[CANCER RESEARCH 58, 5337–5339, December 1, 1998]

Advances in Brief

A Human Placenta-specific ATP-Binding Cassette Gene (*ABCP*) on Chromosome 4q22 That Is Involved in Multidrug Resistance[1]

Rando Allikmets, Lynn M. Schriml, Amy Hutchinson, Vincenzo Romano-Spica, and Michael Dean[2]

Intramural Research Support Program, Science Applications International Corporation-Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201 [R. A., A. H.]; National Cancer Institute, Laboratory of Genomic Diversity, Frederick, Maryland 21702-1201 [L. M. S., M. D.]; and Institute of Hygiene and Public Health, Faculty of Medicine of the Catholic University, 00168 Rome, Italy [V. R-S.]

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 NH_2 terminus and a single COOH-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[3] genes comprise a large superfamily, the protein products of which carry out the transport of many specialized compounds in prokaryotes, eukaryotes, and archaebacteria. 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 represent half transporters with a single TM and single ATP-binding segment (3, 4). Half transporters typically form heterodimers to produce a functional protein (5). Structurally, most eukaryotic transporters have the TM segments NH_2-terminal to the ATP-binding domains (TM-ATP-TM-ATP).

The *white* locus was the first genetic marker described in *Drosophila* and encodes an ABC half transporter (6). The white protein forms complexes with two related transporters, brown and scarlet, to transport guanine and tryptophan, the precursors to eye pigments (7–9). *white* is related to the *Saccharomyces cerevisiae ADP1* gene and a human locus on chromosome 21q22.3, *ABC8* (10–12). Altogether, these genes form an ABC subfamily that includes both half and full transporters (3, 4). In addition, this subfamily includes several drug resistance genes described in *S. cerevisiae*, *S. pombe*, and *Candida albicans* (13–15). However, the endogenous substrates for these proteins have not been established.

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′-CCAGCAAGTTTTTGAATGAACGCTTGG) 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 Dyedeoxy 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.[4]

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.[4] Primers to amplify a portion of the 3′ untranslated region were designed (AbcpF1, 5′-AATCAGGGCATCGAACTGTC; AbcpR1, 5′-GGTAATCAAAGTGCCCAT) using the PRIMER program (http://www-genome.wi.mit.edu) and used to screen a mouse radiation hybrid panel (Research Genetics). The panel was constructed from irradiated mouse embryo primary 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. 1*A*). 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. 1*B*). 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. 1*A*),

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.

[2] To whom requests for reprints should be addressed. Phone: (301) 846-5931; Fax: (301) 846-1909; E-mail: dean@mail.ncifcrf.gov.

[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.

[4] The nucleotide sequence data reported in this study have been deposited at the National Center for Biotechnology Information/GenBank Data Library under accession numbers AF103796 and AF103875.

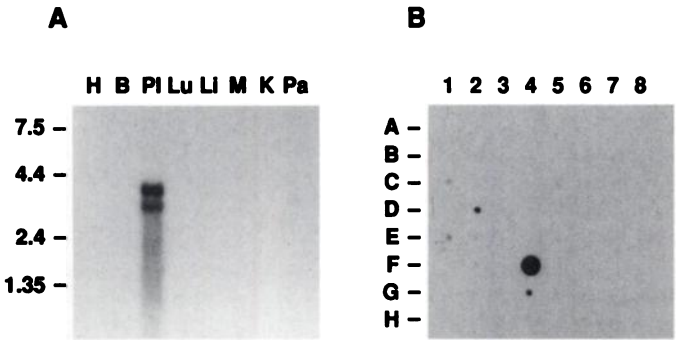

Fig. 1. Expression of the *ABCP* gene. In *A*, a fragment of the *ABCP* gene was hybridized to a Northern blot containing RNA from adult human heart (*H*), brain (*B*), placenta (*Pl*), lung (*Lu*), liver (*Li*), muscle (*M*), kidney (*K*), and pancreas (*Pa*). A longer exposure revealed a weak hybridization signal in the heart but no detectable expression in the other tissues (data not shown). *B*, hybridization of *ABCP* to a dot blot containing RNA from 43 adult (*rows A–F*) and 7 fetal (*row G*) tissues. *Row H* contains negative controls. Significant hybridization was seen to placenta (*F4*), and to lesser extent heart (*C1*), ovary (*D2*), kidney (*E1*), and fetal liver (*G4*).

