

A Long-term Survivor after Congenital Acute Myeloid Leukemia with t(8 ; 16)(p11 ; p13)

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The treatment of patients with congenital leukemia is difficult and often results in a poor prognosis. We present here the case of a female child with congenital acute myeloid leukemia (AML) with t(8 ; 16)(p11 ; p13) who received chemotherapy and survived for more than 10 years without relapse. A novel *MOZ-CBP* chimera was found in her diagnostic sample. Although adult AML patients with *MOZ-CBP* have mainly been reported as having therapy-related AML and showed poor prognoses, the present case supports the idea that AML with *MOZ-CBP* in the pediatric population might show better prognoses.

Key words: congenital leukemia, AML, t(8 ; 16)(p11 ; p13), *MOZ-CBP*

Congenital leukemia has been described as difficult to treat and as showing very poor prognoses. Bresters *et al.* reviewed clinical data of patients with congenital leukemia reported in the literature from 1975 to 2000 and defined the clinical characteristics of 73 patients with congenital acute myeloid leukemia (AML): 63.3% showed complete remission and a 24.4% probability of overall survival at 24 months of follow-up [1]. Ishii *et al.* reported the features and outcomes of 11 neonatal leukemia patients in Japan including 3 neonatal AML patients: 2 of the neonatal AML patients died within 1 month of birth and the third patient had been alive for > 7 years in complete remission as of the time of that report [2]. Congenital leukemia seems to start in utero, and

thus a portion of intrauterine fetal deaths might be explained by congenital leukemia.

Congenital AML is more frequent compared to congenital acute lymphoid leukemia (ALL). Some congenital AML patients who had t(8 ; 16)(p11 ; p13) showed relatively better prognoses compared to other congenital AML patients [3]. The reciprocal chromosomal rearrangement t(8 ; 16)(p11 ; p13) is characterized by disruption and fusion of *MYST3/MOZ* on chromosome region 8p11 to *CBP/CREBBP* on 16p13, which encodes a histone acetyltransferase and a transcriptional co-activator/acetyltransferase, respectively [4, 5]. This rearrangement generates a *MOZ-CBP* fusion protein, and it inhibits AML1-mediated transcription, resulting in a differentiation block. Although the pathogenic pathway of AML with t(8 ; 16)(p11 ; p13)

Received April 22, 2015; accepted September 25, 2015.

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Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

is mostly unclear, *MOZ-CBP* fusion transcripts could play a role—such as leading to disruption in the balance between proliferation and differentiation during hematopoiesis [6].

We present here the case of a female patient with congenital AML with t(8;16)(p11;p13) who survived for more than 10 years without relapse. We found a novel transcript of *MOZ-CBP* fusion in this case. We also discuss the type of *MOZ-CBP* fusion transcripts and its prognosis.

Materials and Methods

Patient. The patient was born at gestational age 37 weeks and 6 days by caesarian section due to maternal severe toxemia of pregnancy (birth weight 1,988 g). Multiple skin nodules were observed (Fig. 1A), and a skin biopsy suggested myeloid sarcoma. A bone marrow smear showed increased CD13⁺ and CD33⁺ monoblasts by flow cytometry (Fig.

1B). A karyotype analysis showed 46, XX, t(8;16)(p11;p13) [7/20] (Fig. 1C). *MLL*-rearrangement was not observed by a fluorescence *in situ* hybridization (FISH) analysis. She was diagnosed with AML FAB M5b, and AML-oriented induction chemotherapy, etoposide, cytarabine and mitoxantrone (ECM), was started in accord with the AML99 study [7]. Complete remission was achieved and followed by five consecutive cycles of consolidation chemotherapy with dose reduction, due to adverse effects. After discharge from the hospital, she was healthy for more than 10 years without relapse or the development of a short stature. Informed consent was obtained from the patient and her parents.

Sequencing analysis. Total RNA was extracted from the patient's first diagnostic bone marrow sample using the QIAGEN RNeasy Mini Kit (QIAGEN, Hilden, Germany), and 1–2 ng total RNA was reverse-transcribed to cDNA using the RETROscript Kit (Ambion, Austin, TX, USA) according to the

