Neonatal congenital leukemia caused by several missense mutations and AFF1-KMT2A fusion: A case report

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Abstract. Neonatal leukemia, a congenital form of leukemia, is a rare and fatal disease occurring in the neonatal period. Its etiology and pathogenesis have remained to be fully elucidated and the clinical manifestations differ due to age variability. Acute myeloid leukemia (AML) occurring after birth indicates genetic abnormalities and possibly intrauterine exposure to radiation, drugs or other toxins. The present report described the case of a premature neonate without phenotypic signs of Down syndrome, but with an elevated white blood cell count, mainly pertaining to the monocytes of peripheral blood. At 31 weeks of gestation, delivery by Caesarean section was performed due to fetal distress; however, the infant died three days after birth. Further laboratory examination indicated pediatric myeloid leukemia. The present case report described a case of fetal AML. According to the results of peripheral blood smear and targeted-panel sequencing, 5 missense mutations with clinical significance and a novel AFF1-KMT2A fusion gene were detected, which may be the main causes of AML and death.

Introduction

Acute leukemia is a malignant clonal disease originating from progenitor or multi-potential progenitor cells (1). Acute leukemia may be classified into either myeloid or lymphoid lineages according to the expression of several key antigens (2). Congenital and neonatal leukemia occur rarely and are associated with high mortality rates; they may be stratified into morphologically or genetically defined subtypes (1). The etiology, pathogenesis and biology have historically been poorly comprehended, reflected in the ambiguity of classification and nomenclature (3). Acute myeloid leukemia (AML) at birth indicates genetic abnormalities and possibly intrauterine exposure to radiation, drugs or other toxins (3). An estimated 21,450 new cases of AML occurred in the US in 2019 and the 5-year survival for patients with AML is 28.3%. Females have a higher risk of developing infant leukemia than males (4). AML diagnosis may be made either from a peripheral blood sample or bone marrow biopsy, depending on white blood count and circulating blasts. Flow cytometric (FCM) analysis is helpful in the diagnosis of AML and useful after treatment to evaluate AML persistence (5). Specific chromosomal rearrangements and certain site mutations have been identified in congenital leukemia. Infants diagnosed with congenital leukemia require thorough investigative workup and extensive supportive care. Although the prognosis is poor, the recent use of high-intensity multiagent chemotherapy regimens has produced promising results (6). The present study reported on a neonate who presented with massive hepatomegaly and various neonatal diseases. Based on ultra-deep sequencing, the patient was finally diagnosed with AFF1-KMT2A fusion-positive AML.

Case report

Clinical presentation. In March 2021, a pregnant 24-year-old female patient was admitted to Shaoxing Women and Children's Hospital (Shaoxing, China) at 31 weeks of gestation due to reduced fetal movement for 3 days. Due to fetal distress in the uterus, a female premature infant weighing ~1,600 g was delivered by Caesarean section with IIIrd degree amniotic fluid contamination. The newborn was in a comatose state with no milk-suckling, no bowel movements, no autonomous respiration, neonatal pneumonia and non-traumatic intracranial hemorrhage with neurological symptoms.

Laboratory findings. Hematologic examination revealed that the number of white blood cells (WBC) was increased to $617.57x10^9$/l [normal range (NR), $15.0\text{-}20.0x10^9$/l], accompanied with a red blood cell count of $3.31x10^{12}$/l (NR, $5.0\text{-}6.4x10^{12}$/l), in addition to a hemoglobin content of 75 g/l (NR, 180-190 g/l), platelet count of $64x10^9$/l (NR, $203\text{-}653x10^9$/l), and a percentage of monocytes, lymphocytes, neutrophil granulocytes, eosinophil granulocytes and basophil granulocytes of 48.6% (NR, 3-10%), 45.6% (NR, 40-60%), 3.1% (NR, 31.0-40.0%), 0% (NR, 0.4-8.0%) and 2.7% (NR, 0-1%), respectively.

