratios, median ages at presentation, the percentages of patients diagnosed $\geq$16 years of age, the main hematologic presenting features, the major non-hematologic features and physical anomalies, including the percentages of patients that may be normal, whether solid tumors may occur in these patients, and an estimate of the risk of developing AML or MDS [5-10, 15-18]. Table **1** also contrasts the genetic features, including the modes of inheritance, the aberrant genes, and the underlying pathogenetic features for these diseases. It is important to remember that the complete set of clinical features that permit a diagnosis of classical IBMF syndromes may be absent in patients who actually do have IBMF, and that these diseases may also present in adults, with the median age at diagnosis for FA and DC as 33 and 42 years, respectively [19]. Although all IBMF patients are at increased risk of developing a malignancy, patients with FA were found to be most likely to develop a malignancy, followed by patients with DC [20]. In that study, the cumulative incidence of leukemia was <10% in DC, and < 5% in FA and SDS, by ages 70, 30, and 20 years, respectively, with no cases of AML reported in the DBA cohort [20]. For MDS, the cumulative incidences were 50% in FA, 20% in DC, 65%, in SDS, and 5% in DBA. The phenotypic spectrum of the IBMF diseases shows variability in penetrance and expression of disease, and therefore, accurate diagnosis requires a high index of suspicion and appropriate genetic testing for inherited genetic abnormalities [20]. Indeed, in a study of all patients referred for HSCT to a single center in the USA with a diagnosis of acquired aplastic anemia or MDS in the

USA, 5.1% of aplastic anemia and 13.6% of MDS patients harbored mutations in known causal and inherited BMF/MDS genes [21].

Notably, the genetic causes of all IBMF patients are constantly being elucidated. In 2014, biallelic mutations in a newly described ERCC excision repair 6 like 2 (*ERCC6L2)* gene, involved in DNA repair and located on chromosome 9q22.32 band [22], were identified in 3 unique BMF patients from consanguineous families in Europe [23]. *ERCCL6* mutations were subsequently identified in additional patients as the cause for their IBMF in a French cohort [24] and 2 of 8 IBMF patients who presented with MDS in another European study [25].

In studies in patients with idiopathic BMF or MDS, massively parallel sequencing by a targeted 86-gene

Table 1: Clinical and Genetic Features of Major Inherited Bone Marrow Failure Syndromes with Significant Increased Risk of Developing Myelodysplastic Syndromes or Acute Myeloid Leukemia [Table Modified from Reference # 15]

Syndromic Disease	FA (OMIM #227650)	DBA (OMIM # 105650)	SDS (OMIM # 260400)	DC	SCN (OMIM # 202700)	AT
Clinical features [5-10, 16-18]						
Male: female	1.2:1	1.1:1	1.5:1	4:1	1:2	0.8:1
Median (range) age, diagnosis	6.6 (0-49) years	0.25 (0-64) years	1 (0-41) years	15 (0-75) years	3 (0-70) years	0.1 (0-11) years
% diagnosed ≥16 y age	9%	1%	5%	46%	13%	0%
Main features, hematologic	Pancytopenia	Anemia, macrocytosis, reticulocytopenia, red cell aplasia	Neutropenia	Pancytopenia	Neutropenia	Thromboc -ytopenia
Major non-hematologic features and physical anomalies [5, 10]	Abnormal thumbs, radii, skin hyperpigmentation, short stature, deafness, bony deformities, congenital dislocation of hips, microcephaly, microphthalmia, gastrointestinal, renal and pituitary anomalies, cardiopulmonary rare, some developmental delay, **~25% normal**	Abnormal thumbs, flat thenars, short stature, webbed neck, fused cervical vertebrae, asymmetric high scapula, hypertelorism, epicanthal folds, cardiac defects, cleft lip, palate, skeletal abnormalities, rare developmental delay, **~70% normal**	Exocrine pancreatic insufficiency, neurodevelopm -ental and skeletal abnormalities	Pigmentation, dysplastic nails, oral leukoplakia, microcephaly, pulmonary fibrosis, esophageal stenosis, liver disease, sparse and early gray hair, osteoporosis, **~10% normal**	Severe infections; no physical anomalies, **all normal**	No physical anomalies ; **all normal**
Solid tumors and lymphoid malignancies	Squamous cell cancer head & neck, anogenital; other solid malignancies in *FANCD2*	Osteosarcoma, colon cancer, female genital cancer; acute lymphoblastic leukemia	Acute lymphoblastic leukemia; no solid tumors	Squamous cell cancer head & neck	No solid tumors	No solid tumors
Development of AML or MDS	Yes; AML may present in undiagnosed FA	Yes	Yes	Yes	Yes	Yes
Incidence or risk of MDS or AML	Incidence MDS: 40% at age 50; AML: 15-20% at age 40 [10]	Observed: expected ratio 287 for MDS, 28 for AML [16]	Risk MDS or AML 19% at 20 years; 36% at 30 years [9,10]	MDS or AML in 7 of 50 patients [9], observed: expected ratio for AML: 195 [9]	11% at 20 years; 22% after 15 yrs of G-CSF [10]	Risk 53% by age 17 [5]
Genetic features [5,8-10, 16-18]						
Major modes of inheritance	AR; XLR rare (*FANCB*) AD rare (*FANCR)*	AD; XLR rare	AR	XLR, AD and AR	AD, AR and XLR	AR

Aberrant genes,* chromosomal locus, % of patients [5, 8-10]	*FANCA,* 16q24.3, ~70% [5] *FANCC,* 9q22.3, ~10% *FANCE,* 6p21.3, ~10% *FANCG*, 9p13, ~10% Rare: *FANCB,* Xp22.31; *FANCD1 (BRCA2),*13q12.3;*FANCD2,* 3p25.3; *FANCF,* 11p15; *FANCI*, 15q25;*FANCJ (BRIP1),* 17q22.3; *FANCL (PHF9),* 2p16.1;*FANCM,* 14q21.3; *FANCN (PALB2),* 16p12.1; *FANCO (RAD51C),*19q22; *FANCP (SLX4),*16p13.3; *FANCQ (ERCC4),*16p13.12; *FANCR (RAD51),*15q15.1; *FANCT (UBE2T),* 1q32.1; *FANCU (XRCC2),* 7q36.1; *FANCV (MAD2L2),* 1p36.22; *FANCW (RFWD3),* 16q23.1	**AD** and *de novo*: *RPS19,* 19q13.2, 25% *RPS24,* 10q22.3, 2%; *RPS17,* 15q25.2, 1%; *RPL35A,* 3q29, 3.5%; *RPL5,* 1p22.1, 6.6%; *RPL11,* 1p36.11, 4.8%; *RPL15,* 3p24.2 *RPL18,* 19q13.33; *RPS7,* 2p25.*3, 1%;* *RPS15A, 16p12.3;* *RPS26,* 12q13.2, 2.6%; *RPS10,* 6p21.31, 6.4%; *RPL26,* 17p13.1, 1%; *RPL27*, 17q21.31; *RPL35, 9q33.3;* *RPS27,* 1q21.3; *RPS28,* 19p13.2; *RPS29,* 14q21.3 **XLR:** *GATA1,* Xp11.23; *TSR2,* Xp11.22 **Genetic causes in ~40% patients unknown**	*SBDS,* 7q11.21, >90%	*DKC1,* Xq28, 36%; [5,9] **AD**: *TERC,* 3q26.2, 10%; *TERT,* 5p15.33, 1%; *TINF2,* 14q12, 15%, includes *de novo*; *RTEL1,* 20q13.33; *ACD*, 16q22.1; **AR**: *NOP10 (NOLA3),* 15q14, <1%; *NHP2 (NOLA2),* 5q35.2, <1%; *WRAP53 (TCAB1),* 17p13.1, rare; *TERT; RTEL1; CTC1,* 17p13.1; *PARN,* 16p13.12; *ACD* 16q22.1; *TERT* all autosomal & *de novo* 5-10%; **Genetic causes in ~30% patients unknown**	**AD:** *ELANE,* 19p13.3, 75%; *GF11,* 1p22.1, < 1%; *SRP54,* 14q13.2; **AR:** *HAX1,* 1q21.3, 1%; *G6PC3,* 17q21.31, <1%; *CSF3R,* 1p34.3, <1%; *VPS45,* 1q21.2; *JAGN1,* 3p25.3 **XLR**: *WAS,* Xp11.23, <1%	*MPL,* 1p34.2; Thrombopoi-etin receptor function absent or reduced
Pathogenetic pathway or effect	FA/BRCA DNA repair defect; spontaneous chromosomal breakage, prolonged G2 cell cycle phase	Ribosome biogenesis defect	Ribosome biogenesis defect	Telomere maintenance defect	Myeloid maturation arrest	Marrow aplasia in 100% before adulthood [18]

* Additional information obtained from respective # OMIM (Online Mendelian Inheritance in Man) webpages at www.omim.org, accessed April 20, 2021.

FA, Fanconi anemia; DBA, Diamond-Blackfan anemia; SDS, Schwachman-Diamond syndrome; DC, Dyskeratosis congenita; SCN, Severe congenital neutropenia; AT, Amegakaryocytic thrombocytopenia; AD, Autosomal dominant; AR, Autosomal recessive; XLR, X-linked recessive.

panel revealed germline alterations in *GATA2, RUNX1*, *DKC1*, or *LIG4* in 8 (11%) of 71 patients, with only 4 of those patients having a history suggestive of an inherited disease. Notably, none of those eight patients had clinical features or physical signs representing the diagnosis revealed by the genetic sequencing studies [26]. Subsequently, Bluteau *et al*. at the French BMF Laboratory service studied 179 pediatric and adult patients from 173 families with IBMF excluding FA but undiagnosed cause and assigned a diagnosis based on causal or likely causal germline variants in 86 (48%) IBMF patients [24]. By applying whole exome sequencing on fibroblast DNA as the germline tissue source, in addition to identifying the aforementioned *ERCCL6* mutations, they identified mutations in the telomere genes (*TERT, TERC, DKC1*, and *RTEL1*) in 29 (34%) of 86 patients, transcription factor genes including *GATA2, RUNX1*, and *MECOM/EVI1* in 21 (24%), and genes involved in ribosome assembly, including *SBDS, SRP72, DNAJC21*, and *RPL5* in 12 (14%) patients. Of note, again, the clinical phenotypes had not suggested the diagnoses revealed by the germline mutational analyses, but 52% of patients remained undiagnosed [24]. Further, in a cohort of 1514 MDS patients who underwent HSCT and were examined for mutations before transplant, 4% of 241 young patients with ages 18 - 39 years were revealed to have clinically unsuspected SDS, often associated with somatic *TP53* mutations [27].

