child at the homozygote state – would be responsible for the phenotype and could explain the absence of amplification of the promoter and the exon 1. To verify the large deletion hypothesis, several couples of primers were designed to amplify 8 small genomic regions around the promoter and the exon 1 (Table 1). These markers were called D01 to D04 from the promoter towards the centromere and D11 to D14 from the exon 1 towards the telomere. Firstly, the deletion was localized between 2680 bp (amplification of D03) upstream and 2794 bp (amplification of D12) downstream from the codon start adenine in exon 1 (Figure 1). Primers surrounding this region were used to determine the exact breakpoints by amplification and sequencing (5' agcaaggacagatatgcaaa 3' on forward and 5' acacctaagcctgactgcac 3' on reverse). A 4591 bp-deletion was characterized in the child and his parents covering 2335 bp in 5'UTR, the exon 1 and 1377 bp in the intron 1-2. Moreover, the sequences of the child and his parents were strongly reorganized in 5' with several sequence alterations such as mutations (10), insertion-deletions (2), duplication (1) and an insertion (23 bp between the breakpoints).

Large deletions are rarely involved in Crigler-Najjar disease but their frequency is probably underestimated as suggested for *CFTR* gene.[7] Gross genomic rearrangements have to be investigated in situations of refractory molecular diagnosis of Crigler-Najjar disease. Quantitative multiplex PCR of short fluorescent fragments should be developed in *UGT1A1* gene analysis in these specific situations.

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Two novel variants of *MOZ-CBP* fusion transcripts in spontaneously remitted infant leukemia with t(1;16;8)(p13;p13;p11), a new variant of t(8;16)(p11;p13)

Translocation t(8;16)(p11;p13) involving the *MOZ/MYST3* gene (8p11) and the *CBP/CREBBP* gene (16p13) is associated with the FAB M4/M5 subtype of acute myeloid leukemia (AML) and a poor prognosis. Five types of *MOZ-CBP* and three types of *CBP-MOZ* fusion transcripts have been identified in adult and adolescent patients by reverse transcriptase-polymerase chain reaction (RT-PCR).[1,2] To date, 6 newborn infants with monocytic malignancies (five AML-M4/M5 and one myelosarcoma) associated with t(8;16)(p11;p13) have been reported.[3-5] In these patients, only one AML patient had a variant of this translocation, t(8;16)(q11;p13).[4] Surprisingly, 2 AML cases, including the case with t(8;16)(q11;p13) and the case of myelosarcoma, underwent spontaneous remission, indicating a more favorable outcome than older patients. Although the involvement of the *MOZ* gene and the *CBP* gene was indicated by fluorescence *in situ* hybridization (FISH) analysis in one patient,[5] the presence of fusion transcripts was not described in any of the 6 cases. Therefore, we analyzed a case of infant AML with a variant of t(8;16)(p11;p13) by RT-PCR to determine whether *MOZ-CBP* and/or *CBP-MOZ* fusion transcripts were also involved in the development of infant leukemia.

A newborn girl presented with multiple skin nodules and hepatosplenomegaly after birth and was referred to our department on day 3 of life. Peripheral blood analysis showed a white blood cell count of 54.18×10^9/L with 6% blasts and 58% immature monocytic cells, a hemoglobin level of 14.1 g/dL, and a platelet count of 54×10^9/L. Bone marrow was hyperplastic with 44% blasts, and a diagnosis of AML (FAB M5b) was made. Surface marker analysis showed that the leukemic cells expressed CD4, CD13, CD14, CD33, and HLA-DR antigen. Cytogenetic study of bone marrow cells revealed an abnormal karyotype of 46,XX,t(1;16;8)(p13;p13;p11) in 17 out of 20 metaphases analyzed (Figure 1A). FISH analysis using probe sets for the centromeric and telomeric regions of the *MOZ* gene (BAC clones RP11-451C05 and RP11-142G13 labeled with Spectrum Green) and the *CBP* gene (RP11-507M07 and RP11-6C20 labeled with Spectrum Orange) demonstrated a fusion signal (Figure 1B). The skin nodules spontaneously regressed, and the patient's clinical condition remained good, so we observed the patient without chemotherapy. She spontaneously underwent complete remission before three months of age and remains in remission 17 months after diagnosis.