Arterial blood gas analysis indicated hypoxemia and acidosis. The concentration of potassium in the blood had

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reached as high as 7.70 mmol/l (NR, 3.5-5.3 mmol/l). The pH value was 7.05 (NR, 7.35-7.45), and the arterial lactic acid concentration was 18 mmol/l. Biochemistry analysis of serum indicated a sharp increase to various degrees in aspartate aminotransferase, adenosine deaminase, alkaline phosphatase, γ-glutamyltransferase, lactic dehydrogenase, total bilirubin total, direct bilirubin and indirect bilirubin, which suggested severe liver damage. Their values were 342 U/l (NR, 13-35 U/l), 141 U/l (NR, 4-18 U/l), 560 U/l (NR, 48-406 U/l), 427 U/l (NR, 7-45 U/l), 8,795 U/l (NR, 120-250 U/l), 46.6 μmol/l (NR, 5.0-21.0 μmol/l), 26.6 μmol/l (NR, <3.4 μmol/l) and 20.0 μmol/l (NR, 1.0-16.0 μmol/l), respectively.

A peripheral blood smear indicating a small number of immature cells revealed the following: Most cells, varied in size and dyed purple on Wright staining, were circular in shape, exhibited cytoplasm reduction, swelling of nucleus. Under oil immersion lens of microscopy, blasts of varying sizes were observed (Fig. 1A) and granules were seen in the blast (labeled by black arrow) (Fig. 1B). It was concluded that the diagnosis of the present case of neonatal leukemia was probably AML.

Targeted panel sequencing and bioinformatics. Sequencing was performed to determine the pathogenesis of neonatal leukemia. Genomic DNA (gDNA) and RNA were isolated from the patient's whole blood specimen using the QIAamp DNA Blood Mini Kit and the PAXgene Blood RNA Kit (both from Qiagen GmbH), respectively. For mutation analysis, gDNA of an adequate quantity and quality was fragmented to a size ranging from 200 to 400 bp, followed by adaptor ligation. Adaptor-ligated DNA underwent hybrid capture using a HEME mutpanel that contained 505 genes related to hematological malignancies. For fusion analysis, a minimum of 1 μg total RNA was subjected to rRNA depletion, followed by canonical RNA-Seq library construction. The resulting cDNA library was hybridized with a capture HEME-fuse panel, consisting of 99 fusion genes. The entire capture process was performed according to the manufacturer's protocol using reagents supplied by Integrated DNA Technologies. The captured libraries were sequenced with a NovaSeq 6000 (Illumina, Inc.) and 150 bp paired-end sequence data were generated for fusion analysis. NGS service consisting 505 genes and 99 fusion genes was provided by MEDx Translational Medicine Co. Ltd.

The sequence data were aligned to the reference human genome (GRCh37) and subjected to adaptor trimming and sequencing quality control. Single nucleotide variants with a variant allele fraction >1%, as well as small insertions and deletions <50 bp in size were detected using Varscan v2.3.9. Possible germline polymorphisms were filtered out if the allele frequency was > 0.1% in the Genome Aggregation Database (http://gnomad.broadinstitute.org/). Fusion events were analyzed using STAR-FUSION v1.5 (https://github.com/STAR-Fusion/STAR-Fusion/wiki). As presented in Fig. 2, five missense mutations, namely MutS homolog 6 (MSH6) K854M (Fig. 2A), Rat sarcoma of NIH3T3 (NRAS) Q61K (Fig. 2B), phospholipase C gamma 2 (PLCG2) T396S (Fig. 2C), tyrosine kinase 2 (TYK2) Y1080C (Fig. 2D) and Runt related transcription factor 1 (RUNX1) S424A (Fig. 2E), as well as AF4/FMR2 family, member 1 (AFF1)-lysine methyltransferase 2A (KMT2A) fusion (Fig. 2F), were detected. All somatic missense site mutations are shown in Table I.

