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Complete Coding Sequence, Promoter Region, and Genomic Structure of the Human ABCA2 Gene and Evidence for Sterol-Dependent Regulation in Macrophages

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Members of the human ABC transporter A subfamily have gained considerable attention based on the recent findings that ABCA1 and ABCR (ABCA4) cause familial HDL-deficiency syndromes and distinct forms of hereditary retinopathies, respectively. Here we report the complete cDNA and the genomic organization of ABCA2, another member of the human ABC A transporter subfamily. The ABCA2 coding region is 7.3 kb in size and codes for a 2436 amino acid polypeptide that bears the typical features of a full-size ABC transporter. Among the known members of the ABC A subfamily ABCA2 shares highest homology with the cholesterol-responsive transporters ABCA1 (50%) and the recently cloned ABCA7 (44%). The ABCA2 gene comprises 48 exons which are localized within a genomic region of only 21 kb. Analysis of the putative ABCA2 promoter sequence revealed potential binding sites for transcription factors that are involved in the differentiation of myeloid and neural cells. Gene expression analysis in human macrophages showed that ABCA2 mRNA is induced during cholesterol import indicating that ABCA2 is a cholesterol-responsive gene. Our results suggest a potential role for ABCA2 in macrophage lipid metabolism and neural development.

ATP binding cassette (ABC) transporters constitute a gene family of multispan transmembrane proteins which translocate a variety of substrates across cellular membranes (1–3). Among the known substrates transported by ABC molecules are lipids, lipophilic compounds such as xenobiotics, ions, amino acids, peptides, and carbohydrates. Structurally, ABC transporter molecules are subdivided into two classes, the half-size and the full-size transporters. Full-size ABC transporters are characterized by the presence of two nucleotide binding folds (NBF) with conserved Walker A and B motifs, signature sequences and two multispan transmembrane domains (TMD), whereas half-size transporters are composed of only one NBF and one TMD (3). The specificity for the substrates translocated by ABC transporters appears to be determined by the transmembrane domains, while the energy required for the transport activity is provided by hydrolysis of ATP at the NBF (1–3). For quite some time, ABC transporters have been implicated in multidrug resistance in cancer therapy, however, during the past few years convincing evidence has accumulated to suggest that a significant portion of the ABC transporter family members is involved in the transmembrane transport of endogenous lipids (4–6). This concept has been supported by recent work from our laboratory and others which demonstrated that ABCA1, the prototypic member of the ABC A subfamily, is a major regulator of plasma HDL and functions as a facilitator of cellular phospholipid and cholesterol export (7–10).

ABCA2 is the second member of the ABC A subfamily which was originally coidentified with ABCA1 from mouse tissue (11). The recently cloned rat ABCA2 mRNA is of 8 kb size and predicts a 2434 amino acid polypeptide with a molecular mass of 250 kDa (12). The human ABCA2 gene has been assigned to chromosome 9q34 by using a murine genomic probe (11). Although GenBank database entries exist which report partial human ABCA2 cDNA sequences, complete characterization of the human ABCA2 transcript has not yet been reported. ABCA2 is predominantly expressed in the brain, however, ABCA2 transcripts are also detectable in kidney, uterus, thymus, and heart (11, 12).

Sequence data from this article have been deposited with the NCBI-GenBank Data Library under Accession Nos. AF327657–AF327705, respectively.

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Currently, only little is known about the structure and function of human ABCA2. We recently demonstrated that ABCA2 is expressed in human monocytes and upregulated during monocyte differentiation into macrophages (5). Moreover, a recent study reported the amplification of the ABCA2 gene in a human estramustine-resistant ovarian carcinoma cell line which led the authors to conclude that this transporter may function as a transporter of the estradiol-mustard conjugates (13).

In this study, we report the complete coding region, genomic structure and the promoter region of the human ABCA2 gene and we demonstrate that ABCA2 expression is upregulated by cholesterol in macrophages.

MATERIALS AND METHODS

Cell culture and preparation of enzymatically degraded, nonoxidized LDL. Human monocytes were obtained from healthy donors by leukapheresis and counterflow elutriation (5). Aliquots of 10^8 cells were cultured on plastic Petri dishes in macrophage SFM medium (Gibco BRL, Karlsruhe) and allowed to differentiate into macrophages for 4 days in the presence of 50 ng/ml recombinant human M-CSF (R&D Systems, Wiesbaden) (5). Macrophages were incubated in the presence of enzymatically modified LDL (eLDL, 40 μg/ml) to induce sterol loading for an additional 24 h. LDL (d = 1.006 – 1.063 g/ml) lipoproteins were purified from human plasma obtained from healthy volunteers according to standard protocols (5). The preparation was performed in a Beckman L-70 ultracentrifuge (70 Ti rotor) at 4°C and densities were adjusted with solid KBr. Lipoprotein fractions were dialyzed several times in phosphate-buffered saline (PBS) containing 5 mM EDTA. After the final dialysis step in 0.15 M NaCl, LDL lipoproteins were sterilized using a 0.45 μm sterile filter (Sartorius, Göttingen). Enzymatically degraded, nonoxidized LDL (eLDL) was prepared according to the protocol by Bhakdi *et al.* (14).