Transformation to Myeloid Malignancy in Patients with SDS

SDS patients often have somatic *TP53* mutations in the absence of development to MDS [28]. In comparison with SDS, Li-Fraumeni syndrome is

characterized by heterozygous germline *TP53* mutations that persist in cells throughout life, but in the absence of any toxic stress, the *TP53* mutated cells do not progress to a biallelic *TP53* alteration. In contrast, in SDS, there is an underlying ribosomal dysfunction, which causes additional pressure in cells with a heterogeneous somatic *TP53* mutation to progress to a biallelic *TP53* alteration, which then leads to MDS or AML [29].

In a landmark study published in 2021, the mechanisms of progression to MDS or AML were unraveled in patients with SDS [30], an IBMF syndrome described and included in Table **1**. Key findings in that study had included identification of the presence of specific types of *TP53* mutations that led to MDS or AML, in contrast with other *TP53* mutations that had occurred in other patients (who had not yet progressed to MDS or AML) in order to compensate for the deficiencies in erythropoiesis. Importantly, the latter compensatory types of *TP53* mutations were not malignant or even a harbinger of malignancy, while the former types of mutations did lead to MDS and AML. Specifically, the investigators studied 208 serial samples from 49 SDS patients, and demonstrated that somatic clones existed in *EIF6, TP53, PRPF8* and *CSNK1A1* in patients with SDS that emerged in an attempt to compensate for the ribosome dysfunction in SDS [30]. Importantly, they showed that the presence of high variant allele frequencies (VAFs) for *TP53*, or the number of *TP53* clones, or how long the clones were present did not correlate with progression to malignancy. Notably, by using single cell sequencing, they showed that it was the presence of biallelic *TP53* alterations that led to *TP53* inactivation, which led to malignancy in patients with SDS, and that bulk sequencing could not have identified those deleterious *TP53* mutated clones at an earlier time point [30].

Interestingly, similar to SDS patients developing myeloid malignancy after bilallelic *TP53* inactivation, a publication from Finland described AML patients with *ERCCL6* mutations that were associated with *TP53* mutations in all patients at leukemia diagnosis [31]. Collectively, although these are rare diseases, there are similarities in the manner in which progression to a malignancy or AML occurs in these conditions, which provides an understanding of the principles that could likely be applied to other similar predisposing conditions or carrier states.

INHERITED OR FAMILIAL AML AND MDS

Syndromic Familial AML

The genetic basis for familial AML was first identified in 1990 in the Li-Fraumeni syndrome [32], which is caused by a loss of tumor suppressor function due to germline genetic defects in *TP53*, which predispose to the development of multiple malignant neoplasms, including sarcomas, neoplasms of the brain, breast, and hematolymphoid tissues, including acute lymphoblastic leukemia (ALL), MDS, and AML. Numerous publications exist for this subject that cannot be reviewed for this article, and the reader is referred to earlier publications [33-35].

Other rare cancer predisposing syndromes with predisposition to develop AML include the constitutional mismatch repair deficiency syndrome (CMMRD), Werner syndrome, Bloom syndrome and Nijmegen breakage syndrome, reviewed in a recent publication [36]. CMMRD is a rare, autosomal recessive, highly aggressive disease that occurs due to homozygous germline mutations in DNA mismatch repair genes, *MLH1, MSH2, MSH6, PMS2*, and *EPCAM* and typically presents with high grade non-Hodgkin lymphoid malignancies, brain and colonic tumors, and rarely as AML [15, 37, 38]. CMMRD surveillance guidelines were recently updated [39, 40]. The clinical features of CMMRD may include café au lait macules, and can be reminiscent of neurofibromatosis type 1, which is caused by loss of tumor suppressor NF1 function that may also cause myeloid neoplasms. Other syndromic causes of AML include Down syndrome-associated AML and Noonan syndrome, as described in the WHO classification [11].

Non-Syndromic Familial AML

As defined in a previous publication [15], "**non-syndromic familial AML** may be defined to include multiple genetically and clinically heterogeneous disorders, each caused by genetic aberrations in a single gene, including *CEBPA* and *DDX41* (both with no pre-existing disorders), or *RUNX1, ANKRD26,* and *ETV6* (all with pre-existing platelet disorders or inherited thrombocytopenia), all of which co-segregate in family members leading to an increased and heritable predisposition to develop AML" [15]. The following sections describe these non-syndromic types of AML, including information from that previous publication [15] and other publications cited in the respective sections.

FAMILIAL *CEBPA* MUTATED AML

AML with germline mutated *CEBPA* was first identified in 2004 by Smith *et al.* in a family with several members in different generations presenting with AML [41]. As previously reviewed, this is the purest form of familial AML, with no antecedent MDS phase described in the detailed reports of patients with germline *CEBPA* mutated AML. Table **2** shows the demographics and genetic findings of the first 42 patients and their family members examined for germline *CEBPA* mutations from the 17 earliest reported families, both with and without a family history of AML [15, 41-54]. A family history of AML was present in all patients with N-terminal germline *CEBPA* mutated AML, which comprised 70% of the 17 families. In those families, the median age for AML diagnosis was 25 (range 1.75-46) years for N-terminal *CEBPA* mutated AML, lower than the median age of 36 (2.8-69) years for C-terminal *CEBPA* mutated AML, and closer to the overall median age of 26.5 (1.75-69) years for all germline *CEBPA*-mutated AML patients. The disease is autosomal dominant, with nearly complete penetrance for N-terminal *CEBPA* mutated AML in contrast with incomplete penetrance for C-terminal mutated *CEBPA* [53, 54]. Importantly, as shown in Table **2**, unaffected family members who harbored the germline mutation did not manifest as AML until at least 88 years of age [53] for the C-terminal, and 41 years of age for the N-terminal germline mutations [51].

The clinical presentation is virtually indistinguishable for germline and sporadic *CEBPA* mutated AML [55, 56]. Pathologic findings include FAB subtypes M1, M2, M4, often with Auer rods, aberrant expression of CD7, normal cytogenetics by karyotype, and frequent co-occurrence of *GATA1* and *WT1* somatic mutations at diagnosis [51]. Nevertheless, anticipation is present in germline *CEBPA* mutant AML, wherein the disease presents with increasing severity or at earlier ages in successive generations as previously described in familial AML [43, 57]. Table **2** also shows that anticipation was present with both paternal and maternal inheritance in all of 10 families with confirmed germline *CEBPA* mutations. One family harboring C-terminal germline mutations showed both modes of inheritance [53]. The mechanism for anticipation, however, is not yet elucidated in familial AML. Further, the natural history of familial germline *CEBPA* mutated AML after remission shows relapse of AML, which occurs due to a somatic mutation different from the somatic mutation present at initial AML diagnosis [51].

The "CCAAT/enhancer binding protein (C/EBP), alpha" gene (*CEBPA*), a single exon gene located on chromosome 19q13.1 band, encodes for a transcription factor that is crucial for maturation of hematopoietic myeloid cells. There are no mature granulocytes in *cebpa*-mutant mice and CEBPA is downregulated in the t(8;21) translocated AML [15, 58-60]. Most commonly, frameshift mutations in the transactivation domain in N-terminal *CEBPA* lead to premature termination, and a dominant-negative, smaller 30 kDa protein is formed in the absence of the normal, full-length 42 kDa protein. Biallelic *CEBPA* mutated AML develops in the carrier individuals when a second somatic *CEBPA* mutation, often in the C-terminus, is acquired by the cells [44, 51], although a second N-terminal somatic *CEBPA* mutation derived from a family donor has also been reported to lead to AML [49].

The incidence and prevalence of germline *CEBPA* mutated AML is not known. Our current understanding of this prevalence is likely to be underestimated, contributed by the difficulties in evaluating the GC rich *CEBPA* gene. Prevalence might also be different due to ethnicity or geographical differences. In cohorts from the Western world, *CEBPA* mutated AML comprise up to about 11% of all AML, reviewed in [61]. In a study of 149 AML patients, familial *CEBPA* mutated AML occurred in a 74-year-old patient as 1 of 2 *CEBPA* mutated AML [61]. Of interest, in the cohort by Green *et al*, the youngest (15-29 years of age) AML patients comprised the highest percentage among all AML patients with biallelic *CEBPA* mutations [1, 55, 61]. Further, in a large study from the USA, *CEBPA* mutations were more prevalent (>10%) in Asians [62]. Additionally, studies in Chinese cohorts have shown *CEBPA* mutated AML to be the most common (or as common as *NPM1* mutated) genetic subtype of AML, comprising up to 20% of all AML [63-65]. It must be noted that those studies did not investigate for the nature of mutations as somatic or germline. Even in the USA, at least 100 new AML could potentially be germline *CEBPA* mutated AML, even if these comprise about 0.5-1.0% of all AML, as per the prevalence possible in the Western world by the large study of 1182 cytogenetically normal AML [47]. Although about 25 families with germline *CEBPA* mutated AML have been estimated worldwide [66], greater than half this number of germline *CEBPA* mutated AML families are present in one large AML cohort, notably from only that subset of AML patients in whom germline mutation testing could be performed (written communication, Dr. Christian Thiede, June 15, 2020).

Table 2: Characteristics of 42 AML Patients and the Examined Carrier Individuals from the 17 Earliest Reported Families with Germline *CEBPA* Mutated AML (Table Modified from Reference # 15)