Interventions, outcomes and lessons. All of the tests and clinical manifestation indicated that the newborn was suffering severe asphyxia, shock, neonatal pneumonia, leukemia, hypoxic-ischemic encephalopathy, intracranial haemorrhage, hypoglycaemia and acidosis. The patient was shifted to salvage chemotherapy with blood pressure and blood sugar maintenance, as well as oxygen supply to relieve seizures, cerebral edema and syndrome of the brain stem. Intubation and ventilator support were administered after parental informed consent. In spite of accurate corresponding emergency rescue measures implemented in a timely manner, such as correction of acidosis and anemia, as well as vitamin K1 and calcium gluconate injection, the neonate died two and a half days after birth. FCM and bone marrow biopsy were all missed. In general, FCM analysis of peripheral blood and bone marrow biopsy may be used for making a definite diagnosis of leukemia; low age and a high initial WBC count are high-risk factors (6). Combined analysis of morphology, immunology, cytogenetics and molecular biology for leukemia typing were insufficient. The remaining blood samples were subjected to deep sequencing for analysis of site mutations and fusion genes. The Institutional Review Board of Shaoxing Women and Children's Hospital (Shaoxing, China) approved this retrospective case study.

Discussion

Due to reduced fetal movement for 3 days and fetal distress in the uterus, the mother was admitted to hospital at 31 weeks of gestation and gave birth to a premature female infant by Caesarean delivery. According to the hospitalization records, the expectant mother denied any history of exposure to toxins or radiation and prenatal genetic testing indicated a low risk. From the first trimester on, 0.4 mg folic acid was supplemented daily. The results of regular prenatal detection and three-dimensional ultrasonic imaging revealed that maternal nutrition and fetal development were all normal until 3 days prior to hospitalization due to decreased fetal movement.

The infant was born prematurely with a body weight of 1.6 kg and IIIrd degree amniotic fluid contamination. The neonate was weak due to numerous types of neonatal disease, as mentioned earlier. Of note, the infant's WBC in the peripheral blood outdistanced the normal range, particularly the monocyte count, with a sharp increase. Follow-up routine peripheral blood smear indicated that both monocytes and lymphocytes were all atypical and immature. Hyperactive monocyte proliferation suggested a high probability of monocytic leukemia. Children presenting with multiple leukemias were more likely to suffer from genetic predisposition. In order to determine the cause of the pathology of the present case, gene detection of the hematologic tumor, was performed and 505 genes and 75 fused genes were analysed using targeted panel sequencing. K854M of MSH6, Q61K of NRAS, T396S of PLCG2, Y1080C of TYK2, S424A of RUNX1 and AFF1-KMT2A fusion were detected.

K854M in the MSH6 gene was reported to be associated with hereditary nonpolyposis colorectal cancer (7). In a recent study, NRAS mutations were discovered in 13% of patients with AML (152 of 1,149), and Q61K and Q61R substitutions of NRAS frequently occurred (8). Mutations in PLCG2 are

Table I. List of all site somatic mutations of the patient.

