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Original Article

The importance of targeted next-generation sequencing based genomic profiling at diagnosis of childhood acute myeloid leukemia: a single center experience

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ABSTRACT

Background. The management of pediatric acute myeloid leukemia (AML) is based on the prognostic risk classification of initial leukemia. Targeted next-generation sequencing (NGS) is a reliable method used to identify recurrently mutated genes of pediatric AML and associated prognosis.

Methods. In this study, we retrospectively evaluated the prognostic, and therapeutic utility of a targeted NGS panel covering twenty-five genes, in 21 children with de novo and 8 with relapsed or secondary AML.

Results. Variants were detected in 44.8% of patients, and 63.2% of them were in the signaling pathway genes. The number of variants per patient and diversity increased with age. The panel results affected hematopoietic stem cell transplantation decisions, especially in core binding factor AML, and allowed the categorization of diseases according to current classifications. Panel results also pointed out predisposition to germline leukemia to the extent of the panel coverage. No targeted therapy was used based on the variants, and none of the variants were used to monitor minimal residual disease.

Conclusions. Targeted NGS results, along with well-known genetic aberrations and treatment responses, can guide treatment modalities. The coverage of the routine panels should include proven mutations of childhood AML and germline leukemia predisposition genes.

Key words: acute myeloid leukemia, children, mutation, next-generation sequencing.

Acute myeloid leukemia (AML) is a molecularly and clinically heterogeneous clonal disease characterized by uncontrolled proliferation of immature myeloid cells due to complex genetic alterations that impair hematopoiesis. Pediatric AML comprises less than 20% of childhood leukemia and, nearly 40% of patients relapse after first-line therapies.[1-3]

Childhood AML development is generally enhanced by chromosomal rearrangements that create chimeric fusion genes, which often involve transcription factors. Subsequently, acquired mutations in other pathways, often tyrosine kinase or RAS, cooperate with chromosomal rearrangements.[4] The Cancer Genome Atlas network reported 23 genes that recurrently

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mutated in adult AML patients, which were grouped into nine functional categories including; transcription-factor fusions (18% of cases), nucleophosmin 1 (*NPM1*) mutations (27%), tumor suppressor genes (16%), DNA-methylation-related genes (44%), activated signaling genes (59%), chromatin-modifying genes (30%), myeloid transcription-factor genes (22%), cohesin-complex genes (13%), and spliceosome-complex genes (14%).[5]

Management of pediatric AML patients is constructed on the prognostic risk classification of leukemia. The morphologic, immunophenotypic evaluation and specific cytogenetic and molecular abnormalities are critical prognostic indicators. Most genetic methods widely used in developing countries, such as karyotype analysis, fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR) do not provide adequate information for the current prognostic classifications and targeted treatment modifications. Targeted next-generation sequencing (NGS) is a rapid, precise, and cost-effective prognostic method that is routinely used in adult AML patients.[6] Since the genetic profiles of adult and pediatric AML patients are diverse with similar phenotypes, the applicability of molecular genetic results of adult AML to children is limited. The value of NGS for pediatric AML patients has not yet been entirely determined.

This study aimed to determine the prognostic and therapeutic benefits of panel-based targeted NGS results for pediatric AML patients.

Materials and Methods

Study design and patient recruitment

Fifty children with pediatric AML were diagnosed in Ankara Bilkent City Hospital Pediatric Hematology Clinic between November 1st, 2019 and July 31st, 2023. Diagnoses were determined according to morphological, immunophenotypic, and genotypic criteria. At diagnosis, cytogenetic analysis by conventional G-banding, fluorescence in situ hybridization (FISH) analysis for t(8;21), t(15;17), t(9;22), inv16, 5q del, 7q del, 11q23, *TP53* (17p13) del and PCR analysis for *RUNX1-RUNX1T1*, *PML-RARA*, *CBFB-MYH11*, *KMT2A-AFF1*, and *BCR-ABL* were performed in all patients. The targeted NGS myeloid panel testing was performed for 21 de novo AML and 8 relapsed or secondary AML patients. None of the relapsed patients had an NGS myeloid panel study at their primary diagnosis. De novo AML patients were treated according to the Berlin-Frankfurt-Munster (BFM) AML 2019 protocol. Patients with relapsed AML received various combination therapies, such as idarubicin - fludarabine - cytarabine, gemtuzumab - ozogamicin, and venetoclax.