Extraction of RNA and genomic DNA. Total monocyte/macrophage RNA was prepared for RT-PCR using Trizol (Gibco Life Technology, Karlsruhe). Genomic DNA was extracted from human venous blood utilizing the Qiamp DNA blood midi kit (Qiagen, Hilden). Yields and purity of RNA and DNA were determined spectrophotometrically and by gel electrophoresis, respectively.

RT-PCR. Aliquots of 1 μg total monocyte/macrophage RNA were reverse-transcribed (RNA PCR Core Kit, Perkin Elmer, Langen) and PCR amplified for 40 cycles (cycle profile: 94°C 30 s, 55°C 45 s (annealing temperature differs in certain primer-combinations), and 72°C 90 s). For RT-PCR cloning of the ABCA2 5′ coding region a set of degenerate primers were used derived from regions that were localized at the 5′ end of ABCA1, ABCA7, and ABCR, respectively, which exhibited maximum homology among these transporters. The degenerate forward primers were used in combination with two ABCA2 specific reverse primers (5′CCTGGGTGCACAGCCTCAAT3′ and 5′CAGCCAGTAGGACTTCTGCA3′; respectively) which were located at the 5′ end of the KIAA1062 sequence. Semiquantitative RT-PCR was performed as previously published (5, 15). For this, the ABCA2 specific primers were used (forward 5′GCCATGGGCTTCCTGCACCAGCT3′; reverse 5′CGGGTTTCTGGCCACCGAGTCCAGA3′) yielding a diagnostic fragment of 459 bp size. Equal amounts of input RNA were reverse-transcribed and amplified for 25, 30, and 35 cycles, respectively, to monitor that the PCR was in the exponential phase. Amplification of the housekeeping gene GAPDH served to normalize input RNA amounts (not shown).

PCR amplification of intron sequences and 5′ RACE. PCR based cloning of intron sequences was carried out using the Expand High Fidelity PCR System (Roche, Mannheim). ABCA2 cDNA specific oligonucleotide primers for ABCA2 were used that were likely to be located in exons. The reaction mix (50 μl) was composed of 300 nmol oligonucleotide primers, 1.5–3 mM $MgCl_2$, 200 μM dNTP, 10× Expand HF buffer, 3 units of Expand High Fidelity PCR DNA polymerase mix (Roche, Mannheim), and 100–500 ng aliquots of genomic DNA. After a 2 min denaturation step (94°C), reaction mixtures were PCR amplified for 10 cycles using the following cycle profile: 94°C for 15 s, 60–65°C for 30 s, and 68°C for 7 min. In a subsequent second amplification round (25 cycles) the elongation time was extended by 5 s after each cycle. To assess the ABCA2 mRNA transcription start point 5′ RACE was performed using the Marathon Ready spleen cDNA kit (Clontech, Palo Alto) according to the manufacturer's protocol. The gene specific primers used were 5′GCTGTTCTTTATCCTGCTGGGGCT3′ and 5′GGCTCCGGCGTTTGAGCGTCACGT3′, respectively. Template DNA was denatured at 94°C for 1 min and the PCR cycling conditions were 94°C for 30 s and 72°C for 4 min (5 cycles), followed by 94°C for 30 s and 68°C for 4 min for an additional 35 cycles. All PCR amplification products were separated on ethidium bromide stained agarose gels. Bands of interest were excised and the DNA was extracted using the GFX PCR DNA purification kit (Amersham, Piscataway) for subsequent sequence analysis.

ARC-PCR. The ABCA2 5′ gene flanking region was cloned using the *Alu* repeat consensus-based polymerase chain reaction (ARC-PCR) method (16). This approach relies on the fact that human promoter regions are typically flanked by upstream Alu repeat elements at a distance of 0.5–5 kb from their protein-coding areas. With this approach unknown 5′ flanking regions can be rapidly amplified by PCR using specific primers derived from specific *Alu* repeat consensus sequences and gene specific reverse primers. In ARC-PCR the *Alu* repeat consensus specific primers were used as previously published (16). For the cloning of the ABCA2 5′ upstream sequence, exon 1 specific primers 5′AGCTGGTGCAGGAAGCCCATGG 3′ and 5′ GTTCTTCCAGAGCAGCAGCTGCAGC 3′ were utilized, respectively. 100 ng aliquots of genomic DNA were amplified in a 100 μl reaction volume which contained 25 nmol oligonucleotide primers, 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 25 mM dNTP, and 5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Überlingen). Reaction mixtures were prepared at 4°C and subjected to 30 amplification cycles at 94°C for 25 s, 60°C for 35 s, and 72°C for 5 min. A denaturation step (94°C for 1 min) was included before PCR amplification.

DNA sequencing. Amplified genomic segments were purified and both strands were sequenced on an ABI Prism 310 capillary sequencer (PE Applied Biosystems, Weiterstadt) as previously described (15, 17). Cycle-sequencing reactions were performed with BigDye terminators (DNA sequencing kit, PE Applied Biosystems), purified on CentriSep spin columns and analyzed by capillary electrophoresis.

Computer sequence analyses. DNA computer analyses were performed utilizing the WWW[2] HUSAR GCG software package (DKFZ, Heidelberg, http://genius.embnet.dkfz-heidelberg.de/menu/w2h/w2hdkfz/index.shtml). The FASTA and MOTIFS were used for sequence comparison and protein alignment applications. Potential transcription factor binding sites were identified using the TESS (transcription element search system) software (University of Pennsylvania, http://www.cbil.upenn.edu/cgi-bin/tess).