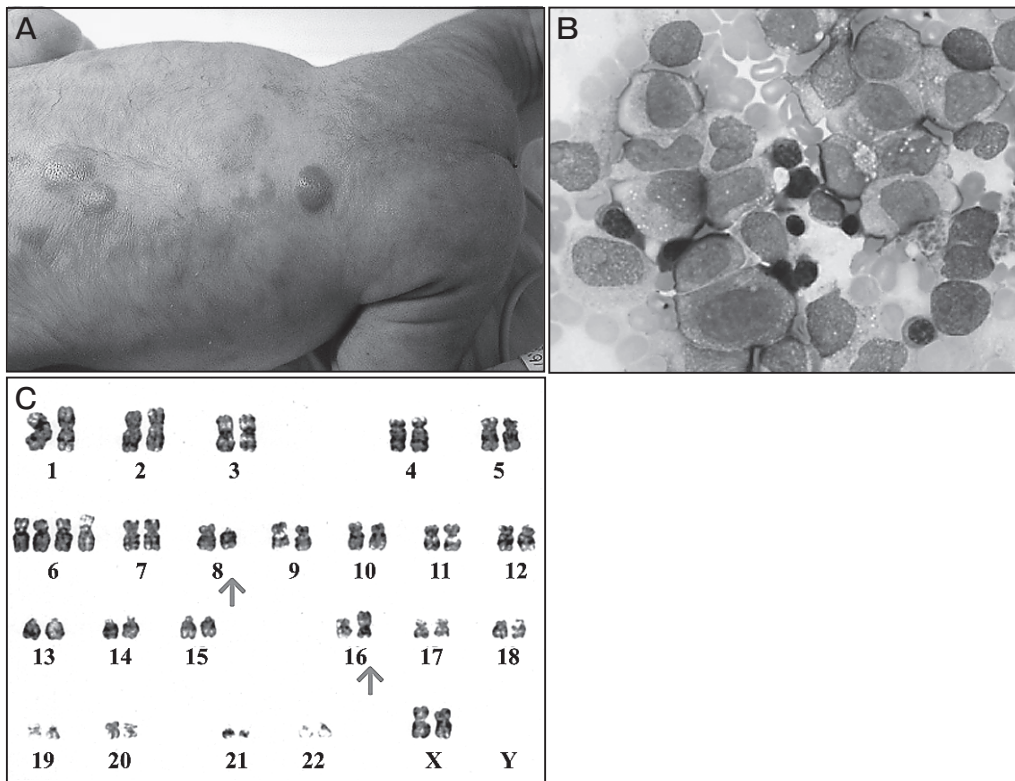


Fig. 1 (A) Multiple skin nodules were found on the patient's back and superior limb. Spontaneous regression was observed after chemotherapy. (B) Pictures of blasts in her bone marrow smear. May-Giemsa staining $\times 100$ showed the increase in monoblasts in the bone marrow smear. (C) Karyotype by the G-banding method showed t(8;16)(p11;p13) in this patient.

manufacturer's instructions. Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using the AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions except for the use of 1.3 μg cDNA. The primers for RT-PCR were MOZ4024F (5'-GTTGAGCCAAAAGAAGACATGCC-3'), a new primer, and CBP1201R (5'-GTTGC AATTGCTTGTGTGGGTAC-3'). PCR-amplified fragments were purified using the QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. DNA sequencing of the PCR-amplified fragments was performed using the Big Dye terminator v3.1 Cycle Sequencing Kit (Applied

Biosystems), the illustra AutoSeq G-50 Dye Terminator Removal Kit (GE Healthcare UK, Buckinghamshire, UK), Hi-Di Formamide (Applied Biosystems), and a 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. The sequencing analysis and alignment were performed using BLAST software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

The results of the sequence analysis showed that base pair (bp) 4,198 of MOZ exon 17 (accession number U47742.1) was fused to bp 1,007 of CBP exon 3