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  1  MSSSNVEVFI PVSQGNTNGF PATVSNDLKA FTEGAVLSFH NICYRVKLKS

 51  GFLPCRKPVE KEILSNINGI MKPGLMAILG PTGGGKSSLL DVLAARKDPS

101  GLSGDVLING APRPANFKCN SGYVVQDDVV MGTLTVRENL QFSAALRLAT

151  TMTNHEKNER INRVIEELGL DKVADSKVGT QFIRGVSGGE RKRTSIGMEL

201  ITDPSILSLD EPTTGLDSST ANAVLLLLKR MSKQGRTIIF SIHQPRYSIF

251  KLFDSLTLLA SGRLMFHGPA QEALGYFESA GYHCEAYNNP ADFFLDIING

301  DSTAVALNRE EDFKATEIIE PSKQDKPLIE KLAEIYVNSS FYKETKAELH

351  QLSGGEKKKK ITVFKEISYT TSFCHQLRWV SKRSFKNLLG NPQASIAQII

401  VTVVLGLVIG AIYFGLKNDS TGIQNRAGVL FFLTTNQCFS SVSAVELFVV

451  EKKLFIHEYI SGYYRVSSYF LGKLLSDLLP MRMLPSIIFT CIVYFMLGLK

501  PKADAFFVMM FTLMMVAYSA SSMALAIAAG QSVVSVATLL MTICFVFMMI

551  FSGLLVNLTT IASWLSWLQY FSIPRYGFTA LQHNEFLGQN FCPGLNATGN

601  NPCNYATCTG EEYLVKQGID LSPWGLWKNH VALACMIVIF LTIAYLKLLF

651  LKKYS
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Fig. 2. Open reading frame of *ABCP*. The predicted open reading frame of the *ABCP* gene is shown with the ATP-binding domain in *boldface* and the six putative TM segments *underlined*.

where two equally strong transcripts are present. Both transcripts contain the same open reading frame of 658 amino acids (Fig. 2) that consists of an ATP-binding domain at the NH_2 terminus and six predicted TM segments in the COOH-terminal portion of the molecule. The amino acid sequence of ABCP is 31% identical to the *Drosophila* white protein, and it represents a member of an ABC gene subfamily that also contains the *Drosophila brown* and *scarlet* genes, as well as the yeast *ADP1* and two other human *white*-related genes (Fig. 3). Phylogenetic analysis revealed that ABCP is most closely related to ADP1 and YOL075 and is outside the ABC8 and white/brown/scarlet groups. However, given the sparse knowledge of substrates for these transporters and the considerable divergence in their TM domains, it is hard to speculate on the possible function(s) of ABCP based on phylogenetic studies.

The *ABCP* gene was mapped to human chromosome 4q22, between the markers *D4S2462* and *D4S1557*, with the GeneBridge 4 radiation hybrid panel (Fig. 4). No other *ABC* gene has previously been mapped to human chromosome 4. A partial mouse *Abcp* cDNA was sequenced and used to map *Abcp* in murine radiation hybrids. *Abcp* mapped to chromosome 6, 28–29 cM from the centromere. Only one other gene, the delta 2 ionotropic glutamate receptor (*GRID2*), has been localized to the human 4q22 region and to mouse chromosome 6. Thus, *Abcp* and *Grid2* define a new syntenic group between human chromosome 4 and murine chromosome 6.

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.

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).

