A Human Placenta-specific ATP-Binding Cassette Gene (ABCP) on Chromosome 4q22 That Is Involved in Multidrug Resistance

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Abbreviations: EST, expressed sequence tag; RACE, rapid amplification of cDNA ends.

Abstract

We characterized a new human ATP-binding cassette (ABC) transporter gene that is highly expressed in the placenta. The gene, ABCP, produces two transcripts that differ at the 5' end and encode the same 655-amino acid protein. The predicted protein is closely related to the Drosophila white and yeast ADP1 genes and is a member of a subfamily that includes several multidrug resistance transporters. ABCP, white, and ADP1 all have a single ATP-binding domain at the N\textsubscript{H\textsubscript{2}} terminus and a single COO\textsubscript{H}-terminal set of transmembrane segments. ABCP maps to human chromosome 4q22, between the markers D4S2462 and D4S1557, and the murine gene (Abcp) is located on chromosome 6 28-29 cm from the centromere. ABCP defines a new syntenic segment between human chromosome 4 and mouse chromosome 6. The abundant expression of this gene in the placenta suggests that the protein product has an important role in transport of specific molecule(s) into or out of this tissue.

Introduction

A universal requirement for all organisms is the transport of molecules across cellular membranes. The ABC\textsuperscript{C} genes comprise a large superfamily, the protein products of which carry out the transport of many specialized compounds in prokaryotes, eukaryotes, and archaeabacteria. ABC transporters are one of the few superfamilies abundant in all three kingdoms and are characterized by an extensive conservation of the ATP-binding domains throughout evolution (1, 2). A functional ABC transporter consists of two ATP-binding domains and two sets of TM domains. In eukaryotes, most ABC genes either have all four domains in a single open reading frame (full transporters) or a single COOH-terminal set of transmembrane segments. ABCP has two sets of TM domains. In eukaryotes, most ABC genes either have all four domains in a single open reading frame (full transporters) or a single COOH-terminal set of transmembrane segments. ABCP defines a new syntenic segment between human chromosome 4 and mouse chromosome 6. The abundant expression of this gene in the placenta suggests that the protein product has an important role in transport of specific molecule(s) into or out of this tissue.

Materials and Methods

Sequence Analysis. Searches of the EST database (http://www.ncbi.nlm.nih.gov) were performed with the BLAST program (16) using the HuEST157481 sequence. Phylogenetic analysis was performed using the PHYLIP package (http://evolution.genetics.washington.edu/phylip.html).

cDNA Cloning. Primers were designed from the sequences of the EST clones from 5' and 3' regions of the gene and used to link the EST cDNA sequences by RT-PCR with placenta QUICK-Clone cDNA (Clontech) as a template. Primers ABCP-R1 (5'-CCACAACTGTTTTGAATGACGCT-ATCAAAGTGCCCAT) and ABCP-R2 (5'-AGGTGGTGTAGCTGATCTCCTTGAAGACTG) were used for 5' RACE reactions using RACE-ready cDNA (Clontech). PCR products were cloned into the pGEM-T vector (Promega). Sequencing was performed with the Taq Dyedex Terminator Cycle Sequencing kit (Applied Biosystems), according to the manufacturer's instructions. Sequencing reactions were resolved on an ABI 373A automated sequencer. The sequence of the ABCP cDNA has been deposited.

Northern Hybridization. DNA fragments used as probes were purified on a 1% low-melting temperature agarose gel. DNA was labeled directly in agarose with the Random Primed DNA labeling kit (Boehringer Mannheim) and hybridized to a multiple tissue Northern blot and a Master blot (Clontech), according to the manufacturer's instructions.

Genetic Mapping. The human ABCP (also designated ABC15) was mapped in the GeneBridge 4 radiation hybrid panel. A murine ABCP-related sequence was identified through searches of the mouse EST database. A representative clone (AA008579) was obtained and sequenced from both the 5' and 3' ends. Primers to amplify a portion of the 3' untranslated region were designed (AbcpFl, 5'-AATCAGGGCATCGAACTGTC; AbcpRl, 5'-GGTAA-ATCAAAGTGCCCAT) using the PRIMER program (http://www-genome.mit.edu) and used to screen a mouse radiation hybrid panel (Research Genetics). The panel was constructed from irradiated mouse embryonic cells (129aa) fused with a hamster cell line (A23). Chromosome location was determined by The Jackson Laboratory Mapping Group.

