Molecular Genetics of Pediatric Acute Myeloid Leukemia

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KEYWORDS

• Acute myeloid leukemia • Pediatric oncology • Molecular genetics • Rearrangement • Next-generation sequencing

KEY POINTS

- Pediatric acute myeloid leukemia (AML) has drivers that are unique to both infants and children as well as drivers found in common with adult AML.
- Genetic profiling of pediatric acute myeloid leukemia can identify drivers that are diagnostic, prognostic, or predictive in a large majority of patients.
- Profiling of pediatric megakaryoblastic leukemia identifies genetic alterations associated with a wide range of patient outcomes.

INTRODUCTION

Acute myeloid leukemia (AML) is a biologically and genetically heterogeneous malignancy that accounts for approximately 20% of pediatric acute leukemias.[1] With the development of optimized therapeutic regimens and allogeneic hematopoietic stem cell transplantation, overall survival rates of pediatric AML have approached 70% but still significantly lower than that of pediatric acute lymphoblastic leukemia (ALL).[2] As with other hematological malignancies, pediatric AML has been evolving from a morphologic classification scheme to a genetically based one, aided by the rapid progression of molecular detection methods, such as next-generation sequencing (NGS).

The World Health Organization (WHO) currently recognizes several types of AML with recurrent genetic abnormalities, the extent of which is certain to grow with the explosion of genomic profiling studies in recent years. Although many of the recurrent genetic drivers of myeloid leukemia are found in common between pediatric and adult patients, the prevalence and clinical significance of these drivers differ depending on the age of the patient.

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AML often is defined by the presence of recurrent chromosomal rearrangements that create chimeric fusion genes that promote AML development and progression. These fusions are diagnostic, prognostic, and, in some cases, predictive biomarkers that drive clinical management. These so-called class II alterations often involve transcription factors that serve to block differentiation of hematopoietic progenitor cells, which subsequently acquire cooperating mutations in other pathways, often tyrosine kinase or RAS, which are progrowth pathway mutations (class I mutations).[3]

This review focuses on genetic variants found in AML that are most germane to clinical management of pediatric patients, while acknowledging that variants found more commonly in adults still can be found in pediatric patients.

DISCUSSION

Classes of Genomic Variation in Pediatric Acute Myeloid Leukemia

NGS has enabled the description of all classes of somatic genomic variation in a single tumor. These studies can reveal the underlying mutational processes that manifest as a particular mutational signature and can facilitate the understanding of the natural history of a given tumor. The Children's Oncology Group–National Cancer Institute Therapeutically Applicable Research to Generate Effective Treatments AML initiative has characterized the genomes of approximately 1000 pediatric AML cases using whole-genome, transcriptome, and epigenetic profiling, providing the most extensive characterization of pediatric AML to date.[4] This study and others have begun to reveal both similarities and differences between adult and pediatric AML.

Although adult AML is defined by a low tumor mutational burden, all subtypes of pediatric AML have an even lower rate of somatic mutations, averaging less than 1 somatic mutation per megabase of genomic sequence.[4–6] Tumor mutational burden is lowest in infants and increases with age of onset.[4] Genes affected by somatic mutations are diverse, but there are few recurrent somatic mutations, with only 5 genes harboring mutations in more than 5% of subjects (FLT3, NPM1, WT1, CEBPA, and KIT).[4] This contrasts with somatic variants in adult AML, where alterations TP53, DNMT3, IDH1, and IDH2 are common, although they are rare in children.[4–6] In contrast to somatic sequence variants, pediatric AML exhibits a higher rate of chromosomal rearrangements than observed in adult AML, with rearrangements involving RUNX1, CBFB, and KMT2A alone found in more than 35% of subjects.[7] The prevalence of structural rearrangements is highest in infants and decreases with age of onset[4] (**Table 1**). Although most recurrent rearrangements are observed in both adult and pediatric AML, there are several that appear with much higher prevalence or are even unique to pediatric subjects, discussed later.

As with sequence variants, copy number variation is low across most subtypes of pediatric AML, with approximately a third of cases exhibiting no identifiable copy number losses or gains.[8] Many of the identified copy number variants are the result of focal microdeletions and amplifications that frequently occur near the breakpoints of chromosomal rearrangements and display a similar age-dependent distribution to structural rearrangements.[4,8–10] Recurrent focal codeletions of MBNL1 and ZEB2 and deletions of ELF1 have been reported, although their clinical significance has yet to be demonstrated.[4] Similarly, copy neutral loss of heterozygosity is identified at a much lower rate in pediatric AML than other malignancies and was identified in 13% of AML cases. Copy neutral loss of heterozygosity typically has been observed in genomic regions with known molecular drivers and tumor suppressors, such as FLT3 internal tandem duplications (FLT3-ITDs) and CDKN2A/B.[8] In contrast to most forms of pediatric AML, acute megakaryoblastic leukemia (AMKL) exhibits a higher rate of copy number variants.[8,11]