The results were analyzed according to the AML subgroups and age groups, as follows; infants (<3 years), children (3-14 years), and adolescents (15-18 years).

Next-generation sequencing analysis

The QIAact Myeloid DNA UMI Panel (Qiagen, Hilden Germany) was performed with GeneReader NGS System at diagnosis. The study material was fresh bone marrow in all patients. The panel included a total of 25 genes, seven of which with whole coding region (*CEBPA, DNMT3A, EZH2, MPL, RUNX1, TET2,* and *ZRSR2*); and the remaining 18 genes with specific exons (*ASXL1, CALR, CBL, CSF3R, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, NPM1, NRAS, SETBP1, SF3B1, SH2B3/LNK, SRSF2, TP53,* and *U2AF1*). This panel accurately and sensitively determines significant insertion/deletion (InDel) mutations and single nucleotide variants. Utilizing unique molecular index technology allows the detection of low-frequency variants. Variants were analyzed with Qiagen Clinical Insight Software, including population frequency databases, public variant databases, and in-silico prediction tools. The actionable variants were evaluated in the light of the 2017 recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American

Pathologists, which is a four-tiered system to categorize somatic sequence variations based on their clinical significances. These include: Tier I variants with strong clinical significance; Tier II, variants with potential clinical significance; Tier III, variants of unknown clinical significance; and Tier IV, variants deemed benign or likely benign.[7] As a principle of routine clinical practice, written informed consent was obtained from the legal guardians of patients for NGS analysis and other genetic tests, at the time of AML diagnosis. The ethics committee of Ankara Bilkent City Hospital approved the study (E2-23-3712).

Statistical analysis

Mean, standard deviation, frequency, and percentage were used for descriptive statistics. Analyses were conducted using SPSS (version 20.0).

Results

The electronic medical records of 50 children with 38 de novo AML and 12 relapsed or secondary AML were examined. Among these patients, 21 de novo and 8 relapsed/secondary AML patients were evaluated with the NGS AML panel at diagnosis and included in the study. The mean age of de novo AML patients was 8.3±6.2 years, and 38.1% (n=8) were male, whereas the mean age of relapsed/secondary AML patients was 10.8±6.1 years and 75% (n=6) were male. In 11 patients, metaphases could not be obtained for karyotype analysis. Demographic features, disease characteristics, karyotype analysis, FISH, PCR, and targeted NGS results of de novo AML patients are shown in Table I and relapsed or secondary AML patients are shown in Table II. Twenty patients (69%) had cytogenetic anomalies detected either by karyotype analysis or FISH. By NGS panel study, 19 variants were detected in 13 patients (44.8%), two of whom didn't have a cytogenetic anomaly. The 63.2% of variants (12/19) were in the signaling pathway genes.

Eight patients (27.6%) were under 3 years of age, twelve patients (41.4%) were between 3 and 15 years of age, and nine patients (31%) were 15 years or older. Patients younger than 3 years had 0.38 (3/8) variants per patient, whereas patients between 3 and 15 years had 0.50 (6/12) and patients older than 15 years old had 1.11 (10/9). The variants detected in patients younger than three years of age were in the signaling pathway (*NRAS, KRAS*) and spliceosome (*U2AF*) genes. Patients between 3 and 15 years of age had mutations in the signaling pathway (*FLT3, KIT)* and tumor suppressor genes *(TP53*). However, the variants detected in patients older than 15 years were seen in a wide variety of functional categories, such as chromatin-modifying (*ASXL1)*, signaling pathway (*FLT3, KIT, NRAS*), myeloid transcription factor (*RUNX1, CEBPA*), and DNA methylation genes (*DNMT3A*).

The NGS results were also assessed according to AML subgroups. Five patients had t(8;21) and four of them had variants detected by the NGS panel study. Two de novo AML patients had *KIT* variants (Patient #9 and Patient #11), one de novo AML (Patient #19) had copartner *RUNX1* and *DNMT3A* mutations and one relapsed patient (Patient #26) revealed an *NRAS* variant. Allogeneic hematopoietic stem cell transplantation (HSCT) was performed, considering *KIT* exon 17 mutation (N228K) in Patient #11 and copartner mutations in Patient #19. They had also experienced suboptimal (<3 log reduction in the bone marrow transcripts) *RUNX1-RUNX1T1* transcript reduction after consolidation chemotherapy.

