Meeting was organized in 2002, and efforts to update the contents are still ongoing, its driving force is apparently decreasing. The mission first proposed by the authors to IPCS was in a sense fulfilled at the time of the second IFCS Meeting, because of the advances of the Internet and its quick worldwide acceptance. IOMC, which once adopted this project from IPCS after the first IFCS, has already lost its interest, for its member agencies have already provided their websites and put their information contents on them. Though GINC shifted its focus to the Asian region, many East Asian countries have also provided their own websites for their information contents. Therefore, the capacity building mission of GINC in this area seems to be fulfilled too. Even though the project is still supported by WHO/IPCS and the Japanese MHLW, it may now be the time to redesign the concept of the project and set new goals.

Chemical safety information is classified into two categories, regulatory information and scientific information. While regulatory information is provided by administrative organizations, scientific information such as toxicological data are mostly produced by research institutions of developed countries or by collaborations among them. Scientific information forms the basis of regulatory information, yet it is different in nature from regulatory information. Developing countries can adopt regulatory information produced by developed countries or international organizations like IPCS, Food and Agriculture Organization (FAO), or OECD, but they cannot play important roles in providing scientific information.

The information contents that GINC aims to cover are both regulatory information relevant for government regulatory actions and scientific information that provide the basis of the argument for regulations. If toxicological data become available to researchers in developing countries and if these researchers learn how to handle these data by computer, the levels of chemical safety and toxicology will undoubtedly be raised. This means, however, that researchers in these countries can easily access to the Internet and can easily find relevant data and manipulate them. Moreover, information for regulation and for scientific research often overlap, and thus there should be some one-stop-shopping site or portal site from where one can easily navigate both regulatory and scientific information. We have developed an example for such a system on the endocrine disruptor problem (http://www.nihs.go.jp/hse/endocrine-e/index.html) (Kaminuma et al., 2000).

Since the IT field is still rapidly advancing and changing, updating an information system requires the continuous efforts of dedicated experts. Such experts must improve their skills continuously. For example, XML is emerging as the new mark-up language for the next generation Internet. XML is useful for integrating distributed systems such as databases and file servers. It is not only necessary to train information experts to adapt to this new technology, but some negotiation is needed to standardize terminologies and codes. Meetings like GINC where experts on such topics convene are useful platforms to discuss these matters among collaborating groups.

Therefore, it is the authors' opinion and hope that the GINC project should not be terminated but rather it should be re-considered and restructured into a new international collaborative project for chemical safety that is not only for regulatory purposes but also for aiding collaboration among toxicology researchers and supporting toxicology information experts in the world.

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Biographies

Tsuguchika Kaminuma, Ph.D. Dr Kaminuma is currently working as a freelance researcher. He is also a board member of the Chem-Bio Informatics Society and the president of his own company, Biodynamics Inc. Dr Kaminuma did undergraduate work at International Christian University in Tokyo, received a Master of Science degree in Physics from Yale University in 1966 and a Ph.D. in Physics from the University of Hawaii in 1970. He worked on Pattern Recognition as a research assistant to Professor Michael Satoshi Watanabe from 1966 to 1971 at the University of Hawaii. He worked on computer application to biomedicine at a Laboratory of Hitachi Inc. (1971–1976), at Tokyo Metropolitan Institute of Medical Science (1976–1989), and at the NIHS (1989–2001). He retired from NIHS in March 2001. He has taught at several universities, founded the Chem-Bio Informatics Society and its journal, and worked for IPCS as a coordinator of Japanese researchers and a Program Advisory Board member.

Kotoko Nakata, Ph.D. Dr Nakata is currently working as Head of Second Section, Division of Chem-Bio Informatics, NIHS in Tokyo. She is also a board member of the Chem-Bio Informatics Society. Dr Nakata did undergraduate work at Saitama University, received a Master of Science degree in Physics from Ochanomizu University in 1970 and a Ph.D. in Biophysical Engineering from Osaka University in 1980. She worked on computer application to various Biosciences at Mitsubishi-Kasei Institute of Life Sciences (1980–1982) and at CSIRO (Australia, 1982–1983). She started working on DNA and protein sequence analyses at NCI, NIH (USA, 1983–1985), and continued work on that at NCI-FCRF (USA, 1985–1989) and at Mount Sinai School of Medicine (USA, 1989–1990). Since 1991, she has been working on protein structural analyses, and various database systems relating Drugs and Endocrine Disruptor Chemicals at NIHS, Tokyo.

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Molecular and cytogenetic characterization of the mouse ATP-binding cassette transporter Abcg4☆

Megumi Yoshikawa[a,b], Hikaru Yabuuchi[a], Asato Kuroiwa[c,d], Yoji Ikegami[b], Yoshimichi Sai[e], Ikumi Tamai[e], Akira Tsuji[e], Yoichi Matsuda[c,d], Hisahiro Yoshida[b], Toshihisa Ishikawa[a,*]

[a]*Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama-shi, Kanagawa 226-8501, Japan*

[b]*Department of Drug Metabolism and Disposition, Meiji Pharmaceutical University, Noshio 2-522-1, Kiyose-shi, Tokyo 204-8588, Japan*

[c]*Laboratory of Cytogenetics, Division of Bioscience, Graduate School of Environmental Research Science, Hokkaido University, North 10 West 8, Kita-ku, Sapporo-shi, Hokkaido 060-0810, Japan*

[d]*Chromosome Research Unit, Faculty of Science, Hokkaido University, North 10 West 8, Kita-ku, Sapporo-shi, Hokkaido 060-0810, Japan*

[e]*Faculty of Pharmaceutical Sciences, Kanazawa University, Takaramachi 13-1, Kanazawa-shi, Ishikawa 920-0934, Japan*

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Abstract

We have cloned a new mouse ATP-binding cassette (ABC) transporter, Abcg4, from a complementary DNA (cDNA) library of mouse brain. The cloned Abcg4 cDNA encodes a protein consisting of 646 amino acids and including one ATP-binding cassette and six transmembrane domains. The Abcg4 protein exhibits high identity (96%) with human ABCG4 in terms of the amino acid sequence. Fluorescence in situ hybridization with mouse and rat chromosomes has revealed that the *Abcg4* gene is located on chromosomes 9A5.3 and 8q22 distal in mouse and rat, respectively. In these loci on mouse and rat chromosomes, conserved linkage homologies were hitherto identified with human chromosome 11q23, which involves the human *ABCG4* gene. The mouse *Abcg4* gene as well as the human *ABCG4* gene each has a total of 14 exons to encode its respective protein. High transcript levels of mouse Abcg4 were detected in mouse brain, spleen, eye, and bone marrow. Taken together, our data on the chromosomal location, gene homology, protein structure, and phylogenetic relationships strongly support the idea that mouse Abcg4 is orthologue to the human ABCG4. By functionally analyzing the mouse Abcg4 protein, we may better understand the biological role of the human ABCG4 transporter. © 2002 Published by Elsevier Science B.V.

Keywords: ATP-binding cassette (ABC) transporter; Mouse chromosome; Fluorescence in situ hybridization; ABCG2 (BCRP/MXR/ABCP); ABCG4

1. Introduction

The ATP-binding cassette (ABC) transporter family comprises a large group of proteins with over 100 members identified in eukaryotes and prokaryotes alike. Based on the arrangement of such molecular structural components as the ATP-binding cassette and the topology of transmembrane domains, human ABC transporters are classified into 7 different gene sub-families (A–G) (Klein et al., 1999; Dean et al., 2001; http://gene.ucl.ac.uk/nomenclature/genefamily/abc.html). ABC transporters have regions of highly conserved amino acids, namely the Walker A, Walker B, and signature C motifs, within the ATP-binding cassette (Higgins, 1992; Walker et al., 1982). In mammalian cells, ABC transporters are localized in the plasma membrane or in cellular organelles such as endoplasmic reticulum, the Golgi complex, peroxisomes, and mitochondria. The ABC transporters perform the function of transporting a wide variety of exogenous and endogenous compounds across the cell membrane (Higgins, 1992; Childs and Ling, 1994; Dean and Allikmets, 1995). Overexpression of some types of ABC transporters has been reported to confer multidrug resistance upon tumor cells (Childs and Ling, 1994; Shen et al., 1986; Doyle et al., 1998; Ishikawa et al., 2000). On the other hand, disrupted and/or reduced functions caused by mutations of certain ABC transporter genes are reportedly

Abbreviations: ABC, ATP-binding cassette; EST, expressed sequence tag; FISH, fluorescence in situ hybridization; ORF, open reading frame; PCR, polymerase chain reaction; SSC, saline-sodium citrate solution (0.15 M NaCl and 0.015 M sodium citrate, pH 7.6)

☆ The mouse Abcg4 cDNA cloned in this study was submitted to the GenBank on June 18, 2001, and is registered with the accession number of AY040865.

* Corresponding author. Tel.: +81-45-924-5800; fax: +81-45-924-5838.
E-mail address: tishikaw@bio.titech.ac.jp (T. Ishikawa).

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responsible for some human genetic diseases. In this regard, studies on mouse models constructed to have specific ABC transporter gene disruptions have provided evidence for the biological function of such human ABC transporters as ABCA4, ABCB1, ABCB4, ABCC1, and ABCC7 (Weng et al., 1999; Schinkel et al., 1994; Smit et al., 1993; Schultz et al., 2001; Dorin et al., 1992).

Some recently discovered human ABCG sub-family members (i.e. ABCG1, ABCG2, ABCG4, ABCG5 and ABCG8) are so-called 'half-transporters', because they have one ATP-binding cassette and six transmembrane domains. Accumulating evidence suggests that those ABC transporters operate as homo- or hetero-dimers. It has been reported that transporters of the ABCG sub-family play a significant role in various physiological events: for example, ABCG1 involved in macrophage cholesterol metabolism (Klucken et al., 2000); ABCG2 in stem cell differentiation (Zhou et al., 2001); and ABCG5 and ABCG8 in absorption and excretion of sitosterols (Berge et al., 2000).