RESULTS

Cloning of the Human Full-Length ABCA2 cDNA

The human ABCA2 cDNA sequence has as yet not been cloned to completeness. GenBank database search revealed two entries, U18235 and KIAA1062

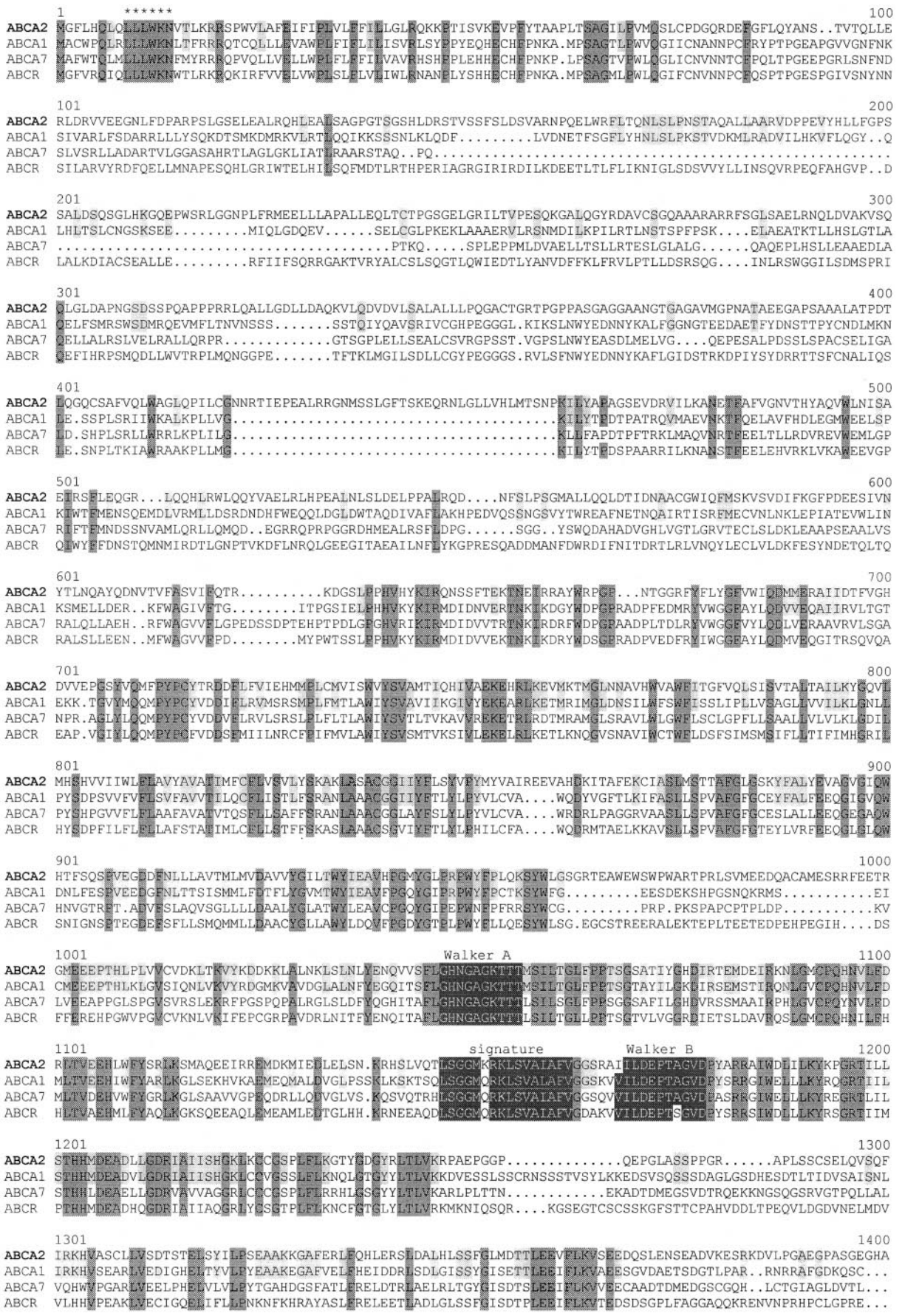
(AB028985), which resulted from large-scale gene screening efforts (18, 19). These share considerable sequence homology with murine ABCA2 (11) and are referred to as partial ABCA2 mRNA. The 0.5 kb U18235 sequence is completely identical with the 5.3 kb KIAA1062 sequence strongly suggesting that the latter represents the human ortholog of murine ABCA2. Importantly, sequence alignment with the human ABC A subfamily members ABCA1 and ABCA7 which we recently cloned (15, 20) raised the possibility that the 5′ sequence of the human ABCA2 cDNA was significantly longer than that indicated by the previous screening studies which report transcripts of 0.5 and 5.3 kb lengths, respectively (18, 19). To test for the existence of an extended human ABCA2 transcript, we performed an RT-PCR approach employing sets of degenerated primers that were based on homologous regions identified at the very 5′ end of the coding regions of human ABCA1, ABCA7, and ABCR, respectively. Using these degenerate primers in combination with nested primers located at the 5′ end of the KIAA1062 sequence we amplified a PCR product of app. 2.7 kb length from human macrophage RNA (not shown). Sequence analysis revealed that the 3′ end of the PCR segment was completely identical with the 5′ end of the KIAA1062 sequence indicating that the sequence represented the 5′ portion of the human ABCA2 mRNA. The 2.7 kb amplification product contained an open reading frame encoding a polypeptide which exhibited significant homology with the N-terminal amino acid sequence of human ABCA1 and ABCA7, respectively, a start codon, however, was absent. To determine the definitive 5′ end of the ABCA2 coding region 5′ RACE was performed utilizing the Marathon Ready spleen cDNA kit (Clontech). This resulted in the identification of the canonical start codon and the transcription initiation site (see below). The thus obtained ABCA2 full-length coding region comprises 7311 bp which encode a 2436 amino acid polypeptide. The detailed ABCA2 cDNA sequence has been submitted to the GenBank database (Accession No. AF327657). Amino acid sequence alignment of ABCA2 with other characterized ABC A transporters demonstrated that it shares highest homologies with ABCA1 (50%) and the recently cloned ABCA7 (44%), respectively (Fig. 1).