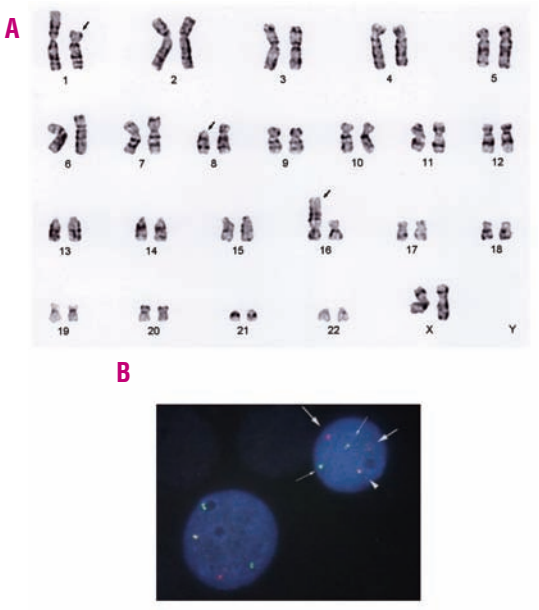

Figure 1. Cytogenetic analysis of the patient's bone marrow cells. (A) The G-banded karyotype of the leukemic cells showing 46, XX, t(1;16;8)(p13;p13;p11). Three arrows indicate the rearranged chromosomes. (B) Dual-color FISH analysis using probe sets for the centromeric and telomeric regions of *MOZ* (labeled with Spectrum Green) and CBP (labeled with Spectrum Orange) demonstrated a fusion signal (a yellow signal indicated by an arrow head). Thin arrows and thick arrows indicate the signals for *MOZ* and *CBP* respectively. Each probe set was composed of two BAC clones: RP11-451C05 (centromeric) and RP11-142G13 (telomeric) for *MOZ*, and RP11-507M07 (centromeric) and RP11-6C20 (telomeric) for *CBP*.

