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Cloning and chromosomal mapping of a novel ABC transporter gene *(hABC7)*, a candidate for X-linked sideroblastic anemia with spinocerebellar ataxia

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Abstract We isolated a novel human ATP-binding cassette (ABC) transporter cDNA, determined its nucleotide sequence, and designated it human ABC7 (hABC7). The nucleotide sequence was highly homologous to the *ATM1* gene in yeast, which encodes an ABC transporter (yAtm1p) located in the mitochondrial inner membrane. The deduced human product, a putative half-type transporter, consists of 752 amino acids that are 48.9% identical to those of yAtm1p. A computer-assisted protein structural and localization analysis revealed that the mitochondrial targeting signal of yAtm1p is conserved in the N-terminal region of the primary sequence of the hABC7 protein, and therefore this product is also likely to be located in the mitochondrial inner membrane. The evidence strongly suggests that the *hABC7* gene is a counterpart of *ATM1* and that its product is probably involved in heme transport. We mapped the *hABC7* gene to chromosome Xq13.1–q13.3 by fluorescence in-situ hybridization. As band Xq13 has been implicated in X-linked sideroblastic anemia with spinocerebellar ataxia, *hABC7* becomes a candidate gene for this heritable disorder.

Key words ABC transporter · Mitochondria · Heme · Xq13 · X-linked sideroblastic anemia with spinocerebellar ataxia

Introduction

The heterogeneous features of sideroblastic anemias (MIM no. 301300) include microcytic, hypochromic anemia, and an abnormal accumulation of iron in mitochondria of bone marrow erythroblasts. Sideroblastic anemias are inherited in an X-linked recessive or, in very few reported cases, an autosomal manner (Bottomley 1982; Kasturi et al. 1982; May and Fitzsimons 1994). Hereditary spastic parapareses and ataxias (MIM no. 301310) may be autosomal or X-linked; however, each can be expressed either separately or in a form complicated by other abnormalities (Harding 1983).

In early 1996, the entire nucleotide sequence of the budding yeast, *Saccharomyces cerevisiae*, genome was published (Goffeau et al. 1996). By a screening for human homologues using our private database of human expressed-sequence tags (ESTs) (Fujiwara et al. unpublished data) against the yeast genomic sequence deposited in GenBank and the European Molecular Biology Laboratory (EMBL, Heidelberg), we obtained an EST homologous to yeast *ATM1*, an ATP-binding (ABC) transporter gene (Leighton and Schatz 1995) and a partial sequence of the murine ABC7 *(mABC7)* gene. Recently, the complete sequence of *mABC7* was cloned and reported by Savary et al. (Savary et al. 1997). This EST fragment of coding sequence therefore seemed likely to represent a novel member of the ABC superfamily.

Most of the products of ABC superfamily genes are transmembrane proteins that take part in transporting a wide variety of substrates across cell membranes (Dean and Allikmets 1995; Ortiz et al. 1995; Steiner et al. 1995). Several ABC transporters in yeast, in addition to *ATM1*, have been identified and characterized (Kuchler et al. 1989; McGrath and Varshavsky 1989; Balzi et al. 1994; Bissinger and Kuchler 1994; Hirata et al. 1994; Szczypka et al. 1994; Shani et al. 1995).

ATM1 encodes a half-type transporter that is located in the mitochondrial inner membrane. Typical ABC transporters consist of two transmembrane regions and two ATP binding domains, and these regions independently function in transporting their substrates. In contrast, the half-type transporters consist of a single transmembrane region and an ATP binding domain, and each such region properly functions as a homo- or heterodimer in the same manner (Hyde et al. 1990). The protein yAtm1p is one of only a few members of the mitochondrial ABC transporter family that have been identified; it is essential for normal cellular

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growth, as disruption of the gene results in a major deficiency of cytochromes in mitochondria and instability of mitochondrial DNA. Thus, yAtm1p is assumed to be a heme transporter (Leighton and Schatz 1995). However, the biochemical functions and substrates of yAtm1p are not yet fully understood.

In humans, diseases caused by defective ABC transporters include cystic fibrosis (CFTR; Riordan et al. 1989), Zellweger syndrome (PMP70; Kamijo et al. 1990; Gartner et al. 1992), adrenoleukodystrophy (ALD; Mosser et al. 1993), and Stargardt macular dystrophy (ABCR; Allikmets et al. 1997). Interestingly, the PMP70 and ALD proteins are located in the membranes of peroxisomes (Kamijo et al. 1990; Contreras et al. 1994) which, like mitochondria, are organelles.