Families with germline *CEBPA* mutations, year reported, ethnic origin	Germline *CEBPA* mutations in family members with AML	Generation: number of family members with AML	Gender, age in years at onset of AML	Anticipation in AML family, paternal or maternal inheritance	Cytogenetic karyotype with clonal abnormality at AML diagnosis	Somatic *CEBPA* mutations at AML diagnosis	N carriers of germline mutation/ N healthy members tested	Ages in y of carrier (generati-on)
Familial AML with germline N-terminal *CEBPA* mutations								
Smith *et al*. [41] 2004	c.212delC	II:1 father of III:1 & 5	Male, 10		Not done	NA	0/5	None
	c.212delC	III:1 proband	Male, 30	Present, II:2 & III:1, maternal	Normal	c.1054-1089dup [51]		
	c.212delC	III:5	Female, 18		Normal	c.1063_1089dup [51]		
	c.212delC	IV:1 son of III:5	Male, 2			c.1087_1089dup [51]		
Sellick *et al.* [42] 2005, De Lord *et al.* [43] 1997, Caucasian	NA	III:1 father of IV:1 & 2	Male, 34	Present, III:1 & IV:1, III:1 & IV:2 paternal; IV:1 & V:1, paternal	NA	NA	NA/None tested	NA
	c.218_219insC[71]	IV:1 father of V:1	Male, 25		del(6)(q21)	c.1075_1081delinsCTGGAGGCCA [51]		
	c.218_219insC[71]	IV:2	Male, 24		Normal	c.1075_1077dup [51]		
	c.217insC	V:1	Male, 4		Normal	NA		
Pabst *et al*. [44] 2008, pedigree A	c.291delC	I:1 mother of II:1	Female, 46	Present, I:1 & II:1, maternal	Monosomy 7	NA	1 male/3 children of II:1	19 (III)
	c.291delC	II:1	Female, 40		Normal	c.1085_1087dup [51]		
Pabst *et al*. [44] 2008, pedigree B	c.464_465insT	I:1 father of II:1	Male, 42	Present, I:1 & II:1, paternal	NA	c.G1207C; c.A1210C [51]	NA	NA
	c.464_465insT	II:1 proband	Female, 27		Normal	c.1087_1089dup [51]		
Renneville et al. [45] 2009	c.218insC	I:1 mother of II:1	Female, 23	Present, I:1 & II:1, maternal	Normal	c. c.991_992insGA [51]	None tested/2	NA
	c.218insC*	II:1	Male, 5		Normal	c.1067_1068insGCG [51]		
Nanri et al. [46] 2010, Japanese	c.351_352insCTAC [51]	I:1, father of II:1	Male, 39	Present, I:1 & II:1, paternal	NA	c.1067_1068insGGCCCTCGCCCCCCCGCG [51]	1 male/1	24 (II)
	c.351_352insCTAC [51]	II:1	Male, 26		NA	c.1087_1089dup		
Taskesen et al. [47] 2011	c.308delG [51]	I:1 proband	Female, 25	NA	NA	c.1126_1127ins 1079_1227 [51]	NA; 1 obligate [51]	NA
	NA, 2 others	NA	Female & male, NA	NA	NA	NA	NA	NA
Taskesen et al. [47] 2011, *Stelljes et al.* [48] 2011	c.338delC	I:1, mother of II:1	Female, 28	Present, I:1 & II:1, maternal	Normal	c.1087_1089dup [51]	0/3	None
	c.338delC	II:1	Female, 2		Normal	c.1076_1087dup [51]		
Xiao et al. [49] 2011 Chinese study	c.584_589dup	I:1 proband	Male, 36	NA	del(9)(q11q34)	c.247dupC (N-terminal) c.914_916dup	1/1, sister, donor for transplant	33

Debeljak et al. [50] 2013	c.297_315del [51]	I:1 twin A mono-zygotic	Female, 1.75 (21 months)	NA	Normal	c.1087_1089dup [51] c.1061_1210del [51]	NA	NA
	c.297_315del [51]	I:2 twin B	Female, 15	NA	Normal	c.1087_1089dup [51]		
Tawana et al.† [51] 2015	NA	I:1, mother of II:1	Female, 32	Present, I:1 & II:1, maternal	NA	NA	1/1, aunt of II:1	41
	NA	I:2, sister of I:1	Female, 3		NA	NA		
	c.218_219insC	II:1 proband	Female, 18		NA; failed	c.1047_1088dup		
Yan et al. [52] 2016, Vietnamese	c.134insC	I:1	Male, 33	NA	del(9)(q13q22)	insertion of 33 bases c.937_938	NA	NA
Familial and non-familial (no family history) AML patients with germline C-terminal *CEBPA* mutations								
Taskesen et al. [47] 2011, patient 1	C-terminal c.T1096C	No family history	51, gender NA	NA	NA	N-terminal c.478_485del	NA/none tested	NA
Taskesen et al. [47] 2011, patient 2	C-terminal c.G1164A	No family history	33, gender NA	NA	NA	None in *CEBPA*‡	NA/none tested	NA
Taskesen et al. [47] 2011, patient 3	C-terminal c.G1036T	No family history	69, gender NA	NA	NA	C-terminal c.1086insAAG	NA/none tested	NA
Pathak et al. [53] 2016	NA	II:5	Male, 62	Present II:8 & III:7, maternal; III:2 & IV:1,2,3, 4, maternal; III:5 & IV:7, maternal; IV:7 & V:2, paternal	NA	NA	7/12; III:2 mother of IV:1,2,3,4; III:5 mother of IV:7; III:10 brother of III:8; IV:5,6, twin sons of III:2; IV:11, sister of IV:7; V:1 sister of V:2	44 at death§ (III:2); 88 (III:5); III:10 dead at 80; 54 (IV:5,6); 60 (IV:11); 27 (V:1)
	NA	II:8 sister of II:5	Female, 53		NA	NA		
	NA	III:7, son of II:8	Male, 36		NA	NA		
	c.A1932C	III:8, son of II:8	Male, 58		NA	NA		
	c.A1932C	IV:1	Female, 20		Normal	NA		
	NA	IV:2	Male, 2.8 (34 mo)		NA	NA		
	NA	IV:3	Male, 6		NA	NA		
	c.A1932C; p.Q311P	IV:4 proband	Female, 11		Normal	NA		
	NA	IV:7 father of V:2	Male, 41		NA	NA		
	c.A1932C	V:2	Female, 22		Normal	NA		
Ram et al. [54] 2017	c.G442T	I:1	Female, 36, proband	Present, maternal	Normal	c.68dupC	2 (mother & sister)/4	66 & 37

AML: Acute myeloid leukemia; N: Number; NA: Information not available; mo: months; * Not present 2 years after receiving allogeneic transplant [45], †Mutations reported by Tawana *et al.* [51] as per *CEBPA* transcript: ENST00000498907 (hg19); ‡Germline *CEBPA* mono-allelic mutated AML does not qualify for a diagnosis of *CEBPA* mutated AML by the 2016 WHO classification of AML; §Death due to carcinoma with no history of any leukemia.

FAMILIAL PLATELET DISORDERS WITH A PROPENSITY TO MYELOID MALIGNANCIES INCLUDING MDS OR AML, DUE TO GERMLINE MUTATIONS IN *RUNX1* (OMIM 601399)

The *RUNX1* gene, located on chromosome 21q22.12 is critical for embryogenesis and hematopoiesis. Germline *RUNX1* mutations were identified in 1999 as the underlying inherited abnormality in families with thrombocytopenia in the familial platelet disorders with a propensity to myeloid malignancies including AML (FPD/AML) [67]. The true incidence and prevalence of FPD/AML are unknown. It has been estimated that approximately 5500 families with FPD/AML may exist worldwide [68]. The main clinical feature is mild to moderate thrombocytopenia,

in conjunction with platelet functional or structural or both abnormalities, and a propensity to develop hematologic malignancies, most commonly AML or MDS, less frequently lymphoid malignancies, T-ALL, B-ALL, and rarely hairy cell leukemia [15]. Additionally, eczema, arthritis and solid tumors have been reported in FPD/AML families, including tumors of the breast, prostate, bone, stomach, pancreas, and skin in 3 of 10 families in one study [69]. In the studies by Zhang *et al.* and Bluteau *et al*, germline *RUNX1* mutations were identified by targeted gene and whole exome sequencing, respectively, in rare families with inherited IBMF with previously unknown genetic causes [24]. Also notably, Simon *et al.* recently identified germline *RUNX1* mutations in 30% of all *RUNX1* mutated AML [70]. Of note, the authors used rigorous methods to exclude contamination by somatic neoplastic cells before categorizing the mutations as germline [70].

The germline *RUNX1* mutations are of various types, including missense, nonsense, frameshift, deletions, insertions, and splice site mutations, leading to challenges in diagnosis since some types of mutations (deletions) may not always be detected by next generation sequencing assays [15, 69, 71]. The reader is referred to a recent publication by Brown *et al.* for a detailed description of the types of germline *RUNX1* mutations for about 130 reported families with FPD/AML [69].

FPD/AML is inherited as an autosomal dominant disease, with an incomplete penetrance since the heterozygous germline mutation is insufficient to cause myeloid malignancy. The disease characteristics include variation among family members for developing a malignancy, and some *RUNX1* mutation carriers may never develop a malignancy. AML develops in 20-60% of all carriers with germline *RUNX1* mutations [15, 72-77]. Interestingly, germline *RUNX1* carriers were found to develop clonal hematopoiesis at an earlier age than other age-matched healthy individuals [78], in whom clonal hematopoiesis is detected in an age-related manner [79-81]. However, clonal hematopoiesis has not been shown to progress to a myeloid neoplasm in germline *RUNX1* carriers, as previously reviewed [15].

Biologically, mutated *RUNX1* may inhibit wild type *RUNX1*, and therefore, *RUNX1* mutations that are dominant-negative instead of those that cause haploinsufficiency are more likely to lead to AML [82, 83]. Nevertheless, FPD/AML may also occur due to allelic loss of *RUNX1* in patients with constitutional syndromic features due to microdeletions in chromosome 21 in the absence of mutations in the wild type *RUNX1* allele, with progression to MDS or AML [67, 84, 85]. The transforming events in allelic *RUNX1* loss include acquired trisomy 21 and additional acquired mutations [86].

In contrast, the transforming events are considered to be different in *RUNX1* mutations. Progression to AML occurs after a latency period of 33 years age as the average age at diagnosis, due to the acquisition of a second somatic mutation, either in *RUNX1* [69, 75, 87, 88] or *FLT3*, *MLL*-PTD [86,87] and other genes, including *ASXL1, IDH1, IDH2, TET2* [89-91], *NFE2* or *NRAS* [92] or chromosomal abnormalities. In a recent study, tumor-associated somatic abnormalities in *PHF6, SH2B3, TET2, MEIS1, BCOR1, BCORL1, KRAS,* and *EZH2* were found across 10 FPD/AML families [69]. Biallelic *RUNX1* mutations are often present in familial FPD at the time of diagnosis of AML [66, 72, 87, 88, 92], indicating that the presence of biallelic *RUNX1* mutations in AML should prompt evaluation for germline mutated *RUNX1*. Of interest, Simon *et al.* found a difference in the secondary mutations in germline versus somatic *RUNX1* mutated AML [70]. When germline *RUNX1* was mutated, secondary mutations in AML were often present in *NRAS* and other signaling pathway genes, in contrast with acquired *RUNX1* mutated AML, wherein mutations of *ASXL1* were more common [70]. Also of note, at least four patients from 3 families with FPD/AML also harbored co-existing germline *RUNX1* and *CEBPA* mutations [69, 70].

Further, in a Japanese study, acquired *CDC25C* mutations were found in 7 of the 13 patients from 7 FPD/AML families, followed by the emergence of a *GATA2* mutation in 3 of the 7 with *CDC25C* mutations, which were described as key findings for progression to AML [93]. Acquisition of *CDC25C* mutations in pre-leukemic or leukemic clones in FPD/AML has not been identified in studies from other countries, including from France [75, 87], Ireland [91], and USA [88]. Whether ethnic or geographic differences might exist in FPD/AML evolution to AML remains to be determined.