We have previously demonstrated that ABCG2 transports SN-38, an active metabolite of DNA topoisomerase I inhibitor CPT-11 and thereby suggested the association of ABCG2 with cancer drug resistance (Nakatomi et al., 2001). To develop mouse patho-physiological models, we have been seeking new mouse ABC transporters that belong to the ABCG sub-family. Meanwhile, Engel et al. (2001) have recently suggested that human ABCG4 is potentially involved in lipid metabolism. In the present study, we have undertaken the cloning of a new mouse ABC transporter, named Abcg4, to characterize its chromosomal localization, protein structure, and tissue-specific expression in comparison with those of the human ABCG4. Our data strongly suggest that the cloned mouse Abcg4 is orthologues to the human ABCG4. Therefore, the mouse Abcg4 complementary DNA (cDNA) obtained in the present study would be a useful tool to elucidate the physiological role of the human ABCG4.

2. Materials and methods

2.1. Cloning of mouse Abcg4 cDNA

Expressed sequence tag (EST) clones (GenBank Accession numbers: BG298084 and BE861999) were extracted from the currently available EST database by using the BLASTN program. To screen and clone cDNA of mouse Abcg4, the following polymerase chain reaction (PCR) primers were designed: the forward primer: 5′-CGCTGAGCATCCGGGTCGAA-3′ and the reverse primer: 5′-TTCCTGCCCAGAAGAGGTAGGAG-3′. PCR was performed with the TaKaRa Ex Taq™ polymerase (Takara Shuzo Co., Shiga, Japan), where the PCR reaction consisted of 35 cycles of 95°C for 30 s, 64°C for 30 s, and 72°C for 2 min. We have screened a panel of mouse multiple tissue cDNAs (MTC™, Clontech, Palo Alto, CA, USA) and found that Abcg4 is highly expressed in mouse brain and spleen. We then cloned Abcg4 from a mouse brain cDNA library (Clontech) by PCR in the same manner. The resulting PCR product was extracted from the electrophoresis agarose gels by using the QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA, USA), and subjected to TA cloning (Original TA cloning Kit, Invitrogen Co., Carlsbad, CA, USA). The sequence of cDNAs, thus cloned, was analyzed by automated DNA sequencing (TOYOBO Gene Analysis, Fukui, Japan).

2.2. Detection of mouse Abcg4 and human ABCG4 transcripts in different tissues

The transcripts of *Abcg4* and *ABCG4* genes were detected by PCR, where mouse and human cDNA panels from normal and fetal tissues as well as mouse embryo were purchased from Clontech. The PCR primers to detect mouse Abcg4 were the same as those described above, whereas the following primers were used for the detection of human ABCG4: the forward primer: 5′-GCTGCTTCCAACTCCCTACA-3′ and the reverse primer: 5′-GACTTGACCCGGTAACGCAG-3′. The PCR reaction for ABCG4 consisted of 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 2 min.

2.3. Data analysis

DNA sequences were analyzed with the GENETYX-MAC software version 11 (Software Development Co., Ltd. Tokyo, Japan) and compared with other ABCG transporter genes registered in the NCBI database. The hydropathy profile of the protein deduced from the cDNA sequence was calculated with the Kyte and Doolittle (1982) hydropathy algorithm, and the SOSUI program (http://sosui.proteome.bio.tuat.ac.jp/sosuimenu0.html) was used to predict transmembrane domains. Phylogenetic relationships were calculated by using the GENETYX-MAC software of the distance-based neighbor-joining method (Saitou and Nei, 1987). Mouse genome data (Celera Genomics Discovery System) was from the Celera Genomics, Inc.

2.4. Preparation of mouse and rat chromosomes and fluorescence in situ hybridization (FISH)

Preparation of R-banded chromosomes and FISH were performed to assign the *Abcg4* gene's chromosomal location in the mouse and rat as described by Matsuda et al. (1992) and Matsuda and Chapman (1995). Mitogen-stimulated mouse and rat splenocyte cultures were synchronized by thymidine blockage, and treated with 5-bromodeoxyuridine for incorporation during the late replication stage to enable differential replication staining after the release from excessive thymidine. R-band staining was performed by exposing the chromosome slides to ultraviolet (UV) light after staining with Hoechst 33,258.

The chromosome slides were fixed at 65°C for 2 h, dena-

tured at 70°C in 70% formamide/2 × saline-sodium citrate solution (0.15 M NaCl and 0.015 M sodium citrate, pH 7.6, SSC), and then dehydrated in a 70–85–100% ethanol series at 4°C. The mouse 2.0 kb cDNA fragment inserted in the pCR2.1 TOPO vector was used for chromosomal mapping. The cDNA fragment was labeled by nick translation with biotinylated 16-dUTP (Roche Diagnosis Co., Indianapolis, IN, USA) by the standard protocol. The labeled DNA fragment was ethanol-precipitated with salmon sperm DNA and *Escherichia coli* tRNA, and then denatured at 75°C for 10 min in 100% formamide. The denatured probe was mixed with an equal volume of hybridization solution to make a final concentration of 50% formamide, 2 × SSC, 10% dextran sulfate, and 2 μg/μl of bovine serum albumin (BSA) (Sigma-Aldrich, Tokyo, Japan). The mixture (20 μl) containing 250 ng of labeled DNA was pipetted onto the dehydrated slide, covered with parafilm, and incubated overnight at 37°C. The slides were washed for 20 min in 50% formamide/2 × SSC at 37°C, and in 2 × SSC and 1 × SSC for 20 min each at room temperature. After rinsing in 4 × SSC, they were incubated with goat anti-biotin antibodies (Vector Laboratories, Burlingame, CA, USA) at a 1:500 dilution in 1% BSA/4 × SSC under the cover-slip for 1 h at 37°C. They were washed with 4 × SSC, 0.1% Nonidet P-40/4 × SSC, 4 × SSC for 5 min each and then stained with fluoresceinated donkey anti-goat IgG (Nordic Immunological Lab., Tilburg, Netherlands) at a 1:500 dilution for 1 h at 37°C. After washing in 4 × SSC, 0.1% Nonidet P-40/4 × SSC, 4 × SSC for 10 min on a shaker, the slides were rinsed in 2 × SSC and stained with 0.75 μg/ml of propidium iodide. FISH images were photographed with a Nikon fluorescence microscope using Nikon filter sets B-2A and UV-2A.

3. Results and discussion

3.1. Cloning and characterization of mouse Abcg4 cDNA

The mouse Abcg4 cDNA was cloned from the mouse brain cDNA library by PCR, where specific primers were designed based on the mouse EST data for BG298084 and BE861999 (Fig. 1A). A 2056 bp fragment of Abcg4 cDNA was obtained, containing a 1938-bp open reading frame. The cDNA encodes a single peptide consisting of 646 amino acids, which exhibits high identity (96%) with the human ABCG4 amino acid sequence (Fig. 1B). Based on our data, mouse Abcg4 has an extension of 19 amino acids at the amino terminus as compared with the human ABCG4 sequence previously registered in the GenBank (AJ300465). In this respect, our finding is consistent with the most recent report of Engel et al. (2001) regarding human ABCG4.

Motif analyses revealed the existence of one ATP-binding cassette; namely, Walker A, Walker B and signature C motifs were recognized at positions of 102–109, 221–225, and 201–215 in the amino acid sequence, respectively (Fig. 1B). Six transmembrane domains were also found at positions of 381–403, 427–449, 474–496, 502–524, 534–556, and 618–639 in the amino acid sequence (Fig. 1B). Fig. 2A shows the hydropathy profile of the mouse Abcg4 protein, and Fig. 2B depicts a putative structure for the mouse Abcg4 protein. The deduced protein exhibits characteristic features of the ABCG sub-family with one ATP-binding cassette on the amino terminal part and six transmembrane domains on the carboxyl terminal part (Fig. 2B). Fig. 2C demonstrates the phylogenetic relationships between the Abcg4 protein and other members of the ABCG sub-family hitherto reported in mouse, rat, and human. These results suggest that the mouse Abcg4 protein is closely related to Abcg1 in mouse and rat as well as with ABCG4 and ABCG1 in human.

3.2. FISH analysis for chromosome mapping of Abcg4 in mouse and rat

The chromosomal location of the mouse *Abcg4* gene was determined by FISH with our cloned Abcg4 cDNA. Fig. 3A (panels a–c) shows the *Abcg4* gene loci on the mouse chromosome 9A5.3, and Fig. 3B schematically illustrates mouse chromosomes according to Evans (1996) and Matsuda et al. (1992). Because of the high identity between mouse and rat Abcg4, we could map the *Abcg4* gene on the rat chromosome 8q22 distal (Satoh et al., 1989) by using our mouse cDNA (panels d–f in Fig. 3A). In human, the *ABCG4* gene is reportedly located on chromosome 11q23 (Engel et al., 2001), which contains organizations homologous with regions of mouse chromosome 9A5.3 and rat chromosome 8 (Serikawa et al., 1998). In this context, it is concluded that mouse and rat *Abcg4* genes as well as the human *ABCG4* gene exist in the chromosomal regions where a conserved linkage homology has been identified among these species.

Fig. 3C shows the exon-intron structure of the *Abcg4* gene on mouse chromosome 9. The *Abcg4* gene spans about 15 kb and consists of 15 exons, where the translation-start codon (i.e. ATG) was found in exon 2. The Walker A motif was found in exon 3, whereas the Walker B motifs as well as the signature C were in exon 6. Table 1 summarizes the exons and introns of the mouse *Abcg4* gene and shows the partial sequences at splicing sites to confirm that all splicings in this gene follow the conventional GT-AG rule.

3.3. Tissue-specific expression of mouse Abcg4 and comparison with human ABCG4

The transcript of the *Abcg4* gene was detected in various tissues of the adult mouse, including brain, spleen, bone marrow, eyes, smooth muscle, and stomach (Fig. 4). In particular, high expression levels were observed in the brain and spleen. Interestingly, expression of Abcg4 in the mouse embryo seems to be enhanced during embryonic development, as exemplified by increasing transcript levels on days 7, 11, 15, and 17 (Fig. 4).

