A neonate with a unique non-Down syndrome transient proliferative megakaryoblastic disease

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Abstract
Transient myeloproliferative disorder (TMD) is a leukemia type that occurs typically in newborns. In Down syndrome, TMD is referred to as transient abnormal myelopoiesis (TAM). Recently, transientness has also been reported in acute myeloid leukemia patients with germline trisomy 21 mosaicism, and even in cases with somatic trisomy 21, with or without GATA1 mutations. TMD cases without trisomy 21 are rare, and recurrent genetic aberrations that aid in clinical decision-making are scarcely described. We describe here a TMD patient without trisomy 21 or GATA1 mutation in whom single-nucleotide polymorphism analysis of leukemic blasts revealed a novel combined submicroscopic deletion (5q31.1–5q31.3 and 8q23.2q24).

KEYWORDS
Down syndrome, EXT1, GATA1, non-Down syndrome, transient abnormal myelopoiesis, transient myeloproliferative disorder, transient leukemia, transient myeloproliferative disease, trisomy 21

1 | INTRODUCTION

Transient myeloid leukemia occurs typically in newborns with Down syndrome (DS) and is classified by the WHO as transient abnormal myelopoiesis (TAM).²⁸,³² Clinical characteristics of TAM vary from asymptomatic presentation to serious conditions with hepatosplenomegaly, breathing problems and hemorrhage, based on megakaryocytic hyperproliferation, leukocytosis, anemia and thrombocytopenia.¹,²⁰,²⁸,³¹,³⁴ Germine trisomy 21 and prenatal acquired somatic GATA1 mutations are considered to be pathognomic for TAM.¹–³⁶,₂⁹,³¹ More recently, transient leukemia has also been reported in patients with germline trisomy 21 mosaicism and even with somatic trisomy 21, with or without GATA1 mutations.²,³

Non-DS transient myeloid leukemia without germline or somatic trisomy 21 is very rare, and these cases are challenging with regard to clinical decision-making, as the alternative to a watch-and-wait strategy is highly intensive treatment. TMD has mainly been described in hyperproliferative germline RAS-mutated transient juvenile myelomonocytic leukemia (JMML) or oligoclonal monoblastic TMD cases with germline THPO gene mutations.⁴,²⁰ In addition, transient myelomonocytic leukemia harboring t(8;16) has been reported.⁵ In transient non-DS acute myeloid leukemia (AML) M7 cases without any trisomy 21, only trisomy 12 and subclonal deletions of chromosome 7, 8 and 13 have been reported. We describe here a non-DS TMD patient without (subclonal) trisomy 21 in whom single-nucleotide polymorphism (SNP) analysis of leukemic blasts revealed a novel submicroscopic genetic aberration.

2 | CASE REPORT

We screened the patient (35.5/7 weeks gestation) at the age of 12 days with myeloid blasts in peripheral blood (PB) (Fig. 1), without phenotypical DS suspicion, hepatosplenomegaly, or other clinical leukemia signs. Mild dysmorphic signs included straight eyebrows, merging into the lateral hairlines, a long philtrum and thin upper vermilion of the

Abbreviations: AMKL, acute megakaryoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CN-LOH, copy-neutral loss-of-heterozygosity; CN-loss, copy number loss; DS, Down syndrome; FISH, fluorescence in situ hybridization; JMML, juvenile myelomonocytic leukemia; PB, peripheral blood; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; TAM, transient abnormal myelopoiesis; TMD, transient myeloproliferative disorder; WBC, white blood cells.
lip. Details on blood counts and immunophenotyping of PB and bone marrow (BM) at birth and day 12 are depicted in Fig. 1.

Conventional karyotyping (32 metaphases) and fluorescence in situ hybridization (FISH) analysis using a chromosome 21-specific probe LSI 21 (Abbott, Downers Grove, Illinois) (100 metaphases) on phytohemagglutinin stimulated PB T-lymphocytes revealed normal female karyotype (46,XX) without (mosaic) trisomy 21; t(15;17), t(8;21), inv(16), t(16;16), t(4;11), t(9;11), t(11;19), t(10;11) and young age-related translocations t(8;16), t(7;12) and KMT2A-fusion transcripts were absent. In addition, no GATA1, THPO, NPM1, FLT3-ITD, C-KIT, CEPBA, WT1 or RAS gene mutations were identified. Specific acute megakaryoblastic leukemia (AMKL) related fusion genes (i.e. NUP98/KDM5A, NUP98/NSD1, KAT6A/CREBBP, CBF2/RS1/GUS2, RBM15/MKL1 and MNX/ETV6) were not found. GATA1 mutation analysis (with detection limit of 5% in our hands) was negative.

The premature state of the child, the absence of clinical signs and the absence of genetic/molecular aberrations associated with AML/MMML encouraged us to choose the careful watch-and-wait strategy by consensus of the pediatric oncology team. The non-DS AMKL clone resolved spontaneously and at the age of 4 months, blood counts had completely recovered and no clone could be identified by SNP analysis anymore (Fig. 1). The cytogenic clone was no longer present at days 83 and 132 (BM).