Table 1
Common structural rearrangements and sequence variants identified in pediatric acute myeloid leukemia patients

Genetic Alteration	Frequency	Subgroup	Prognostic Implication	Cooperating Mutations
KMT2A rearrangements	20%	Infants (60%)	Neutral; partner imparts influence	NRAS, KRAS, FLT3
RUNX1-RUNX1T1	15%	Children	Favorable	KIT, NRAS, KRAS, FLT3, chromatin modifying genes, cohesins
CBFB-MYH11	10%–15%	Children	Favorable	KIT, NRAS, KRAS, FLT3
NUP98 rearrangements	6%–10%	Children	Poor	FLT3-ITD, WT1
PML-RARA	5%–10%	Older children	Favorable	FLT3-ITD, WT1
DEK-NUP214	<2%	Older children	Poor	FLT3-ITD (70%)
ETS rearrangement	1%	Infants	Poor	Few
CEBPA	4%–9%	Older children	Favorable	GATA2, FLT3, CSF3R
NPM1	4%	Older children	Favorable	FLT3-ITD
FLT3	30%	Children, older children	Poor (FLT3 alone is intermediate among FLT3-ITD group)	NPM1, WT1, NUP98-NSD1
FLT3-ITD, NPM1	—	—	Most favorable among FLT3-ITDs	—
FLT3-ITD, WT1	—	—	Poor among FLT3-ITDs	—
FLT3-ITD, NUP98-NSD1	—	—	Poor among FLT3-ITDs	—
RUNX1 Mutation	3% (AML)	Older children, AMKL (10%)	Poor (AML), excellent (AMKL)	JAK, cohesins (AMKL)
CBFA2T3-GLIS2	15%–20% (AMKL)	AMKL	Poor	Few
HOX gene rearrangement	15% (AMKL)	AMKL	Favorable	MPL
RBM15-MKL1	10% (AMKL)	AMKL	Favorable	Few
NUP98-KDM5A	12% (AMKL)	AMKL	Poor	RB1

World Health Organization Recurrent Genetic Abnormalities in Acute Myeloid Leukemia and Common Co-occurring Mutations

KMT2A rearrangement

KMT2A (also known as mixed lineage leukemia [MLL]) is the most commonly rearranged gene in both adult and pediatric leukemias.[12,13] It is most prevalent in pediatric AML, where it is observed with greatest frequency in infancy.[14,15] The KMT2A gene encodes a lysine methyltransferase that mediates methylation of histone 3 lysine 4. KMT2A translocations involve a large number of partner genes and typically involve the N-terminus of KMT2A fused to the C-terminus of its partner gene, with more

than 90 reported to date.[13] Despite the large number of partners, KMT2A partners most commonly are part of the AF4/FMR2 family, which function in the superelongation complex, and KMT2A fusions are thought to drive inappropriate expression of KMT2A target genes, notably HOX genes.[12] Despite this complexity, approximately 70% of pediatric AML KMT2A rearrangements are with 4 partners. The KMT2A-MLLT3 (t[9;11] [p21;q23]) fusion is the most common (43%), followed by KMT2A-MLLT10 (t[10;11]p12;q23); 13%), KMT2A-AFDN (t[6;11] [q27;123]; 5%), and KMT2A- MLLT1(t[11;19] [q23;p13]; 8%).[16]

AML with KMT2A-MLLT3 rearrangement is given its own category in the WHO diagnostic classification system because it is the most clinically homogeneous, whereas other rearrangements of KMT2A are diagnosed as AML, not otherwise specified.[17] The KMT2A fusion partner is relevant for prognosis, because KMT2A-MLLT11 (t[1;11] [q21;q23]) has a better prognosis whereas KMT2A-AFDN, KMT2A-ABI (t[10;11] [p11.2;q23] and KMT2A-AFF1 (t[4;11] [q21;q23]) have a poor prognosis.[18]

Consistent with the typical very young age of onset, pediatric patients with KMT2A fusions had fewer somatic mutations than tumors without these fusions.[4] Cooperating somatic mutations, when present, are recurrent in NRAS, KRAS, and FLT3, whereas GATA2 and CEBPA mutations typically are not found. The identification of cooperating mutations in KMT2A-MLLT3 tumors has been associated with a negative prognostic impact, although further study is needed to confirm this association.[19] In addition, deletion of MBNL1 and ZEB2 show frequent co-occurrence.[4]

Given the significant diversity of KMT2A rearrangement partners, their rarity and the heterogeneous outcomes associated with them, it will continue to be a challenge to integrate risk stratification of these uncommon drivers in clinical practice. When KMT2A rearranged AML is examined morphologically and immunophenotypically, they commonly have a monoblastic phenotype.