Here, we report the nucleotide sequence, deduced amino acid sequence, expression pattern, and chromosomal localization of a novel human gene of the ABC transporter family, designated *hABC7*, whose sequence is significantly homologous to *ATM1*. We believe this gene to be a good positional and functional candidate for X-linked sideroblastic anemia with spinocerebellar ataxia.

Materials and methods

DNA cloning and sequencing

We have determined the nucleotide sequences of approximately 30,000 expressed sequence tags (EST) that were randomly selected from human fetal brain, placenta, and aorta cDNA libraries constructed at the Otsuka GEN Research Institute, Otsuka Pharmaceutical Co., Ltd. On the basis of these EST sequences (Fujiwara et al. unpublished data), we established an original EST database whose sequences we compared with the complete genome of *S. cerevisiae* deposited in GenBank and EMBL. Homology search was carried out by means of the TfastA program of the UWGCG package (Pearson and Lipman 1988). We isolated and sequenced an EST, designated GEN-535D01, which was highly homologous to the yeast *ATM1* gene. DNA sequencing was performed by the dideoxynucleotide chain termination method using an A.L.F. (Pharmacia, Uppsala, Sweden) or a 377A DNA sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequence data reported here will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases with accession number AB005289.

Mixed oligonucleotide primed amplification of cDNA (MOPAC)

Mixed oligonucleotide primed amplification of cDNA (MOPAC) was applied in our experiments according to the conventional method (Lee et al. 1989), with slight modifications. We designed a mixed oligonucleotide primer, MABC7-P1: 5′-CAGCAGAGATGGGG(ATCG)AA-(AG)GA(TC)AA(TC)TC-3′, based on a corresponding segment of predicted amino acids in murine ABC7 (mABC7): QQRWGKDNS at residues 8–16 (GeneBank accession No. U43892; Savary et al. 1997). We carried out polymerase chain reaction (PCR) amplification with primer MABC7-P1 and a gene-specific primer, 535D01P01: 5′-AGGCTTTCCAGGCTCACATC-3′ (antisense), which had been constructed on the basis of the nucleotide sequence determined for the GEN-535D01 clone. After the PCR products were purified and subcloned into pT7Blue(R) T-Vector (Novagen, Madison, WI, USA), their nucleotide sequences were determined. With this procedure we targeted PCR product GEN-535D01M04 for isolation.

Plaque hybridization and cloning

Approximately 500,000 plaques in a λgt10 human fetal brain library (oligo-dT- and random hexamer-stretched; Clontech, Palo Alto, CA, USA) were screened by plaque hybridization. For the probe, a DNA fragment was amplified from GEN-535D01M04 by PCR with specific primers MABCT7-P1 and 535D01P12 (5′-AAGCTCCCGTCTGTCTGCTC-3′, antisense), purified, and labeled with [^{32}P]dCTP. Nylon membranes were prehybridized at 65°C for an hour in a solution of 0.7% polyethylene glycol (PEG 8000)/10% sodium dodecylsulfate (SDS) containing 100 μg/ml of denatured salmon sperm DNA, and hybridized overnight in the same solution containing the probe. The membranes were washed twice with 2 × sodium saline citrate(SSC)/0.1% SDS at room temperature for 5 min and twice with 0.1 × SSC/0.1% SDS at 65°C for 15 min, then exposed at −80°C for 6 h. Positive plaques were picked up and suspended n 50 μl of SM buffer. Inserts in positive plaques were amplified by PCR using a flanking primer (λgt10-PRV2: 5′-GCAAGTTCAGCCTGGTTAAG-3) at the *Eco*RI cloning site of the λgt10 phage and the gene-specific primer 535D01P12. After cloning and sequencing the MOPAC products, we selected a clone designated GEN-535D01F20.