Six patients had acute promyelocytic leukemia (APL) and the NGS panel detected aberrations in two of them. A *FLT3-ITD* mutation was detected in a de novo APL patient (Patient #14) which was not cause to change treatment protocol. The other variant was a *KIT* mutation which was detected in a 17-year-old secondary APL patient (Patient #28) whose initial diagnosis was precursor B acute lymphoblastic leukemia (pre-B ALL) followed by AML with inversion 16, five years later she relapsed as APL. This *KIT* mutation, with unknown clinical significance and 43% variant allele frequency (VAF),

Table I. The clinical and genetic characteristics of de novo AML patients.

Patient number	Age at diagnosis (years)	Gender	Disease subtype	Karyotype	FISH	Variant (NGS), VAF, classification	HSCT in CR1	Outcome	Overall survival (months)
1	0.7	M	FAB M0	43-47, XY, del(7q), del(17p)	TP53 deletion	None	Planned	Remission, alive	4
2	0.8	F	FAB M5	46, XX	*KMT2A* rearrangement	*KRAS* 35%Tier 1A *U2AF* 35% Tier 2C	No	Dead, refractory disease	10
3	1	F	FAB M7	46, XX	None	None	Yes	Remission, alive	30
4	1	F	FAB M7	51-54, XX, t(1;22) (q12;p11.2)	None	NRAS 3% Tier 1A	No	Dead (CR)	4
5	1.5	F	FAB M5	46, XX	None	None	No	Remission, alive	15
6	2	F	FAB M7 Down syndrome	Unsuccessful	None	None	No	Remission, alive	5
7	2	M	FAB M2	46, XY, t(7;9;11)	None	None	Yes	Remission, alive	11
8	3	F	FAB M4	Unsuccessful	None	None	No	Remission, alive	6
9	6.5	M	FAB M0	Unsuccessful	t(8;21)	KIT c.1254_1255delCG 26% Tier 3	Planned	Remission, alive	4
10	7	M	FAB M1	Unsuccessful	t(8;21)	None	No	Remission, alive	7
11	10	F	FAB M1	Unsuccessful	t(8;21)	KIT N822K 22% Tier 2C	Yes	Remission, alive	23
12	10	F	FAB M2	46, XX	None	None	No	Remission, alive	18
13	11	F	FAB M2	Unsuccessful	5q deletion	FLT3-ITD 13% Tier 2A	Yes	Dead (CR)	11
14	11	F	FAB M3	46, XX	t(15;17), inversion 3	FLT3-ITD 39% Tier 2A	No	Remission, alive	32
15	12	F	FAB M3	Unsuccessful	t(15;17)	None	No	Remission, alive	22
16	14	M	FAB M3	46, XY	t(15;17)	None	No	Remission, alive	23
17	15	F	FAB M3	Unsuccessful	t(15;17)	None	No	Remission, alive	24
18	15	F	FAB M0	Unsuccessful	Monosomy 7	*ASXL1* 15% Tier 2C *NRAS* 66% Tier 2C	Yes	Remission, alive	17
19	17	M	FAB M1	46, XY	t(8;21)	*RUNX1* 8,6 % Tier 1A *DNMT3A38* % Tier 1A	Yes	Dead (CR)	12
20	17	M	FAB M1	46, XY	None	*CEBPA* %39 Tier 1A *NRAS* %46 Tier 1A	Yes	Remission, alive	28
21	17.5	M	Myeloid sarcoma	46, XY	None	None	No	Remission, alive	9

CR: complete remission; F: female; FAB: French–American–British classification; FISH: fluorescent in situ hybridization; HSCT: hematopoietic stem cell transplantation; *KMT2A*: Lysine methyltransferase 2A gene; M: male; NGS: next generation sequencing; VAF: variant allele frequency.

Table II. The clinical and genetic characteristics of relapsed or secondary AML patients.