Genomic Organization and Exon/Intron Boundaries of Human ABCA2

ABCA2 candidate intron segments were amplified by long template PCR (Expand High Fidelity PCR, Roche). Genomic DNA obtained from human leukocytes served as template. Exon-specific primers which flanked putative ABCA2 exon/intron junctions were used whose locations were based on the comparison of the known gene structures of human ABCA1 (21) and ABCA7 (17), respectively, the ABC A transporters that display highest cDNA sequence homology with ABCA2. The exon/intron organization of ABCA2 was characterized by direct sequencing of the respective PCR products. The flanking regions of all intron spanning amplification products showed complete sequence identity with their respective ABCA2 cDNA counterparts. We identified a total of 48 exons for the ABCA2 gene (Table 1). Their complete sequences have been deposited in the GenBank database (Accession Nos. AF327658–AF327705). The ABCA2 exon sizes range from 61 to 700 bp. The majority of the introns are smaller than 0.3 kb in size and only two introns (intron 1 and 2) are extend 1 kb (Table 1). The initial and terminal dinucleotides of all ABCA2 introns displayed the characteristic GT-AG configuration which is diagnostic of bona fide splice junctions (22). Surprisingly, the entire ABCA2 gene spans only a 21 kb genomic region. It thus exhibits an unusually compact genomic organization within the class of full-size ABC transporters.

Cloning of the ABCA2 5′ Gene Flanking Region and Characterization of the cDNA 5′ End

The 5′ end of the ABCA2 transcript was completed by 5′ RACE utilizing the Marathon Ready spleen cDNA kit (Clontech). The reverse primers used for this were specific for the 5′ end of the cloned 2.7 kb amplification product (see above) which represented the largest portion of the missing 5′ end of the ABCA2 coding region. The putative transcription start site was identified 148 bp upstream of the translation initiation point (Fig. 2). It was confirmed by PCR mapping using sequential primers specific for the genomic region that flanks the identified transcription start site in combination with an exon 1 specific primer (not shown). The ABCA2 5′ flanking region was cloned from genomic DNA by Alu repeat consensus-based PCR (ARC-PCR) (16). Using multiple combinations of ARC specific primers and ABCA2 exon 1 specific oligonucleotides a band of 1.9 kb size could be amplified. Direct sequencing demonstrated identity of the 3′ end of the amplification product with the 5′ end of the ABCA2 cDNA indicating that the amplified sequence represented the ABCA2 5′ gene flanking region. The 5′ end of the 1.9 kb ARC-PCR amplification product showed high homology with a forward oriented Alu repeat. This confirmed the expected presence of an Alu element (16) ~1.7 kb upstream of the ABCA2 translation start point (data not shown). The detailed 5′ UTR sequence representing the putative ABCA2 gene promoter is shown in Fig. 2. Computer assisted analysis of the ABCA2 5′ upstream region for the presence of transactivator target sites revealed a number of potential binding sites for transcription factors with roles in the myeloid and neural system (25–35). These include target sites for