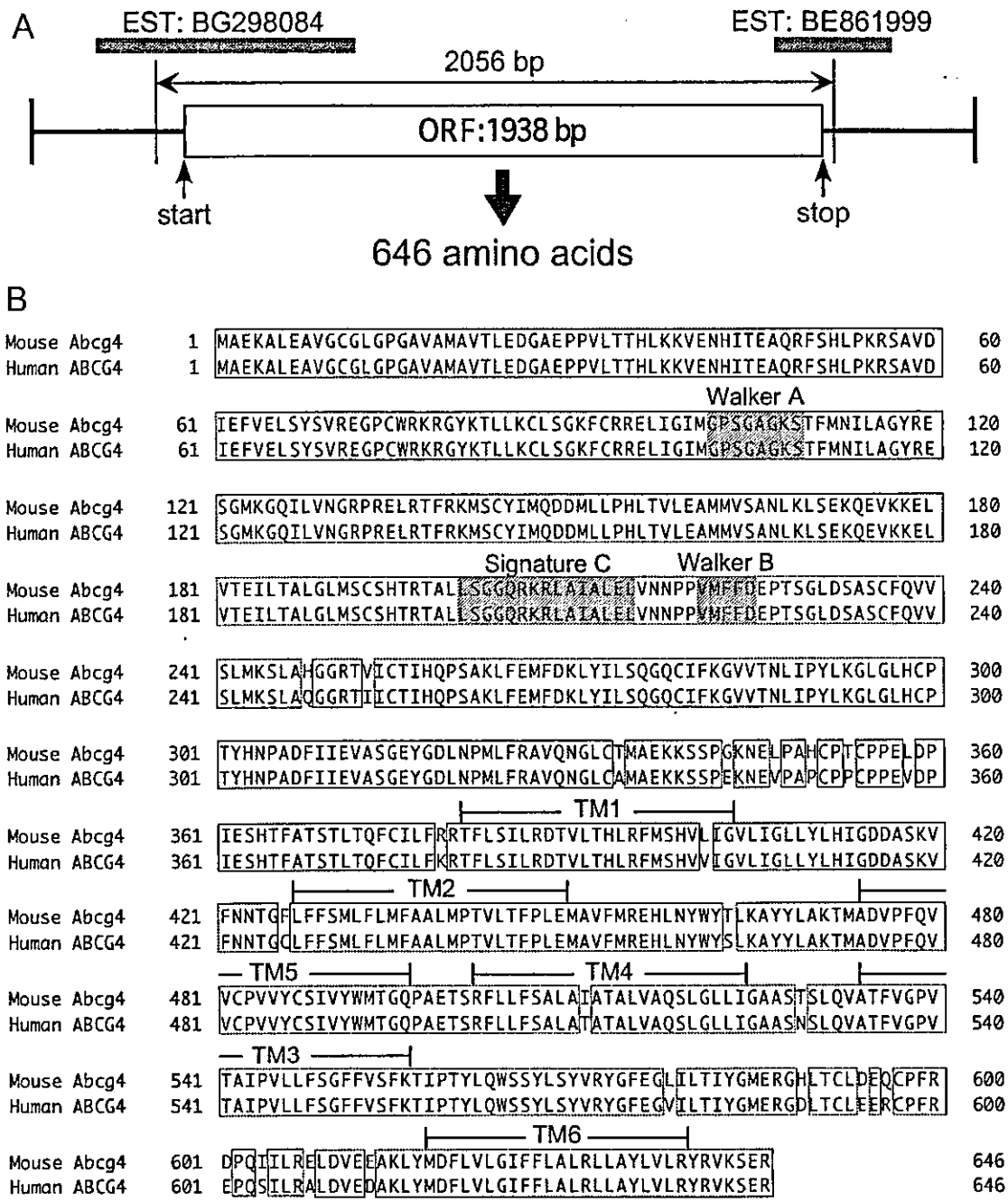

Fig. 1. Strategy for the cloning of mouse Abcg4 cDNA (A); and the alignment of mouse Abcg4 and human ABCG4 proteins (B). (A) Mouse Abcg4 cDNA was cloned by PCR as described in Section 2. (B) Amino acid sequences were aligned by using the GENETYX-MAC program, and identical sequences between mouse Abcg4 and human ABCG4 are indicated by boxes. Walker A and B motifs as well as the signature C are shaded. TM, transmembrane domain.

For comparison with the expression profile of the mouse *Abcg4* gene, the expression profile of the *ABCG4* gene in human tissues has been examined by PCR (Table 2). The human ABCG4 transcript was observed in various adult tissues, including brain, spleen, liver, testis, thymus, ovary, and small intestine. In the fetus as well, the transcript of human ABCG4 was detected in brain, lung, liver, kidney, and spleen. While further studies should be needed to complete the expression profiles for mouse Abcg4 and human ABCG4, it could be concluded from our data that these genes are expressed at high levels in the adult spleen of both mouse and human.

3.4. Potential functions of mouse Abcg4 and other members in the ABCG sub-family

ABCG1 and ABCA1 are known to be involved in cholesterol and phospholipid export. The expression of these genes is induced by activation of the hetero-dimer nuclear receptor of the liver specific X receptor (LXR) and retinoid X recep-

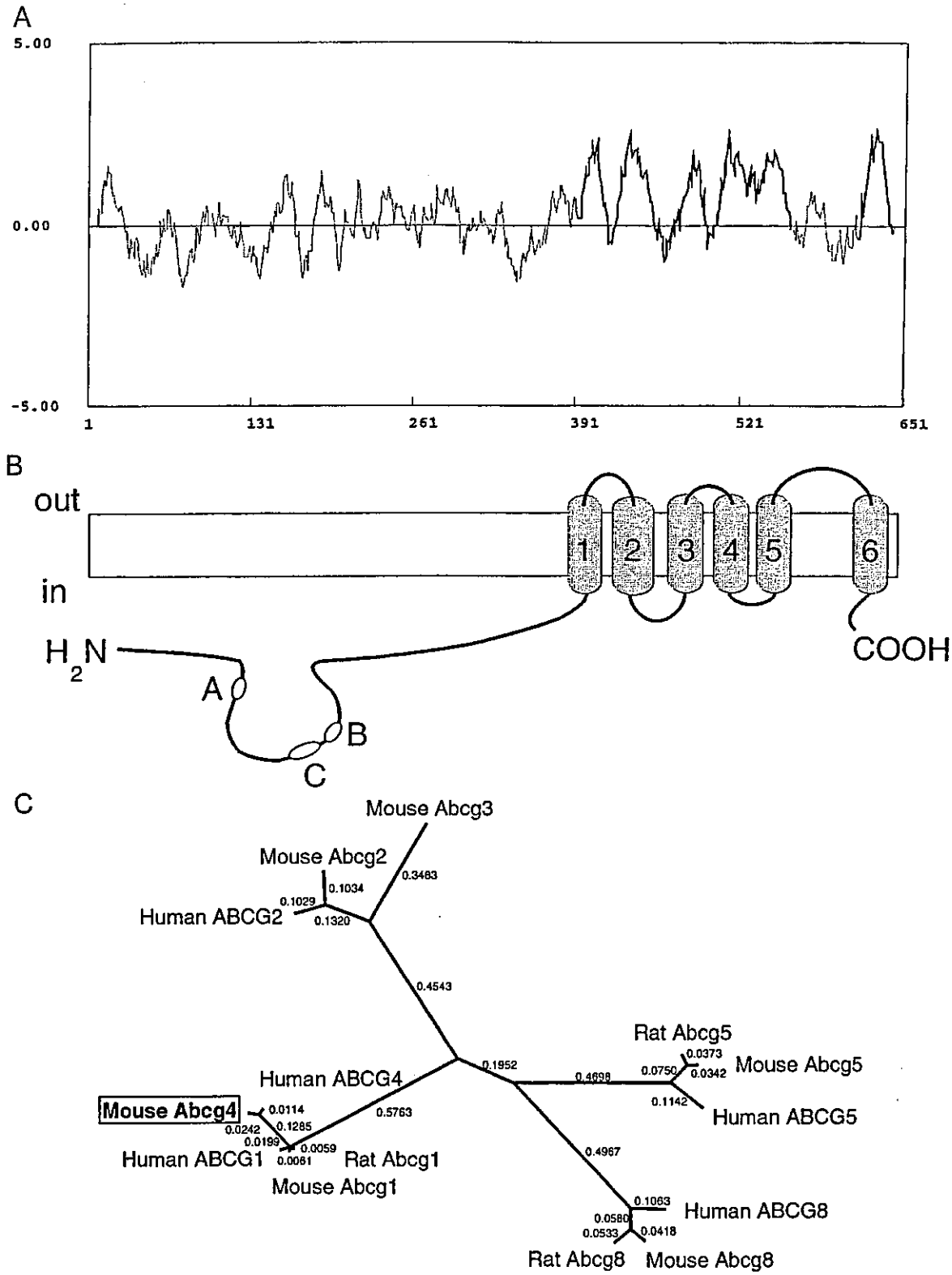

Fig. 2. The hydropathy plot (A); and the schematic illustration of the putative protein of mouse Abcg4 (B) as well as the phylogenetic relationship among members of the ABCG sub-family (C). (A) The hydropathy profile was calculated by the Kyte and Doolittle algorithm. Bold plots correspond to the area of transmembrane domains. (B) Walker A and B as well as signature C are indicated by A, B and C, respectively. Transmembrane domains are indicated by numbers. (C) The phylogenetic relationships among members of the ABCG sub-family in mouse, rat and human were calculated by using the distance-based neighbor-joining methods (Saitou and Nei, 1987).