Patient number	Age at diagnosis (years)	Gender	Diagnosis	Disease subtype	Karyotype	FISH	Variant (NGS), VAF, classification	HSCT in CR2	Outcome	Overall survival (months)
22	2	M	Relapsed AML (After HSCT)	FAB M5	46XY, ins(15;2)(q21q22,q14.3q21)	None	None	No	Dead, refractory disease	2
23	5	M	Relapsed AML	FAB M7	51-52, XY	None	None	No	Dead (CR)	10
24	5	F	Relapsed AML (After HSCT)	FAB M4	Unsuccessful	None	*TP53* p.R248Q 24% Tier 1A *TP53* p.G279fs*27 21% Tier 1A (2 different mutations in TP53)	Yes	Remission, alive	26
25	10	M	Secondary AML (Previously Pre-B ALL)	FAB M3	46, XY	t(15;17)	None	No	Remission, alive	28
26	15	M	Relapsed AML	FAB M0	45, XY-19 / 45, XY-14	t(8;21)	*NRAS* 47 % Tier 2C	Yes	Dead (CR)	8
27	16	M	Therapy related AML (Previously Ewing sarcoma)	FAB M4	Unsuccessful	*KMT2A* rearrangement	*FLT3* c.2505T>G 7% Tier 1A *FLT3* c.2516A>G 5% Tier 1A	No	Dead, refractory disease	1
28	17	F	Secondary AML (Previously Pre-B ALL, AML with inv16)	FAB M3	46, XX	t(15;17), TP53 deletion	*KIT* p.R804Q 43% Tier 3	No	Remission, alive	22
29	17	M	Secondary AML (Previously T ALL)	FAB M0	46, XY	None	None	No	Relapsed, alive	23

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CR: complete remission; F: female; FAB: French–American–British classification; FISH: fluorescent in situ hybridization; HSCT: hematopoietic stem cell transplantation; *KMT2A*: Lysine methyltransferase 2A gene; M: male; NGS: next generation sequencing; VAF: variant allele frequency.

was confirmed as a germline heterozygote mutation after remission. Four patients had acute megakaryoblastic leukemia (AMKL). The myeloid NGS revealed an *NRAS* variant with a low VAF in addition to hyperdiploidy and t(1;22) in one of them (Patient #4).

De novo AML patients, one with 5q deletion (Patient #13) revealed a *FLT3-ITD* mutation and one with monosomy 7 had both *ASXL1* and *NRAS* mutations (Patient #18). They underwent HSCT based on high-risk cytogenetics. Another de novo AML patient (Patient #20) with both *CEBPA* and *NRAS* mutations did not provide any metaphases or didn't have any abnormal results with FISH or PCR techniques. However, based on 39% VAF of the *CEBPA* variant suggesting a monoallelic expression, the case was classified in the intermediate-risk group and HSCT was performed due to induction failure in the first complete remission.

Two patients had *KMT2A* rearrangements accompanied by variants detected by the NGS panel, and both of them died due to refractory disease. One (Patient #2) was an infant with de novo AML and had both *KRAS* and *U2AF* gene mutations. The other was an adolescent, who had therapy-related AML (Patient #27) with two different *FLT3* variants. A 5-year-old girl who relapsed after HSCT (Patient #24) also had two distinct variants in the same gene, *TP53*, while peripheral blood chimerism was 95%. Another secondary AML patient (Patient #29) previously diagnosed with T cell ALL revealed no mutations detected by the NGS myeloid panel. Of interest, his sister also had pre-B ALL, and whole exome sequencing (WES) revealed a pathogenic splicing *ETV6* mutation in a heterozygote state in both siblings indicating germline autosomal dominant *ETV6*-related leukemia predisposition.

No targeted therapy, even *FLT3* inhibitors, had been used based on the detected variants, and none of the variants were used for minimal residual disease (MRD) monitoring in our study group.

Discussion

Following the identification of genetic structural abnormalities and balanced chromosomal translocations in AML, the classification of leukemia evolved from a morphology-based classification to genetic-based criteria. With the advances in sequencing techniques, awareness of changes in specific genes has increased over the last decade. The Therapeutically Applicable Research to Generate Effective Treatments (TARGET) AML project revealed that somatic mutations in children are lower than in adults but increase with age. These mutations were commonly found in transcriptional regulators and signaling mediator genes.[8] Recently, European Leukemia Net (ELN) recommended screening the mutations in *FLT3, IDH1, IDH2, NPM1, CEBPA, DDX41, TP53, ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1,* and *ZRSR2* genes at diagnosis of adult AML to establish the diagnosis, prognosis and identify therapeutic targets.[6,9] We identified an increasing number and diversity of variants by age, mostly in signaling pathway genes (*FLT3*, *KIT*, *NRAS*, *KRAS*), almost in half of the patients by an NGS panel covering most of the ELN recommended genes. In a more comprehensive study, NGS of >150 cancer-related genes, Ishida et al.[10] evaluated 27 pediatric AML patients and similar to our results, reported the most frequent variations in *KRAS*, *NRAS*, *KIT*, and *FLT3* genes. Although we didn't use any variants to detect MRD, the detection of molecular MRD was associated with a significantly higher relapse rate; except *DNMT3A, TET2*, and *ASXL1* mutations which are often present in persons with age-related clonal hematopoiesis.[11]