Lastly, germline mutations in *RUNX1* comprise a part of the range of genetic abnormalities that lead to inherited thrombocytopenias, introduced in the next section. For the complete spectrum of inherited thrombocytopenias, the reader is referred to excellent reviews by the investigators of these disorders in Italy [94, 95].

GERMLINE *ANKRD26* MUTATED AML OR MDS

The pathogenetic causes of inherited thrombocytopenias include (1) defects in megakaryocyte differentiation, with diseases including amegakaryocytic thrombocytopenia and thrombocytopenia absent radii, (2) deficiencies in megakaryocyte maturation, including germline mutations in three genes, *RUNXI, ANKRD26* and *ETV6*, and (3) defects in platelet release, which include *MYH9*-related disease, likely the commonest cause of inherited thrombocytopenia worldwide, and the second most common in Italy [95]. Among all genetic causes for inherited thrombocytopenias, germline mutations in 3 genes, *RUNX1, ANKRD26*, and *ETV6*, are known to predispose to hematologic malignancies, including MDS and AML. All inherited thrombocytopenias due to germline mutations in *RUNX1, ANKRD6* and *ETV6* are autosomal dominant, and 10-40% patients with all of these 3 inherited thrombocytopenias progress to a hematologic malignancy [18].

The *ANKRD26* (ankyrin repeat domain 26) gene (OMIM #610855) is located in chromosome 10p12.1 band. Germline mutations in the *ANKRD26* gene cause thrombocytopenia 2 (THC2, OMIM # 188000). In this familial thrombocytopenia, in 2011, the most frequent mutations were reported to cluster in a highly conserved 22-nucleotide region in the 5' untranslated (UTR) region of the *ANKRD26* gene. The mutations occurred only in affected members and were absent in all healthy family members [96-98]. *ANKRD26*-related inherited thrombocytopenia (*ANKRD26*-RT) was diagnosed in 23 (10%) cases in 215 subjects with inherited thrombocytopenias [98], comprising the 3rd most common cause of inherited thrombocytopenias in Italy [95], and also including families from Spain, France, The Netherlands, Canada, USA, Argentina, and Japan [97,98]. However, as previously reviewed [15], prevalence may be different with differences in ethnic or geographic origin. A Japanese study showed only 1 (2%) *ANKRD26*-RT among 43 families with inherited platelet disorders [99]. In 2013, among 118 individuals with *ANKRD26*-RT, Noris *et al.* reported 8.5% (n=10) *ANKRD26*-RT patients who developed myeloid malignancies, including 4 AML, 4 MDS, and 2 chronic myeloid leukemia (CML) [98]. After combining 104 previously reported *ANKRD26*-RT cases with the 118 cases, they reported 4.9% cases to develop acute leukemias, 2.2% MDS, and 1.3% CML [98].

Conversely, *ANKRD26* mutations were found in 4 (1.6%) AML patients in a cohort of 250 consecutive non-familial AML, and 1 of those 4 AML patients was a member of a family with typical but previously undiagnosed *ANKRD26*-RT [100], indicating that *ANKRD26*-RT may be diagnosed for the first time when patients present with AML. The latter finding may occur because the bleeding tendency is mild in *ANKRD26*-RT. The significant risk in *ANKRD26*-RT is the predisposition to myeloid malignancies, which is why the genetic abnormality must be recognized, including to screen family members for HSCT. In addition, these patients can be misdiagnosed as MDS due to dysmorphic megakaryocytes in the BM [101], which are present in the pre-malignant baseline state in the carriers [102]. Alternatively, the patients may be misdiagnosed as acquired immune thrombocytopenia.

ETV6 MUTATED AML OR MDS

The "ETS translocation variant gene 6" (*ETV6*) gene, located in chromosome 12p13.2 band, is well-known to be translocated in myeloid and lymphoid leukemias, with *ETV6-RUNX1* (previously *TEL-AML1*) fusion as the most common genetic abnormality in pediatric B-lymphoblastic leukemia [15]. In 2015, *ETV6* mutations were identified in families with inherited thrombocytopenia 5 [THC5, OMIM # 616216], and found to be associated with diverse hematologic malignancies, including most frequently, ALL, and less often, AML, MDS, polycythemia vera, multiple myeloma, and non-hematologic cancer [103, 104]. *ETV6*-related thrombocytopenia (*ETV6*-RT) was reported in 2 (8.6%) of 23 [104], and 7 (2.6%) of 274 European families [105], with no *ETV6*-RT identified in 43 Japanese families with inherited thrombocytopenia [99].

Table **3** provides characteristics of 27 families with germline pathogenic *ETV6* mutations from 10 publications [103-112], including the heterogeneous cohort characteristics from where the families were identified, the ethnic origin, if available, or the study locations, the germline *ETV6* mutations and the hematologic features of the individuals in the families, and any malignancies or other features reported in the families. The germline *ETV6* mutations included missense, nonsense, frameshift or deletions, and were located primarily in the highly conserved C-terminal DNA binding domain, except for the p.P241L mutation that occurs in the linker region between the N-terminus and the C-terminus to indirectly affect DNA binding [103-106, 108]. In one patient at progression from thrombocytopenia to MDS, there were somatic mutations in *BCOR* and *RUNX1*, and additionally, in

Table 3: Characteristics Reported for 27 Families with *ETV6* Germline Mutations and Predisposition to Hematologic Malignancies

Publication, year, ethnic origin or study location	*ETV6* germline alterations reported	Study design/ original cohorts from where these families were identified	N Families reported	Individuals in families, hematologic features	Malignancy developed (at age in y)	Other characteristics
Zhang *et al.* [103], 2015 German & Native American	c.1195C>T encoding p.Arg369Gln	After 1st family A identified, studied 55 individuals with familial leukemia or MDS (all lacking germline *GATA2, RUNX1, CEBPA & PAX5* mutations) & 153 individuals with idiopathic cytopenias and/or bone marrow failure, which led to the identification of families B and C	Family A, included 4 individuals positive for germline *ETV6*^{mut}, including proband III-2, and III-1, III-3, and II-5	II-5, Thrombocytopenia, neutropenia	Colorectal cancer (45) & multiple myeloma (51)	Myopathy (n=2), GI symptoms (n=2), developmental delay (n=1), seizures (n=1), delayed puberty (n=1); all present in III-2, the proband; myopathy and GI symptoms in III-3
				III-1, thrombocytopenia	Precursor B-ALL (age 7)	
				Proband, III-2, easy bruising in infancy and menorrhagia, pancytopenia	MDS: Refractory anemia (age 9), RAEB-1 (age 21)	
				III-3, Anemia and thrombocytopenia	No malignancy	
Zhang *et al.* [103], 2015, Scottish	p.Arg399Cys		Family B: 6 germline *ETV6*^{mut}, 3 WT *ETV6*; 2 DNA unavailable	Thrombocytopenia with petechiae and epistaxis	I-1, CMML (82) & skin cancer, DNA unavailable; III-3 (WT): Skin cancer; III-8, Stage 4 colon cancer (age 43)	Reading disability (n=3), GERD (n=4), esophageal stricture (n=2)
Zhang *et al.* [103], 2015, African-American	p.Pro214Leu		Family C, one with germline *ETV6*^{mut}	Proband: Long history of nosebleeds, menorrhagia, not responsive to ITP therapy	T/myeloid mixed phenotype acute leukemia (age 50)	Mother of proband WT *ETV6*; colon cancer (at age 68 y)
Noetzli *et al.* [104], 2015, United States	c.641C>T p.Pro214Leu	After 1st family, screened 23 European families with AD thrombocytopenia, high red cell MCV and some with increased leukemia incidence; that screen by Sanger sequencing identified families 2 and 3	F1: 5 members germline *ETV6*^{mut}	Platelets 67–132 k/uL, MPV normal; Red cell MCV high 92.5–101.5 fL	Precursor B cell ALL in III-1 (age 3) and II-7 (age 37)	Bone marrow dysmorphic megakaryocytes and erythroid precursors
Noetzli *et al.* [104], 2015, Czech Republic & Italy	c.641C>T p.Pro214Leu		F2: 3 members *ETV6*^{mut}	44,000–115,000 platelets/uL, MCV 88–97 fL	I-2: ALL (age 14)	
	c.1252A>G, p.Arg418Gly		F3: 2 members *ETV6*^{mut}	99,000–101,000 platelets/uL, MCV 93–98 fL	No malignancy	
Topka *et al.* [106], 2015, Polish Moroccan	L349P	F1 and F2 identified as part of a pediatric leukemia study; then sequenced exons 5-8 *ETV6* in 27 families with a history of ALL, and screened a cohort of 588 pediatric leukemia patients; that screen identified 2 germline *ETV6* VUS: V37M (chr12:11905459G>A) and R181H (chr12:12022436 G>A)	F1: 13 members, 9 with *ETV6*^{mut}, 4 not sequenced, one with ALL not tested for *ETV6*	11 members thrombocytopenic, concurrent anemia in 2; red cell MCV not elevated in 3 with thrombocytopenia in the absence of ALL (other MCV values not known)	Precursor B-ALL (n= 4, including 1 also with MDS) II-3: CD19+,CD20+, CD22+,CD34+,TdT+ ALL; II-9: CD10+, CD20+, CD22+, TdT+; III-13:CD10+, CD20+, CD79a+, TdT+, DNA not sequenced, also pancytopenia, MDS	Cleft lip/palate (n=2, including III-13); renal cell ca (n=1) in not sequenced individual; duodenal adenocarcinoma (n=1) in not sequenced individual
Topka *et al.* [106], 2015, Western European/Native American	N385fs		F2: 4 members, 2 *ETV6*^{mut}, 2 not tested	Proband's mother, maternal aunt and maternal grandfather had thrombocytopenia	ALL, MDS, AML (n=1) CD10+,CD20+, CD79a+,TdT+ ALL	The patient with ALL (proband) had craniofacial /skeletal dysmorphisms
Moriyama *et al.* [107], 2015, European ancestry	p.P359X	Family at St. Jude Children's Hospital; then screened 4405 newly diagnosed pediatric ALL for *ETV6*	One family, 5 members studied, 4 *ETV6*^{mut}, 1 WT	Thrombocytopenia (n=2), no cytopenia (n=3); II-1 was *ETV6*^{mut} and negative for ALL at age 11 yr	3 *ETV6*^{mut} ALL; the mother (I-2) at age 9, her 2 daughters (II-2 at age 3 and II-3 at age 2)	II-2: Turner syndrome II-3: Learning disability; No MDS/AML