Walker A
signature
Walker B

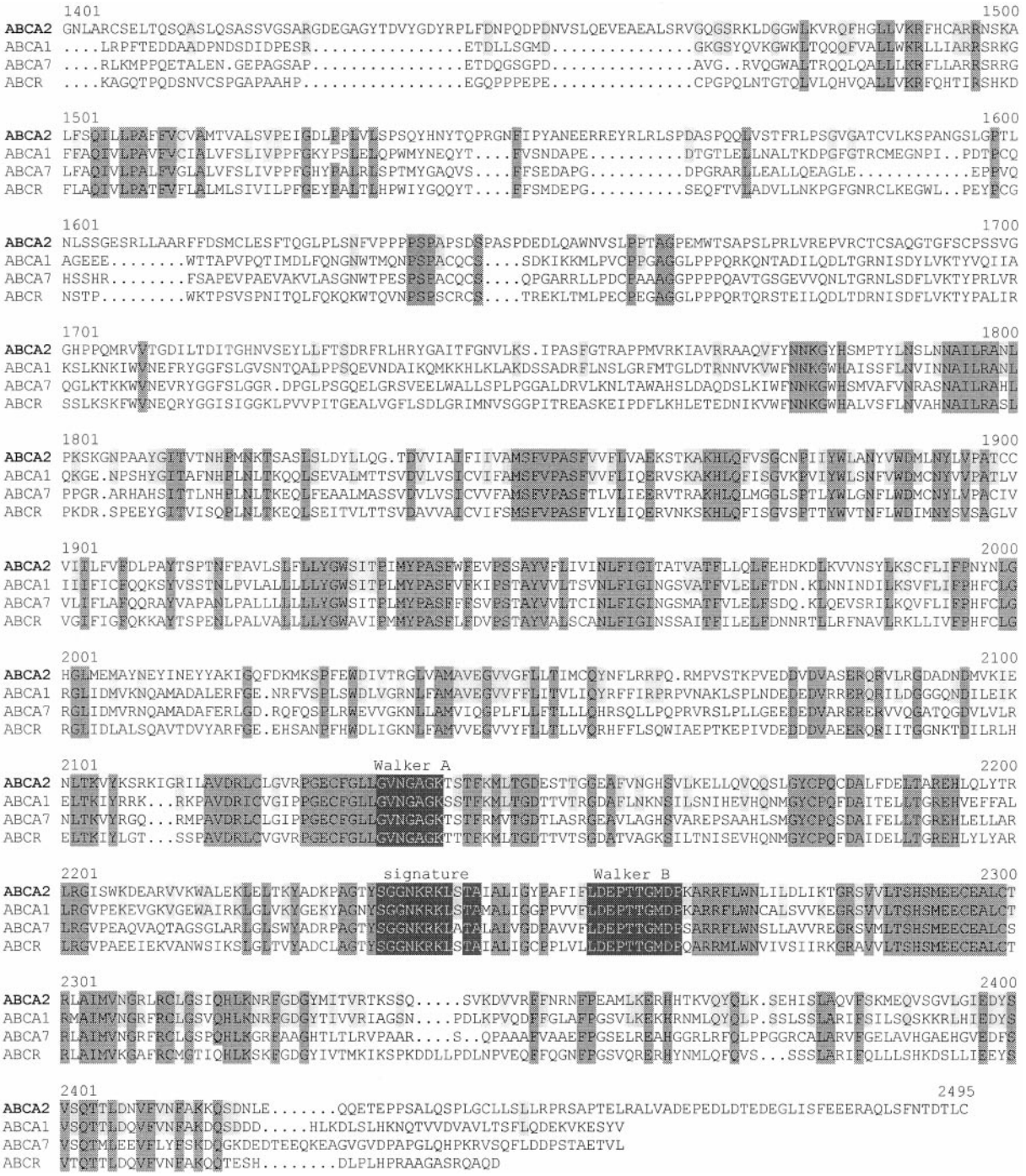


FIG. 1—*Continued*

FIG. 1. Amino acid sequence of human ABCA2 aligned with known members of the ABC A subfamily (ABCA1, ABCA7, and ABCR, respectively). Amino acids are shown in the single letter code, gaps due to optimal alignment are represented by dots. Annotations designate Walker motifs and the signature sequence of each of the two nucleotide binding domains. Gray shaded amino acids highlight identities among the various ABC transporters (light gray shading, identity between ABCA2 and ABCA1; medium gray shading, identity between ABCA2, ABCA1, ABCA7 and ABCR; dark gray shading, Walker A, B, or signature sequence). Asterisks indicate the N-terminal LLLWKN heptapeptide diagnostic of ABC A transporters. The complete cDNA sequence for ABCA7 has been deposited at the NCBI-GenBank (Accession No. AF327657).