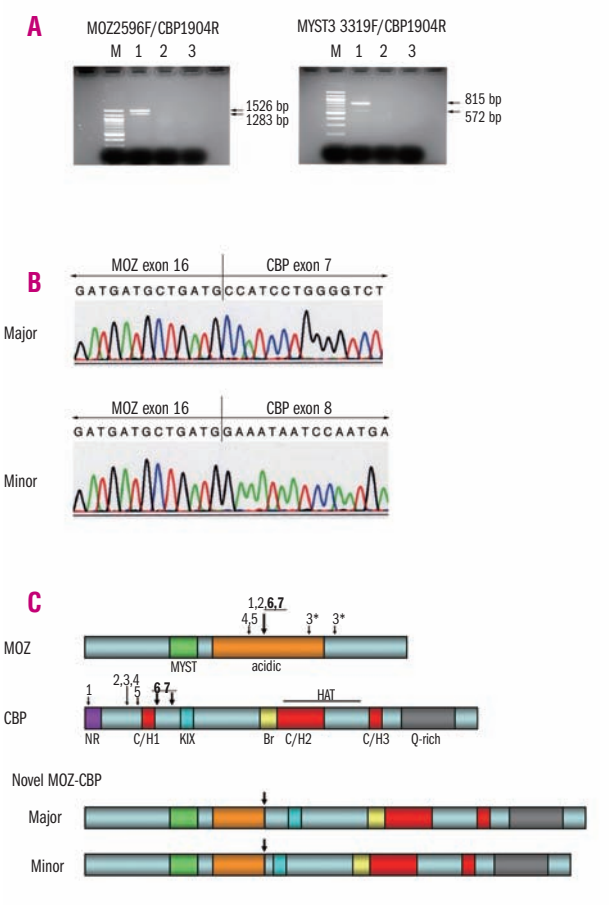

Figure 2. Analysis of the *MOZ-CBP* fusion transcripts. (A) RT-PCR using a primer set of MOZ2596F and CBP1904R amplified a major fragment of 1,526 bp and a minor of 1,283 bp (left). Two fragments of 815 bp and 572 bp were also amplified with a primer combination of MYST3 3319F and CBP1904R (right). M, size marker; lane 1, present case; lane 2, negative AML case; lane 3, no template. (B) Sequencing analysis revealed that *MOZ* exon 16 was fused in-frame to *CBP* exon 7 in the major fusion transcript (top) and also in frame to *CBP* exon 8 in the minor (bottom). (C) Predicted novel MOZ-CBP fusion proteins lack C/H1 domain of CBP compared to previously reported ones, but retain most of the other functional domains, including the bromodomain and the HAT domain. The thick arrows with the bolded and underlined numbers 6 and 7 indicate the breakpoints of the novel fusions. The arrows indicate the breakpoints of the type I-V MOZ-CBP with the numbers 1-5. MYST: MYST domain; acidic: acidic domain; NR: nuclear receptor binding domain; C/H 1-3: cysteine/histidine-rich domains 1-3; KIX: KIX domain; Br: bromodomain; Q-rich: glutamine-rich domain; HAT: histone acetyltransferase domain. *Two breakpoints in *MOZ* exon 17 were reported in the type III fusion.

Total RNA was extracted from bone marrow cells and cDNA was prepared. Since RT-PCR using previously published primer sets, MOZ3558F and CBP1201R, MOZ3536F and CBP404R,[1] and MYST3 3319F and CBP1201R[2] were negative for *MOZ-CBP* fusion, we designed a new primer set in exon 13 of the *MOZ* gene (MOZ2596F: 5'-cgaatgctggacttccgtagtg-3') and a reverse primer in exon 9 of the CBP gene (CBP1904R: 5'-ccagagttggagccatcgttc-3'). Two DNA fragments, a major fragment of 1,526 bp and a minor of 1,283 bp, were successfully amplified using this primer set (Figure 2A, left). RT-PCR using a primer combination of MYST3 3319F and CBP1904R also amplified two fragments of 815 bp and 572 bp (Figure 2A, right). Sequencing analysis revealed two novel in-frame fusion transcripts, a major fusion transcript between *MOZ* exon 16 and *CBP* exon 7 and a minor between *MOZ* exon 16 and *CBP* exon 8, indicating alternative splicing at the *CBP* gene (Figure 2B). The fusion transcripts were still positive at the age of one month but negative at two months. To detect reciprocal *CBP-MOZ* fusion transcripts, nested RT-PCR with the primer sets of CBP96F and MOZ3953R (first step) and CBP174F and MOZ3844R (second step) were performed as previously described.[1] However, no amplification products were obtained.

To our knowledge, these fusion transcripts will be the sixth and seventh types of *MOZ-CBP*, demonstrating the heterogeneity of the fusion transcripts. Although amplification of *MOZ-CBP* and *CBP-MOZ* fusion transcripts by RT-PCR has been reported to be difficult,[1,2] some of the cases negative for RT-PCR may have fusion transcript variants that could not be detected by previously reported primers. Our primer combinations may help to detect *MOZ-CBP* fusion transcripts in more AML cases with t(8;16)(p11;p13). *CBP-MOZ* fusion transcripts have not always been detected in *MOZ-CBP*-positive patients, indicating that *MOZ-CBP* is responsible for the leukemogenesis. However, it remains unclear whether *CBP-MOZ* fusion transcripts contribute to the development of leukemia because it has not been excluded that *CBP-MOZ* transcripts failed to be detected for technical reasons.[6] *CBP-MOZ* fusion transcripts were also negative in our patient, but false negatives caused by technical problems