Based on the lack of recurrent aberrations by standard analysis, an extended search for genomic alterations was performed using CytoSNP-850K SNP array analysis (build Hg 19 BlueGnome®, Illumina Inc., San Diego, California) of PB (day 17/day 83) and BM (day 26/day 132). Copy number loss (CN-loss) of a 10.6 Mb region on chromosome 5 (breakpoints, bands 5q31.1 and 5q31.3; minimally deleted region 130,813,377-141,386,638 (hg19), including the EGR1 gene) and CN-loss of a 17.0 Mb region on chromosome 8 (breakpoints, bands 8q23.1 and 8q24.13; minimally deleted region 108,408, 409-125,438,835, including the EXT1 gene) were both identified in approximately 30% of PB blasts (day 17) and 10% of BM blasts (day 26) (Fig. 2 and Supplementary Fig. S1). FISH analysis using 5q31(EGR1) and 8q24(EXT1) probes on metaphases (cultured PB, day 17) demonstrated cryptic del(5)q and del(8)q to be within the same clone (data not shown). At days 83, 132 and 211, there was no longer evidence in this patient for these deletions (PB and BM), demonstrating the somatic nature of these subclonal, potential drivers of malignant hematopoiesis and consistent with the transient nature of the AMKL.

At the age of 11 months, the patient developed AML M7 (47% leukemic blasts, PB). Using chromosome banding, FISH and SNP analyses (BM), the 8q24 clone was identified with a clonal evolution (additional trisomy chromosome 6 and trisomy chromosome 19, 20% mosaicism), as well as a second independent clone with copy-neutral loss-of-heterozygosity (acquired uniparental disomy) of the long arm of chromosome 5 (from band 5q11.2 to 5qter), with ~25% mosaic (Fig. 2c), but 5q31.1-5q31.3 negative. Currently, 4 months later, the patient is in complete remission and MRD-negative after two cycles of standard AML induction treatment.

3 | DISCUSSION

The differential diagnosis of myeloid hyperproliferation in infants includes JMML, AML (mainly FAB-M4, M5 and M7), TAM (DS) and other types of TMD. The challenge in clinical practice is to separate transient from aggressive infant leukemia types that require immediate intensive treatment. This discrimination can be hard in patients who do not reveal typical genetic aberrations.

In our patient, a megakaryoblastic clone (AML-FAB-M7) was identified in the absence of a GATA1 mutation and (mosaic) trisomy 21. The possible option of transient leukemia (i.e. TMD) always needs to be considered in such young children, especially in DS cases, in which case, it is referred to as TAM. In non-DS TMD, only a few other recurrent somatic or germline aberrations have been described. In patients with non-M7/non-DS TMD, these include somatic t(8;16), trisomy 12 with GATA1 mutation, del(8)(q23.2q24), del(5q) and del(13)(q13;q31), as well as germline THPO mutations and del(13)(q12.11) aberrations. In our patient, TMD-related aberrations and lack of clinical features allowed to carefully watch-and-wait, but only after excluding typical somatic translocations associated with aggressive AML M7.

Currently, a clinical guideline for suspected TMD cases including genetic aberrations is being developed by the International Berlin-Frankfurt-Müster-AML Study Group, based on a worldwide collection of clinical and genetic data and reviewed literature (Supplementary Table S1). We here show that, in addition to conventional genetic testing such as karyotyping and mutational analyses, further genome-wide analysis can aid in the confirmation of monoclonality in rare individual non-DS TMD cases.

In our patient SNP array analysis at presentation revealed a somatic cryptic deletion(5)(q31.1-q31.3), including the EGR1 gene. It is conceivable that the submicroscopic EGR1 gene size hampered the detection of this aberration by conventional karyotyping. Future evaluations of
more infant AML cases are necessary to reveal the recurrence and relevance of this gene for leukemogenesis in TMD. The other identified aberration was del(8)(q23.2q24), which was previously described once in TMD. This deletion harbors the EXT1 gene, which encodes a glycoprotein that enhances cellular growth, adhesion, invasiveness and metastatic potential. EXT1 is considered to be a tumor suppressor gene, and EXT1 downregulation by hypermethylation of the EXT1 promoter has been shown to reduce heparan sulphate synthesis, thereby deregulating hematopoiesis. Interestingly, one previous study in DS patients addressed the role of EXT1 hypermethylation in the progression of TMD to DS-AMKL. The fact that clonal evolution (+6,+19) at time of AML recurrence in our patient at the age of 11 months occurred in the clone harboring 8q(q23.2q24) is suggestive of a leukemia-driving role of aforementioned genes at that hotspot. This is underscored by the fact that the AML M7 presented transiently with one clone containing both the 5q- and 8q-, whereas at reemergence the leukemia was biclonal: one clone with a de novo acquired uniparental disomy 5q (25% mosaic) and one clone with 8q- (no 5q-, +19,+6 [20% mosaicism]).

Hence, we add combined 8q(q23.2q24) and 5q(q31.1q31.3) deletions to the list of recurrent aberrations in which "watch-and-wait" could be considered in neonatal AMKL cases (Supplementary Table S1) without trisomy 21 and mutated GATA1. It needs to be confirmed whether such cases are at substantial risk to develop leukemia at a later stage and what drives this clonal evolution. Therefore, ongoing international collaboration remains important.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

REFERENCES


SUPPORTING INFORMATION

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