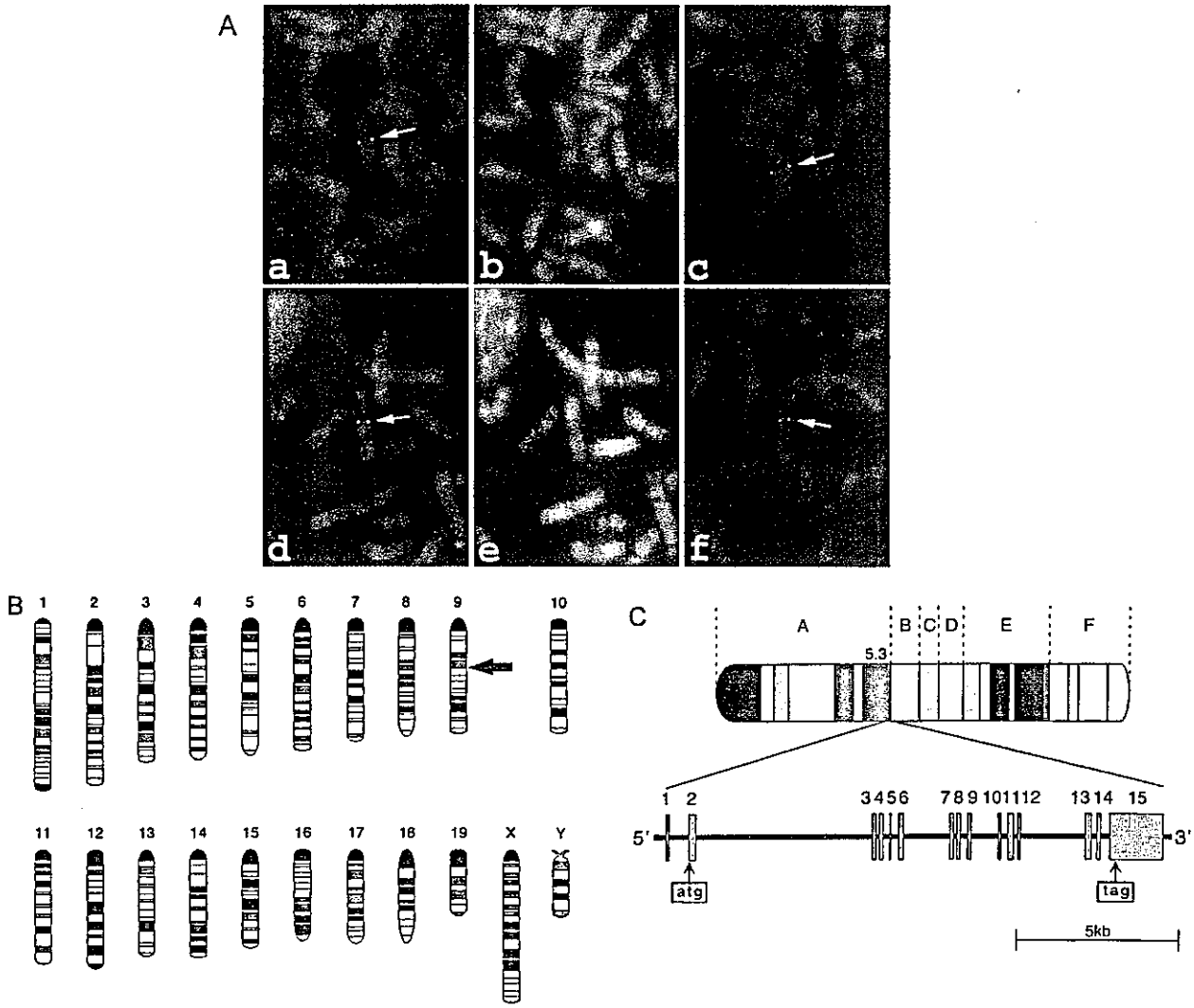

Fig. 3. Chromosomal location of the *Abcg4* gene (A); schematic illustration of mouse chromosomes (B); and the exon-intron structure of the mouse *Abcg4* gene located on chromosome 9 (C). (A) The *Abcg4* gene locus detected by FISH on the chromosomes of mouse (a–c); and rat (d–f). Arrows point out the hybridization signals. R- and G-banded patterns are demonstrated in (a, c, d, f); and (b, e), respectively. (B) The bold arrow points out the locus of the mouse *Abcg4* gene. (C) Exons are numerically indicated. 'atg' and 'tag' are translation start and stop codons, respectively.

tor (RXR) (Repa et al., 2000; Venkateswaran et al., 2000). It has recently been shown that the human *ABCG4* gene is also regulated by oxysterols and retinoids, active ligands of LXR and RXR (Engel et al., 2001). In the tissue-specific expression profiling, the human *ABCG4* gene was highly expressed in the liver (Table 2). Linkage analysis data suggest that a locus on human chromosome 11q23 is linked to hypoalphalipoproteinemia in many Utah families (Wagner et al., 2000). Since the *ABCG4* gene is located at the 11q23 region, it is tempting to speculate that ABCG4 is involved in the transport of lipid metabolites. On the other hand, *ABCG4* gene expression was also detected in human spleen and thymus, while the *Abcg4* gene is expressed in the mouse spleen and bone marrow (Table 2). Interestingly, mouse Abcg4 expression increased during the development of the embryo. At the present time, however, the biological function of Abcg4 in those tissues and in the embryo is not known.

So far, the ABCG sub-family members have been reported to operate as either homo- or hetero-dimers. For example, ABCG5 and ABCG8 are suggested to form a hetero-dimer to play a role in the absorption and excretion of dietary sterols (Berge et al., 2000). In terms of the amino acid sequence, the identity of mouse Abcg4 with Abcg1 was calculated to be 68.8%, the highest value among mouse ABCG sub-family members (Fig. 2C). High expression levels of both ABCG1 and ABCG4 are observed in the human brain, (Yoshikawa et al. unpublished work for ABCG1; Table 2 for ABCG4). ABCG1 was first described as a homologue of the *Drosophila* white protein (Chen et al., 1996). The *Drosophila* white protein is involved in the formation of eye color pigments as a hetero-dimer with

Table 1
Intron/exon boundaries of the mouse *Abcg4*

Exon	Exon size (bp)	Intron/exon	Exon/intron	Intron size (bp)
1	110	aggtgaagag AGCCGGAGTG	ACGCGTCAAG gtgaccaggc	545
2	250	tctcctgcag GCCGGCTGCG	CGCAAACGGG gtaggtggca	5485
3	118	cttgatgtag GTTACAAGAC	CAGGGTACAG gtgagcttga	112
4	133	ctccctgcag GGAGTCGGGG	GGCCATGATG gtgaggagag	111
5	51	acttgtttag GTCTCTGCCA	GAAGGAACTG gtgagtgggt	169
6	146	gtgcctgcag GTGACAGAGA	AGCCTACCAG gtagctccct	1423
7	124	ctctggacag CGGTCTAGAC	GTTTGACAAG gtcagtatcg	117
8	115	tctgcctcag CTCTACATCC	GCTGACTTCA gtaagtgagg	188
9	143	gtcccctag TCATTGAGGT	TTGCCCTCCG gtgagtgggg	841
10	99	tctcccctag GAGCTGGATC	CAGGGACACG gtgagatgtc	205
11	169	cgggccatag GTCCTGACCC	GTGCTCACCT gtgagctgat	108
12	92	gttcccttag AGATGGCGGT	GCCCTTCCAG gtgagtcttg	2014
13	159	tgctccccag GTGGTATGCC	CTCCTTGCAG gtgggaaagt	115
14	119	tctccttcag GTGGCCACTT	CCTATGTTAG gtgggtgtgg	158
15	1675	gtttatctag GTATGGCTTT	ACGCGTCAAG gtgaccaggc	

brown protein and in combination with scarlet protein (Ewart and Howells, 1998). The present study shows that the mouse *Abcg4* gene is highly expressed in mouse brain and eye (Table 2), suggesting a potential relationship between the *Abcg4* and *Abcg1* genes.

3.5. Concluding remarks

In the present study, we have cloned and characterized the mouse Abcg4 cDNA, to conclude that the mouse Abcg4 is an orthologue of human ABCG4. This conclusion is supported by the following observations: (1) the high identity (96%) of their amino acid sequences; (2) their chromosomal localization in the regions with conserved linkage homology between these species; and (3) similar exon-intron structures, namely, a total of 14 exons encoding the corresponding protein. We have recently established an expression system for the mouse Abcg4 protein (Yoshikawa et al., unpublished data), and the molecular mass of the expressed protein was estimated as 62 kDa, consistent with the value deduced from the mouse Abcg4 cDNA. As the next step, it would be of interest to study the function of Abcg4 by using the expression system. Moreover, in the present study, the chromosomal locus of the mouse *Abcg4* gene has been identified. Therefore, Abcg4-null mice, when established, would provide a useful tool to elucidate the biological function of mouse *Abcg4* and human *ABCG4* genes and their potential link to genetic diseases.

3.6. Additional note

Annilo et al. (2001) have most recently reported human and mouse orthologues of ABCG4. The present study on the sequence of mouse Abcg4 cDNA and the chromosomal localization of *Abcg4* gene strongly support their findings. In particular, our study is the first report that, by means of FISH, directly demonstrates the localization of the *Abcg4* gene on chromosomes 9A5.3 and 8q22 distal in mouse and rat, respectively (Fig. 3A). The tissue-specific expression profile of mouse Abcg4 (Fig. 4) provides evidence that the

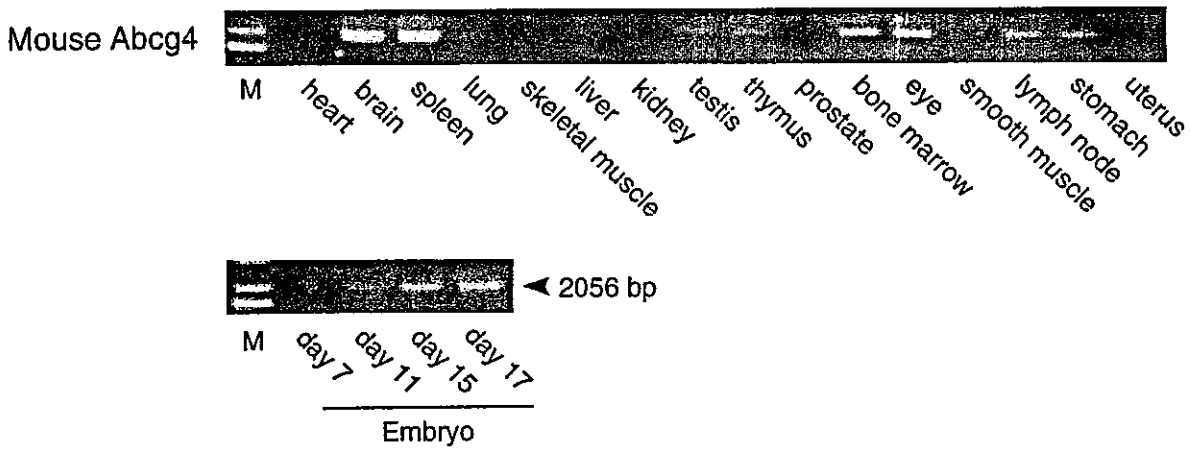

Fig. 4. The levels of Abcg4 transcript in different tissues of both the adult and embryo-mouse. The transcript was detected by PCR as described in Section 2. Lane M, DNA size markers (1.5 and 2.0 kb).