Northern analysis

To examine expression of the gene represented by GEN-535D01 in human adult tissues, northern-blot hybridization was carried out using the Human Multiple-Tissue Northern (MTN) blot system (Clontech) according to the manufacturer's protocol. For the probe, a DNA fragment was amplified from GEN-535d01 by PCR with primers 535D01PN1: 5′-AACAGTGGTTGATGCAGATG-3′ (sense) and 535D01PN2: 5′-ATGTGACTTAGCACG-AACAG-3′ (antisense). The product was purified and labeled with [^{32}P]dCTP. The membrane was prehybridized for 2 h and then hybridized with the probe for 20 h at 42°C in 50% formamide/5 × Denhardt's solution/5 × SSC/2% SDS containing 100 μg/ml denatured salmon sperm DNA. The membrane was washed twice with 2 × SSC/0.1% SDS at room temperature for 5 min, and twice with 0.1 × SSC/0.1% SDS at 65°C for 15 min, then autoradiographed at −80°C for 20 h and read again after a 42-h exposure.

Bacterial artificial chromosome cloning and chromosomal localization by direct R-banding fluorescence in-situ hybridization

A bacterial artificial chromosome (BAC) genomic clone containing the *hABC7* gene was screened from the human BAC DNA library (Research Genetics, Huntsville, AL, USA) by PCR with primers 535D01PB1: 5′-ATACACAGAGCAGCCGTGTG-3′ (sense) and 535D01PB2: 5′-ATGTGACTTAGCACGAACAG-3′ (antisense), according to the manufacturer's protocol. Direct R-banding fluorescence in-situ hybridization (FISH), a technique based on FISH combined with replicated prometaphase R-bands (Takahashi et al. 1990, 1991), was

Fig. 1 Nucleotide and deduced amino acid sequences of the *hABC7* gene. The 2416 nucleotides of total sequence and the translated 752 amino-acid open reading frame are indicated. Nucleotide positions are *numbered*, and the deduced amino acids are shown in standard single-letter symbols *below* each codon. The mitochondrial targeting sequence is indicated by a *shaded zone*

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                                                        ATCTAGGC      8
TCAGTTATGTTTACTAACATGAATCTTACTTAACAGCATGAGGCAAGATCTACGCTCAAG     68
ATGGCGCTGCTCGCGATGCATTCTTGGCGCTGGGCGGCCGCGGCGGCTGCTTTCGAAAAG    128
M  A  L  L  A  M  H  S  W  R  W  A  A  A  A  A  A  F  E  K      20

CGCCGGCACTCCGCGATTCTGATCCGGCCTTTAGTCTCTGTTAGCGGCTCAGGTCCGCAG    188
R  R  H  S  A  I  L  I  R  P  L  V  S  V  S  G  S  G  P  Q      40

TGGAGGCCACATCAACTCGGCGCCTTGGGAACCGCTCGAGCCTACCAGATTCCAGAGTCA    248
W  R  P  H  Q  L  G  A  L  G  T  A  R  A  Y  Q  I  P  E  S      60

TTAAAAAGTATCACATGGCAGAGATTGGGAAAAGGCAATTCAGGACAGTTCTTAGATGCT    308
L  K  S  I  T  W  Q  R  L  G  K  G  N  S  G  Q  F  L  D  A      80

GCAAAGGCTCTCCAGGTATGGCCACTGATAGAAAAGAGGACATGTTGGCATGGTCATGCA    368
A  K  A  L  Q  V  W  P  L  I  E  K  R  T  C  W  H  G  H  A     100

GGAGGAGGACTCCACACAGACCCAAAAGAAGGGTTAAAAGATGTTGATACTCGGAAAATC    428
G  G  G  L  H  T  D  P  K  E  G  L  K  D  V  D  T  R  K  I     120

ATAAAAGCAATGCTTTCTTATGTGTGGCCCAAAGACAGGCCAGATCTACGAGCTAGAGTT    488
I  K  A  M  L  S  Y  V  W  P  K  D  R  P  D  L  R  A  R  V     140

GCCATTTCGCTGGGATTTTTGGGTGGTGCAAAGGCCATGAATATTGTGGTTCCCTTCATG    548
A  I  S  L  G  F  L  G  G  A  K  A  M  N  I  V  V  P  F  M     160

TTTAAATATGCTGTAGACAGCCTCAACCAGATGTCGGGAAACATGCTGAACCTGAGTGAT    608
F  K  Y  A  V  D  S  L  N  Q  M  S  G  N  M  L  N  L  S  D     180

GCACCAAATACAGTTGCAACCATGGCAACAGCAGTTCTGATTGGCTATGGTGTATCAAGA    668
A  P  N  T  V  A  T  M  A  T  A  V  L  I  G  Y  G  V  S  R     200