Nucleoporin 98kD rearranged

Nucleoporin 98kD (NUP98) rearrangements are relatively common in pediatric AML (6%–10%) and much rarer in adult disease (1%–2%).[20–22] They typically involve the fusion of the NUP98 N-terminus to the C-terminus of at least 31 different partner genes, with the t(5;11) (q35;p15) (NSD1) and t(11;15) (p15;q35) (KMT5A) rearrangements preferentially found in pediatric AML.[23] Most NUP98 rearrangements are not detectible by conventional cytogenetics and require an RNA sequencing approach on a practical basis. NUP98 encodes a structural component of the nuclear pore complex, but more recent data indicate that the N-terminus can act as a transcriptional activator through the recruitment of the chromatin modifying complexes.[24–26] NUP98 rearrangements are associated with a poor prognosis and often are identified with FLT3-ITDs and WT1 cooperating mutations.[20,27] NUP98-KDM5A is preferentially identified in pediatric AMKL, found in approximately 10% of pediatric cases, and characteristically have RB1 mutations that decrease protein expression.[11]

The DEK-NUP214 fusion is the result of t(6;9) (p23;q34.1) and is a relatively rare diagnosis in pediatric AML (<2%).[28,29] This rearrangement typically is associated with older age of onset and basophilia, and outcomes are poor, with high rates of relapse and lower rates of remission.[15,29] FLT3 mutations are common in DEK-NUP214 cases, with FLT3-ITD mutations found in up to 70% of pediatric cases, although the presence of FLT3-ITD mutations do not significantly influence outcomes.[29,30]

ETS rearranged

The recurrent t(7;12) (q36;p13) rearrangement is rare in all pediatric AML cases (approximately 1%) but is more common in infants with AML and is associated with

poor clinical outcomes.[4,31] This rearrangement juxtaposes the MNX1 gene with ETV6, but there is debate as to the molecular mechanism by which this alteration drives oncogenesis, because the chimeric MNX1-ETV6 transcript not always is detected in tumors with the chromosomal rearrangement and a fusion protein has not been identified. Oncogenesis likely is mediated, at least in part, by MNX1 overexpression, and a recent study showed MNX1 overexpression could impair hematopoietic differentiation.[32] Genomic landscaping studies have reported only a small number of cases, and recurrent cooperating somatic mutation have not been cataloged. Other rare pediatric rearrangements from the ETS family include ERG rearrangements.[4]

Core binding factor rearranged

Core binding factor (CBF) leukemias drivers fusions include RUNX1-RUNX1T1 t(8;21)(q22;q22) and CBFB-MYH11 inv(16) (p13q22), which commonly are found in pediatric AML cases (20%–30%).[4,15,33] The RUNX1 and CBFB genes are transcription factors that heterodimerize to bind DNA and recruit transcription factors that regulate hematopoiesis.[34] The resulting fusion products block myeloid differentiation through transcriptional repression. These fusions are associated with favorable outcomes, with 90% of patients achieving complete remission with chemotherapy and 70% overall survival, although approximately 30% of patients relapse.[15,33,35,36] The incidence of CBF rearrangements peaks in older children, and when controlled for age, have a higher mutational burden than expected for their age.[4] Often considered similar entities, CBFB-MYH11 and RUNX1-RUNX1T1 rearranged leukemias exhibit divergent patterns of cooperating mutations. Although both commonly exhibit mutations in NRAS, KIT, FLT3, and KRAS, RUNX1-RUNX1T1 cases display a dramatic enhancement of mutations in chromatin modifying genes and the cohesion complex compared with CBFB-MY11 cases.[37] The presence of cooperating mutations does not appear to influence outcomes in CBF rearranged AML.

Somatic mutations in the KIT receptor tyrosine kinase are common alterations in pediatric AML, found in approximately 12% of all pediatric AML cases. They are significantly enriched in CBF-rearranged AML, where they are found in up to 36% of cases.[4] Cooperating KIT mutations are associated with poor outcomes in adult CBF AML, but prognostic significance has been less clear in pediatric patients. Recent studies indicate exon 17, but not exon 8 KIT, mutations may impart a poor prognosis.[38–41] CBFB fusion-positive myeloid blasts often show a myelomonocytic phenotype and marrows of affected patient typically have abnormal eosinophils with basophilic granules.