TABLE 1

Exon–Intron Boundaries of the Human ABCA2 Gene

Exon number	Exon size (bp)	5′ splice site	3′ splice site	Intron size (kb)
1	212		GGAGCCCGgtgagccc	3.9
2	94	ctttccagTGGGTCCT	GAAGGAAGgtgagtgc	1.1
3	112	tctgccagTCCCCTTC	AACTCCACgtgagtgc	<0.1
4	164	tggtgcagGGTCACGC	ATCCACAGgtgtgccc	0.2
5	128	ctgtccagTGTCTTCC	CGCCCGAGgtaaggca	0.4
6	111	cctcctagGTCTACCA	GGATGGAGgtgagggc	0.2
7	219	catcccagGAGCTGCT	CCCAGCAGgtgaggcc	0.3
8	368	cccctcagCTGGGCCT	AACAACCGgtaggtgg	0.2
9	160	taccccagCACCATTG	TCCTCAAGgtgcacgg	1.0
10	129	ccctgcagGCCAACGA	TGCAGCAGgtgccaag	0.1
11	168	accccaagTATGTAGC	TGTCCAAGgtgagcgg	<0.1
12	97	cccgctagGTGAGCGT	TTTTGCCAgtgagctg	0.4
13	174	ggccccagGTGTGATC	GATCCAGGgtgaggag	0.1
14	103	acccacagACATGATG	CGCGATGAgtgagtgc	<0.1
15	115	ccccgcagCTTCCTGT	TCAAGGAGgtgcgcgg	<0.1
16	191	acccgcagGTGATGAA	ATGTTCTGgtgagcgc	0.2
17	151	ccacccagCTTCCTGG	GCATCGCGgtgagggt	<0.1
18	205	cttctcagTCCCTCAT	GCACCCAGgtactggc	0.2
19	165	acccccagGCATGTAC	GCGCTTTGgtgaggct	0.1
20	181	tggtgcagAGGAGACC	ACCACCATgtgagtgt	0.1
21	213	ctacccagGTCCATCC	ATGGACAAgtgggtgg	<0.1
22	197	tgtcccagGATGATCG	CAAGCCAGgtgagtgg	<0.1
23	183	tgtcctagGCCGCACC	CCCCCAAGgtctgtgt	0.3
24	185	ccccacagAGCCAGGG	TCTTCCAGgtgtgagg	0.1
25	121	ccctgcagCACCTGGA	TGAGGCCGgtgagggg	0.3
26	237	tgcggcagATGTGAAG	CCTGCAAGgtgggggt	0.1
27	207	ctgcacagAGGTGGAG	GGAGATTGgtaggcca	0.3
28	103	cctgccagGTGATCTG	GAGTACCGgtgaggcc	0.1
29	317	gcccgcagGCTGCGGC	TGGGCCAGgtagtggt	0.1
30	214	gcccacagAAATGTGG	CTGCACCGgtgagctg	0.1
31	106	ctgcccagGTATGGGG	CTGCCCAGgtgagcga	0.1
32	112	ttctgcagGTTTTCTA	GGCTTACGgtgagctg	0.1
33	61	gtgtgcagGCATCACC	GATTACCTgtaagtgt	<0.1
34	175	tgccccagGCTGCAGG	GGGACATGgtgcgccc	<0.1
35	116	ccctgcagCTCAACTA	CTCTATGGgtaagcgg	<0.1
36	148	gcccgcagGTGGTCCA	ACGACAAGgtggggcg	<0.1
37	124	ccccgcagGACCTGAA	CAAGATTGgtgagggg	<0.1
38	133	ccccgcagGCCAGTTT	CGGCCACAgtgagtgg	0.2
39	118	cccccaagGCGCATGC	TGACCAAGgtgggctc	0.2
40	179	ctggccagGTCTACAA	GGACACAGgtggggcg	<0.1
41	142	gtccccagCGTGCTGA	AGGCCCGGgtgaggat	<0.1
42	135	ctggccagGTGGTGAA	TCTTCCTGgtaagtcc	0.1
43	104	atccccagGACGAGCC	TCACACAGgtgagggc	<0.1
44	93	tcccacagCATGGAGG	AAGAACCGgtgagccg	0.3
45	103	cccaccagGTTTGGAG	TGCTCAAGgtgcgccg	<0.1
46	138	ccctgcagGAGCGGCA	TGGACAATgtgagcgc	0.1
47	207	cccaccagGTGTTCGT	AGGAGCGGgtgagcag	0.5
48	>700	gtgcacagGCCCAGCT		

the transcription factors Ets-2, Sp1, NF-1, ETF, EGR2, and WT1, respectively (Fig. 2).

Cholesterol-Responsive Regulation of ABCA2 mRNA

The two ABC transporters that show highest homology with ABCA2, ABCA1, and ABCA7, show sterol-dependent regulation in macrophages (15, 20). To test the hypothesis that ABCA2 is also regulated by cholesterol, monocytes were allowed to differentiate into macrophages for 4 days according to published protocols (5). Subsequently, cells were subjected to sustained cholesterol loading in the presence of enzymatically modified LDL. Using a semiquantitative RT-PCR approach (5), we observed that ABCA2 mRNA expression is upregulated during cholesterol loading of human macrophages indicating that ABCA2 is a cholesterol-responsive ABC transporter gene (Fig. 3).

DISCUSSION

In this study, we present the complete coding sequence, promoter region and the genomic structure of the human ABC transporter ABCA2 and demonstrate cholesterol-dependent regulation of ABCA2.

Using a homology based RT-PCR approach a 7.3 kb cDNA containing the complete coding region of the human ABCA2 gene was obtained. The open reading frame comprises 7308 bp and encodes a 2436 amino acid polypeptide with a calculated molecular weight of 240 kDa. The predicted ABCA2 polypeptide contains two highly conserved ATP-binding cassettes including Walker A and B motifs and signature sequences and thus conforms to the model of a full-size transporter.

The human ABCA2 cDNA exhibits 87% homology with the recently reported rat (12) and the incomplete murine ABCA2 cDNA (11), respectively, strongly suggesting that these sequences represent orthologous genes. During the preparation of this manuscript an unpublished GenBank entry (AF178941) was released reporting an extended cDNA relative to the previous entries for human ABCA2. The indicated coding region is identical with the one reported here and thus confirms our sequence data. Sequence comparison revealed highest overall homology of the predicted ABCA2 peptide sequence with the cholesterol-responsive ABC A subfamily transporters ABCA1 (50%) (20) and ABCA7 (44%) (15). Beside amino acid homologies in the highly conserved ATP binding cassette domains, ABCA2 exhibits significant sequence identity at its N-terminus with ABCA1, ABCA7, and ABCR, respectively (Fig. 1). Interestingly, amino acid sequence comparison revealed an N-terminal heptapeptide motif, LLLWKN (amino acids 9–14), which is present in all as yet cloned ABC A transporters and thus may be a characteristic structural feature of the ABC A transporter subfamily (Fig. 1).