GCTGGAGCTGCTTTTTTTAACGAAGTTCGAAATGCAGTATTTGGCAAGGTAGCCCAGAAT    728
A  G  A  A  F  F  N  E  V  R  N  A  V  F  G  K  V  A  Q  N     220

TCAATCCGAAGAATAGCCAAAAATGTCTTTCTCCATCTTCACAACCTGGATCTGGGTTTT    788
S  I  R  R  I  A  K  N  V  F  L  H  L  H  N  L  D  L  G  F     240

CACCTGAGCAGACAGACGGGAGCTTTATCTAAGGCTATTGACAGAGGAACAAAGGGTATC    848
H  L  S  R  Q  T  G  A  L  S  K  A  I  D  R  G  T  K  G  I     260

AGTTTTGTCCTGAGTGCTTTGGTATTTAATCTTCTTCCCATCATGTTTGAAGTGATGCTT    908
S  F  V  L  S  A  L  V  F  N  L  L  P  I  M  F  E  V  M  L     280

GTCAGTGGTGTTTTGTATTACAAATGCGGTGCCCAGTTTGCTTTGGTAACCCTTGGAACA    968
V  S  G  V  L  Y  Y  K  C  G  A  Q  F  A  L  V  T  L  G  T     300

CTTGGTACATACACAGCATTCACAGTTGCAGTCACACGGTGGGGAACTAGATTTAGAATA   1028
L  G  T  Y  T  A  F  T  V  A  V  T  R  W  G  T  R  F  R  I     320

GAAATGAACAAAGCAGATAATGATGCAGGTAATGCTGCTATAGACTCACTGCTGAATTAT   1088
E  M  N  K  A  D  N  D  A  G  N  A  A  I  D  S  L  L  N  Y     340

GAAACTGTGAAGTATATTAATAATGAAAGATATGAAGCACAGAGATATGATGGATTTTTG   1148
E  T  V  K  Y  I  N  N  E  R  Y  E  A  Q  R  Y  D  G  F  L     360

AAGACGTATGAGACTGCTTCATTGAAAAGTACCTCTACTCTGGCTATGCTGAACTTTGGT   1208
K  T  Y  E  T  A  S  L  K  S  T  S  T  L  A  M  L  N  F  G     380

CAAAGTGCTATTTTCAGTGTCGGTTTAACAGCTATAATGGTGCTCGCCAGTCAGGGAATT   1268
Q  S  A  I  F  S  V  G  L  T  A  I  M  V  L  A  S  Q  G  I     400

GTGGCAGGTACCCTTACTGTTGGAGATCTAGTAATGGTGAATGGACTGCTTTTTCAGCTT   1328
V  A  G  T  L  T  V  G  D  L  V  M  V  N  G  L  L  F  Q  L     420
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Fig. 1 Continued

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TCATTACCCCTGAACTTTCTGGGAACTGTATATAGAGAGACTAGACAAGCACTCATAGAT  1388
S  L  P  L  N  F  L  G  T  V  Y  R  E  T  R  Q  A  L  I  D    440

ATGAACACCTTGTTTACTCTACTCAAGGTAGACACCCAAATTAAAGACAAAGTGATGGCA  1448
M  N  T  L  F  T  L  L  K  V  D  T  Q  I  K  D  K  V  M  A    460

TCTCCCCTTCAGATCACACCACAGACAGCTACCGTGGCCTTTGATAATGTGCATTTTGAA  1508
S  P  L  Q  I  T  P  Q  T  A  T  V  A  F  D  N  V  H  F  E    480

TACATTGAGGGCCAGAAAGTCCTTAGTGGAATATCCTTTGAAGTCCCTGCAGGAAAGAAA  1568
Y  I  E  G  Q  K  V  L  S  G  I  S  F  E  V  P  A  G  K  K    500

GTGGCCATTGTAGGAGGTAGTGGGTCAGGGAAAAGCACAATAGTGAGGCTATTATTTCGC  1628
V  A  I  V  G  G  S  G  S  G  K  S  T  I  V  R  L  L  F  R    520

TTCTATGAGCCTCAAAAGGGTAGCATTTATCTTGCTGGTCAAAATATACAAGATGTGAGC  1688
F  Y  E  P  Q  K  G  S  I  Y  L  A  G  Q  N  I  Q  D  V  S    540