Core binding factor (CBF) AML, shares a common pathogenic mechanism involving rearrangements of the CBF transcriptional complex characterized by the presence of either t(8;21) (q22;q22) or inversion 16(p13q22)/t(16;16). There is growing evidence for considerable genotypic heterogeneity in this group. Activating mutations of tyrosine kinase/RAS pathway genes such as *NRAS*, *KIT,* and *FLT3* are commonly found in CBF-AML. Exon

17 *KIT* mutations, including D816V, D816H, D816Y, and N822K, confer poor outcomes. Also, mutations in genes of the methylation group (i.e., *DNMT3A, TET2*) had a strong negative prognostic impact in CBF-AML.[12] The contribution of c*KIT* N822K mutation influenced the HSCT decision in Patient #9 and Patient #11 underwent HSCT considering the co-occurrence of *RUNX1* and *DNMT3A* mutations in addition to t(8;21). *DNMT3A* mutations have also been reported as frequent concurrent mutations in *RUNX1*-mutated AML with a negative impact on overall survival.[13] Furthermore, those CBF-AML patients did not have sufficient *RUNX1-RUNX1T1* transcript level decline, which is recommended for MRD monitoring to predict relapse and guide treatment in some reports.[14,15] *NRAS* variants are the most frequently observed in t(8;21) positive AML and are associated with improved outcomes, most likely due to sensitivity to higher doses of cytarabine.[14,16] We observed an *NRAS* variant in a relapsed patient (Patient #26).

Acute promyelocytic leukemia is a distinct type of leukemia, and *FLT3 ITD* mutation frequency is reported as 27% in de novo APL. Somatic mutations are also found in *WT1, NRAS, KRAS, ARID1B*, and *ARID1A* genes whereas, the mutations of other common AML genes, including *DNMT3A, NPM1, TET2, ASXL1, IDH1/2*, and *KIT* are usually absent.[17] In our study, *FLT3-ITD* mutation was detected in a de novo APL patient who had a high leukocyte count and inversion 3 (Patient #14). Besides being a poor prognostic factor in combination with a chromosomal abnormality like inversion 3, the high allelic burden of *FLT3 ITD* is associated with high leukocyte counts.[18,19] Interestingly, an adolescent APL patient (Patient #28) who had first experienced pre-B cell ALL and then AML with inv16 had an unusual *KIT* mutation with a high VAF (43%), which was later confirmed as a germline variant.

Acute megakaryoblastic leukemia is a genetically heterogeneous disease. Non-Down syndrome AMKL patients have several rearrangements other than t(1;22) and can be classified into risk groups according to cytogenetics.[20,21] In addition to rearrangements, recurrent mutations in *GATA1*, *JAK* kinase, *STAT*, cohesion, *CTCF*, *RAS* pathway genes, and cytokine receptor genes, mostly *MPL*, have also been reported in children with AMKL.[22] Non-Down syndrome AMKL patients harboring *GATA1* mutations may have similar biology with Down syndrome AMKL and reduced intensity chemotherapy might be efficient in these patients.[23] Besides, patients with Down syndrome can also develop AML without *GATA1* mutations with a poorer prognosis, necessitating intensified chemotherapy.[24] Bone marrow aspiration may be difficult due to extensive myelofibrosis in the course of AMKL. Next-generation sequencing is a sensitive method to detect variants in the presence of low blast percentage in fibrotic bone marrow.[25] We detected an *NRAS* variant with a very low VAF in one AMKL patient (Patient #4), which didn't affect our treatment modality.