Gene	Transcript no.	Nucleotide	Amino acid	Exon location	Variation pattern	Variation ratio (%)
KDR	NM_002253.3	c.3724A>G	p.Ile1242Val	Exon 28	Missense mutation	51.54
CD79B	NM_001039933.1	c.221A>C	p.Asn74Thr	Exon 3	Missense mutation	51.17
NTRK3	NM_001012338.1	c.278C>T	p.Thr93Met	Exon 4	Missense mutation	49.47
PLCG2	NM_002661.1	c.1187C>G	p.Thr396Ser	Exon 13	Missense mutation	49.20
MLH3	NM_001040108.1	c.1879T>C	p.Phe627Leu	Exon 2	Missense mutation	48.33
KCNT1	NM_020822.1	c.3139G>A	p.Val1047Ile	Exon 27	Missense mutation	47.66
TYK2	NM_003331.4	c.3239A>G	p.Tyr1080Cys	Exon 23	Missense mutation	47.62
CROCC	NM_014675.4	c.3635G>A	p.Arg1212His	Exon 24	Missense mutation	47.26
GRIK4	NM_014619.4	c.664T>G	p.Ser222Ala	Exon 7	Missense mutation	46.96
DLC1	NM_182643.2	c.1664T>C	p.Val555Ala	Exon 9	Missense mutation	46.04
KRT79	NM_175834.2	c.266G>A	p.Gly89Asp	Exon 1	Missense mutation	45.85
MLLT10	NM_004641.3	c.2552C>T	p.Thr851Ile	exon2 1	Missense mutation	45.68
NSD1	NM_022455.4	c.1852A>G	p.Lys618Glu	Exon 5	Missense mutation	45.58
MSH6	NM_000179.2	c.2561A>T	p.Lys854Met	Exon 4	Missense mutation	43.96
NRAS	NM_002524.4	c.181C>A	p.Gln61Lys	Exon 3	Missense mutation	43.65
HYDIN	NM_001270974.1	c.11803C>T	p.Gln3935*	Exon 70	Nonsense mutation	43.65
CSMD3	NM_198123.1	c.7520A>G	p.Lys2507Arg	Exon 48	Missense mutation	42.53
CACNA1G	NM_018896.4	c.4382G>A	p.Arg1461Gln	Exon 23	Missense mutation	42.32
CACNA1B	NM_000718.3	c.2990C>T	p.Thr997Met	Exon 19	Missense mutation	22.14
RUNX1	NM_001754.1	c.1270T>G	p.Ser424Ala	Exon 9	Missense mutation	11.25
MLLT1	NM_005934.3	c.805A>C	p.Lys269Gln	Exon 6	Missense mutation	3.85

A total of 505 genes related to hematological malignancies were analysed by targeted panel sequencing and bioinformatics; all variations consisting of 20 missense mutations and 1 nonsense mutation were listed and arranged in the order of the variation ratio.

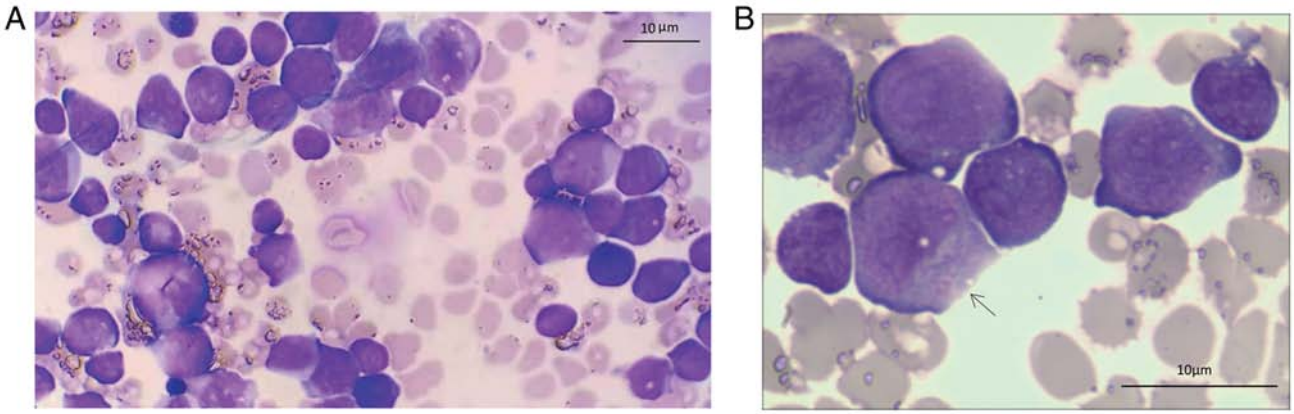

Figure 1. (A) Peripheral blood smear with Wright staining. Under oil lens microscopy, blasts of varying sizes were observed. (B) Enlargement of blasts with granules was present (black arrow; Wright staining) (scale bars, 10 μm).

found in most patients with chronic lymphocytic leukemia and have been assumed to be the causative drivers of ibrutinib resistance (9). TYK2 is a member of the Janus kinase family involved in cytokine signal transduction in immune and haematopoietic cells. TYK2 variants were found in 25.8% of cases of B-acute lymphoblastic leukemia, which is the most frequent childhood cancer and accounts for 25% of adult acute leukemias (10). The RUNX1 gene, a member of the transcription factor family, has a critical role in myeloid differentiation and hematopoietic stem cell emergence and regulation (11). Mutations in RUNX1 were detected in 9.1% of AML and 13.9% of myelodysplastic syndrome cases (12).