CTGGAAAGCCTTCGGAGGGCAGTGGGAGTGGTACCTCAGGATGCTGTCCTCTTCCATAAT  1748
L  E  S  L  R  R  A  V  G  V  V  P  Q  D  A  V  L  F  H  N    560

ACTATTTATTACAACCTCTTATATGGAAACATCAGTGCTTCACCTGAGGAAGTGTATGCA  1808
T  I  Y  Y  N  L  L  Y  G  N  I  S  A  S  P  E  E  V  Y  A    580

GTGGCAAAATTAGCTGGACTTCATGATGCAATTCTTCGAATGCCACATGGATATGACACC  1868
V  A  K  L  A  G  L  H  D  A  I  L  R  M  P  H  G  Y  D  T    600

CAAGTAGGGGAACGAGGACTCAAGCTTTCAGGAGGAGAAAAGCAAAGAGTAGCAATTGCA  1928
Q  V  G  E  R  G  L  K  L  S  G  G  E  K  Q  R  V  A  I  A    620

AGAGCCATTTTGAAGGACCCCCCAGTCATACTCTATGATGAAGCTACTTCATCGTTAGAT  1988
R  A  I  L  K  D  P  P  V  I  L  Y  D  E  A  T  S  S  L  D    640

TCGATTACTGAAGAGACTATTCTTGGTGCCATGAAGGATGTGGTCAAACACAGAACTTCT  2048
S  I  T  E  E  T  I  L  G  A  M  K  D  V  V  K  H  R  T  S    660

ATTTTCATTGCACACAGATTGTCAACAGTGGTTGATGCAGATGAAATCATTGTCTTGGAT  2108
I  F  I  A  H  R  L  S  T  V  V  D  A  D  E  I  I  V  L  D    680

CAGGGTAAGGTAGCCGAACGTGGTACCCACCATGGTTTGCTTGCTAACCCTCATAGTATC  2168
Q  G  K  V  A  E  R  G  T  H  H  G  L  L  A  N  P  H  S  I    700

TATTCAGAAATGTGGCATACACAGAGCAGCCGTGTGCAGAACCATGATAACCCCAAATGG  2228
Y  S  E  M  W  H  T  Q  S  S  R  V  Q  N  H  D  N  P  K  W    720

GAAGCAAAGAAAGAAAATATATCCAAAGAGGAGGAAAGAAAGAAACTACAAGAAGAAATT  2288
E  A  K  K  E  N  I  S  K  E  E  E  R  K  K  L  Q  E  E  I    740
GTCAATAGTGTGAAAGGCTGTGGAAACTGTTCGTGCTAAGTCACATAAGACATTTTCTTT  2348
V  N  S  V  K  G  C  G  N  C  S  C                            752

TTTTGTTGTTTTGGACTACATATTTGCACTGAAGCAGAATTGTTTTATTAAAAAAATCAT  2408
ACATTCCC                                                       2416
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applied for determining the chromosomal localization. Labeling, hybridization, rinsing, and detection were carried out in a routine manner (Takahashi et al. 1991). Signals were detected and analyzed with a Cytovision imaging device (Applied Imaging, Santa Clara, CA, USA).

Results

cDNA cloning and sequencing

We obtained a cDNA clone, GEN-535D01, whose deduced amino acid sequence revealed a high degree of homology with yAtm1p. However, when compared with the nucleotide sequence of *ATM1*, GEN-535D01 appeared to be lacking the 5′ portion. We performed MOPAC and plaque hybridization procedures to obtain the missing segment, and obtained two clones containing the 5′ end of the gene. We determined the entire nucleotide sequence of hABC7 cDNA by sequencing the overlapping cDNA clones GEN-535D01, 535D01M04, and 535D01F20. The assembled cDNA of hABC7 consisted of 2416 nucleotides containing an open reading frame of 2256 nucleotides. The size of this cDNA plus an estimate of polyA (usually 100–200 bases in eukaryotes) was in good agreement with the value observed by northern blotting (~2.6 kb). Figure 1 shows the nucleotide and deduced amino acid sequences of hABC7. The ATG at nucleotides 69–71 appeared to be the start codon because the sequence (AAGATGG) surrounding it was similar to the Kozak consensus sequence for translational initiation (Kozak 1991) and because it was preceded by an in-frame stop codon upstream. No polyadenylation signals (AATAAA) were found in the short 3′-noncoding region of available sequence (Fig. 1). The open reading frame

