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Assignment of the canalicular multispecific organic anion transporter gene (CMOAT) to human chromosome 10q24 and mouse chromosome 19D2 by fluorescent in situ hybridization

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Abstract. Rabbit epithelial basolateral chloride conductance regulator (EBCR) and rat canalicular multispecific organic anion transporter (Cmoat) are found to be homologues based on protein sequence comparison and Northern blot analysis. EBCRis, therefore, renamed as rabbit Cmoat. The gene encoding CMOAT, a transporter possibly involved in Dubin-Johnson syndrome in humans, is mapped on human chromosome 10q24 and mouse chromosome 19D2.

Recently, a rabbit cDNA was isolated which encodes a novel member of the ATP-binding cassette (ABC) superfamily of transporters (Van Kuijck et al., 1996). Using immunohistochemistry, this protein was located in liver parenchymal cells, in epithelial cells lining the villus tip of small intestine, and in the basolateral domain of renal distal tubular cells. In Xenopus laevis oocytes expressing this protein, a cAMP-activated chloride conductance was demonstrated. This protein was, therefore, baptized epithelial basolateral chloride conductance regulator (EBCR). At the same time, a rat liver ABC transporter, named canalicular multispecific organic anion transporter (Cmoat) (Paulusma et al., 1996) or canalicular multidrug resistance protein (cmMrp) (Büchler et al., 1996), was identified, which appeared to be absent in rats suffering from a defect in the ATP-dependent hepatobiliary excretion of bilirubin glucuronides (Jansen et al., 1985; Jansen et al., 1987; Nishida et al., 1992). In analogy, it was suggested that the dysfunctional human homologue may underlie the Dubin-Johnson syndrome (Zimniak 1993), which is a congenital form of jaundice originally described in 1954 (Dubin and Johnson, 1954; Sprinz and Nelson, 1954). As ABC transporters usually consist of more than 25 exons and as human biopsies from liver, kidney or small intestine are not easily obtained, chromosomal localization of human CMOAT is highly desirable in order to be able to couple diseases like Dubin-Johnson syndrome to CMOAT dysfunctioning.

In this study we show that rabbit EBCR is identical to rat Cmoat and assign the CMOAT gene to human chromosome 10q24 and mouse chromosome 19D2.

Materials and methods

Poly(A)* RNA isolation and Northern blot analysis
Poly(A)* RNA isolation and Northern blot analysis were performed as described previously (Van Kuijck et al., 1996). 3 μg of duodenum poly(A)* RNA (D) was loaded and other samples were adjusted to the glyceraldehyde-3-phosphate dehydrogenase signal of this sample. Using stringent hybridization and washing conditions, the Northern blot was first hybridized with an [α-32P]dCTP labeled (Feinberg and Vogelstein, 1983) XhoI-KpnI cDNA fragment of 1737 bp, consisting of rabbit CMOAT coding sequence (Van Kuijck et al., 1996), followed by a hybridization with a labeled rat HindIII-AnfI
Fig. 1. Northern blot analysis of various rabbit tissues with EBCR and CMOAT probes. Duodenum (D), jejunum (J), ileum (I), colon (C), stomach (S), kidney (K), and liver (L) are indicated. Exposure times were 24 h (lanes D, J, I) or 3 days (lanes C, S, K, L). RNA size marker in kb is indicated on the left of each blot.

Fig. 2. Human CMOAT is assigned to chromosome 10q24 (top-left figure) and mouse Cmoat to chromosome 19D2 (bottom-left figure). Locations are indicated on corresponding ideograms (right top and bottom).

cDNA fragment of 1 kb (Paulusma et al., 1996). Between the two hybridizations the Northern blot was stripped and checked for possible remnant radioactive signals.

Screening of genomic libraries
A supercos human genomic library was screened, using an [α-32P]dCTP-labeled (Feinberg and Vogelstein, 1983) cDNA fragment as a probe, which consisted of 346 bp of the 3'-untranslated region and 810 bp of the rabbit CMOAT coding sequence (Van Kuijck et al., 1996). The hybridization conditions were 250 mM PO4 (pH 7.2), 7% SDS, 1 mM EDTA at 65°C, while final washing was performed in 100 mM PO4 (pH 7.2), 0.1% SDS, 1 mM EDTA at 65°C. After one round of rescreening one positive clone was isolated. A 400-bp HindIII-BamHI fragment, which hybridized with an [α-32P]dCTP-labeled 5.2-kb cDNA fragment as a probe containing the complete open reading frame of rabbit CMOAT, was ligated into pBluescript KS (Stratagene) and sequenced (Sanger et al., 1977). The nucleotide sequence of human CMOAT has been submitted to Genbank/EMBL under accession number U49248.