A 3,969 aa nuclear protein encoded by KMT2A/mixed-lineage leukemia (MLL) is divided into two parts through proteolysis by Taspase1 and then dimerizes to generate the functional unit, which is essential for normal hematopoiesis (13). MLL rearrangements (MLL-r) originated *in utero* is a devastating malignancy with a dismal prognosis, which exhibits a clear correlation with age. It accounts for ~70% of acute leukemias in infants. MLL-r occurs in 5% of childhood

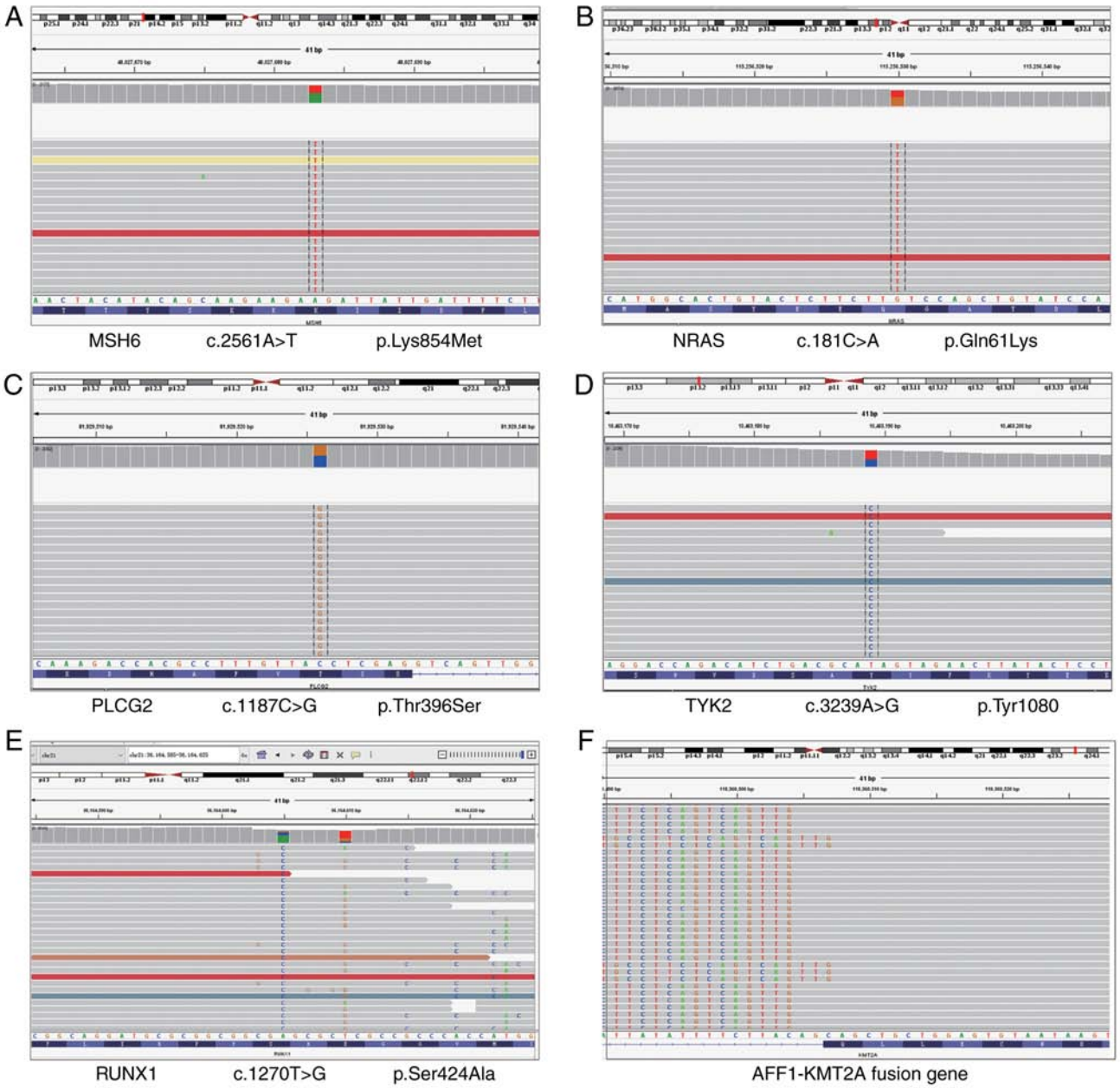

Figure 2. Representative mutations and fusion genes with clinical significance. (A) MSH6 K854M, (B) NRAS Q61K, (C) PLCG2 T396S, (D) TYK2 Y1080C, (E) RUNX1 S424A and (F) AFF1-KMT2A fusion. MSH6, MutS homolog 6; NRAS, rat sarcoma of NIH3T3; PLCG2, phospholipase C gamma 2; TYK2, tyrosine kinase 2; RUNX1, Runt-related transcription factor 1; AFF1, AF4/FMR2 family, member 1; KMT2A, lysine methyltransferase 2A.

ALL cases, 70-80% of ALL in infants, 15-20% of childhood AML and 50% of infant AML cases. MLL-r leukemia has long been suspected to originate from an uncommitted precursor (14). MLL-r results in the fusion of the N-terminus of MLL with the C-terminus of a partner. A total of 79 different MLL partner genes have now been identified. In infant ALL, 4 partner genes account for 93% of cases: AF4 (49%), eleven-nineteen leukemia (22%), AF9 (17%) and AF10 (5%). In infant AML, 3 partner genes account for 66% of cases: AF9 (22%), AF10 (27%) and ELL (17%) (4). MLL and AF4 are fused in a balanced recombination event to cause the generation of the two fusion genes MLL-AF4 and AF4-MLL. Fusion proteins bind directly to their target gene and upregulate gene expression by increasing H3K79me2 through disruptor of telomeric silencing 1-like recruitment (15). The most frequent rearrangement is MLL-AF4, which is relatively common in ALL but uncommon in AML (16).