We found that sustained cholesterol influx mediated by modified LDL induces an upregulation of ABCA2 mRNA levels in human macrophages suggesting a role for ABCA2 in lipid homeostasis of these cells. A similar regulatory response to cholesterol loading in macrophages was also observed for ABCA1, ABCA3, and ABCA7 (5, 15, 20). This raises the possibility that the induction of ABCA2, like that of other ABC A transporters, may be part of a complex lipid export machin-

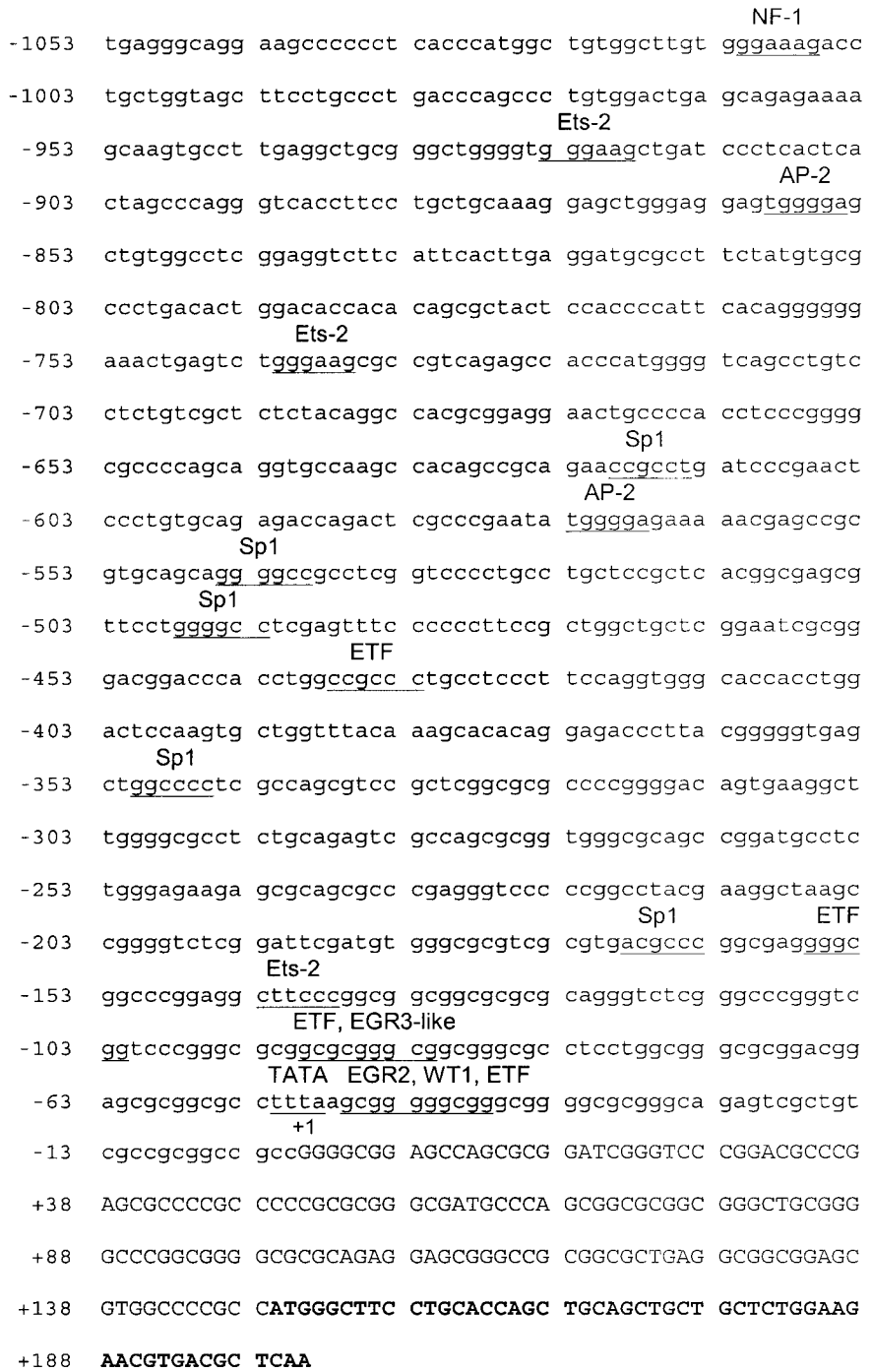


FIG. 2. Putative ABCA2 promoter sequence with predicted transactivator binding sites. Potential binding sites for transcription factors that are involved in myeloid cell activation and the regulation of neural differentiation are underlined. A potential TATA box sequence is indicated. The 5′ end of the primary transcript (start site: +1) is shown in capital letters and the ABCA2 open reading frame is indicated in bold face.

ery that is activated upon excessive cholesterol influx to maintain lipid homeostasis in macrophages. The molecular mechanisms which mediate the cholesterol-dependent regulation of ABCA2 in macrophages are currently unknown. Further work will be required to address this important question in detail. In a previous survey, in which cholesterol influx into macrophages was mediated by acetylated LDL, we observed no regulation of ABCA2 mRNA expression (5) which indicates that the cholesterol-dependent regulation of ABCA2 depends critically on the nature of the cholesterol carrier particle that is used to induce cholesterol