Melazzini *et al.* [105], 2016, Italy	c.641C>T p.P214L	From an Italian cohort of 274 consecutive familial thrombocytopenia, screened 130 molecularly undiagnosed probands; identified 20 *ETV6*^{mut} individuals in 7 families designated A to G	A: 3 members *ETV6*^{mut}	Thrombocytopenia diagnosed at birth (n=6), and at ages of 1, 2, 3 (n=2), 4, 5, 7, 8, 14, 20 (n=2), 30, 38, 47 years; MPV normal with mild platelet anisocytosis; red cell MCV high >98 fL (reference range 82-98 fL) in 4 of 20 individuals but red cell MCV not elevated in the 4 patients with ALL or PV; iron deficiency anemia (n=1), neutrophil counts within normal limits (n=20)	Common ALL, at age 7 (n=1)	
	c.641C>T p.P214L		B: 3 members *ETV6*^{mut}		B/I-2: Pre B ALL, at age 15 (n=1)	B/I-2*: breast fibroadenoma (35 y), meningioma (at age 42 y)
	c.1105C>T p.R369W		C: 2 members *ETV6*^{mut}		*JAK2* V617F PV, 37y (n=1); common ALL (age 7) (n=1)	
	c.1105C>T p.R369W		D: 2 *ETV6*^{mut}		No malignancy	
	c.1138T>A p.W380R		E: 5 *ETV6*^{mut}		Common ALL (age 3) (n=1)	
	c.1153-1_1165del p.N385Vfs*7		F: 3 members *ETV6*^{mut}		No malignancy	F/II-2: breast fibroadenoma (at age 14 y)
	c.1252A>G p.R418G+ p.N385Vfs*7		G: 2 members *ETV6*^{mut}		No hematologic malignancy	G/I-2: breast carcinoma (at age 49 y)
Poggi *et al.* [108], Feb 2017, European	p.P214L	DNA samples from 957 patients with unexplained bleeding and platelet disorders from the BRIDGE-BPD project, and 8 patients from a French Cohort; identified 6 families with germline *ETV6*^{mut}	F1: 12 members; 9 *ETV6*^{mut}, 3 WT *ETV6*	IV-3, proband, 7 yr age, normocytic anemia, MCV not high 83 fL, low platelets 50 x 10^9/L, MPV high >10 fL	No malignancy; bone marrow hypolobated megakaryocytes	Circulating CD34 positive levels showed 4-6 fold increase in 5 carriers in this family
				Proband's mother: low platelets, MCV not high, MPV high (>12 fL)	No malignancy, previous history of splenectomy	
				II-2, Proband's grandfather: red cell MCV high (101 fL), platelets 44 x 10^9/L, MPV not high (7.2 fL)	MDS: RAEB-2 at age 70	
	p.A377T		F2: 4 members	Platelets 60-111 x 10^9/L, MPV not high, MCV elevated (n=1)	MDS: RAEB (n=1)	
	p.Y401N		F3: 6 members	All 6 with MPV elevated (9.3 – 10.4 fL), *ETV6*^{mut} in 3/3	No malignancy	Circulating CD34 positive levels increased (n=2)
	p.I358M		F4: 5 members (4 carriers, 1 WT *ETV6*)	Low platelets & MPV elevated (9.8 - 11.7 fL) all 4 *ETV6*^{mut}, but MPV not elevated in WT *ETV6*; MCV not high in all (n=5)	Presentation with AML M0 (n=1) at age 8, treated, low platelets post-therapy	
	p.R396G		F5: 3 members	Low platelets & elevated MPV (n=3)	No malignancy	
	p.Y401H		F6: Mother & daughter	Mother *ETV6* WT, platelets 199 x 10^9/L; daughter 77 x 10^9/L platelets, *ETV6*^{mut}	No malignancy	Mother and daughter with storage pool deficiency
Dirse *et al.* 2017 [109], Caucasian, study from	p.W72Ter	Clinical report of adult leukemia in one family	3 members *ETV6*^{mut}; I-2 (mother), II-2 (sister)	All 3 negative for thrombocytopenia; II-3: Proband, platelets 123 x 10^9/L at	T/myeloid mixed phenotype acute leukemia (n=1), at age 19	*NOTCH1* P2377L germline variant in proband's

Lithuania			& II-3	leukemia diagnosis		brother & father
Duployez *et al.* [110], 2018, Europe	p.R378Ter	One family with high hyperdiploid ALL	3 members *ETV6*^{mut}	III-2: low platelets II-2: Father	III-2: ALL (age 13) II-2: PV (age 51)	
Yoshino *et al.* [111], 2020, Tokyo, Japan	c.641C > T p.Pro214Leu	Initial diagnosis ITP at 11 mos age, at 17 y age short stature	2 members *ETV6*^{mut}; father II-1 Proband I-1	I-1, at 11 mo age, platelets 3.4 x 10^9/L, then 9-10 x 10^9/L II-1 low platelets	ALL (age 4), *ETV6-NTRK3* positive salivary gland MASC (age 11)	At age 17 y, in remission, platelets 4.0-8.0 x 10^9/L
Nishii *et al.* 2021 [112], USA	p.R386fs	Comprehensive study of 31 B-lineage ALL & 2 AML samples from 32 unique patients from SJCRH, DFCI and COG	Family A (suppl. Fig 10 in ref.)	Proband and mother *ETV6*^{mut}; mother thrombocytopenic	Proband: ALL (22 months), therapy-related AML (4 y)	
	p.R399H		Family B	Proband and mother *ETV6*^{mut}; mother thrombocytopenic	Proband: AML (3 y), mother 31 y ALL (at age 3 y)	

GI, gastrointestinal; MDS, myelodysplastic syndromes; *ETV6*^{mut}, *ETV6*^{mutated}; WT, wild type; ALL, acute lymphoblastic leukemia; RAEB, refractory anemia with excess blasts; CMML, chronic myelomonocytic leukemia; GERD, gastroesophageal reflux disease; AD, autosomal dominant; MPV, Mean platelet volume; MCV, red blood cell mean corpuscular volume; AML, acute myeloid leukemia; PV, polycythemia vera; ITP, immune thrombocytopenia; MASC, mammary analog secretory carcinoma; SJCRH, St. Jude Children's Research Hospital; DFCI, Dana Farber Cancer Institute; COG, Children's Oncology Group.

*In Melazzini *et al*. the age for last follow-up 43 for B/I-2, for the female patient with a breast fibroadenoma [105].

In Poggi *et al*. all 5 families except for F6 (the storage pool deficiency family) showed decreased platelet aggregation response to arachidonic acid [108].

KRAS at further progression to higher grade MDS [103].

As shown in Table **3**, the *ETV6* germline mutations do seem to cause ALL most frequently, but MDS or AML or other myeloid malignancies are not uncommon, including polycythemia vera (n=2, separate families), and T/myeloid mixed phenotype acute leukemia (n=2, separate families). Moreover, intriguingly, even a carcinoma harboring the *ETV6-NTRK* oncogene fusion has been reported in a Japanese patient with one of the common germline *ETV6* mutations in the linker region [111]. That patient was initially diagnosed with immune thrombocytopenia in infancy with a platelet count less than 5 x 10^9/L, progressed to ALL at 4 years of age, and then developed the salivary gland neoplasm harboring the *ETV6-NTRK* fusion at the age of 11 years [111]. That patient's father had thrombocytopenia but no malignancy [111].

Moreover, in a recent whole genome sequencing study of 31 B-ALL and 2 AML samples from 32 patients, one patient had developed both ALL and post-therapy AML [112]. In another family in the same study, one patient had AML, and the patient's mother had developed ALL, both at 3-year-age. The study findings had suggested that it is the nature of the somatic (acquired) mutations that determine the lineage (lymphoid or myeloid) of the malignancy that develops in the individual harboring a germline mutation [112].

Intriguingly, however, the same deleterious mutation when present in another individual, even at an older age, and in the same family does not necessarily lead to malignancy, as evidenced by findings in multiple families included in Table **3**, including two families reported by Zhang *et al.* [103], two by Topka *et al.* [106], 4 by Melazzini *et al*. [105], 3 by Poggi *et al.* [108], 1 by Dirse *et al.* [109], 1 by Duployez *et al.* [110], 1 by Yoshino *et al.* [111], and 1 with both ALL and AML by Nishii *et al.* [112]. Collectively, the occurrence of the *ETV6-NTRK* oncogene fusion in an epithelial malignancy (carcinoma) after a lymphoid leukemia, and a myeloid malignancy occurring after ALL in individuals with germline *ETV6* mutations further suggest that it is likely that it is the acquired events that lead to malignant progression, and not the mere presence of a germline deleterious mutation in an individual.

Notably, the *ETV6* germline mutations identified to date are most commonly located in the C- terminal DNA binding domain, with a single mutation reported in the N- terminal domain in one family [109], and the p.P214L mutation in the linker identified to co-segregate in 7 of 27 families. Table **3** shows 20 germline *ETV6* mutated patients from 14 (~50% of 27) families who progressed to ALL, one patient with multiple myeloma, and 13 germline *ETV6* mutated patients from 9 (33%) of 27 families with myeloid malignancy, including MDS, chronic myelomonocytic leukemia, and AML (combined n= 9), T/myeloid mixed phenotype acute leukemia (n=2), and polycythemia vera (n=2). Of note, both myeloid and lymphoid malignancies developed in 9 (33%) of 27 families, including in the same individuals in 5 families, either MDS or AML following ALL (n=3), or as T/myeloid leukemia (n=2), and among different members in 4 families (Zhang *et al.* family A [103], Topka *et al.* family 1 [106], and one each in Duployez *et al.* [110] and Nishii *et al.* [112]). All reported features to date,

including in Table **3**, suggest that the spectrum of *ETV6* germline mutations is wide and has yet to be completely characterized, particularly for the types of malignancies that may arise, hematologic and non-hematologic, and the reasons why any given neoplasm might arise in any individual with that deleterious germline mutation.

Similarly, the incidence or prevalence of *ETV6* germline mutations are currently unknown. In the Italian study of 274 consecutive families with inherited thrombocytopenia, 25% (5 of 20) patients in 5 of 7 *ETV6*-RT progressed to a hematologic malignancy [105]. In these individuals, myeloid malignancies may present at any age, with range 3-82 (median 28) years in 10 reported patients in 27 families in the publications shown in Table **3** [103-112]. Notably, although individuals with *ETV6*-RT may present with mild or moderate thrombocytopenia and mild or minimal bleeding tendencies as in the Italian cohort [105], thrombocytopenia may also be severe with platelet counts less than 10 x 10^9/L at diagnosis, as was reported in the family from Japan [111] and by Topka *et al.* [106]. These patients may have been initially diagnosed as immune thrombocytopenia. Concurrent anemia or pancytopenia may be present. Platelet size is often normal but may show variability, with mild but significant platelet anisocytosis and reduced ability of platelets to spread with collagen noted in the Italian study [105]. Red blood cell macrocytosis was reported by Noetzli *et al.* [104], but of note, the identified patients were selected from a cohort of inherited thrombocytopenia patients with high red blood cell mean corpuscular volume (MCV). In the studies that did not pre-select for high MCV, the individuals who progressed to MDS showed elevated MCV (Poggi *et al.* family 1) [108]. In the Italian study, MCV was high in 4 of 20 patients, but not elevated in the 4 patients with ALL or polycythemia vera [105]. Of note, BM examination may show both dysmegakaryopoiesis and dyserythropoiesis in the absence of a myeloid malignancy [104]. Interestingly, megakaryocytes may also be normal in morphology as reported by Yoshino *et al.* after completion of treatment for ALL in the pediatric patient in remission but with persistent *ETV6*-RT [111].