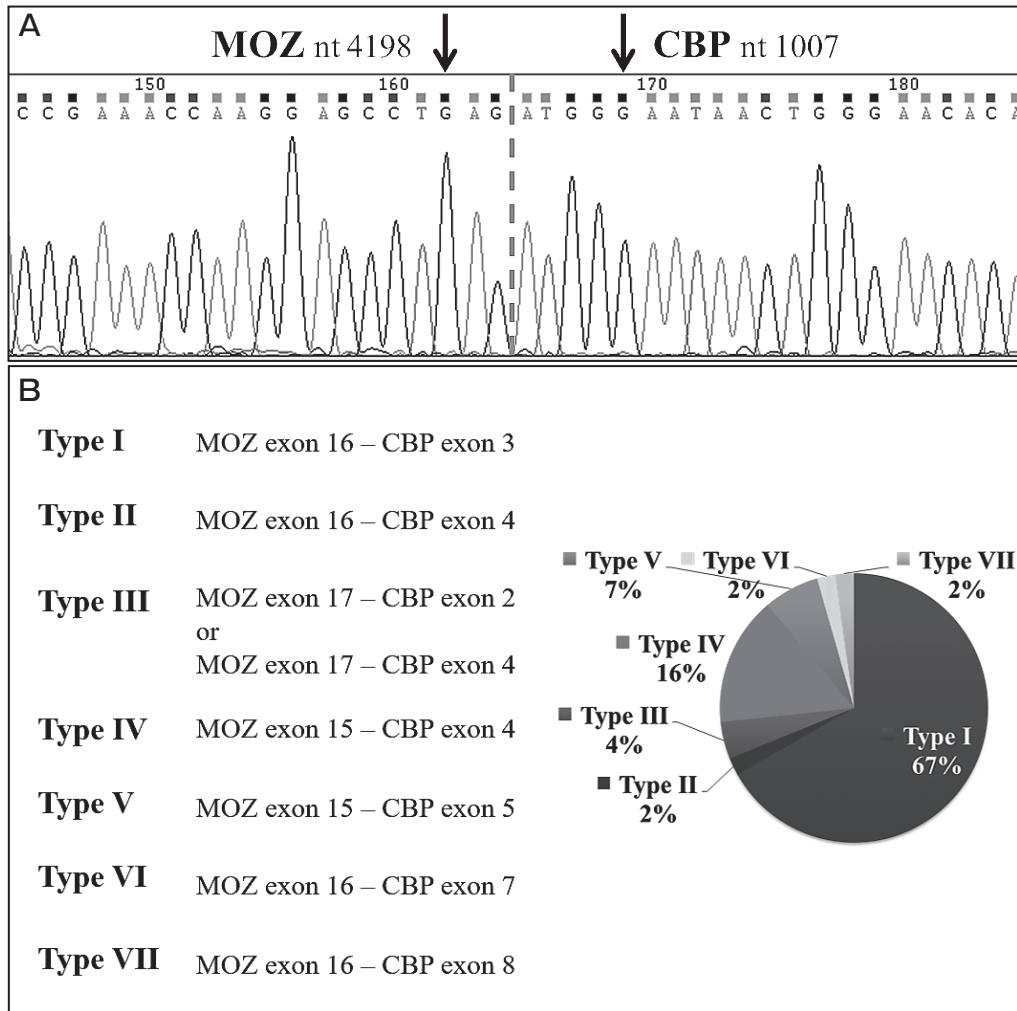


Fig. 2 (A) Partial sequence chromatogram showing the junction of MOZ and CBP genes (arrows). MOZ-CBP fusion transcript was detected in the patient's first diagnostic bone marrow sample. (B) Types and frequency of MOZ-CBP fusion transcripts.

(acc. no. NM_004380.2) (Fig. 2A). At this junction, there was a 6-bp stretch of an unknown fragment, which may correspond to base pairs 4,199–4,204 of MOZ exon 17 or base pairs 4,199–4,200 of MOZ exon 17, which were fused to base pairs 1,003–1,006 of CBP exon 3.

Discussion

The reciprocal chromosomal rearrangement t(8;16)(p11;p13) is rare in both adult and pediatric AML patients, accounting for 0.2–0.4% of de novo AML cases. Several studies reported that pediatric AML cases with t(8;16)(p11;p13) are a distinct clinical and biological entity. For example, Coenen *et al.* reported a cohort study of 62 pediatric AML cases with t(8;16)(p11;p13); they observed that AML cases with t(8;16)(p11;p13) had M4 or M5 (97%), erythrophagocytosis (70%), leukemia cutis (58%), and disseminated intravascular coagulation (39%), and they also reported that the AML cases with t(8;16)(p11;p13) had a higher frequency of congenital cases compared to other AML cases [8]. Diaz-Beya *et al.* reported that AML cases with t(8;16)(p11;p13) had a distinct signature characterized mainly by the down-regulation of multiple microRNAs, some of which are responsible for the high *RET* proto-oncogene levels [9]. Camos *et al.* reported the distinctive gene expression profile of *MOZ-CBP* AML, with overexpression of *RET* and prolactin (*PRL*) and a specific pattern of homeobox (*HOX*) gene expression [10].

The majority of adult AML cases with t(8;16)(p11;p13) are secondary to therapy and poor prognosis [11, 12]. The pediatric AML cases with t(8;16)(p11;p13) showed relatively good prognosis in recent reports. Daifu *et al.* reported that congenital AML cases with *MOZ-CBP* treated only with induction chemotherapy showed long-term remission [3]. Terui K. *et al.* reported that an infant AML case with *MOZ-CBP* spontaneously resulted in complete remission [13]. Wu *et al.* reported a congenital AML case with t(8;16)(p11;p13) who spontaneously resulted in complete remission [14].

To date, 7 types of *MOZ-CBP* fusion transcripts have been identified (Fig. 2B). Type I is the most common transcript found in adults with poor prognosis, and types VI and VII have been detected only in the pediatric population [13]. The difference in the prog-

nosis of AML cases with t(8;16)(p11;p13) between the adult and pediatric populations might be a result of the difference in the type of *MOZ-CBP* fusion transcripts.

The present case supports the idea that pediatric AML cases with *MOZ-CBP* which is a rare type of *MOZ-CBP* and not type I show a relatively good prognosis. More AML t(8;16)(p11;p13) cases, both pediatric and adult, must be analyzed to clarify the clinical utility of the types of *MOZ-CBP* fusion transcripts.

Acknowledgments. We thank for all the medical staff who participated in the patient care. This work was supported in part by a Grant-in-Aid for Cancer Research and a grant for Clinical Cancer Research and Research on Children and Families from the Ministry of Health, Labor and Welfare of Japan.

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