Fig. 2 Alignment of predicted amino acid sequences of human ABC7, murine ABC7 protein, and yAtm1p. "|" and ":" indicate identical and similar amino acid residues, respectively. Mitochondrial targeting sequences are indicated by *underlining*. The *shaded zone* and *bold-face* type indicate respectively the predicted amino acid sequences of the open reading frame of murine ABC7 and the partial human ABC7 sequence reported by Savary et al. (Savary et al. 1997). The *outlined "E"* indicates the amino acid residue different from the "V" reported by Savary et al.

would encode 752 amino acids with a calculated molecular weight of 82,598 daltons.

Structural characteristics

The nucleotide and deduced amino acid sequences of hABC7 cDNA, compared with those of *ATM1*, showed 58.1% and 48.9% identities, respectively. Furthermore, a BLASTX homology search (Altschul et al. 1990) in GenBank and EMBL revealed that this gene is highly homologous (91.6% identity) to the deduced amino acid sequence of murine ABC7 (mABC7) (GenBank accession No. U43892; Savary et al. 1997). Figure 2 shows the alignment of the deduced amino acid sequences for hABC7, mABC7, and Atm1p. Hydropathy analysis (Kyte and Doolittle 1982) revealed that hABC7 protein contains four transmembrane domains, which occur in N-terminal and hydrophilic C-terminal regions (data not shown). Prediction of Protein Sorting Signals and Localization Sites in Amino Acid Sequences (PSORT) protein structural and localization analysis (Nakai 1991; Nakai and Kanehisa 1992) demonstrated the presence of a mitochondrial targeting sequence (QRLGKG) at amino acids 65–70 in the N-terminal region of hABC7 (cf. Figs 1,2), and the predicted protein is likely to be located in mitochondrial membrane.

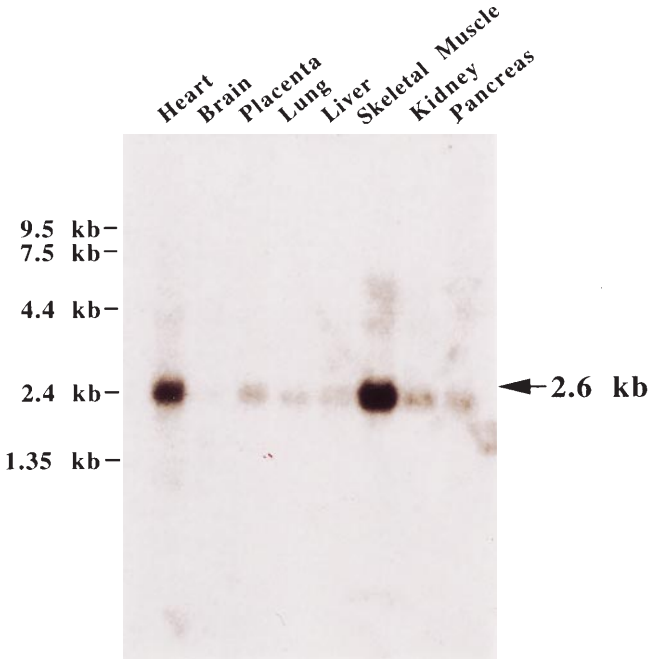

Fig. 3 Northern-blot analysis of the human *ABC7* gene in various human tissues after 20 h of exposure. Size markers are shown on the *left*

Expression of *hABC7*

Northern-blot analysis indicated a single transcript of 2.6 kb, which was expressed strongly in skeletal muscle and was detectable in heart (Fig. 3). Prolonged exposure demonstrated weak expression of a transcript of the same size in all other tissues examined (data not shown).

Chromosomal localization by direct R-banding FISH

We performed direct R-banding fluorescence in situ hybridization (FISH) to determine the chromosomal location for a BAC clone containing hABC7 genomic DNA. The signals were localized to the q13.1–q13.3 band of chromosome X, and no twin spots were observed in any other chromosomes (Fig. 4).