DDX41 MUTATED AML OR MDS

The DEAD-box proteins, named after the strictly conserved sequence Asp (D)-Glu (E)-Ala (A)-Asp (D), are putative RNA helicases implicated in several cellular processes. The DEAD-box helicase 41 (*DDX41*) gene, located on chromosome 5q35.3 band, encodes for a DEAD-box protein specifically expressed in the germ cell lineage in both sexes and functions in germ cell development [113]. Germline mutations in *DDX41* were first identified in 2015 to co-segregate in familial patients with AML and MDS [114].

Table **4** shows the characteristics of germline *DDX41* mutations in patients and families with hematologic malignancies from 5 large studies [114-118], including the different kinds of cohorts that were investigated to identify these patients, which included selected families with hematologic malignancies, patients selected for high grade myeloid neoplasms, and non-selected patients. The Table also shows characteristics for patients and families with the germline *DDX41* mutations, the associated somatic mutations and reported cytogenetics findings. All except for the Korean study by Choi *et al.* were from Europe, Australia, and North America. The most common germline *DDX41* mutation among the non-Asian cohorts was a loss of function frameshift mutation, c.415_418dupGATG (p.D140Gfs*2) [114, 115].

Notably, striking ethnic differences were observed in the types of germline *DDX41* mutations, with specific mutations exclusive to Caucasians (p.D140Gfs*2) and Asian (p.A500Cfs*9) populations, which have now been confirmed in larger studies [118, 119]. These ethnic differences further suggest that these germline mutations might represent inherited events through generations from a founder germline event instead of Mendelian inheritance [15].

In a large international collaborative study not included in Table 4, 208 (3.6%) patients with *DDX41* variants were identified among 5,609 sporadic patients from different ethnicities and with various myeloid neoplasms [119]. Similar to earlier studies, that large study showed that approximately 50% patients had both germline and somatic *DDX41* mutations. Notably, striking ethnic differences were revealed in germline *DDX41* mutations (A500fs in Japan, D140fs in the USA, Q41* in Germany, G218D in Italy, M1I in Sweden, S21fs in Thailand). The germline mutations included both truncating and missense mutations, and were most common in MDS and AML compared with other myeloid neoplasms [119].

Moreover, Cardoso *et al.* found slightly shorter telomere lengths in 4 families with MDS/AML harboring germline *DDX41* variants compared with age-matched controls, similar to patients with inherited *TERC* or *TERT* mutations in familial MDS/AML. These findings also suggest that instead of inheritance by Mendelian genetics, germline *DDX41* mutations represent a solid

inherited risk factor predisposing to myeloid neoplasia [120].

Finally, as shown in Table **4**, a Korean study investigated *DDX41* mutations in myeloid neoplasms, including patients with idiopathic cytopenia of

Table 4: Characteristics Reported in 5 Large Studies for Families and Patients with Germline *DDX41* Mutations

Publication, year, ethnic origin or study location	Study design/ original cohorts from where these patients were identified	Characteristics for patients and families with reported germline *DDX41* mutation				
		N patients and families	Demographics and HM diagnoses in identified patients and families	*DDX41* germline alterations reported in N families (F) or individual patients	Associated *DDX41* somatic alterations	Cytogenetics & other features in patients with germline *DDX41* mutation
Polprasert *et al.* [114], 2015 USA and Europe	Initial 7 families with strong family history of AML/MDS & germline *DDX41* mutated, then examined 1045 patients with myeloid neoplasms (840 at CC, UM, UC; and 197 from TCGA database)	Total 19 patients: 11 in 7 families and additional 8 (0.7%) patients in 1045 patients with myeloid neoplasms	19 patients with median age 68 (overall 44-88) y; familial age range 44-73 y; identified in high risk MDS/AML; healthy carriers present in families	p.D140fs, in 1 family; p.I396T, in 1 family; p.D140fs (in 4 families and 6 additional patients); p.F183I (in 1 family); c.419insGATG (n=6); p.Q52fs (n=1); p.M155I (n= 1)	Present in 9 (~50%) patients with germline *DDX41* mutation p.R525H in 8 p.A225D in 1	Karyotype normal in 9 of 11 with familial MDS/AML; Abnormalities of chromosomes 7, 8 and 20 in others; also somatic mutations in *TP53, RUNX1*, *LUC7L2*
Lewinsohn *et al.* [115], 2016, Australia and USA	Screened 2 cohorts (Australia and UC) of total 289 families with multiple members having hematologic malignancies	Total 9 (3%) families with 20 individuals with germline *DDX41* mutations	Age of onset of MDS/AML: mean age 57 y; identified in AML (3 of 12 M6)/ MDS, CML, and low grade FL	p.D140Gfs*2 in 3 families; p.R525H in 1 family; novel mutations in 5 families	p.R525H in 1 leukemia	Karyotypes normal in 11 patients; abnormalities of chromosome 5, 8, and 20 in others (other than CML)
Quesada *et al.* [116], 2019, USA	Mainly high grade myeloid neoplasms, at MDACC over a 7 month period; total patients not provided	*34 patients including 5 with familial history of AML/MDS	26 (76%) males, median age 70 y; AML (n=20), MDS (n=10), CMML (n=1) and MPN (n=3)	*Presumed germline based on ~50% VAF; p.M1I in 30% (9 of 30), p.D140Gfs*2 in 13% (4 of 30)	p.R525H in 18 of 27 (67%)	Karyotypes normal in 20 (59%), 1 abnormality in 7 (20%); $\geq$ 3 abnormalities in 5 (15%); 2 abnormalities in 2 (7%); *other mutations
Sébert *et al.* [117], 2019, France	Unselected cohort of 1385 patients with AML or MDS	33 (2.4%) unrelated patients with causal variants (additional 10 VUS positive patients not included in this table)	Median age 69 (36-88) y, 79% males; 9 (27%) FHM; MDS, AA, AML (M2, M1, M0), MDS/MPN; cytopenias in 15 (46%) years before MDS/AML	28 different germline *DDX41* variants in 43 patients, classified as causal (n =21) or VUS (n=7)	A second *DDX41* mutation present in 79%	Karyotype normal in 28 (85%); +8 in 2 (6%); del(20q) in 1 (3%); complex karyotype in 1 (3%)
Choi *et al.* 2021 [118], Korea	457 patients with ICUS (n=75), MDS (n=210), or AML (n=172)	28 (6.1%) patients with germline *DDX41* mutation	Male predominance, old age, germline *DDX41* mutation in 5 (6.7%) ICUS, 19 (9.0%) MDS, and 4 (2.3%) AML	p.V152G (n=10) as unique in Koreans, p.Y259C (n=8), p.A500fs (n=6), p.E7* (n=3), p.L328R (n=1)	27 (96.4%) in second allele; p.R525H in 10 (44%); p.T227M in 5 (22%)	Karyotype normal in 21 (75%), and abnormal in 7 (25%); 6(32%) MDS with IPSS-R < 3.5; 13 (68%) MDS with IPSS-R >3.5

HM, Hematologic malignancy; MDS, myelodysplastic syndromes; AML, Acute myeloid leukemia; CC, Cleveland Clinic; UM, University of Muenster; UC, University of Chicago; TCGA, The Cancer Genomic Atlas; CML, chronic myeloid leukemia; FL, follicular lymphoma; MDACC, MD Anderson Cancer Center; CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasms; VUS, variants of unknown significance; FHM, family history of hematologic malignancy; AA, aplastic anemia; NA, not available; ICUS: idiopathic cytopenia of undetermined significance; IPSS-R, Revised International Prognostic Scoring System.

*Quesada *et al.* [116], 21/34 patients were post-therapy (17 for MDS, 4 for acute leukemia); germline *DDX41* mutation status was confirmed in 5 of 34 patients, including p.D140Gfs*2 (n=3), p.M1I (n=1), and c.572-1G>A splice mutation (n= 1); somatic mutations present in *TP53* in 11 (32%), *ASXL1* in 8(24%), *JAK2* in 4 (12%).

undetermined significance (ICUS). Of note, 5 ICUS patients had causal germline *DDX41* mutations, and 4 of those 5 patients progressed to MDS [118].

Clinically, based on published reports, individuals with germline *DDX41* mutations present with MDS or AML at an older age, similar to the ages for sporadic cases [114, 115]. The latency period can be as long as the 7th or 8th decade of life, and the clinical presentation is very similar to sporadic forms of disease. Family history may not be evident. These individuals may also be predisposed to lymphoid neoplasms, including early onset follicular lymphoma and Hodgkin lymphoma, multiple myeloma [115], and non-hematologic malignancies, including non-small cell lung cancer [121]. In healthy carrier individuals in families with germline *DDX41* mutations, findings may be very subtle, including cytopenia or mild monocytosis [15].

Progression to overt MDS or AML occurs with an acquired mutation in the second allele for *DDX41,* found in the majority of *DDX41* mutated myeloid neoplasms [114]. By itself, the germline *DDX41* mutation does not appear to cause malignancy. That second (acquired) *DDX41* mutation acts as a driver mutation that leads to the development of a myeloid neoplasm [114], similar to the somatic acquisition of a second mutation in familial *CEBPA* mutated AML and *RUNX1* mutated FPD/AML, as described above. However, in contrast with *CEBPA* and *RUNX1*, germline mutations in familial *DDX1* driven AML/MDS have a long latency, with the onset of disease in 4th to 8th decade, similar to somatically driven myeloid neoplasia [15]. Notably, familial germline *DDX41* mutations are also present in healthy carriers, including very rarely in publicly available exome sequencing databases from healthy individuals [114].

Syndromic Familial MDS

In addition to the syndromes referenced above for familial AML, germline alterations in *GATA2,* and *SAMD9* and *SAMD9L* (referred to as *SAMD9/9L)* genes are recognized to present in several syndromic inherited or familial diseases with an increased risk of developing MDS or AML. Germline alterations have also been described in non-syndromic MDS, such that germline *GATA2* and *SAMD9/9L* abnormalities are currently recognized to comprise the most common causes of inherited MDS in pediatric age groups. Both syndromic and non-syndromic forms of MDS due to germline mutated *GATA2* and *SAMD9/9L* genes are described in the following sections.