Other key rearrangements

Acute promyelocytic leukemia (APL) represents approximately 5% to 10% of pediatric AML cases and is defined by the PML-RARA fusion, typically through the balanced translocation t(15;17) (q24.1;q21.2).[14] Rare cases, however, exhibit the clinical and morphologic features of APL without t(15;17), and these patients may have a cryptic PML-RARA rearrangement or a rare variant RARA translocations.[42] Importantly, the variants ZBTB16-RARA and STAT5B-RARA exhibit resistance to all-trans retinoic acid.[42] The incidence of this diagnosis is low in infants, increases in childhood, and peaks in adolescents and young adults. Morphologically, both hypergranular and hypogranular variants exist without apparent correlation to the underlying RARA fusion variant.

CBFA2T3-GLIS2. CBFA2T3-GLIS2 rearrangements, typically the result of a cryptic inversion inv(16) (p13.3q24.3), are identified nearly exclusively in pediatric patients less than 3 years of age.[43] They originally were identified in AMKL, where it is found in 20% to 30% of pediatric AMKL and associated with a dismal outcome.[44]

Subsequently, this fusion has been shown to be enriched in AML with normal cytogenetics but also has been detected as a rare rearrangement in AML with other cytogenetic findings; regardless of the morphologic subtype, this fusion has been associated with adverse outcomes.[11,43,45] Few cooperating mutations are identified in CBFA2T3-GLIS2–positive malignancies, and, interestingly, forced expression of this fusion in cord blood stem cells was sufficient to drive malignant transformation.[45] Importantly, conventional cytogenetics often fails to identify this rearrangement, placing patients in a standard risk category, so appropriate molecular methods are essential to identify this important lesion. CBFA2T3-GLIS2 leukemias have a distinct immunophenotype, known as the RAM phenotype, with high expression of CD56 with absent to dim expression of HLA-DR, CD38, and CD45. Recent data suggest this phenotype alone may be sufficient to identify CBFA2T3-GLIS2–positive cases.[45]

RBM15-MKL1. The RBM15-MKL1 fusion caused by t(1;22) (p13.3;q13.1) typically is identified in young children and is the second most common structural rearrangement in neonatal leukemias.[46] This leukemia commonly has a megakaryoblastic phenotype, accounting for 10% of pediatric AMKL, and can present as a myeloid sarcoma that is CD45 and CD34 negative, which can make diagnosis difficult and easy to mistake for a poorly differentiated tumor of another lineage. RBM15-MKL1 is associated with a favorable prognosis among pediatric AMKL cases.[11,46]

KAT6A-CREBBP. The KAT6A-CREBB fusion caused by t(8;16) (p11.2;p13.3) is a rare finding in pediatric AML, occurring in less than 1% of cases. This leukemia generally has a monocytic or myelomonocytic phenotype. It occurs most commonly in infants and is congenital in approximately 25% of cases.[47] This diagnosis generally is associated with poor outcome, although paradoxically, some neonatal patients experience spontaneous remission.[47–50]

Acute Myeloid Leukemia with Normal Cytogenetics

Cytogenetically normal AML is significantly less common in pediatric AML (15%–25%) compared with adult AML (40%–47%) and is associated with an intermediate prognosis.[51,52] Significant heterogeneity has been noted within this group, likely due to the diversity of subtypes represented herein, and prognosis is altered with identification of cryptic rearrangements, such as NUP98-NSD1, NUP98-KDM5A, or specific somatic mutations, such as variants in NPM1, CEBPA, RUNX1, FLT3, and RAS pathway genes. Those without FLT3-ITDs have favorable prognosis. Younger patients with a normal karyotype and no FLT3-ITDs have a prognosis comparable to that of patients with CBF AML.[53]

NPM1 mutations

Somatic mutations in the last exon of the NPM1 gene are found in 2% to 8% of pediatric AML, significantly rarer than adult AML.[54,55] These mutations, often frameshift insertions and deletions at the far C-terminus, result in NPM1 mislocalization through the removal of 1 or 2 nuclear localization sequences and the creation of a nuclear export signal.[56,57] Patients with NPM1 mutations have a favorable prognosis and typically have a normal karyotype.[54] Coexistent FLT3-ITD mutations are frequent, although patients with both mutations exhibit outcomes similar to NPM1 mutation alone.[4,54]

Biallelic CEBPA

Approximately 4% to 9% of children and young adults with AML carry mutations in the single-exon gene CEBPA, of which there are 2 distinct groups of mutations.[58,59] Truncating mutations in the N-terminal region of CEBPA are located between the primary

translation start site and a second alternative site, which abrogates expression of the longer p42 isoform while preserving translation of the p30 isoform. This p30 isoform has been shown to act in a dominant negative manner, inhibiting activity of p42.[60–62] The second class of CEBPA mutations typically are in-frame deletions and insertions that disrupt the C-terminal basic leucine zipper (bZIP) region and are thought to impair DNA binding and/or homodimerization.[63] Biallelic CEBPA–mutated AML typically exhibits a combination of a truncating N-terminal mutation with a mutation altering the bZIP region.