Results

Previously, we identified over 25 new human ABC genes from the human DNA sequence databases (Ref. 17; data not shown). One of these genes, formerly designated HuEST157481, is highly expressed in the placenta and is found at low to undetectable levels in some other tissues (Fig. 1A). Hybridization of a Masterblot (Clontech) confirmed the very high placental expression, about 100 times more than in other detectable tissues (data not shown) such as heart, ovary, kidney, and fetal liver (Fig. 1B). This gene, ABCP, is represented in the database by over 30 EST clones from placenta, infant brain, fetal liver/spleen, and uterus. Sequencing of cDNA clones, reverse transcription-PCR and RACE products revealed two transcripts that differ by ~200 bp at the 5' end. These data are in agreement with the Northern analysis (Fig. 1A).

Received 10/2/98; accepted 10/19/98.

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1 This project was funded in part with Federal funds from the National Cancer Institute, NIH, under Contract No. NO1-CO-56000.

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3 The abbreviations used: ABC, ATP-binding cassette; ABCP, placenta-specific ABC transporter; TM, transmembrane; EST, expressed sequence tag; RACE, rapid amplification of cDNA ends.
The ABCP gene was mapped to human chromosome 4q22, between the markers D4S2462 and D4S1557, with the GeneBridge 4 radiation cule. The amino acid sequence of ABCP is 31% identical to the sequence of the yeast ADP1 and YOL075 and is outside the ABC8 and white/scarlet groups. However, given the sparse knowledge of subfamily that also contains the Drosophila brown and scarlet genes, as well as several fungal multidrug resistance genes. The transmembrane domain of ABCP is rather distinct from its paralogs. In addition, different members of the same ABC subfamily often transport very different substrates. Identification of the endogenous substrate of ABCP will require additional biological data. In situ hybridization to placental tissue at different stages of development should yield some clues to ABCP location and function. To date, all known ABC half transporters are localized to the membrane of intracellular organelles (5, 9, 18). It will be important to determine whether ABCP is also located inside the cell.

Many ABC genes play a role in human inherited diseases, including cystic fibrosis, adrenoleukodystrophy, diabetes, and retinal degeneration. In addition, mammalian ABC proteins play important roles in drug resistance and peptide and ion transport (1, 19). No obvious candidate phenotypes are found in the regions that ABCP maps to in either human or mouse genomes. Curiously, a genome-wide scan of preeclampsia, a major complication of pregnancy, revealed linkage to chromosome 4, but to a region that would appear to exclude ABCP (20). It is feasible that ABCP can play a role in the placental barrier in vivo, either by protecting the fetus from harmful compounds or by transporting some important, not yet identified, substrate. Most ABC proteins transport very specific molecules, so it is likely that the ABCP pumps a substance(s) that plays an important role in the homeostasis of the placenta. The placenta does contain transporters for some specific substrates, such as glucose (21).

### Discussion

Here we describe a new human ABC gene that is expressed at a very high level in the placenta. ABCP belongs to a subfamily of ABC proteins that includes transporters of guanine and tryptophan, as well as several fungal multidrug resistance genes. However, the transmembrane domain of ABCP is rather distinct from its paralogs. In addition, different members of the same ABC subfamily often transport very different substrates. Identification of the endogenous substrate of ABCP will require additional biological data. In situ hybridization to placental tissue at different stages of development should yield some clues to ABCP location and function. To date, all known ABC half transporters are localized to the membrane of intracellular organelles (5, 9, 18). It will be important to determine whether ABCP is also located inside the cell.

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Recently, Miyake et al. demonstrated that the ABCP gene is overexpressed and amplified in certain human breast and colon cancer cell lines (22) resistant to the chemotherapeutic drugs mitoxantrone and, to a lesser extent, daunorubicin. Mitoxantrone is used in the treatment of acute leukemias and has shown promise in the treatment of breast and ovarian cancers (23, 24). These data suggest that ABCP is a transporter for some chemotherapeutic compounds and that overexpression of this transporter may play a role in drug resistance in some cancers. The ABC gene subfamily that includes ABCP contains a number of full transporters that are involved in yeast multidrug resistance. These pumps (PDR5, SNQ2, CDR1, and CDR2) confer resistance to a wide variety of compounds including cycloheximide, chloramphenicol, brefeldin A, and several antifungal agents (25). Further understanding of the function and regulation of ABCP may be important to effective chemotherapy.

Acknowledgments

We thank Susan Bates and Tito Fojo for helpful discussions and Stan Cervario for oligonucleotide synthesis and assistance with DNA sequencing. Computing resources were provided by the Frederick Biomedical Supercomputing Center.

References

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