An EMBL-3 mouse genomic library was screened using above described hybridization and washing buffers at 55°C. After one round of rescreening one positive clone was isolated. A hybridization positive EcoRI-HindII fragment of 1250 bp was subcloned into pBluescript KS (Stratagene) and sequenced (Sanger et al., 1977).

Fluorescence in situ hybridization
For regional localization of the human or mouse CMOAT genes, FISH was performed on normal human or mouse lymphocyte metaphase chromosomes, respectively, essentially as described (Suijkerbuijk et al., 1991; Sinke et al., 1992; Suijkerbuijk et al., 1992). Briefly, CMOAT cosmid or copy DNA was labeled with biotin-14-dATP (Gibco BRL) using a nick-translation kit (Gibco BRL), and precipitated with 50 x Cot-1 DNA (Gibco BRL). The centromere probe for chromosome 10 (D10Z1) was labeled with fluorolink Cy3-dCTP (BDS Inc.). After denaturation, slides were incubated with probes under coverslips in a moist chamber for 45 h. Detection of hybridizing probes was achieved using avidin fluorocine isothiocyanate (FITC) (Vector Laboratories), rabbit-antiFITC and mouse-anti-rabbit FITC-conjugated antibodies. For evaluation of the chromosome slides a Zeiss epifluorescence microscope was used. Separate images of both CMOAT hybridizing signals and DAPI-counterstained chromosomes were transformed into pseudocolored images. For precise localization and chromosome identification, DAPI-converted banding patterns were generated using the BDS-image™ software package (Oncor). In all cases, more than 20 metaphase spreads were evaluated of which at least 80% showed a signal on both alleles. In the remaining spreads one of the alleles appeared to be positive.
Results and discussion

Comparison of EBCR and CMOAT protein sequences showed a 78.5% identity (data not shown). Furthermore, Northern blot analysis demonstrated that EBCR and CMOAT probes detect only one transcript in several rabbit tissues, which is of identical size and relative abundance (Fig. 1). It is thus very likely that EBCR is the rabbit homologue of rat Cmoat, and hence, we rename, for reasons of unity, EBCR as rabbit CMOAT.

In order to assign the location of the human CMOAT gene, a human genomic library was screened. One hybridization positive clone was isolated from which a 400-bp fragment was subcloned and subjected to sequence analysis. A part of the 400-bp fragment encoded a stretch of 25 amino acids which showed 84% identity to positions 579–603 of rabbit CMOAT (Van Kuijek et al., 1996), 88% identity to positions 577–601 of rat Cmoat (data not shown; Paulusma et al., 1996), and 100% identity to positions 581–605 of human CMOAT (Paulusma et al., manuscript in preparation). Remaining nucleotides did not encode amino acids related to known CMOAT sequences and are presumably part of an intron. The presumed exon-intron boundary conformed to the gt-ag splice consensus sequence. Using the human genomic CMOAT clone, FISH analysis revealed signals on human chromosomes 10q24 and 17p13 (data not shown). In order to discriminate between the two signals, FISH analysis was repeated using a human CMOAT cDNA clone which confirmed the localization on chromosome 10q24 (Fig. 2).

To define the location of the mouse CMOAT gene a mouse genomic library was screened. From one hybridization positive clone a 1250-bp fragment was subcloned and subjected to sequence analysis. A part of this 1250-bp fragment encoded 12 amino acids which showed 92% identity to positions 129–140 of rabbit CMOAT (Van Kuijek et al., 1996) and 83% identity to positions 128–139 of rat Cmoat (data not shown; Paulusma et al., 1996). Since the open-reading frame was lost, the remaining nucleotides are presumably part of an intron. FISH analysis on normal mouse lymphocyte metaphase chromosomes demonstrated that the mouse gene (Cmoat) is located on chromosome 19D2 (Fig. 2). These localizations of human and mouse CMOAT genes underscore the region of synteny between human chromosome 10 and mouse chromosome 19 (Davisson et al., 1991; Searle et al., 1994).

Although several forms of congenital hyperbilirubinemia exist (Isselbacher et al., 1994), only Dubin-Johnson syndrome is characterized by the inability of the liver to excrete a broad range of anionic compounds. A possible linkage between Dubin-Johnson syndrome and chromosome 10q24 would, therefore, directly provide CMOAT as a serious candidate gene to be mutated in this disease.

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References