Data primarily from adult AML indicate that biallelic mutations are associated with favorable outcomes, and the recent WHO classification of AML creates a distinct provisional category for such occurrences in AML.[64–67] A recent study of pediatric CEBPA-mutated AML found no difference in outcomes between monoallelic and biallelic CEBPA mutations; instead, it was the presence of a bZIP mutation alone that was associated with improved event-free survival and overall survival.[68] Whether this association is unique to pediatric AML or extends to adult AML has yet to be determined, although previous studies indicate that monoallelic and biallelic CEBPA–mutated AML exhibit distinct transcriptional profiles and may be distinct entities.[69,70]

Patients with CEBPA-mutated AML typically have a normal karyotype. Cooperating mutations often are detected in GATA2, FLT3, and CSF3R.[71,72] Although GATA2 mutations did not influence outcomes, biallelic CEBPA–mutated pediatric AML with co-occurring CSF3R mutations exhibited significantly inferior event free survival due to high rates of relapse. Approximately 10% of individuals with biallelic CEBPA mutations carry a germline pathogenic variant in CEPBA that is, associated with a hereditary predisposition to AML.[63,69]

RUNX1

Pediatric AML cases carry somatic mutations in RUNX1 much less frequently (3%) than is observed in adult patients (15%), although there is an increased prevalence of RUNX1 mutations in pediatric AMKL (10%).[11,73,74] AML with mutated RUNX1 is associated with inferior outcomes and older age in pediatric cases.[4,75] Although most cases have a normal karyotype, they also can be identified with complex karyotypes, and 1 study found RUNX1-RUNX1T1 rearrangements in 22% of cases harboring a RUNX1 mutation.[75]

FLT3

FLT3 is a receptor tyrosine kinase that is essential for normal hematopoietic development. Somatic activating mutations in FLT3 are among the most common somatic alterations in pediatric AML, found in approximately 30% of cases, and are associated with a normal karyotype.[4,76] The most common activating FLT3 mutation is an internal-tandem duplication in the juxtamembrane domain, which is observed in 10% to 15% of pediatric cases.[4] Point mutations in the activation loop domain, most commonly at codons Asp835/I836, are found in approximately 10% of pediatric AML, and recent evidence suggests that there are pediatric-specific activating mutations in the transmembrane domain and juxtamembrane domain found in 7% of cases and are associated with poor responses to standard therapy.[4,77] Importantly, FLT3-ITD mutations with a high allelic ratio (>0.5) are predictive of adverse outcomes in pediatric AML,[78–80] and patients are directed to consider HSCT at first complete remission. Mutations In the activation loop, however, do not confer the same poor prognosis.[78]

The outcomes of pediatric patients with FLT3-ITD mutations are influenced by cooperating mutations; NPM1 comutations are associated with the best outcomes. In contrast, the presence of a WT1 mutation or NUP98-NSD1 fusion imparted worse

outcomes compared with FLT3-ITDs alone.[4] Importantly, FLT3 is a molecular lesion in AML for which there is a targeted therapy, as the FLT3 tyrosine kinase inhibitors midostaurin and gilteritinib both have been approved for FLT3-mutated adult AML, and trials for FLT3 inhibitors are under way in pediatric AML.[81]

Acute megakaryoblastic leukemia

AMKL is genetically unique in pediatric patients. Individuals with constitutional trisomy 21 have a greatly increased chance of developing lymphoblastic and myeloid leukemia in general, and, when myeloid leukemia occurs, it often has a megakaryoblastic phenotype. The distinction between AMKL occurring in the setting of Down syndrome (DS), versus AMKL occurring in non-DS patients, is important because DS patients with AMKL have a good prognosis.[82] In contrast, non-DS AMKL have a variety of outcomes influenced by the underlying molecular driver.