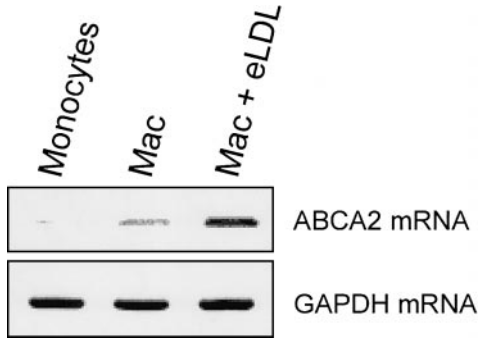


FIG. 3. Upregulation of ABCA2 mRNA in macrophages during sterol import. Shown is the expression of ABCA2 mRNA by semiquantitative RT-PCR in human monocytes and macrophages. Lane 1, Freshly obtained human monocytes. Lane 2, Macrophages (Mac) maintained in culture in the presence of M-CSF for 4 days. Lane 3, Macrophages as in lane 2 incubated with enzymatically degraded LDL (eLDL, 40 μg/ml) for an additional 24 h. Expression of the housekeeping gene GAPDH is shown as an internal standard. The sizes of the diagnostic PCR products are 459 bp (ABCA2) and 280 bp (GAPDH), respectively. RT-PCR was performed for 35 amplification cycles.

influx. This aspect needs to be taken into account in future experiments designed to test ABC transporters for cholesterol-responsive regulation.

Structural analysis of the ABCA2 gene revealed that it consists of 48 relatively small exons which are separated by in most cases equally small intron sequences. The size of the coding region and the number of exons of ABCA2 is similar to that of ABCA1, ABCA7, and ABCR, the three other human ABC A subfamily members whose genetic structure has been characterized to completeness. The ABCA2 gene differs significantly from the genomic sizes of other ABC A transporters in that it is strikingly condensed spanning a genomic region of only 21 kb (Table 2). This structure is unusually compact and contrasts with the genomic sizes reported for other human ABC A transporters whose genomic regions comprise 149 kb (ABCA1), 100 kb (ABCR), and 32 kb (ABCA7), respectively. The gene sizes of known full-size transporters within other ABC subfamilies range from 74 to 250 kb, as evidenced for the multidrug resistance transporter MDR3 (ABCB4) and the cystic fibrosis transmembrane conductance regulator (CFTR) (23, 24). ABCA2 thus displays the most compact genomic organization among all currently known ABC full-size transporters.

ABCA2 is expressed in a limited number of tissues including macrophages (5) and highest expression levels are found in the central neural system (11, 12). Computer-assisted analysis of the ABCA2 5′ gene flanking region revealed multiple potential binding sites for transcription factors with roles in the activation of cells of the myeloid lineage. We identified binding sites for Ets-2, a transcription factor that has been implicated in macrophage activation (25) and several target sites for SP1, a transactivator that is typically expressed in hematopoietic cells (26). In this context, it is interesting to note that SP1 can modulate the promoter activity of the cholesterol-responsive transporter ABCA1, the closest homolog of ABCA2, and that it is also present in the cholesterol-sensitive half-size transporter ABCG1. It is thus conceivable that SP1 is a critical determinant of ABCA2 promoter activity.

Importantly, we found a series of potential binding sites for the transcription factors NF-1, ETF, EGR2, and the Wilms' tumor gene product WT1. The transcription factor NF-1, for example, is highly expressed in the neocortex (27) and has recently been implicated in the regulation of olfactory neuron gene expression (28). WT1 has recently been proposed to play an important role in the differentiation of nerve cells (29). The expression of the transcription factor ETF is strictly regulated in developing embryos and is limited to tissues such as the hindbrain strongly suggesting that it is involved in gene regulation during neural development (30). The Cys2-His2 zinc finger transcription factor early growth response 2 gene (EGR2) (31), also known as Krox20, is thought to play a role in the regulation of peripheral nervous system myelination as evidenced by the observation that Krox20 (EGR2) null mutant mice display hypomyelination of the peripheral nervous system and a block of Schwann cells at an early stage of differentiation (32). Moreover, mutations in the human EGR2 gene have recently been associated with the inherited peripheral neuropathies (33, 34). In addition, the ABCA2 promoter contains several early growth response 3 gene (EGR3)-like binding elements (35). Interestingly, defective EGR3 has been associated with sensory ataxia in mice (36).

Taken together, the presence of target sites for various neurotropic transactivators in the ABCA2 gene promoter supports the view that this transporter may be critically involved in neural development and neuronal differentiation. Further work is required to define the detailed role of ABCA2 in the neural system and in macrophage lipid metabolism.

TABLE 2

Molecular Features of ABCA2 vs Other Fully Characterized Human ABC A Transporters

	ABCA2	ABCA1	ABCA7	ABCR (ABCA4)
Chromosomal locus	9q34	9q22–q31	19p13.3	1p22.3–22.2
Gene size (kb)	21	149	32	100
Exons	48	50	46	50
Coding region (bp)	7308	6783	6438	6819
Amino acids	2436	2261	2146	2273
Reference		(11, 37)	(15, 17)	(38, 39)

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