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## Large deletion in *UGT1A1* gene encompassing the promoter and the exon 1 responsible for Crigler-Najjar type I syndrome

Crigler-Najjar type I syndrome (CN-I, MIM #218800) is due to a complete and non-inductile deficiency of bilirubin-UDP-glucuronosyltransferase activity (EC 2.4.1.17, gene *UGT1A1* located on 2q37.1).[1] Currently, over 90 genetic alterations such as mutations, small insertions or small deletions have been described in the five exons of the *UGT1A1* gene responsible for bilirubin conjugation activity deficiency. Large deletions (>20 bp) are rare genetic alterations in human genetics comprising only 5.8% of all genetic lesions referenced (Human Gene Mutation Database; *http://www.hgmd.cf.ad.uk*). Such genomic rearrangements could be due either to homologous or non-homologous recombination.[2,3] The only large deletion described in the *UGT1A1* gene has been reported by Seppen *et al.* in 1994.[4] The patient was homozygous for a deletion of the exon 2 responsible for CN-I but exact breakpoints have not been characterized.

We report the case of a male CN-I child in whom molecular studies allowed us to identify a large deletion encompassing the promoter and the exon 1 of *UGT1A1* gene. The infant was born at full term after an uneventful pregnancy. During the neonatal period, he presented an elevation of the serum bilirubin to 250-300 µmol/L, entirely unconjugated, with peaks at 600 µmol/L, contrasting with an absence of neurological manifestations. Intensive phototherapy and enzymatic induction by phenobarbital were inefficient in reducing the serum bilirubin concentration. At two weeks, the diagnosis of Crigler-Najjar was suspected and blood was sampled for molecular studies. Blood from parents, who are first cousins, were also sampled. Genomic DNA was extracted from peripheral leucocytes of the child and his parents. The promoter and the five exons with the flanking intron-exon junctions were PCR-amplified as previously described.[5,6] On two different blood samples, no amplification of the promoter and the exon 1 for the child was available. On the other hand, the parents' promoter and exon 1 were correctly amplified and no genetic sequence alteration was observed after sequencing. Since they are consanguineous, an identical large deletion, including at least the promoter and the exon 1, was suspected at the heterozygote state in the parents explaining their *normal* electrophoretic profile. This deletion – transmitted to the

**Table 1.** Genetic markers analyzed to determine the breakpoints of the deletion including the promoter and the exon 1.

| *Marker* | *Primer OLF* | *Primer OLR* | *Size (bp)* | *Position in relation to ATG (bp)* | *Presence in the child* | *Presence in the parents* |
|---|---|---|---|---|---|---|
| D04 | 5' tacactagtaaaggtcactc 3' | 5' ccctctagccattctggatc 3' | 290 | -3496 | + | + |
| D03 | 5' tacactagtaaaggtcactc 3' | 5' tttgcatatctgtccttgct 3' | 816 | -3496 | + | + |
| D02 | 5' tacactagtaaaggtcactc 3' | 5' gttagaaatggtccttgcct 3' | 1266 | -3496 | – | + |
| D01 | 5' ctggccagtgatgtgtatgg 3' | 5' gcaagtattgtgcagccag 3' | 125 | -1545 | – | + |
| D11 | 5' gccaatgggtctgcatgtat 3' | 5' ggttggcacctttcttctca 3' | 111 | 1896 | – | + |
| D12 | 5' tgtaggagaggcaccgaact 3' | 5' ccaaacaaggcaacaacaaa 3' | 112 | 2797 | + | + |
| D13 | 5' agccatttaccaacgctcag 3' | 5' agggtctgcaccacatctct 3' | 108 | 4361 | + | + |
| D14 | 5' gaagggtttcccctggagt 3' | 5' cactgaccagcagaacaacg 3' | 118 | 5939 | + | + |

*Primers were designed with the Primer3 web site (frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Genetic positions were determined with Ensembl Project in relation to the adenine of the first codon of UGT1A1.*

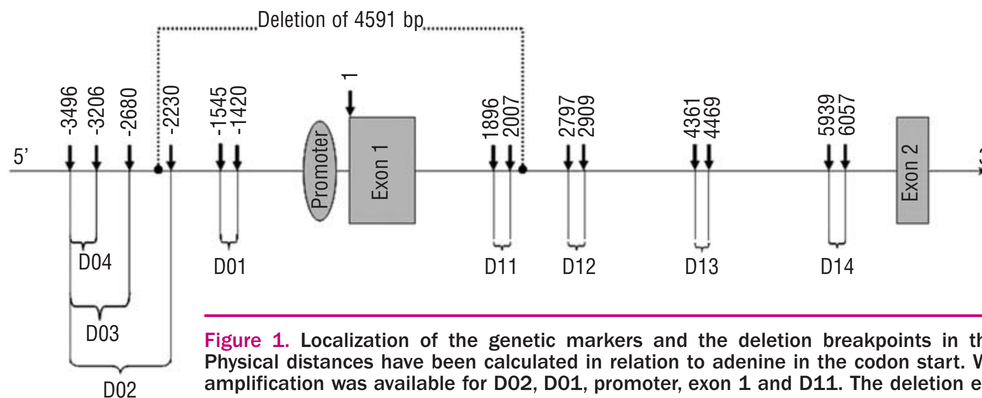


**Figure 1.** Localization of the genetic markers and the deletion breakpoints in the *UGT1A1* genetic region. Physical distances have been calculated in relation to adenine in the codon start. With child genomic DNA, no amplification was available for D02, D01, promoter, exon 1 and D11. The deletion encompasses 4591 bp.

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 $54.18\times10^9$/L with 6% blasts and 58% immature monocytic cells, a hemoglobin level of 14.1 g/dL, and a platelet count of $54\times10^9$/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.