Non–DS AMKL has a high rate of structural variation, with 72% of cases carrying a recurrent chromosomal translocation resulting in a gene fusion, including CBFA2T3-GLIS (18%), NUP98-KDM5A (11.5%), RBM15-MKL1 (10%), and KMT2A fusions (17%) and a new collection of rearrangements involving HOX genes (15%).[11] In cases without a recurrent chromosomal translocation, truncating mutations in exons 2 and 3 of GATA1 were identified in 9% of cases.[11]

Gene expression and clustering analysis indicate these recurrent genomic alterations represent distinct subtypes of non–DS AMKL. Cooperating mutations also were identified in JAK/STAT genes (17%), cohesion or CTCF genes (18%), and RAS pathway genes (18%) and focal deletions/loss of heterozygosity in RB1 (14%) and gains of chromosomes 19 and 21.[11] KMT2A fusions often were associated with mutations in the RAS pathway, whereas GATA1 mutations had coexistent JAK and cohesin mutations. Cases of HOX gene rearrangements are enriched significantly in activating MPL mutations and NUP98-KDM5A cases carried RB1 mutations in nearly all samples.[11]

Clinical outcomes appear to be highly correlated with the genetic subtype of non–DS AMKL, with CBFA2T3-GLIS representing the subtype with the worst survival.[11] KMT2A-rearrangenments and NUP98-KDM5A also represented high-risk subtypes. Patients with GATA1 mutations had excellent outcomes, similar to those with DS AMKL, potentially indicating these represent similar entities. HOX gene rearrangements, RBM15-MKL1, and cases without a recurrent genomic alteration all showed a favorable prognosis.[11]

These recurrent rearrangements are not found commonly in adult AMKL and highlight the need for appropriate molecular testing to triage non-DS AMKL patients to risk-adapted therapy. Both DNA and RNA sequencing with appropriate panels represents the best current method to distinguish among these genetic groups.

Germline Predisposition to Acute Myeloid Leukemia

The WHO recently has included germline variant driven AML as separate disease entities, which becomes important to identify for patient management and family counseling. Recent germline testing studies have demonstrated at least 10% of children with cancer harbored a germline mutation in a cancer predisposition gene, although rates in pediatric hematologic malignancies are lower.[83–85]

Although it long has been recognized that bone marrow failure disorders, such as Shwachman-Diamond syndrome or Diamond-Blackfan anemia, have an increased predisposition to AML, it now is recognized that there are a significant number of other pathogenic germline variants that are associated with hematologic abnormalities, such as thrombocytopenia and predisposition to AML. Although discussion of bone marrow failure disorders is beyond the scope of this article, germline predisposition

due to pathogenic variants in CEBPA, DDX41, RUNX1, ANKRD26, and ETV6 is discussed.

Germline pathogenic variants in CEBPA are associated with a highly penetrant autosomal dominant predisposition to AML.[86] Patients generally present with AML as children or young adults; the diagnosis can be challenging, because no preceding clinical phenotypes are reported and usually is considered upon tumor sequencing when biallelic CEBPA mutations are identified in the tumor.

Among all patients with biallelic CEBPA mutations in AML, it is estimated that approximately 10% carry a germline CEBPA mutation.[63,69] Pathogenic germline CEBPA variants generally are truncating variants located at the N-terminus of the gene, with somatic inactivation of the second allele the result of a truncating or in-frame deletion mutation in the C-terminal region.[60] Prognosis for patients with pathogenic germline CEBPA variants typically is very good, similar to that for sporadic AML with biallelic somatic CEBPA mutations; however, those with pathogenic germline CEBPA variants are at a higher risk of recurrence/relapse.[87] The second somatic CEBPA mutation usually is different upon recurrence, indicating the primary malignancy was cured and that recurrence is due to a new tumor clone and represents a new leukemic episode.[87]

Pathogenic germline DDX41 variants are associated with an autosomal dominant form of hereditary predisposition to AML without additional clinical features, similar to CEBPA.[88] Affected individuals often develop a second mutation on the other DDX41 allele, consistent with its proposed function as a tumor suppressor. Age of onset, however, highly overlaps with sporadic adult disease and has not been reported in children to date.[89,90] Recent data suggest it is fairly common in sporadic adult AML, where a pathogenic germline variant in DDX41 was identified in 2.4% of cases.[90] Although still a newly described entity, DDX41-associated predisposition to AML may not be a pediatric-onset condition; further investigation is needed to understand this condition fully.

Pathogenic germline variants in RUNX1 are causal for familial platelet disorder with predisposition to AML.[91] It is a rare diagnosis, with only 130 reported families to date, despite its first report more than 20 years ago. Affected individuals report mild to moderate thrombocytopenia, platelet dysfunction, although many do not exhibit a clear bleeding history.[92] Approximately 35% of patients develop myelodysplastic syndrome (MDS), and/or AML occurs at an average age of 33 but has been reported in children as young as 6.[93] This disorder is inherited in an autosomal dominant manner, with most reported and de novo germline RUNX1 mutations reported in patients without a family history.[94,95] Clinical presentation can be variable, even within the same family, and some patients initially can present with AML.[93] Prognosis for affected individuals who progress to AML is poor, and allogenic stem cell transplantation typically is recommended in pediatric patients. Asymptomatic RUNX1 mutation carriers develop a clonal hematopoiesis of indeterminate potential with a cumulative risk of greater than 80% by age 50, demonstrating there are additional clinical features in carriers.[96]

Germline RUNX1 mutations are are truncating variants that are distributed throughout the coding region of the gene and missense mutations that are enriched in the RUNT domain.