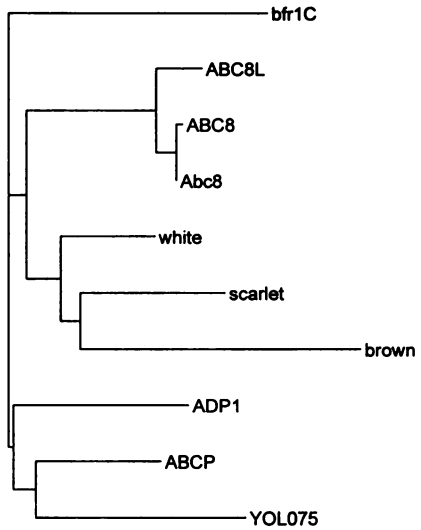

Fig. 3. Phylogenetic tree of ABCP-related proteins. An amino acid alignment of ABCP and several related genes was generated using PILEUP (Genetics Computing Group). The sequence was trimmed to a 673-residue, minimally overlapping segment and used for neighbor-joining analysis. Bootstap analysis of both neighbor-joining and maximum parsimony trees demonstrated that the ABC8/Abc8/ABC8L and white/brown/scarlet clusters are significantly related (data not shown). ABC8, human; Abc8, mouse; ABC8L, human ABC8-like; YOL075, yeast open reading frame; bfr1C, COOH-terminal half of bfr1.

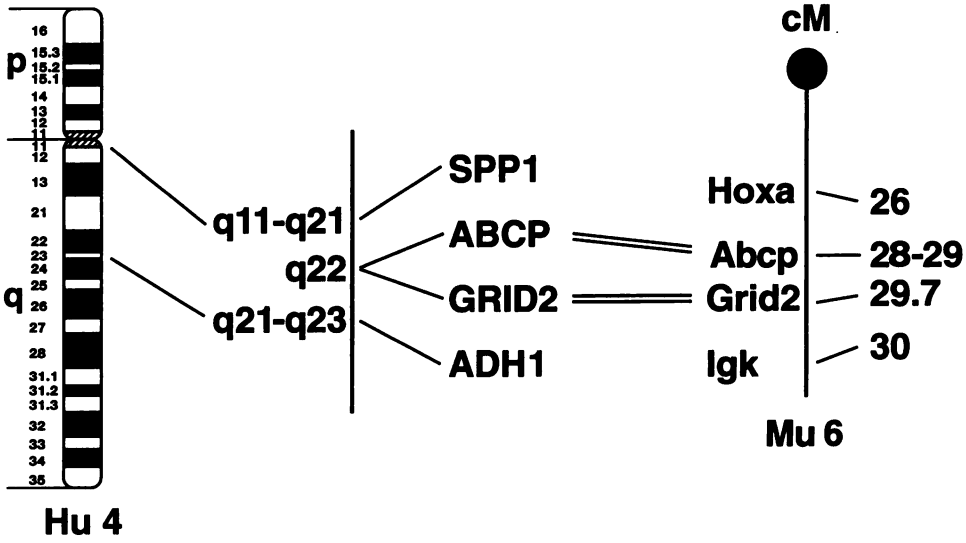

Fig. 4. Location of the *ABCP* and *Abcp* genes. The location of the *ABCP* gene on human chromosome 4q22 and *Abcp* on mouse chromosome 6, 28–29 centimorgans (cM) from the centromere, is shown along with several flanking markers. Both *ABCP* and *GRID2* (glutamate receptor, ionotropic, delta-2) have been localized to 4q22 and mouse chromosome 6. These markers define a short syntenic interval that is flanked in the human by *SPP1* (secreted phosphoprotein-1) and *ADH1* (alcohol dehydrogenase 1), which map to mouse chromosomes 6 and 3, respectively. The mouse chromosome 6 segment is flanked by *Hoxa* (homeo box 1a) and *Igk* (immunoglobulin kappa chain complex) located on chromosomes 7p15-p14 and 2p12, respectively.

Recently, Miyake *et al.*[5] 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 Cevario for oligonucleotide synthesis and assistance with DNA sequencing. Computing resources were provided by the Frederick Biomedical Supercomputing Center.

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Cancer Res 1998;58:5337-5339.