Table 2
Tissue-specific expression of mouse *Abcg4* and human *ABCG4* genes

Tissue	Mouse	Human
Adult		
Heart	−/+	−
Brain	+++ +	+++
Spleen	+++ +	+++
Lung	−	−
Liver	−	+++
Skeletal muscle	−	−
kidney	−	−
Testis	+	+++ +
Thymus	+	+++ +
Prostate	−	++
Bone marrow	+++	N.D.[a]
Eye	+++	N.D.
Lymph node	−	N.D.
Smooth muscle	++	N.D.
Stomach	++	N.D.
Uterus	−	N.D.
Placenta	N.D.	−
Pancreas	N.D.	−
Pvary	N.D.	++
Small intestine	N.D.	++
Colon	N.D.	+
Leukocyte	N.D.	−
Fetal		
Brain	N.D.	+++
Lung	N.D.	++
Liver	N.D.	++
Kidney	N.D.	++
Heart	N.D.	−
Spleen	N.D.	++
Thymus	N.D.	+++
Skeletal muscle	N.D.	+
Embryo 7-day	−	N.D.
Embryo 11-day	+	N.D.
Embryo 15-day	++	N.D.
Embryo 17-day	+++	N.D.

[a] N.D.: not determined.

mouse *Abcg4* gene is expressed not only in the brain and spleen but also in eyes and the bone marrow. The expression in the latter organs was not shown by Annilo et al. (2001). Furthermore, our PCR study (Fig. 4) suggests that mouse Abcg4 is expressed as a single peptide without splicing variants in those organs, being different from human ABCG4 gene products expressed in the thymus (Annilo et al., 2001).

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ABCC13, an unusual truncated ABC transporter, is highly expressed in fetal human liver☆,☆☆

Hikaru Yabuuchi,[a] Shin-ichiro Takayanagi,[a] Keigo Yoshinaga,[b] Naoyuki Taniguchi,[c] Hiroyuki Aburatani,[d] and Toshihisa Ishikawa[a,*]

[a] *Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan*
[b] *Second Department of Surgery, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan*
[c] *Department of Biochemistry and Molecular Biology, Graduate School of Medicine, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[d] *Genome Science Division, Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan*

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Abstract

In the present study, we have cloned the cDNA of ABCC13, a novel ABC transporter, from the cDNA library of adult human placenta. The *ABCC13* gene spans ~70 kb on human chromosome 21q11.2 and consists of 14 exons. The open reading frame of the ABCC13 cDNA encodes a peptide consisting of 325 amino acid residues. The amino acid sequence corresponding to putative membrane-spanning domains was remarkably similar to ABCC1, ABCC2, ABCC3, and ABCC6. The *ABCC13* gene was expressed in the fetal liver at the highest level among the organs studied. While ABCC13 was expressed in the bone marrow, its expression in peripheral blood leukocytes of adult humans was much lower and no detectable levels were observed in differentiated hematopoietic cells. The expression of ABCC13 in K562 cells decreased during cell differentiation induced by TPA. These results suggest that the expression of human ABCC13 is related with hematopoiesis.

Keywords: ABC transporter; Human chromosome 21; Fetal liver; Hematopoiesis; Leukemia cells; Cell differentiation

The ATP-binding cassette (ABC) transporters form one of the largest protein families and play a biologically important role as membrane transporters or ion channel modulators [1]. According to the recently published draft sequence of the human genome, more than 50 of human ABC transporter genes (including pseudogenes) are anticipated to exist in the human genome. Hitherto 48 human ABC-transporter genes have been identified and sequenced (recent reviews: [2–5]). Based on the arrangement of molecular structure components, i.e., the nucleotide binding domain and the topology of transmembrane-spanning domains, human ABC transporters are classified into seven sub-families from A to G (the new nomenclature of human ABC transporter genes: http://gene.ucl.ac.uk/nomenclature/genefamily/abc.html). Mutations of human ABC transporter genes have been reported to be the cause of genetic diseases, such as Tangier disease [6–8], cystic fibrosis [9], Dubin–Johnson syndrome [10], Stargardt disease [11], and sitosterolemia [12].

According to the new nomenclature of human ABC transporter genes, the ABCC gene family comprises the members of multidrug resistance-associated proteins (MRPs) [13], sulfonylurea receptors (SUR) [14], and cystic fibrosis transmembrane conductance regulator (CFTR) [9]. We have provided first evidence that

☆ *Abbreviations:* ABC, ATP-binding cassette; FCS, fetal calf serum, PCR, polymerase chain reaction; GS-X pump, ATP-dependent glutathione *S*-conjugate export pump; MRP, multidrug resistance-associated protein; PKC, paroxysmal kinesigenic choreoathetosis; TM, transmembrane; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

☆☆ The sequence of ABCC13 cDNA has been registered in GenBank under Accession No. of AF418600 on September 7, 2001.

* Corresponding author. Fax: +81-45-924-5838.
E-mail address: tishikaw@bio.titech.ac.jp (T. Ishikawa).

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transport of leukotriene C_4 (LTC_4), a pro-inflammatory mediator, across the cell membrane is mediated by a glutathione *S*-conjugate export pump (GS-X pump) [15–19]. Following studies have proven that the GS-X pump(s) is encoded by ABCC1 (MRP1) and ABCC2 (cMOAT or MRP2) genes [20–22]. ABCC1 (MRP1) and ABCC2 (cMOAT/MRP2) were identified by molecular cloning of cDNA from human multidrug resistant lung cancer cells [23] and from hepatobiliary transport mutant (TR^-) rats [22], respectively. After the discovery of the ABCC1 and ABCC2 genes, five human homologues, i.e., ABCC3 (MRP3), ABCC4 (MRP4), ABCC5 (MRP5), ABCC6 (MRP6), and ABCC10 (MRP7), have been successively identified. Those ABC transporters exhibit a wide spectrum of biological functions and are involved in transport of drugs as well as endogenous substances (see [2–5] for recent reviews).

Most recently, our group [24] and others [25–27] have independently discovered two novel ABC transporters, i.e., human ABCC11 (MRP8) and ABCC12 (MRP9), that belong to the ABCC sub-family. These genes consist of at least 30 and 29 exons, respectively, and they are tandemly located in a tail-to-head orientation on human chromosome 16q12.1 [24,25]. Recent linkage analyses have demonstrated that a putative gene responsible for paroxysmal kinesigenic choreoathetosis (PKC), a genetic disease of infancy, is located in the region of 16p11.2–q12.1 [28,29]. Since ABCC11 and ABCC12 genes are encoded at that 16q12.1 locus, a potential link between the PKC gene and these ABC transporters has been implicated.

In the present study, we have discovered another new ABCC transporter gene, named ABCC13, on the human chromosome 21 and cloned its cDNA from human placenta cDNA library. We herein describe the gene structure of ABCC13 and its expression profile in different organs and cell types. The highest expression of ABCC13 was detected in the fetal liver among different organs from fetal and adult humans. On the other hand, the expression of ABCC13 in leukemia K562 cells was down-regulated during cell differentiation.

Materials and methods

Cloning of cDNA encoding human ABCC13. The draft sequence of the human chromosome 21 (GenBank Accession No. AF130358) was analyzed by using the GENSCAN program (http://genes.mit.edu/GENESCAN.html) to predict the potential exons of ABCC13. EST clones were extracted from the currently available EST database to find partial sequences of the ABCC13 transcript.

To clone the ABCC13 cDNA, we have first screened the human placenta Master Plate of Rapid-Screen Arrayed cDNA Library Panel (OriGene, Rockville, MD, USA) by means of PCR using the following primers: the forward primer (hC13F): 5′-CATATTCCTGGTTTAGC AGA-3′ and the backward primer (hC13B): 5′-GTGAGCATGTTA AAACGTTG-3′). The PCR was performed with *Ex Taq* polymerase (TaKaRa, Japan), where the reaction consisted of 30 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, and 72 °C for 2 min. The resulting PCR products were subjected to 1.2%-agarose gel electrophoresis to identify positive wells. The sub-plate corresponding to the positive wells on the Master Plate was analyzed by 96-well PCR under the same experimental conditions. Pre-transformed *Escherichia coli* cells in the positive wells were taken and plated on the LB amp plates. PCR was subsequently performed on 96 individual colonies using the same primers as described above. Positive colonies were isolated and pCMV6-XL4 plasmids containing ABCC13 cDNA were extracted using the QIAprep Spin Miniprep Kit (Qiagen GmbH, Germany). The sequence of the cloned ABCC13 cDNA was analyzed by automated DNA sequencing (TOYOBO Gene Analysis, Japan). The cDNA sequence of ABCC13, thus obtained, has been deposited to GenBank under Accession No. AF418600.

Data analysis. DNA sequences were analyzed with the GENETYX-MAC software ver.11 (Software Development, Japan) and compared with other ABCC transporter genes registered in the NCBI database. The hydropathy profile of the protein deduced from the cDNA sequence was calculated with the Kyte and Doolittle hydropathy algorithm [30], and transmembrane domains were predicted by using the SOSUI program (http://sosui.proteome.bio.tuat.ac.jp/sosuimenu0.html).

Cell culture and induction of cell differentiation. Human leukemia K562 cells were maintained to grow in RPMI 1640 supplemented with 10% heat-inactivated FCS and penicillin (100 U/ml) and streptomycin (100 μg/ml) in a humidified chamber (37 °C, 5% CO_2). The number of cells was determined in a hemocytometer by trypan blue dye exclusion. Differentiation of K562 cells (2×10^5 cells/ml) was induced by incubation with TPA at a final concentration of 10 nM for 4 days. Cell differentiation was monitored by the observation of morphological changes and the cell proliferation rate. At different incubation times, cells were withdrawn and total RNA was extracted as described below.

