Acute megakaryoblastic leukemia with trisomy 3 and CBFA2T3::GLIS2: A case report

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Abstract
Non-Down-syndrome-related acute megakaryoblastic leukemia (non-DS-AMKL) is a rare form of leukemia that can present with a variety of initial symptoms, including fever, rash, bruising, bleeding, or other more clinically challenging symptoms. Herein, we describe a 19-month-old female patient who presented with left lower extremity pain and language regression who was diagnosed with AMKL, not otherwise specified (NOS), on the basis of peripheral blood and bone marrow analysis, as well as cytogenetic and molecular diagnostic phenotyping. Of note, in addition to this patient’s karyotype showing trisomy 3, a fusion between CBFA2T3 (core-binding factor, alpha subunit 2, translocated to, 3) on chromosome 16 and GLIS2 (GLIS family zinc finger protein 2), also on chromosome 16, was observed. Patients with AMKL who have trisomy 3 with CBFA2T3::GLIS2 fusions are rare, and it is not known if the co-occurrence of these abnormalities is coincidental or biologically related. This highlights the continued need for further expansion of genetic testing in individuals with rare disease to establish the groundwork for identifying additional commonalities that could potentially be used to identify therapeutic targets or improve prognostication.

KEYWORDS
acute megakaryoblastic leukemia, CBFA2T3::GLIS2 fusion, inv(16)(p13.3q24.3), non-Down-syndrome-related acute megakaryoblastic leukemia, trisomy 3

INTRODUCTION
Less common in pediatrics than acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) accounts for approximately 15% of new acute leukemia diagnoses.1-3 Acute megakaryoblastic leukemia (AMKL), a rare subtype of AML in adults, is seen more frequently in pediatric patient, accounting for 4%-15% of newly diagnosed pediatric AML.4-8 AMKL can be subdivided based on patient characteristics into children with Down syndrome (DS-AMKL), children without DS (non-DS-AMKL), and adults (typically without DS).3,9 Compared to AMKL in patients with Down syndrome, non-DS-AMKL carries a poorer prognosis.4 The subtypes of AMKL also differ in their genetic alterations. Patients with Down syndrome will have trisomy 21 in combination with GATA1 mutations, whereas non-DS-AMKL pediatric patients are associated with chromosomal translocations and no association with GATA1 mutations.9

The French-American-British (FAB) classification of AML breaks the disease into categories based on morphology/differentiation, cell...
lineage, and requires a blast percentage of at least 30% in the bone marrow. The FAB classification lists AMKL as M7. The WHO classification of tumors of hematopoietic and lymphoid tissues separates AML into categories based on cell lineage cytogenetic/molecular classification, including the older FAB categories, with the addition of subtypes that correlate with specific cytogenetics, and AML associated with myelodysplasia. WHO guidelines classify our patient as having AMKL not otherwise specified.

Excluded from this definition are:

- AML with myelodysplasia-related changes (AML-MRC)
- Therapy-related AML
- AML with recurrent genetic abnormalities
  - t(1;22)(p13.3;q13.1)
  - inv(3)(q21.3q26.2)
  - t(3;3)(q21.3;q26.2)
- AMKL in patients with Down syndrome.

The combined use of molecular and cytogenetic tools is aiding in identifying subgroups of patients with AMKL to better characterize their prognosis, and potentially identify targeted therapies. Chimeric oncogenes previously identified in non-DS-AMKL have included: RBM15::MKL1, CBFA2T3::GLIS2, KMT2A gene rearrangements, and NUP98::KDM5A. CBFA2T3::GLIS2 is a gene fusion, which results from a cryptic pericentric inversion of chromosome 16. The chromosomal complements in patients with CBFA2T3::GLIS2 fusions are varied, ranging from complex karyotypes to those with complements that appear to be within normal limits. Patients with non-DS-AMKL who have CBFA2T3::GLIS2 fusions tend to be young (less than 2 years old) and have a poor prognosis.

2 | CASE REPORT

At 19 months of age our patient presented to the emergency department with language regression, decreased appetite, somnolence, pallor, hepatosplenomegaly, and clonus upon testing the left patellar reflex. The patient was found to be severely anemic with a hemoglobin of 1.3 g/dl, white blood cell count within normal range, and a platelet count of 7.0 x 10^9/L. Following seizure activity, a non-contrast computed tomography (CT) scan showed areas of bone loss throughout the skull, generalized cerebral volume loss, and soft tissue masses centered within both greater wings of the sphenoid and extending into the lateral aspects of the orbits, the middle cranial fossa, and the temporal fossa (Figure 1A,B).