INHERITED ABNORMALITIES IN *GATA2*

The human *GATA2* gene, located on chromosome 3q21.3, encodes for a major zinc-finger transcriptional factor that is a crucial regulator of hematopoietic stem cells. GATA2 has two zinc-finger domains, through which it can occupy abundant GATA DNA motifs in the genome [122-124]. In 2011, mutations in *GATA2* were identified in patients with the autosomal dominant MonoMac syndrome with monocytopenia and mycobacterial infections [125, 126], defects in dendritic cells, monocytes, and B and NK lymphoid cells [127], familial MDS or AML [128], and in the autosomal dominant primary lymphedema with a predisposition to AML (Emberger syndrome) [129, 130]. Notably, the single *GATA2* gene mutations were similar but led to the above-mentioned various disease phenotypes. Haploinsufficiency of the transcription factor, GATA2 leads to susceptibility to MDS or AML, immunodeficiency, pulmonary disease, and vascular/lymphatic dysfunction [131].

The high prevalence of germline *GATA2* mutations in pediatric MDS was described in a landmark study by the European Working Group of MDS in Childhood (EWOG-MDS) in 2015, wherein Wlodarski *et al.* studied 426 children and adolescents with primary MDS [132]. They identified germline *GATA2* mutations in 15% of advanced and 7% of all primary pediatric MDS patients, including a high prevalence in patients with monosomy 7 (37% all ages, and 72% adolescents). Germline *GATA2* mutations were absent in 82 patients with secondary MDS [132]. Notably, 71% of 57 germline *GATA2* mutations occurred *de novo* (not inherited from either parent) and in the absence of family history. The germline *GATA2* mutated patients were diagnosed with MDS at a median age of 12.3 (5.2-17.4) years, and occurred more often in higher grade MDS than in the *GATA2* wild type MDS patients [132]. Karyotypes showed monosomy 7 in 68%, normal in 16%, der(1;7) leading to a loss of the q arm of chromosome 7 in 7% and trisomy 8 in 9% of patients. In addition to whole or partial gene deletions, they identified 3 main types of *GATA2* mutations: (1) truncating, including frameshift, splice site, and stop gain mutations, (2) missense mutations, primarily in the zinc finger 2 region, and (3) mutations in intron 4 containing the regulatory regions [132]. To date, at least 400 *GATA2* deficient patients have been reported. More recently, in 2020, in another large study of 911 patients by the EWOG-MDS, the authors described the 4th type of mutation as a synonymous substitution in exon 3 of the *GATA2* gene (c.351C>G, p.T117T) that also leads to *GATA2* deficiency by an

allelic loss at the RNA level, indicating that if GATA2 deficiency is suspected and comprehensive DNA testing does not show a mutation, RNA sequencing should be considered [133].

The protean clinical manifestations at presentation of GATA2 deficiency were recently reviewed [134]. Hematologically, patients may have cytopenias prior to developing MDS with variable BM findings. As reviewed by Sahoo *et al*, "approximately 75% of carriers develop a myeloid neoplasm at a median age of about 20 years" [134]. Recently, incomplete clinical penetrance (32.9% by age 40 years) was described in a study of 15 patients with inherited GATA2 deficiency and Mycobacterial infections, with 16 asymptomatic family members with ages 6-78 years, including four members with ages greater than 60 years [135].

Therapeutically, HSCT is currently the only curative treatment with careful surveillance recommended to determine the best time for HSCT, accepted to be before the emergence of complications such as infections [132]. Nevertheless, as stated by Sahoo *et al*, "even careful watching might miss the best opportunity for low risk HSCT" [134].

INHERITED ABNORMALITIES IN *SAMD9* AND *SAMD9L*

In 2009, using microarray comparative genomic hybridization, Asou *et al.* described a microdeletion in the 7q21.3 chromosome band that contained three poorly characterized genes in patients with juvenile myelomonocytic leukemia in Japan [136]. Two of those three genes include the sterile alpha motif domain 9 (*SAMD9*) gene and its paralogue *SAMD9*-like *(SAMD9L),* which encode for 2 proteins with 58% similar amino acids. Since both genes are present only in humans and other higher order primates, while only *SAMD9L* is present in mice, cats, and dogs, and only *SAMD9* in cows and sheep, both gene products are thought to have a common function and to compensate for each other [137, 138]. In 2013, Nagamachi *et al.* created *samd9l*-deficient mice and found that those mice developed MDS similar to that seen in monosomy 7/del(7q) in humans, and died of myeloid disease [137].

In 2016, Narumi *et al.* described 11 patients (6 infants, and five aged 1-51 years) with adrenal hypoplasia and extra-adrenal clinical features, all with a gain of function *SAMD9* missense mutations that inhibit growth [139]. Two infants in that study progressed to MDS via a unique "adaptation-by-aneuploidy" mechanism wherein the *SAMD9*-mutated cells lost the chromosome 7 to overcome the undesirable effects of the mutation, but led to the development of MDS with monosomy 7; those two patients died at ages less than 5 years. The constellation of clinical features was named as the MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) syndrome [139]. In 2017, Buenocore *et al.* studied eight additional patients with similar clinical features. They showed that the somatic alterations included monosomy 7 and other loss of function mutations, which altered the disease phenotype and outcome [140]. Two of their patients also developed MDS, and those patients survived after a bone marrow transplant. The germline mutations were *de novo* in 7 of 8 patients and allowed the possibility of cure with a transplant. Before the development of MDS, cytopenias were reported in the patients with germline mutated *SAMD9* [140].

Additionally, in 2016, Chen *et al.* identified gain of function *SAMD9L* mutations as the underlying cause in a family with clinical features of the autosomal dominant ataxia-pancytopenia syndrome, which is a rare neurologic disorder first described in 1978 in one family with ataxia, cytopenias, development of AML, and monosomy 7 [141]. Subsequently, in 2017, *SAMD9L* germline mutations were described in 2 additional families, 1 Swedish and 1 Finnish, with the ataxia-pancytopenia syndrome, and with hematologic and neurologic manifestations [142, 143]. Similar to the mechanism for development of MDS described for the MIRAGE syndrome patients harboring germline *SAMD9* mutations, patients in the ataxia-pancytopenia families also showed a reversal of the *SAMD9L* mutational abnormality [141-143]. Of note, the age of patients at clinical presentation varied from childhood to older age adults. Notably, all patients with germline *SAMD9L* abnormalities had some neurologic findings except a 4-year-old carrier [143, 144]. MDS developed in members of both families, at the ages of 18 months (with loss of chromosome 7), 4 years (with loss of chromosomes 7 and 22), and at 56 years, with a derivative of chromosome 7 that results in the loss of the 7q arm [142].

For non-syndromic MDS, in an EWOG-MDS multi-institutional cohort of 548 children and adolescents with primary MDS diagnosed between 1998 and 2016 in Germany, germline *SAMD9/9L* pathogenic mutations were identified in 8% of primary pediatric MDS and 22% of MDS with monosomy 7, in the absence of GATA2 deficiency and known IBMF [145]. The

outcome after HSCT was not affected by the presence of germline *SAMD9/9L* mutations in the EWOG-MDS study, with a 5-year-OS of 85% in 35/43 transplanted patients [145]. Collectively, germline mutations of *GATA2* and *SAMD9/9L* are estimated to occur in 30-50% of pediatric MDS. A Japanese study identified germline pathogenic *GATA2* and *SAMD9/9L* alterations in 40% of 25 pediatric with hematologic disorders associated with monosomy 7 [146].

Further, in the French cohort of IBMF patients, Bluteau *et al*. identified 10 patients from 9 families who harbored germline *SAMD9L* mutations. Notably, *SAMD9L* was the most frequently mutated gene identified in that cohort, with 5 of 10 patients having monosomy 7, and significant neurologic abnormalities in 2 patients [24]. In the same study, germline *SAMD9* variants were also present in 6 unrelated patients, with monosomy 7 in 5 of those 6 patients, and 1 having features of the MIRAGE syndrome [24]. In another large cohort of 799 patients with presumed acquired MDS, BMF, and other diseases, germline *SAMD9/SAMD9L* mutations were reported in 4% (21/575) of adult patients with MDS and 3% (3/105) of BMF patients [147]. Interestingly, in contrast with pediatric MDS with a gain of function variants located in the C-terminus, the *SAMD9/9L* variants in adults were loss of function and located in the N-terminus, with a reversion of genetic abnormalities reportedly rare in adults [147].

GERMLINE GENETIC ABNORMALITIES IN ADDITIONAL GENES IN FAMILIAL AML OR MDS

Lastly, in a large cohort of 86 families, with at least two or more members diagnosed with a hematologic disorder including AML, MDS, aplastic anemia or thrombocytopenia, and at least one family member diagnosed with AML or MDS, referred to as “familial AML/MDS”, the investigators identified pathogenic germline variants in known gene loci in 49 (57%) families, including in *CEBPA, DDX41, ANKRD26, RUNX1, ETV6, GATA2, SAMD9, SAMD9L,* and *TP53* [148]. In an additional 37 (43%) families, they identified germline abnormalities in other rare genetic loci, underscoring the genetic heterogeneity underlying familial AML/MDS [148].

CONSIDERATIONS FOR DIAGNOSIS OF GERMLINE PREDISPOSITION TO MDS OR AML

(1) To recognize familial or inherited predisposition, the most important requirement is the awareness of reported findings and the understanding that there is marked intra-familial and interfamilial heterogeneity in the clinical presentation among individuals with germline or familial predisposition to cancer including myeloid malignancies, to the extent that the complete clinical spectrum of these various diseases is likely not yet elucidated. Since germline predisposition may be present even without clinical suspicion for a possible predisposing syndrome or without a family history, each patient with an AML or MDS or any malignancy should be carefully considered by the pathologist or the clinician for whether that patient may have an underlying predisposition to any malignancy or MDS or AML. To that effect, a detailed clinical personal and family history and physical examination, which require time, for signs that may be subtle for any known syndromes or diseases are crucial, followed by laboratory tests, including complete blood counts and careful examination of peripheral blood smear and the BM, including with cytogenetics and molecular genetic tests as essential starting points for diagnosis, in conjunction with specialized tests as per clinical evaluation [149]. Clinical history taking has immense value in conjunction with genomic testing [150], and the use of a questionnaire or tool helps to diagnose pediatric cancer predisposition syndromes, including heritable MDS [151, 152]. Several guidelines for diagnosis, management, and follow-up have been published [35, 40, 149, 153-156].