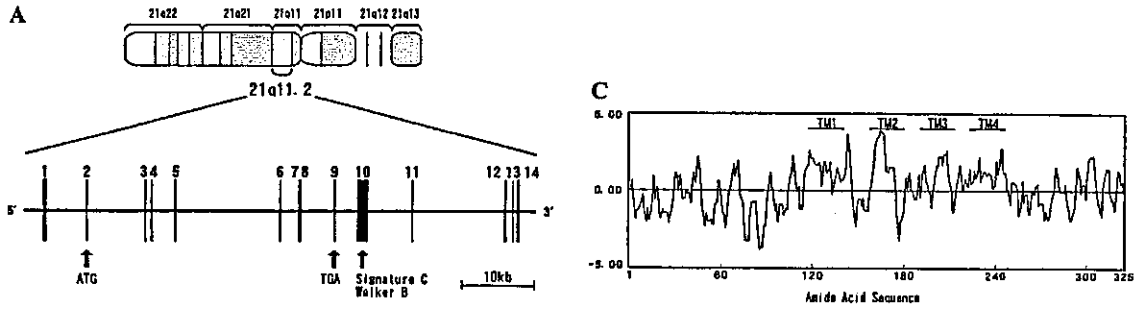
RNA extraction and preparation of cDNA. Total RNA was extracted from cultured cells using the ISOGEN RNA extraction solution (WAKO Pure Chemical Industries, Japan) according to manufacturer's protocol. cDNA was prepared from the extracted RNA in the reverse transcriptase reaction using Superscript II RT (Invitrogen, Gaithersburg, MD, USA) and oligo(dT) primers according to manufacturer's instruction.

Quantitative PCR analysis. Quantitative PCR was performed using cDNA from several cell lines and human tissues as well as Multiple Tissue cDNA (MTC) Panels (Clontech, Palo Alto, CA, USA). Two μl of each human cDNA was used for each 25 μl of the Ex Taq R-PCR Version (TaKaRa, Japan). The above-mentioned ABCC13-specific primers, i.e., hC13F and hC13B, were used for the quantitative PCR analysis. Each primer was used at a final concentration of 300 nM in the reaction mixture. Quantitative PCR analysis was performed using a Smart Cycler system (TaKaRa, Japan) with SYBR Green I (Bio-Whittaker Molecular Applications, Rockland, ME, USA) as a fluorescence indicator. The PCR consisted of 40 cycles of 95 °C for 15 s, 58 °C for 15 s, 72 °C for 15 s, and 85 °C for 7 s. The resulting PCR product was a 442-bp fragment. The expression levels of ABCC13 were normalized with β-actin mRNA levels. For this purpose, PCR was carried out to detect β-actin expression by using the following primers: the forward primer, TGAAGTACCCCATCGAGCACG and the backward primer, CAAACATGATCTGGGTCATCTTCTC. The PCR consisted of 40 cycles of 95 °C for 15 s, 58 °C for 15 s, 72 °C for 15 s, and 90 °C for 7 s. The resulting PCR product was 174-bp long.

Results and discussion

Gene structure of human ABCC13

A novel ABCC transporter gene, *ABCC13*, was discovered by database search on the human chromosome



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                                                       M  L  S  S  T  Q  N  A  G  G  S  Y  Q  R  V  R  G  A  L  D  T  Q  K  23
AATGCAGTCCAGAGAAAAGTGCCTCATTTTTCAGTAAAGTGACATATTCCTGGTTTAGCAGAGTAATTACTTTAGGCTATAAGAGACCTTTGGAAAGAGAGGATCTTTTTGAACTAAAGG 480
  C  S  P  E  K  S  A  S  F  F  S  K  V  T  Y  S  W  F  S  R  V  I  T  L  G  Y  K  R  P  L  E  R  E  D  L  F  E  L  K  E  63
AAAGTGATTCCTTCTGCACTGCGTGTCCCATCTTTGAAAAACAATGGAGAAAGGAAGTTTTAAGGAATCAAGAGAGGCAAAAAGTAAAGGTATCTTGTTATAAAGAGGCACATATCAAGA 600
  S  D  S  F  C  T  A  C  P  I  F  E  K  Q  W  R  K  E  V  L  R  N  Q  E  R  Q  K  V  K  V  S  C  Y  K  E  A  H  I  K  K  103
AACCATCTCTACTCTATGCATTGTGGAACACCTTTAAATCCATCCTGATTCAAGTTGCCTTATTCAAAGTGTTTGCTGATATTTTGTCCTTCACTAGCCCACTCATAATGAAGCAAATTA 720
  P  S  L  L  Y  A  L  W  N  T  F  K  S  I  L  I  Q  V  A  L  F  K  V  F  A  D  I  L  S  F  T  S  P  L  I  M  K  Q  I  I  143
                                                                                    TM1
TCATTTTCTGTGAACACAGCTCAGATTTTGGCTGGAATGGCTATGGCTATGCAGTGGCACTTCTTGTTGTAGTCTTTTTGCAAACTCTGATTCTTCAGCAATATCAACGTTTTAACATGC 840
  I  F  C  E  H  S  S  D  F  G  W  N  G  Y  G  Y  A  V  A  L  L  Y  V  V  F  L  Q  T  L  I  L  Q  Q  Y  Q  R  F  N  M  L  183
                                                                                 TM2
TCACCTCAGCAAAAGTTAAGACAGCTGTAAATGGACTGATCTACAAAAAGGTGTCCCTGGCAACCTTGTGTGTCTATTTCTTACTGGATGAAGGAAACATTTTAACAGCCACTAAAGTGT 960
  T  S  A  K  V  K  I  A  V  N  G  L  I  Y  K  K  V  S  L  A  T  L  C  V  Y  F  L  L  D  E  G  N  I  L  T  A  T  K  V  F  223
                                                  TM3
TCACATCGATGTCTTTGTTTAATATTTTAAGGATTCCTCTGTTTGAGTTACCAACCGTGATCTCAGCTGTGGTCCAGACAAAGATATCCCTGGGCCGTTTGGAAGACTTTCTCAACACTG 1080
  T  S  M  S  L  F  N  I  L  R  I  P  L  F  E  L  P  T  V  I  S  A  V  V  Q  T  K  I  S  L  G  R  L  E  D  F  L  N  T  E  263
                                                     TM4
AGGAGCTTCTTCCTCAAAGTATTGAAACGAACTATACAGGAGATCATGCTATTGGGTTTACAGATGCTTCTTTCTCCTGGGATAAAACAGGAATGCCAGTTCTAAAAGAGGCTCTGTGGC 1200
  E  L  L  P  Q  S  I  E  T  N  Y  T  G  D  H  A  I  G  F  T  D  A  S  F  S  W  D  K  T  G  M  P  V  L  K  E  A  L  W  L  303
TTATGTTTCTCAGCAGGCCTGGATTCAGAATTGCATTTTGCAAGAAAACATTCTCTTTGGCTCCATCATGAagaaagagttttatgagcaagtattggaagcctgtgctctcctttccaga 1320
  M  F  L  S  R  P  G  F  R  I  A  F  C  K  K  T  F  S  L  A  P  S  *                                                    325
tttggagcagttgccaaagggagatcaaactgaGATTGGAGAAAGAGTAAGGAAACGGCTGTGAATATAAGTGGGGGCCAGCAGCATCGAGTAAGCCTGGCCAGAGCTGTCTACAGTGGG 1440
                                  D  W  R  K  S  K  E  T  A  V  N  I  S  G  G  Q  Q  H  R  V  S  L  A  R  A  V  Y  S  G  29
                                                                                 Signature C
GCCGACGTCTACCTCCTGGATGATCCCTTGTCTGCTATTGATGTTCATGTTGGAAAGCAGCTTTTTGAAAAAGTGATAGGGTCCTTGGGCCTTTTGAAAAACCGGGTAGCCATAGTTTCA 1560
A  D  V  Y  L  L  D  D  P  L  S  A  I  D  V  H  V  G  K  Q  L  F  E  K  V  I  G  S  L  G  L  L  K  N  R  V  A  I  V  S  69
                  Walker B
TTTATGCTTTCTTGTGGGGCAGGGGATATATCTAGCACTGTGCCTAAAATTCCATTTATTTTGTTTATTTGTTGAagcaaagtagtcacatttcacatgaaaaaatgggatgctcagat 1680
F  M  L  S  C  G  A  G  D  I  S  S  T  V  P  K  I  P  F  I  L  F  I  C  *                                              93
tgcaatttcacaggcaaatgtcaatgggtaatttttgaagttttacttacaagtttactctggtgacaatttatgtgattgttatctcttcatctaataccatatcctaaggggatgtat 1800
gtgccataatgaattgttgttgactcaagtgatttttacaatggtatttggtgcacaccaaaatattaataaaattttatattattaacatagcaatttgacattattatcacagcaat 1920
catagtgattcagctgttttgttaaagggccaagaagatgaaaattagtgtttaccagtacatgccatatttcacagggtaaattagttcaatccctatttcacacataaaggagctcag 2040
ggctcacaatgacttgatataggttgctcaacttaagaacaatagagatttttaaaagtatgtctgactccaaagccacattcttcataatatgtgtttatggaattattaatatagaga 2160
actttgaaaatatatgtatataaattataaaatatatgtctcagtaaaaataaacaaacatctttatatattgacataaacccaagattaaaaatgtcacacttaattgtatgtatatgta 2280
taaaataatatgaatgatttaaaaataaggactatgaattatgacttggattaaacactttttttgtaatggtactgtatatttaaaaataagcaagaatgacatatatgaaattgtttt 2400
aaatttgcaattaaattgattcatttctaatgaacgaaaaaatacccgaactaaagtgttaaaaatcacatttagaaactatttctcatctagcagcatgtcattattccttgtaaaat 2520
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atttttttgaagcagcacttcacagaagtcgaggtgaccaggattgacatgcaagttctaagagacaggccctcattggatcaaggaaaacagctctcaatgaaaaaagaaaagatccctg 3120
ttggtgggttgaagttctccatcattctgcagtacctccaagcctttggctggctctgggtgtggctgactgtggtcacttacttagggcagaatttggtgagcattggacagaatctat 3240
ggcttagtgcatgggcaaaagaagcaaaaaacatgaatgagttcacagaatggaagcaaataagaagcaataaacttaatatctatggattattgggattaataaaaggtaaaagaaaat 3360
gtaaaaaaaaaaaaaaaaaaaaaaaaaaa 3389
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Fig. 1. (A) The genomic structure of *ABCC13* gene on the human chromosome 21. The cytogenetic location of the *ABCC13* gene as well as the structures of exons and introns were analyzed by BLASTN search on the Human Genome Project Working Draft (http://genome.cse.ucsc.edu/). (B) cDNA nucleotide and amino acid sequences of human ABCC13. An in-frame TGA translation stop (over-lined) is present 69 nucleotides upstream of the first methionine codon. The asterisk indicates the translation stop codon. (C) Hydropathy plot of the predicted ABCC13 peptide. Transmembrane domains were predicted using the SOSUI program (http://sosui.proteome.bio.tuat.ac.jp/sosuimenu0.html) and are indicated by TM1–TM4.