The age at diagnosis is an important predictor of prognosis, regardless of the therapeutic approach (1). Infant leukemia refers to acute leukemia diagnosed prior to 1 year of age. Infant leukemia is rare but requires much attention from clinicians to due to aggressive clinical presentation and poor prognosis (1). The patient of the present study was prematurely delivered at our hospital and diagnosed with a series of severe neonatal illnesses of the central nervous system, respiratory system and hematologic system. According to the progress notes, prenatal screening indicated no Down's syndrome (DS) and other congenital genetic defects in the first trimester. Pediatric patients with DS have an increased risk of both ALL and AML (17). Children are vulnerable to the neurotoxic effects of

chemicals, radioactive exposure, certain elements and heavy metals, air pollutants and amniotic fluid contamination, particularly in the prenatal period. The mother denied any history of the above-mentioned items, and the mother and her fetus were healthy in the early pregnancy. The etiology of infant leukemia is not fully clarified and cannot be fully explained; single-gene and chromosomal defects are only partially responsible for the pathology of the present case. In the present study, sequencing was used to gain insight into the cellular and molecular factors that drive neonatal leukemia. Based on all the available data, AFF1-KMT2A fusion may be the key factor, while missense mutations at other sites may have contributed to the pathogenesis. Contaminated amniotic fluid and poor living conditions may also contribute to progression and exacerbation. All mothers-to-be must avoid contact with any harmful factors mentioned above.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BQ and JD designed the study and obtained funding support. BQ, XD and JD performed the research; BQ analyzed data and wrote the manuscript. BQ, XD and JD confirm the authenticity of all the raw data All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study involving human participants was reviewed and approved by the Institutional Ethics Committee of Shaoxing Maternity and Child Health Care Hospital (Shaoxing, China).

Patient consent for publication

Written informed consent was provided by the infant's mother.

Competing interests

The authors declare that they have no competing interests.

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