Pathologically, BM examination may show morphologically abnormal megakaryocytes, including increased hypolobated forms, particularly in the 3 disorders associated with inherited thrombocytopenia, including germline *ANKRD26, RUNXI* or *ETV6* mutations associated MDS or AML [88, 104, 157, 158]. Importantly, such abnormal morphologic features must not be misdiagnosed as having evolved to an MDS. Even dysmorphic erythroid precursors may be present in *ETV6*-RT patients [104]. Patients may have been previously diagnosed as immune thrombocytopenia without a response to therapy. Frequently reported cytogenetic abnormalities include loss of chromosome 7, trisomy 8, trisomy 21, del(20q), del(7q), dup(1q), dup(3q), and complex abnormalities. The germline mutations may also occur *de novo*, involve coding and non-coding genomic regions, and be of different types, necessitating appropriate tests that would detect the different types of mutations. As an example, if a germline *RUNX1* variant might be a diagnostic possibility but is not identified, it would be important to

ensure that the test requested can detect the various possible types of mutations.

(2) Testing for germline predisposition requires informed consent with genetic counseling [159-161]. The perspectives of the patient and the family must be respected when considering germline testing for variants that may predispose to cancer, including myeloid malignancies. As presented in April 2021 at a pediatric genomics conference at the Rady Children's Hospital in San Diego, California, most pediatric patients and families tend to have similar views toward genetic testing for predisposition to cancer. However, barriers remain for testing in those who may benefit from such testing, including due to racial or ethnic origin, and concerns about losing privacy or health insurance or both in the United States. Genetic counseling from individuals who understand these aspects and have experience with patients with malignancies is critical and must be a component for care for such patients and individuals. While testing for those individuals and patients who may benefit from germline testing must be encouraged with appropriate support measures within a process that ensures privacy and security, unnecessary testing for germline mutations must also be avoided.

The gold standard tissue for germline testing is cultured skin fibroblasts; however, this process requires a fresh skin biopsy and may take several weeks before a result is obtained and can be expensive. Tissue types that have been successfully used for germline testing include buccal swabs, peripheral blood collected when the patient is in remission, nail clippings, hair bulbs, sorted T cells, and direct skin biopsy.

(3) Definitive differentiation is critical between somatic or inherited germline causal variants when a HSCT is being considered for the patient with a family member who could harbor the germline mutation as the potential donor. It is important to remember that molecular genetic analysis performed to detect somatic mutations may, or may not, detect germline mutations [161]. Germline variants may even be present at low (<30%) VAFs closer to the VAFs seen in somatic mutations. Conversely, VAFs of 40-60% may represent somatic variants. Therefore, even if a germline abnormality or variant is detected in any analytical test meant for somatic mutations, it is not possible to be sure that the detected abnormality is indeed germline solely based on testing by a panel designed for somatic mutation testing. However, additional observations can help determine the abnormality as germline or somatic. If assessments at multiple time points during therapy show consistent VAF values, for example, 50% VAF for any suspected germline abnormality at multiple time points in the same individual, or the same suspected abnormality present in multiple family members would indicate a germline variant.

Also crucial is to determine that the variants are causal (pathogenic or likely pathogenic) [162]. Even in genes such as *TP53*, where mutations usually imply poor prognosis, there can be compensatory benign variants in addition to pathogenic alterations, as was shown in patients with SDS [30]. The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) have published guidelines for interpreting and reporting germline *TP53* variants [163]. Similarly, based on the previous guidelines established by the AMP and ACMG expert panels, guidelines were optimized for hematologic malignancies for the diagnostic reporting of *RUNX1* germline variants, by a ClinGen American Society of Hematology committee [68].

FUTURE PERSPECTIVES AND CONCLUSION

(1) Inherited or germline predisposition to MDS and AML is not uncommon and occurs due to various genetic causes as described above, including fewer reported examples in other genes [164-167], and certainly, additional studies will reveal more causes of germline predisposition.

A major challenge ahead is to unravel why someone with a germline predisposition develops a malignancy or an AML or another cancer, and why someone else who harbors the same mutation can live until the 9th decade of life and never develop any malignancy. Those contributing factors could include not only the molecular genetics of the non-neoplastic cells carrying the germline mutation, but also the interactions with the cellular microenvironment [168, 169], and the individual host factors with examples including inflammation, obesity, smoking, immunological dysfunction [170-172], and features unique to the geographical or ethnic origin and surrounding environmental influences. As was discussed by Dr. Akiko Shimamura at the European School of Hematology conference for erythropoiesis control and ineffective erythropoiesis in March 2021, not necessarily in these exact words, "we monitor individuals with germline predisposition to cancer or a myeloid malignancy, including a complete blood count or bone marrow examination, but we have no way of

knowing before the development of the myeloid malignancy, when any of those tests might show a progression to a myeloid neoplasm."

Nevertheless, similar to the mechanisms of progression to malignancy unraveled in patients with SDS by Dr. Shimamura and colleagues [30], and described in an earlier section, specific molecular events that eventually lead to malignant progression need to be identified and distinguished from compensatory molecular mechanisms that are clonal but benign, in the various other causes of inherited predisposition to myeloid malignancies. Such studies could even lead to a greater understanding of the reasons for development and progression in the sporadic forms of myeloid malignancies.

(2) Specifically for pathologic examination, in the current WHO classification of AML, there are several genetically defined subtypes of AML [2, 11]. Careful application of the 2016 WHO AML classification was a pre-requisite in identifying patients with a novel subtype of familial AML with mutated *NPM1*, and a 74-year-old patient with familial AML with biallelic mutated *CEBPA* [61].

In contrast, the current 2016 classification of MDS is based primarily on morphologic features, essentially similar to the French-American-British classification, except for the addition of *SF3B1* mutation as a criterion for diagnosing MDS with ringed sideroblasts [2, 11]. MDS have been recognized for decades to be a highly heterogeneous group of myeloid neoplasms that vary remarkably from very low to very high prognostic risk [173]. Moreover, in the last decade since 2011 [174], many studies have investigated molecular genetic abnormalities in sporadic MDS [175-179], and molecular genetic analysis is clinically recommended for deciding treatment for MDS and AML [180-183]. In 2020, a large study showed that biallelic *TP53* alterations were associated with the poor prognosis in sporadic MDS, and for monoallelic *TP53* variants, the prognosis was similar to that of wild type *TP53* MDS [184]. Further, treatment strategies are currently in development to target underlying genetic mutations, including *RUNX1* and *TP53* [185-188], with deleterious *TP53* variants known to have a poor prognosis regardless of the type of malignancy. Importantly, in addition to guiding regarding prognostic risk and current and emerging treatment options for any individual patient with MDS, moving to a genetic-based sub-classification of the highly heterogeneous MDS in

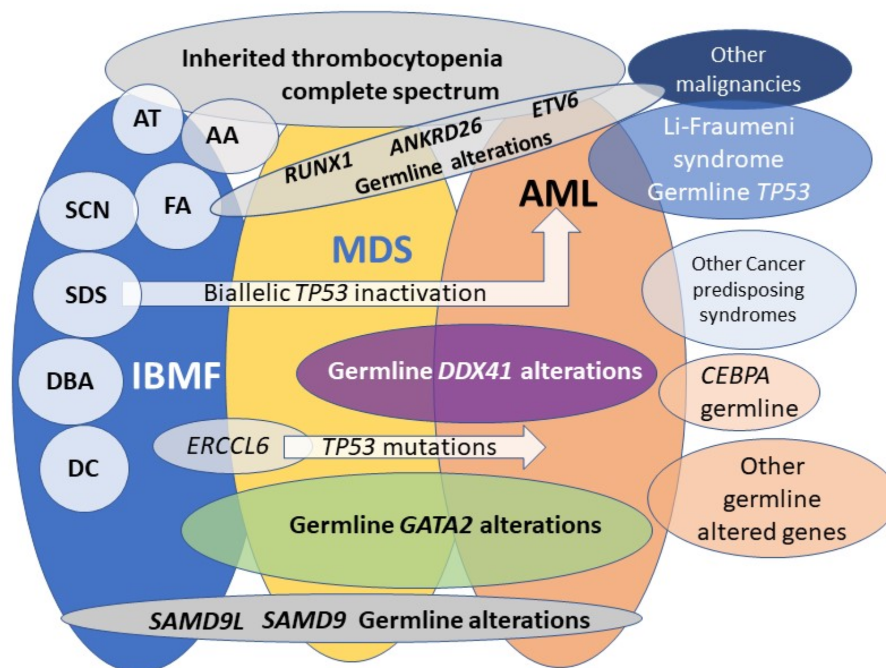

Figure 1: A schematic diagram showing an overview and interrelationships of the various groups of diseases that harbor germline predisposition to developing AML or MDS. Each of the four large oval shapes represents the entire spectrum of inherited thrombocytopenia, IBMF syndromes, MDS, AML, including acquired and inherited causes, and unknown genetic causes. The smaller ovals depict significant groups of germline alterations that lead to germline predisposition to MDS or AML.

IBMF, inherited bone marrow failure; AA, Acquired aplastic anemia; FA, Fanconi anemia; SDS, Schwachman-Diamond syndrome; DBA, Diamond-Blackfan anemia; DC, Dyskeratosis congenita; SCN, Severe congenital neutropenia; AT, amegakaryocytic thrombocytopenia; MDS, myelodysplastic syndromes; AML, acute myeloid leukemia.

the next update of the WHO classification would also facilitate an increased recognition of MDS with an underlying inherited or germline predisposition to malignancy.

In conclusion, a germline predisposition to MDS or AML is harbored by individuals and patients with multiple groups of diseases, which may occur as a constellation of findings or be non-syndromic, familial, or without a family history, and include common or less common and rare genetic disorders, the complete spectrum for which is constantly being further characterized. These groups include constitutional or IBMF syndromes, familial cancer predisposition syndromes, including the Li-Fraumeni and CMMRD syndromes, inherited thrombocytopenias, inherited or familial MDS, and inherited or familial AML. Figure **1** schematically depicts an overview of these groups of diseases with the overlapping features among different groups. Accurate diagnosis of germline predisposition to MDS or AML requires notably, the awareness that additional germline defects in genes and their clinical phenotypes have yet to be identified even in known familial cases in all of the above-mentioned groups of diseases, and that the next patient with MDS or AML that anyone encounters could have an underlying germline predisposition. In the current era of precision medicine, for the individual carriers of deleterious germline variants and the patients and families with these inherited diseases with predisposition to MDS and AML or any other malignancy, moving from the current uncertainty about whether or not progression will occur to malignancy or when it might occur, to hope, and eventually cure, requires tremendous collaboration in the future.

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CONFLICT OF INTEREST

The author does not have any relevant conflict of interest. The author is a consultant for Astellas; however, this paper is solely the author's work and has no connection with the author's role as a consultant for Astellas.

ORCID ID

Rina Kansal (https://orcid.org/0000-0003-4289-3057).

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