21 working draft (GenBank Accession No. AF130358) using the BLASTN program. The *ABCC13* gene is located on human chromosome 21q11.2 and consists of at least 14 exons (Fig. 1A) as predicted from the sequence of the cDNA cloned in this study. Fig. 1B shows the cDNA sequence (3389 bp) of ABCC13 and its deduced amino acid sequences. The longest open reading frame was 978 bp (between +294 and +1271) and had a partial Kozak consensus initiation sequence for translation [31] around the first ATG region, namely, 5′-CGGACC ATG C-3′. This open reading frame encodes a protein consisting of 325 amino acid residues with the starting methionine residue (Fig. 1B). Based on hydropathy and SOSUI analyses, it is suggested that the protein has four transmembrane domains (Figs. 1B and C). Furthermore, protein motif analysis predicted that this peptide has one N-glycosylation site (Asn273) and three protein kinase C phosphorylation sites (Thr21, Thr113, and Ser185).

Fig. 2 shows the partial alignments of the protein with the amino acid sequences of four ABCC trans-

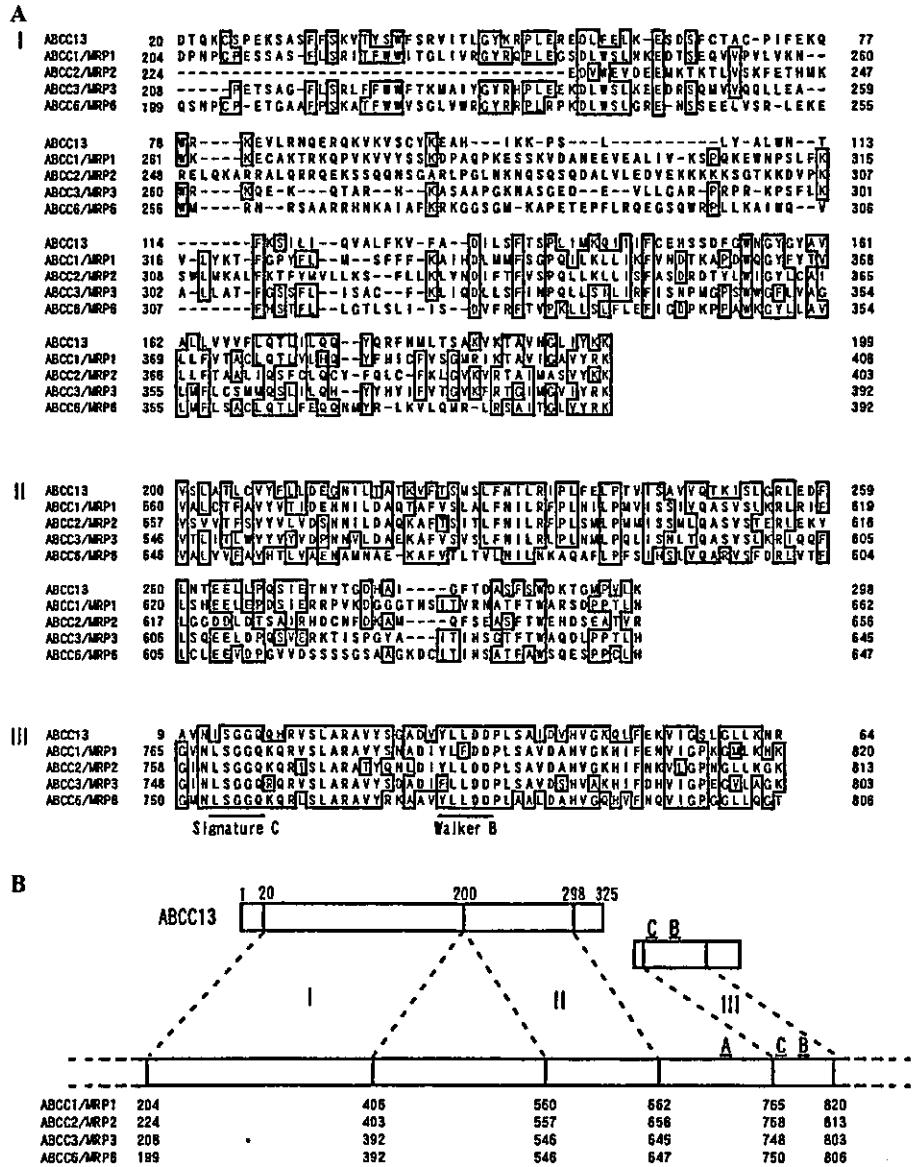

Fig. 2. Multiple alignments of the ABCC13 protein and the putative peptide with ABCC1, ABCC2, ABCC3, and ABCC6. (A) Numbers indicate the positions of amino acid residues. Homology analysis was performed using GENETYX-MAC Version 11 and identical amino acid residues are indicated by boxes. (B) The domains I and II of the ABCC13 protein, as well as the domain III of the putative peptide deduced from the non-coding region (1354–1635) of the ABCC13 cDNA are correlated with those ABC transporters. A, B, and C in the figure indicate Walker A, Walker B, and signature C motifs, respectively. The amino acid sequences of human ABC transporters were acquired from the NCBI database (Accession No. in parentheses): ABCC1/MRP1 (NM004996), human ABCC2/MRP2 (NM000392), human ABCC3/MRP3 (NM003786), and human ABCC6/MRP6 (NM001171).

porters, i.e., ABCC1 (MRP1), ABCC2 (MRP2), ABCC3 (MRP3), and ABCC6 (MRP6). The amino acid sequence form 20 to 298 of the ABCC13 protein is related to those ABC transporters of the sub-family C (domains I and II in Figs. 2A and B). However, the ABCC13 protein lacks Walker A, Walker B, and signature C motifs within the deduced amino acid sequence.

Interestingly, if the region between +1354 and +1635 in the non-coding sequence of the ABCC13 cDNA was translated in a different frame shift, a putative short peptide (93 amino acid residues) could emerge (Fig. 1B). The peptide exhibits remarkably high identities with ABCC1 (MRP1), ABCC2 (MRP2), ABCC3 (MRP3), and ABCC6 (MRP6) and has signature C and Walker B motifs (domain III in Figs. 2A and B). We have searched potential nucleotide sequences corresponding to the Walker A motif throughout the *ABCC13* gene up to the exon 10, however, there is no relevant sequence. In this context, the *ABCC13* gene encodes a non-functional ABC transporter.

Expression profile of human ABCC13

The tissue-specific expression of human ABCC13 was studied by quantitative PCR analysis using human Multiple Tissue cDNA panels (Clontech). As shown in Fig. 3, the mRNA level of ABCC13 was the highest in the fetal liver among the organs studied. Next to the fetal liver, relatively high expression of ABCC13 was detected in the fetal spleen (Fig. 3). It is noteworthy that the expression level of ABCC13 in the fetal liver was approximately 20 times as high as that in the adult liver. Likewise, the expression of ABCC13 in the fetal spleen is 20 times higher than that detected in the adult spleen.

In adult human tissues, colon is the organ where ABCC13 was highly expressed. Lower mRNA levels were observed in several other tissues, including brain, placenta, lung, liver, pancreas, and ovary (adult panel in Fig. 3). In the digestive system, ABCC13 was widely expressed from ileum to rectum, whereas especially highly expression was detected in the colon ascending and transverse (digestive system panel in Fig. 3).

While ABCC13 was expressed in bone marrow cells, its expression in peripheral blood leukocytes of adult humans was several-fold lower. In particular, the expression of ABCC13 was under detection levels in differentiated hematopoietic cells, such as mononuclear cells, resting CD4+ cells, resting CD8+ cells, resting CD14+ cells, resting CD19+ cells, activated mononuclear cells, activated CD4+ cells, activated CD8+ cells, and activated CD19+ cells (blood fraction panel in Fig. 3).