Discussion

ABC transporters constitute a superfamily of proteins responsible for transporting a variety of substrates across cell membranes. Dysfunctions of ABC transporters lead to various diseases such as cystic fibrosis, Zellweger syndrome, adrenoleukodystrophy, and Stargardt macular dystrophy. For this reason, ABC transporters have been the subject of intensive studies with respect to many other diseases (Allikmets et al. 1996; Decottignies and Goffeau 1997). In the work reported here, we isolated the human ABC7 cDNA through its high degree of homology to *ATM1* by screening our private human EST database against the archived yeast genome. In view of its predicted primary structure, hABC7 protein seemed to be a half-type ABC transporter located in the mitochondrial inner membrane, as is yeast Atm1p; thus we consider the *hABC7* gene as the human counterpart of *ATM1*. This type of ABC transporter probably consists of a transmembrane region and an ATP-binding cassette.

During preparation of the present manuscript, Savary et al. (1997) reported the full-length cDNA sequence of murine *ABC7*, a partial sequence of the human cDNA, and chromosomal localizations of both clones. Their predicted primary sequence of hABC7 protein corresponds to the codons represented by nucleotides 413–752 of Fig. 1. Our chromosomal mapping of the *hABC7* gene corresponds to the location reported by the Savary group. However, the amino acid sequences they reported for mABC7 and hABC7 proteins seemed to lack the N-terminal positions in comparison with ours, i.e., amino acids 1–65 and 1–412 respectively; moreover, at position 542 of the hABC7 protein, our sequence shows glutamic acid instead of valine (Fig. 2). The mitochondrial targeting sequence is found at positions 9–14 in the predicted primary sequence of the mABC7 protein. This signal sequence is conserved among human, mouse, and yeast (Fig. 2).

The substrate of yAtm1p has not been clarified, but Leighton and Schatz (1995) proposed that this protein is involved in the transport of heme across the mitochondrial membrane. Yeast ABC protein families have been classified into six major clusters and ten subclusters. This cluster-

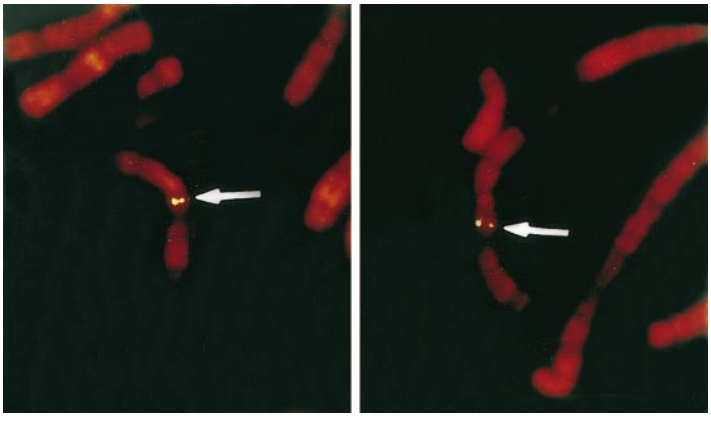

Fig. 4 Partial R-banded metaphase plates after fluorescence in-situ hybridization (FISH) with the *hABC7* gene. *Arrows* indicate the signals on chromosome Xq13.1–q13.3