were excluded because der(16)t(8;16) encoding *CBP-MOZ* was not generated in this three-way translocation. Translocation t(8;18;16)(p11;q21;p13), another three-way translocation variant of t(8;16)(p11;p13) found in a 15-month-old boy with AML M5b, also has a structure that could not generate *CBP-MOZ* but *MOZ-CBP*, while RT-PCR was not performed in this case.[7] Moreover, Murati *et al*. reported a 72-year-old man with AML M5a having a complex t(8;16)(p11;p13), in whom only *MOZ-CBP* but not *CBP-MOZ* was detected as expected because of the insertion of 8q material between 16p and 8p on the der(16).[2] These results clearly show that the *CBP-MOZ* fusion transcripts are not essential for the development of AML with t(8;16)(p11;p13) and its variants.

We find for the first time that *MOZ-CBP* fusion transcripts are expressed in a case of infant leukemia with a variant of t(8;16)(p11;p13), and the fusion transcripts were shown to be two novel variants. Although predicted *MOZ-CBP* fusion proteins lack a protein interaction domain, C/H1 (cysteine/histidine-rich domain 1), compared to previously reported ones, these fusion proteins retain most of the other functional domains of CBP, including the bromodomain and the HAT domain (Figure 2c) which were reported to be important for *MOZ-CBP* to inhibit the Runx1-mediated transcription and myeloid cell differentiation.[8] This suggests that the novel *MOZ-CBP* proteins are also leukemogenic and contribute to leukemic development in this case. While the reason for the favorable outcome of infant leukemia with t(8;16)(p11;p13) is not clear, secondary mutations may be required to develop aggressive disease as observed in adult patients. The difference of *MOZ-CBP* structures between infant and adult patients might also be related to the different outcomes. To clarify these questions, more cases of both infant and adult AML with t(8;16)(p11;p13) need to be analyzed.

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Pulmonary extramedullary hematopoiesis in patients with myelofibrosis undergoing allogeneic stem cell transplantation

We examined the lung function of 11 patients with intermediate/high risk myelofibrosis undergoing allogeneic hematopoietic stem cell transplantation (HSCT). In 3 patients, chest computerized tomography (CT) scans revealed multiple pulmonary nodules with extramedullary hematopoiesis that disappeared after transplantation. Pulmonary extramedullary hematopoiesis in patients with myelofibrosis rapidly regresses after allogeneic HSCT.

Primary myelofibrosis (PMF) or myelofibrosis secondary to polycythemia vera (PV-MF) or essential thrombocythemia (ET-MF) are clonal myeloproliferative disorders often characterized by pancytopenia, bone marrow fibrosis, leukoerythrocytosis, teardrop poikilocytosis and splenomegaly.[1] Splenomegaly, in particular, is the result of extramedullary hematopoiesis. Due to the extramedullary hematopoiesis, patients with myelofibrosis can develop pulmonary hypertension secondary to hematopoietic infiltration, portal hypertension, thrombocytosis, hypercoagulability, and left ventricular failure.[2,3] Radiographic findings such as ground glass appearance, effusions, septal thickening on chest computerized tomography have been also described in PMF patients.[4] In patients with intermediate/high risk myelofibrosis,[5] allogeneic hematopoietic stem cell transplant (HSCT) is the only known curative therapy and the development of reduced intensity conditioning has allowed a decrease in transplant related mortality while inducing long-term remission, especially in older patients[6-9] In particular, HSCT can restore a normal hematopoiesis, and allows the resolution of marrow fibrosis[10] as well as the progressive reduction of splenomegaly.[11]

In this study, we evaluated the chest computerized tomography (CT) and pulmonary function tests (PFTs) of 11 consecutive patients with primary myelofibrosis (n=5), PV-MF (n=3), or ET-MF (n=3) who received an allogeneic hematopoietic stem cell transplantation (HSCT) to assess the presence of pulmonary extramedullary hematopoiesis. An informed consent was signed prior to transplant and the transplants were performed according to the protocol approved by the University of Illinois at Chicago Institutional Review Board. All patients received an HLA matched graft from related (n=7) or unrelated (n=4)