Since the fetal liver expressed ABCC13 at predominantly high levels like the oncofetal α-fetoprotein, we assumed that the expression of this gene might be elevated in cancer. We therefore examined the mRNA levels of ABCC13 in several tumor tissues. Contrary to our expectation, the expression of ABCC13 was not significantly enhanced in lung carcinoma, colon adenocarcinoma, prostate adenocarcinoma, ovarian carci-

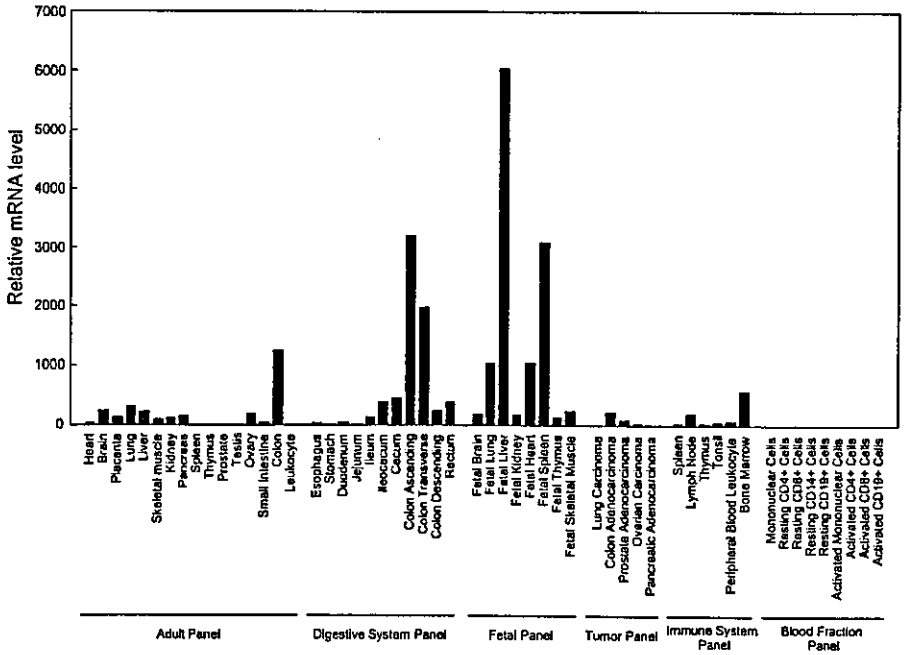

Fig. 3. Relative mRNA levels of ABCC13 detected in different tissues and cell types. Quantitative PCR analysis was carried out in a Smart Cycler system (TaKaRa) using Multiple Tissue cDNA (MTC) Panels, i.e., adult tissues, fetal tissues, digestive system, immune system, tumor, and blood fraction panels (Clontech). The ABCC13-specific primers, i.e., hC13F and hC13B, were used for the PCR (see Materials and methods).

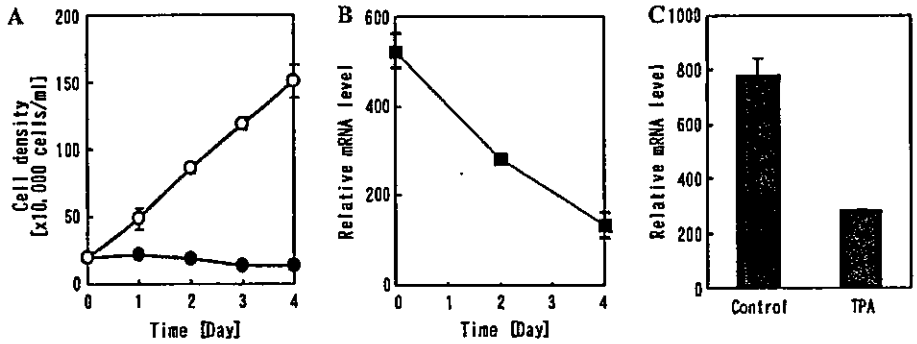

Fig. 4. The effect of TPA on the expression of ABCC13 in K562 cells. (A) Time courses of the growth of K562 cells incubated with (closed circles) and without (open circles) 10 nM TPA. (B) The time course of the ABCC13 mRNA level in K562 cells during incubation with 10 nM TPA. (C) Comparison of relative mRNA levels between TPA-treated and control K562 cells after 2 days of incubation. Results are expressed as means ± SD in triplicate experiments. The levels of ABCC13 mRNA were normalized with β-actin mRNA levels.

noma or pancreatic adenocarcinoma (tumor panel in Fig. 3).

To further examine the expression of ABCC13 in human hepatocarcinoma, we analyzed mRNA levels in tumor and non-tumor regions of biopsy samples by means of quantitative PCR. The mRNA levels of ABCC13 in cancerous regions did not alter, as compared to those in non-cancerous regions in four patients of hepatocellular carcinoma (data not shown). We also examined the expression levels of ABCC13 between tumor regions and non-tumor regions of surgically excised tissue samples from colon carcinoma patients. However, because of wide variations in the expression levels among non-tumor samples, statistically significant results were not obtained.

Expression of ABCC13 in human leukemia K562 cells

Human leukemia K562 cells were found to express ABCC13 at high levels. To study the effect of cell differentiation on the mRNA level of ABCC13, K562 cells were incubated with 10 nM TPA for 4 days. Cell growth was arrested (Fig. 4A) and morphological changes were observed (data not shown). Fig. 4B shows the time course of relative levels of ABCC13 mRNA measured by quantitative PCR. In TPA-treated K562 cells, ABCC13 mRNA level decreased in a time-dependent manner (Fig. 4B), whereas it was maintained almost constant in the control cells during the incubation period. After 2 days, the mRNA level of ABCC13 in TPA-treated K562 cells was approximately one-third of the level in the control cells (Fig. 4C). These results suggest that the expression ABCC13 is down-regulated by cell differentiation in certain leukemia cells.

Concluding remarks

As demonstrated in the present study, the *ABCC13* gene located on chromosome 21q11.2 encodes an unusual truncated peptide that has four transmembrane domains homologous to the ABCC sub-family, but it lacks Walker A, Walker B, and signature C motifs (Figs. 1 and 2). Thus, the *ABCC13* gene encodes a non-functional ABC transporter. Nevertheless, extremely high mRNA levels of ABCC13 in the fetal liver and spleen were noteworthy (Fig. 3). The expression of ABCC13 was detected in bone marrow cells in adult humans. In addition, the mRNA level of ABCC13 in human leukemia K562 cells substantially decreased during cell differentiation (Fig. 4). It has recently been reported that Abcg2, a murine ABC transporter, is expressed at high levels in primitive hematopoietic stem cells and that its expression was sharply down-regulated with cell differentiation [32]. Our preliminary analysis of the promoter region in the 5-kb upstream of the *ABCC13* gene revealed the existence of AML1, GATA-1, and GATA-2 binding motifs (Matsubara and Ishikawa, unpublished work). AML1 is reportedly the target of multiple chromosomal translocations in human leukemia and plays an essential role in fetal liver hematopoiesis [33]. GATA-1 and GATA-2 are critically involved in the development of hematopoietic cells [34–37]. In this context, it is strongly suggested that the expression of the *ABCC13* gene is related with hematopoietic processes in humans.

Acknowledgments

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Regular Article

*Does ABCG2 Need a Heterodimer Partner? Expression and Functional Evaluation of ABCG2 (Arg 482)**

Megumi YOSHIKAWA[1,2], Shiho KASAMATSU[1], Masa YASUNAGA[1], Guizhi WANG[1,3], Yoji IKEGAMI[2], Hisahiro YOSHIDA[2], Shigeki TARUI[3], Hikaru YABUUCHI[1] and Toshihisa ISHIKAWA[1]

[1]*Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Kanagawa, Japan*
[2]*Department of Drug Metabolism and Disposition, Meiji Pharmaceutical University, Kiyose, Tokyo, Japan*
[3]*GS PlatZ Co. Ltd., Nihonbashi, Chuo-ku, Tokyo, Japan*

Summary: Accumulating evidence suggests that several ATP-binding cassette (ABC) transporters mediate the elimination of anticancer drugs from cancer cells and thereby confer drug resistance. SN-38-selected PC-6/SN2-5H human lung carcinoma cells were shown to overexpress ABCG2 with the reduced intracellular accumulation of SN-38, the active metabolite of irinotecan. We have recently demonstrated that plasma membrane vesicles prepared from those cells transported SN-38 in an ATP-dependent manner, and it was suggested that ABCG2 is involved in the active extrusion of SN-38 from cancer cells. In the present study, we have cloned the cDNA of ABCG2 from PC-6/SN2-5H human lung carcinoma cells, expressed ABCG2 in Sf9 insect cells, and characterized its function. Sequence analysis has revealed that the cloned ABCG2 has an arginine at the amino acid position 482, as does the wild type. Expression of the cloned ABCG2 in Sf9 cell membranes was detected by immunoblotting with the BXP-21 antibody. Contrary to our expectation, however, ATPase activity in the cell membranes expressing ABCG2 was stimulated by neither SN-38 nor rhodamine 123. It is suggested that there is a partner protein of ABCG2 required for heterodimer formation to exhibit transport activity toward SN-38.

Key words: ABC transporter; ABCG2; BCRP; SN-38; camptothecin; insect cells

Introduction

Development of drug resistance in cancer cells is the major obstacle to long-term, sustained patient response to chemotherapy. There is accumulating evidence that active export of anticancer drugs from cells is one of the major mechanisms of drug resistance. It has been convincingly documented that several ATP-dependent drug transporters can cause drug resistance in cancer cells by actively extruding the clinically administered chemotherapeutic drugs. By far, the best known major drug transporters, ABCB1 (P-glycoprotein or MDR1) and ABCC1 (MRP1 or GS-X pump), have been characterized in detail with respect to their structure and function.[1-4] These drug transporters belong to the ATP-binding cassette (ABC) transporter gene family.[5-7]

The ABC transporters form one of the largest protein families and play a biologically important role as membrane transporters or ion channel modulators.[8] Until now more than 48 human ABC-transporter genes have been identified and sequenced. Based on the arrangement of their molecular structural components, such as the nucleotide binding domain and the topology of transmembrane domains, human ABC transporters are classified into seven different sub-families (A to G).[5-7]

Recently, a novel ABC transporter, breast cancer resistant protein (BCRP), has been discovered in doxorubicin-resistant breast cancer cells.[9] The same transporter has also been found in the human placenta[10] as well as in drug-resistant cancer cells selected in mitoxantrone and in DNA topoisomerase I inhibitors.[11-18] The newly found ABC transporter is a so-called "half-transporter" bearing six transmembrane domains and one ATP-binding cassette. This ABC transporter protein is now named ABCG2 and has been classified in the G-subfamily of human ABC transporter genes according to the new nomenclature.

Overexpression of ABCG2 reportedly confers cancer

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To whom correspondence should be addressed: Toshihisa ISHIKAWA, *Ph.D. Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta 4259, Yokohama, Kanagawa 226-8501, Japan. Tel. +81-45-924-5800, Fax. +81-45-924-5838, E-mail: tishikaw@bio.titech.ac.jp*