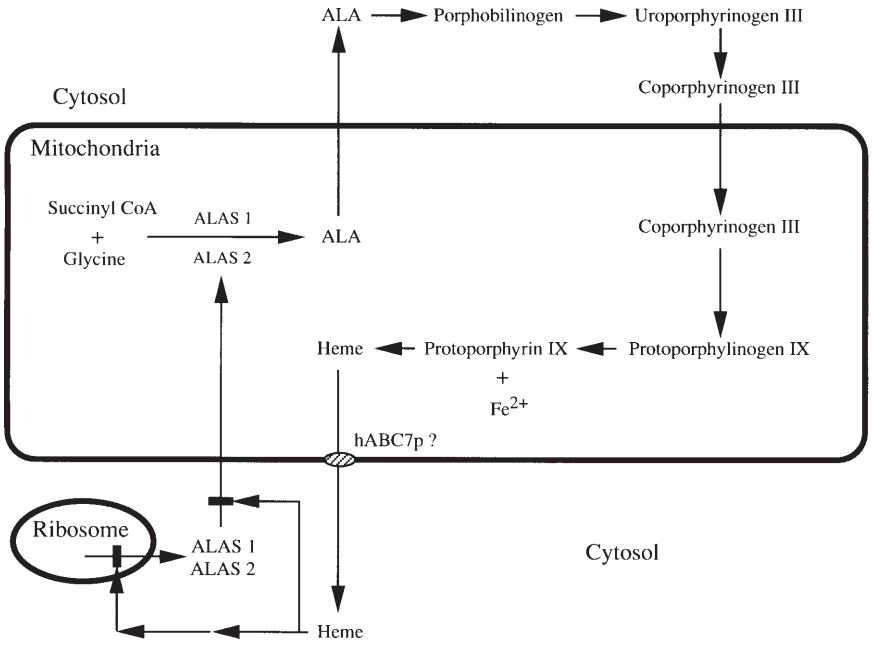

Fig. 5 Pathway of heme synthesis and its regulatory system (Harada et al. 1995). δ-aminolevulinate synthase (ALAS) is synthesized in ribosomes and transferred into mitochondria where it catalyzes production of δ-aminolevulinate from succinyl CoA and glycine. The δ-aminolevulinate is then exported to the cytosol and enzymatically processed to coproporphyrinogen III by way of porphobilinogen and uroporphyrinogen III. Coproporphyrinogen III is introduced into mitochondria and transformed to protoporphyrinogen IX. In the course of heme production, protoporphyrinogen IX is converted to protoporphyrin IX and then combined with ferrous ions. Synthesis of ALAS is enhanced by cytosolic heme in its own pathway. On the other hand, cytosolic heme inhibits the transportation of ALAS into mitochondria

ing may be associated with functional similarities; e.g., proteins belonging to the subcluster that contains yAtm1p may be involved in transportation of anionic compounds (Decottignies and Goffeau 1997). Given the high degree of homology between hABC7 and yAtm1p in amino acid sequences that may determine functional characteristics, and considering the pattern of expression, the activity of the *hABC7* gene probably correlates with the level of cytochrome *c*, a heme derivative, in various human tissues. We suggest that the hABC7 protein may play a role in transporting heme or anionic heme-related compounds across mitochondrial membranes.

In humans, a decrease in activity of δ-aminolevulinate synthase (ALAS) triggers an increase in heme biosynthesis in patients with X-linked sideroblastic anemia. Nonerythroid (ALAS-N) and erythroid-specific (ALAS-E) isozymes of ALAS are encoded by the *ALAS1* and *ALAS2* genes respectively (Bishop et al. 1981); these genes have been localized respectively to chromosomes 3p21 and Xp11.21-p21.3 (Bishop et al. 1990). Several independent missense mutations of the *ALAS2* gene have been found in pedigrees segregating an allele responsible for X-linked sideroblastic anemia (Cotter et al. 1992, 1994; Cox et al. 1994; Prades et al. 1995). However, one linkage study suggested that another gene is responsible for a syndrome of X-linked sideroblastic anemia with spinocerebellar ataxia; its likely location was chromosome Xq13 (Raskind et al. 1991), the region where we have mapped the *hABC7* gene.

The products of the *PMP70* and *ALD1* genes, whose mutations are responsible for Zellweger syndrome and adrenoleukodystrophy respectively, are found in the membrane of another type of organelle, the peroxisome (Kamijo et al. 1990; Contreras et al. 1994). We speculate that dysfunction of the hABC7 protein is involved in X-linked sideroblastic anemia with spinocerebellar ataxia. We propose a hypothesis that the hABC7 protein is involved in transport of heme from the mitochondria to the cytosol (Fig. 5).

The transcription of ALAS1 mRNA is suppressed under the influence of the cytosolic heme molecules. In contrast, ALAS2 mRNA transcription is enhanced in the same conditions (Sassa and Nagai 1996). Furthermore, it is reported that δ-aminolevulinate causes ataxia in the rat when injected into the brain (Sasaki et al. 1995). These observations suggest the following hypothesis. Dysfunction of the hABC7 protein inhibits the transport of heme molecules into the cytosol, and therefore, heme molecules accumulate in mitochondria, resulting in their depletion in the cytosol. This change in relative heme molecule concentration results in an increase in the transcription of ALAS1 mRNA and a decrease in the transcription of ALAS2 mRNA. Low activity of ALAS-E in the erythrocyte (ALAS-E) plays a major role in causing sideroblastic anemia. On the other hand, high activity of ALAS-N induces the accumulation of δ-aminolevulinate in the brain, causing the patient to develop X-linked sideroblastic anemia with spinocerebellar ataxia.

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