AML samples from affected individuals often show somatic mutations affecting the second RUNX1 allele but otherwise show few additional somatic mutations. When they are identified, ASXL1, PTPN11, STAG2, BCOR, DNMT3A, and GATA2 have been reported.[93,96]

ANKRD26 is associated with one of the most common inherited thrombocytopenias, with patients exhibiting lifelong mild to moderate thrombocytopenia, normal

platelet size, and a mild bleeding phenotype, although some may present initially with a myeloid malignancy.[97,98] Studies demonstrate an increased risk for developing acute leukemias (4.9%), MDS (2.2%), and chronic myeloid leukemia (CML) (1.3%).[99] Pathogenic ANKRD26 variants primarily are substitutions located within a discrete region of the 5′ UTR of ANKRD26, and additional studies indicate these variants result in upregulation and persistent expression of ANKRD26 through a loss of the repressive binding of RUNX1 and FLI1.[97] Penetrance for thrombocytopenia is nearly complete and diagnosed in adulthood, and transformation to myeloid malignancies has been reported as early as 30 years, suggesting this may not be a pediatric-onset malignancy; however, with only 230 affected individuals reported to date, pediatric onset cannot be excluded.[98,100]

ETV6 is associated with an autosomal dominant inherited thrombocytopenia in which affected individuals display an increased risk for a variety of hematological malignancies, including ALL, CML, and MDS/AML.[101,102] Thrombocytopenias in affected individuals are highly variable, although bleeding symptoms typically are mild and accompanied by either normal or large platelets.[103] Pathogenic variants typically are missense or frameshift alterations in the C-terminal ETS domain that confer a dominant negative effect through binding and mislocalization of wild-type ETV6.[101,102] Among the germline mutation carriers published, ALL, typically pre–B-cell leukemia, is the most common malignancy identified, followed by myeloid malignancies. Age of presentation for malignancies ranges from 2 years to 82 years, demonstrating this is a pediatric-relevant cancer predisposition syndrome.[103]

Finally, patients with pathogenic germline variants in GATA2 have variable syndromic presentations characterized by immunodeficiency, bone marrow failure, and an autosomal dominant predisposition to development of MDS/AML.[104–107] Affected individuals typically exhibit B-cytopenia, DC-cytopenia, NK-cytopenia, and monocytopenia and show susceptibility to nontuberculosis mycobacterial, fungal, and viral (in particular, Epstein-Barr virus and human papillomavirus) infections. Presentations are highly variable in terms of age of onset and disease severity and also can include additional phenotypes, such as primary lymphedema, deafness, and aplastic anemia.[104,108] Pathogenic loss of function variants typically are truncating alterations upstream of zinc finger 2, missense variants within zinc finger 2, or noncoding variants within intron 4 that disrupts a transcriptional enhancer.[104]

Myeloid neoplasms develop in up to 75% of patients with an age of onset ranging from 3 years to 78 years with a median of 20 years.[109] Because a majority of pathogenic germline variants are de novo, most patients do not have a suggestive family history. Furthermore, myeloid malignancies can present in the absence of preceding hematological phenotypes, demonstrating the utility of genomic testing for this diagnosis.

Clinical Genomic Testing

Advances in genomic technologies have altered the landscape of clinical genetic testing for hematological malignancies dramatically. The diversity and prominence of chromosomal rearrangements and cooperating somatic sequence variants in pediatric AML necessitate the diagnostic testing for both types of genomic variants.

Structural variants historically have been evaluated by traditional cytogenetics, including karyotype and fluorescence in situ hybridization. These approaches later were augmented with targeted polymerase chain reaction (PCR)-based molecular methods, including quantitative real-time PCR and qualitative PCR, which can query a small number of known rearrangement partners, often with superior sensitivity.

NGS-based methodologies have the advantage of assessing many or even all genomic regions for structural variation simultaneously. NGS-based methods for the detected of chromosomal rearrangements can query DNA or RNA. DNA-based methods typically rely on the enrichment of known breakpoint regions through either hybrid capture or amplicon-based methods. This approach has the advantage that it can be added to a gene panel for the detection of somatic sequence variants, enabling the detection of both variant types in 1 assay. DNA-based fusion detection requires prior knowledge of the DNA breakpoints, which typically reside within intronic regions, are highly variable, and can be composed of repetitive or nonunique sequences that are challenging to specifically capture, sequence, and bioinformatically map to the appropriate genomic region. Thus, DNA panel–based methods typically exhibit lower sensitivity for known fusions and offer limited ability to identify novel rearrangements. Additionally, this approach cannot determine whether the identified fusion is transcribed or whether it is likely to result in a functional protein, because novel or unexpected patterns of RNA splicing often are observed. Whole-genome sequencing theoretically can identify any known or novel structural variant, but its clinical utility is limited by the high cost, lower depth of sequence recovered, and computational burden related to the large amount of data generated by this method.