Peripheral smear analysis showed an abundance of small round lymphoid cells with high nucleus to cytoplasm ratio (N:C) and normal appearing neutrophils (Figure 1C). Further inspection revealed many atypical mononuclear cells with smooth chromatin and cytoplasmic blebs (Figure 1D). The aspirate and clot sections showed a monotonous population of medium to large cells with varying N:C, smooth chromatin, prominent nucleoli, and cytoplasmic projections (Figure 2A–D). Few red blood cell precursors and fewer myeloid cells were also observed. The bone marrow core showed evidence of fibrosis and a monotonous blast cells population which was used as the primary block for immunohistochemical (IHC) staining. IHCs were partially positive for CD117 (KIT) and diffusely positive for CD33. Flow cytometry showed a blast population expressing CD33, CD117, CD56, CD41a, CD61, while negative for CD1a, CD2, surface and cytoplasmic CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD25, CD34, CD38, HLA-DR, MPO, nTdT, CD22, glycophorin A, CD36, CD123.
FIGURE 2  
(A, B) Aspirate smears demonstrating a monotonous population of atypical lymphoid cells with a relative lack of normal bone marrow complement; 
(C, D) clot sections demonstrating a monotonous population of atypical lymphoid cells with a relative lack of normal bone marrow complement, images courtesy of Virginia Commonwealth University Health System Department of Pathology.

FIGURE 3  
(A) GTG-banding studies showing trisomy 3 as the sole finding (karyotype was: 47,XX,+3 [13]/46,XX [7])
Initial GTG-banding cytogenetic studies completed on the bone marrow specimen showed trisomy 3 as the sole finding (Figure 3A) in 13 of the 20 metaphase spreads analyzed (47,XX,+3 [13]), with the remaining cells having a normal female complement (46,XX [7]). Fluorescence in situ hybridization (FISH) studies showed no clear abnormalities for the targeted regions evaluated (AML, acute lymphocytic leukemia, and myelodysplastic syndrome probe sets); however, it did show equivocal for loss (1 signal) of \( ABL1 \) in 2.5% of cells. Oncogenomic Heme v2 next-generation sequencing (NGS) panel studies showed no pathogenic single-nucleotide variants, indels, or copy number variants. However, a fusion involving \( CBFA2T3 \) (core-binding factor, alpha subunit 2, translocated to, 3) and \( GLIS2 \) (GLIS family zinc finger protein 2) was observed (Figure 4A–C), with the chimeric fusion transcript resulting from a fusion between exon 11 of \( CBFA2T3 \) and exon 3 of \( GLIS2 \). Subsequent cytogenetic testing at 4 weeks, and later at 5 months following initial diagnosis, showed normal chromosomal analysis with the exception of equivocal FISH studies for loss (1 signal) of \( ABL1 \) in 0.6% and 2.5% of cells, respectively. Follow-up NGS panel studies demonstrated no clinically significant variants (specifically, no \( CBFA2T3:GLIS2 \) fusion was detected) at 3 months. The patient later underwent allogenic stem cell transplantation at 7 months, remained asymptomatic without evidence of minimal residual disease for 6 months, then relapsed with FISH studies again showing equivocal loss for \( ABL1 \) and later a gain of \( BCL6 \) locus in 7.5% of cells.

3 | DISCUSSION

Overall, pediatric AML has a 3-year survival rate of 70%.\(^{14}\) DS-AMKL has a 100% 3-year survival rate, whereas non-DS-AMKL patients have only 34% 3-year survival on currently available therapies.\(^{14}\) Clinical presentation of AMKL varies greatly and can include anemia, thrombocytopenia or thrombocytosis, normal or moderately elevated white cell count, lytic bone lesions, hepatosplenomegaly, and can present as a mass and mimic sarcomas or metastatic solid tumors as seen in our patients orbits and cranium.\(^{15,16}\) Bone marrow aspiration is essential for diagnosing AMKL. However, it can be difficult to interpret due to the frequently concurrent extensive myelofibrosis.\(^{15}\) Once an adequate sample is obtained the observed blasts often remain small with high N:C ratio, resembling lymphoblasts.\(^{3}\) Patients may also present with both small and large blasts simultaneously. Additional findings which may be present in a peripheral smear include circulating micro-megakaryocytes, megakaryoblast fragments, dysplastic large platelets, and hypogranular neutrophils.\(^{3}\) Due to the varying clinical presentations and wide variety of observable cellular abnormalities, ancillary testing is essential and may include IHC staining, flow cytometry, cytogenetics, and molecular diagnostics.