KMT2A rearrangements are common cytogenetic abnormalities in AML and have intermediate to adverse prognosis depending on the various partner genetic aberrations. *KRAS* mutations have been reported to coexist with high-risk *KMT2A* fusions, with significantly lower event-free and overall survival.[26] A *KRAS* mutation was detected in a refractory infant patient (Patient #2) suggesting her worse prognosis. Our other patient with *KMT2A* rearrangement (Patient #27) had two distinct mutations in *FLT3* and was refractory to chemotherapy. The interaction of menin protein to *KMT2A* is critical in leukemia generation. Combined menin and *FLT3* inhibition represents a novel and promising therapeutic strategy for patients with *KMT2A* rearranged AML with concurrent *FLT3* mutation.[27] It may be possible to add *FLT3* inhibitors to menin inhibitors in the future in the management of patients like Patient #27.

Myeloid malignancies with *TP53* mutations are distinct entities associated with complex cytogenetic abnormalities, advanced age, chemoresistance, and poor outcomes. They are frequently detected in AML related to increased genomic instability, therapy-related AML, or

AML with myelodysplasia.[28] Myeloid neoplasms with mutated *TP53* (AML and myelodysplastic syndrome) are classified as a separate disease category in the 2022 International Consensus Classification (ICC) of myeloid neoplasm because of their similar aggressive behavior irrespective of the blast percentage.[29] And recently, *TP53* alterations, either mutations or deletions, were found to be associated with the most dismal prognosis in pediatric AML patients and the importance of concurrent *TP53* mutation and deletion analysis has since been underlined.[30] In addition to *TP53* deletions detected by FISH in two patients (Patient #1 and Patient #28), two different mutations in the *TP53* gene were detected in Patient #24 during the relapse after HSCT. The presence of multi-hit *TP53* mutations, as it occurred in Patient #24, can be confirmed by the presence of two or more distinct *TP53* mutations and corresponds to a highly aggressive disease.[31,32]

AML with myelodysplasia-related genetic alterations is designated by the ICC and World Health Organization (WHO) 2022 classification. According to WHO classification, AML, myelodysplasia-related (AML-MR) is defined as a neoplasm with ≥20% blasts expressing a myeloid immunophenotype with either one or more cytogenetic abnormality [complex karyotype, 5q deletion, monosomy 7, 7q deletion, 11q deletion, 12p deletion, monosomy 13, 17p deletion, isochromosome 17q or idic(X)(q13)] or somatic mutations in *ASXL1*, *BCOR EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2* genes.[33] With similar cytogenetic abnormalities and the same mutations, ICC also includes *RUNX1* mutations for this category.[29] Patient #18, who had *ASXL1* (VAF 15%) and *NRAS* (VAF 66%) mutations in addition to monosomy 7, can be classified as AML-MR according to both groups.

Our study evaluated the contribution of targeted AML NGS myeloid panel in managing childhood AML. However, the panel was created mainly for adult AML and covered the genes most recommended by the ELN. The current WHO classification system introduced "myeloid neoplasms with germline predisposition" as a new class of myeloid neoplasms.[33] In addition to detecting somatic mutations in myeloid neoplasms, panels containing germline gene variations will be helpful. Non-hematopoietic tissue or post-treatment remission samples should be tested with NGS to confirm germline pathogenic mutations associated with hematological malignancies. Our panel did not cover the most common germline mutations, such as *ANKRD26* and *ETV6*. It also did not cover the *WT1* gene. *WT1* mutations have poor prognosis and its combination with *FLT3-ITD* variants confer a poorer prognosis.[34] Another missing gene in the panel was *GATA1*, which is important for the precise diagnosis of AMKL patients, both with Down syndrome and without Down syndrome. Our study's limitations are the coverage of the used myeloid NGS panel and the small sample size.

In conjunction with well-known recurrent cytogenetic abnormalities, the NGS myeloid panel can identify prognostic risk groups and treatment responses in children with AML. Our NGS results provided information regarding the prognosis and allowed us to guide treatment individually. For some patients, HSCT decisions were strengthened. The coverage of the routinely used AML panels should be expanded with other proven mutations of childhood AML and germline leukemia predisposition mutations. The widespread use of NGS in pediatric hematology-oncology clinics may also obtain precise and reliable data for direct targeted therapies and MRD monitoring.

Ethical approval

The ethics committee of Ankara Bilkent City Hospital approved the study (date: 27/03/2023, number: E2-23-3712).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design:

DK, BÇ, NYÖ, NY; data collection: DK, BÇ, AKY, MI, FBK, FTY, TB, DGG; analysis and interpretation of results: DK, BÇ, NY; draft manuscript preparation: DK, BÇ, NYÖ, NY. All authors reviewed the manuscript and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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