RNA-based fusion detection methods rely on an initial reverse transcription step to produce a cDNA library, and can use an upstream enrichment for known fusion partners through hybrid capture or anchored multiplex PCR.[110–112] Because these enrichment approaches generally target exonic regions, they are able to reduce the burden of sequencing per sample while retaining high sensitivity and the ability to identify novel rearrangement partners. A recent study revealed RNA-based approach exhibited superior performance to DNA, identifying fusions in 15% of lung cancer cases without a significant oncogenic driver.[113] A more unbiased approach, whole transcriptome sequencing, retains sensitivity of other RNA-based approaches but offers the ability to identify completely novel and unanticipated fusion genes. The main limitation of RNA-based methods is that they rely on the production of a chimeric transcript, which is not produced by some rearrangements that rely on the association of an oncogene with a strong promoter element, such as the immunoglobulin rearrangements common in lymphomas. With respect to pediatric AML, most reference laboratories offer assays designed for adult malignancies, with a few academic laboratories offering diagnostics designed for pediatric patients.[114–116] Although there is significant overlap between adult and pediatric AML, there are several pediatric specific and rare rearrangements that may not be assayed by targeted approaches. Thus, nonpediatric specific reference tests that utilize transcriptome sequencing may represent a superior approach.

Molecular genetic testing for somatic mutations has similarly progressed from the targeted analysis of a small number of genomic regions through targeted molecular methods and Sanger sequencing, to the simultaneous sequencing of many genes via NGS. NGS-based diagnostics are able to detect single nucleotide variants and small insertions/deletions (<20 bp) with high sensitivity. The sensitivity for insertions/deletions greater than 20 bp diminishes with increasing length of the variant also can be negatively influenced by homologous sequences elsewhere in the genome or low sequence complexity (repetitive elements). This is an important consideration for somatic mutations that involve tandem duplications, such as FLT3-ITDs, for which custom bioinformatics approaches are needed to specifically identify this mutation and may not match the sensitivity of targeted molecular methods at this time. Larger exon and gene level copy number changes also can be detected by NGS, with greater sensitivity for larger genomic copy number changes and gene amplifications.

Clinical DNA sequencing typically relies on the enrichment for coding exons and other clinically relevant genomic regions by hybrid capture or amplicon-based approaches and can vary significantly in the number of genes assayed. Targeted panels ranging from 10 genes to 100 genes, and in some cases focusing only on mutation hotspots and/or actionable genomic findings may be offered to reduce costs while maintaining clinical sensitivity. The rapid evolution of targeted therapies, however, necessitates frequent content updates that require revalidation of the assay and may mitigate some benefits of focused assays. Many commercial panels use a pan-cancer approach that targets most clinically relevant genes across all cancer types and also can offer reporting of genomic signatures, such as microsatellite instability, tumor mutational burden, and a variety of mutational signatures that are associated with clinical benefit to targeted therapies. It is common practice for diagnostic laboratories to offer solid tumor and hematological tests as separate tests, which can be based on the same wet bench procedure with different bioinformatic/interpretive processes or completely separate wet bench processes. Exome sequencing for oncology diagnostics now is clinically available, providing a more complete mutational landscape for oncology patients, and offers the advantage of being able to identify new and emerging biomarkers.

SUMMARY

Although there likely are additional rare driver genetic lesions to be identified in pediatric AML, the field going forward will focus more on optimizing prognosis for pediatric AML subtypes based on coexistent mutations. The possibility of a germline predisposition to AML always should be considered, particularly when genomic testing of malignancies reveals a suggestive mutation. The development of emerging precision therapies will serve only to increase the utility of genomic testing for this patient population.

CLINICS CARE POINTS

- Given the remarkable heterogeneity of chromosomal and molecular alterations that drive pediatric AML, broad testing methodologies, such as NGS-based panels and exome/genome sequencing, and RNA-based fusion detection should be employed to augment conventional cytogenetics for optimal sensitivity.
- Detection of a diverse set of driver mutations in pediatric AMKL has high prognostic significance.
- Hereditary predisposition to AML, although rare, should be considered when interpreting somatic genomic testing.

DISCLOSURE

The authors are both employees of Caris Life Sciences.

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