Chromosome analysis completed on the initial bone marrow specimen from this patient revealed trisomy 3. A similar case was published in 2018 of a 16-month-old female diagnosed with non-DS-AMKL with concurrent trisomy 3.\(^9\) At presentation, her hemoglobin was 9.7 g/dl and platelet count of 29 \( \times 10^9/\mu l\). She had an additional chromosome 3 and a balanced translocation between chromosome 7 and 17 (karyotype was: 47,XX,+3,t(7;17)(p15;q25) [3]/46,XX [7]).\(^9\) She was treated with the AML-BFM 98 protocol and, at the time of publication, was stable preparing for reinduction chemotherapy.\(^9\) A gene of interest that maps to chromosome 3 EVI I (Ectopic Viral Insertion Site 1), also known as MECOM, is related with poor prognosis when rearranged in patient with AML.\(^{17,18}\)

While uncommon, trisomy 3 has been noted in patients with primary AMKL, as either a sole finding (five of 10 cases [including this current report]), or in conjunction with other chromosomal findings (five of 10 cases, with three of these five patients having complex
Previously reported patients with AMKL who have trisomy 3 with CBFA2T3:GLIS2 fusions are rare. These patients were young (mean of 1.8 years; Table 1) and were also predominantly female (nine females: one male). It is not known if the occurrence of these abnormalities is coincidental or biologically related. Continued testing by clinicians and pooling of data will aid in the characterization of both cytogenetic and molecular findings present in patients with non-DS-AMKL and its subgroups.

Genetically, patients with pediatric non-DS-AMKL are distinct from those who have Down syndrome. There are varieties of chimeric oncogenes associated, including RBM15::MKL1, NUP98::KDM5A, and CBFA2T3::GLIS2, as well as KMT2A gene rearrangements. An increase in bone morphogenetic protein (BMP) signaling due to CBFA2T3::GLIS2 expression has been demonstrated in Drosophila and murine hematopoietic cells, leading to increased self-renewal of hematopoietic progenitor cells. Gruber and Downing also suggest, due to CBFA2T3::GLIS2 expressing cells being growth factor dependent in vitro, that there must be another cooperative mutation involved in growth factor signaling for the development of AMKL. Known examples of the these regions identified in melanoma and T-cell ALL include TERT promoter mutations and super-enhancer formation upstream of the TAL1 oncogene, respectively.

Currently there is no specifically tailored treatment for CBFA2T3::GLIS2 non-DS-AMKL. Depending on the institution, treatment options can include two courses of intensive induction chemotherapy followed by either cytarabine-based consolidation or hematopoietic cell transplantation if remission is achieved. It is our belief that continued investigation into whole-genomic sequencing to aid in the development of gene-specific therapies for clinical trials is essential to improve the poor prognosis associated with non-DS-AMKL. Characterization of both cytogenetic and molecular findings present in patients with non-DS-AMKL could help to identify potential associations of genetic findings that might aid in recognizing subgroups within this heterogeneous condition and shed additional light on its genomic profile. Recognition of the contribution of CBFA2T3::GLIS2 fusion to the leukemogenic process, and targetable drugs for their management, are evolving. For the achievement of this goal, for such a rare disease as AMKL, international cooperation and collaboration between medical communities is paramount.

### CONFLICT OF INTEREST
No conflict of interest is present for all authors. Nothing to disclose.

### DATA AVAILABILITY STATEMENT
The data that support the findings of this study are not publicly available due to privacy or ethical restrictions.

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**TABLE 1** Pediatric patients with non-DS-AMKL and trisomy 3

| Patient | Age (years) | Sex | Diagnosis | WBC ($\times$10^9/L) | Karyotype | CBFA2T3::GLIS2 Fusion | Treatment/ outcome | Reference |
|---------|-------------|-----|-----------|----------------------|-----------|----------------------|-------------------|-----------|
| 1       | 1.7         | F   | AMKL      | 9.8                  | 47,XX,+3[13]/46,XX [7] | +         | 1.2/Stable          | Current case |
| 2       | 2           | F   | AMKL      | NA                   | 47,XX,+3[4]/46,XX [21] | Not tested | 3/Relapsed; Demised | 19         |
| 3       | 0.6         | F   | AMKL      | 10.5                 | 47,XX,+3[2]/46,XX [28] | +         | 4/No relapse; Demised | 12         |
| 4       | 3.3         | F   | AMKL      | 19.6                 | 47,XX,+3[7]/46,XX [13] | +         | 4/No relapse          | 12         |
| 5       | 1.7         | F   | AMKL      | 24.6                 | 47,XX,+3[2]/46,XX [24] | +         | 4/NA                 | 12         |
| 6       | 3.3         | F   | AMKL      | 13.1                 | 49,XX,+3,-6.del(13)[q12q14],+21[11]/46,XX [9] | –         | 4/NA                 | 12         |
| 7       | 1.4         | F   | AMKL      | NA                   | 47,XX,+3,t(7;17)[p15;q25] [3]/46,XX [7] | Not tested | 4/Stable             | 9          |
| 8       | 1.4         | M   | AMKL      | 12.3                 | 47,XY,+3 | –                  | 5/Relapsed; Demised | 20         |
| 9       | 1.7         | F   | AMKL      | 6.1                  | 47,XX,+3,t(11;16;17)[q13;q24;q21]/46,XX [15] | –         | 5/Relapsed; Demised | 20         |
| 10      | 5           | F   | AMKL      | 4.6                  | 52,XX,+X,+3,t(9;11)[p22;q23],+12,+15,+19,+21 | Not tested | NA/NA                | 21         |

*a*, Positive for fusion; *, fusion not detected.

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