CMLS, Cell. Mol. Life Sci. 59 (2002) 1285–1295
1420-682X/02/081285-11

CMLS Cellular and Molecular Life Sciences

Review

ABCA2: a candidate regulator of neural transmembrane lipid transport

G. Schmitz* and W. E. Kaminski

Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Franz-Josef-Strauß-Allee 11, 93042 Regensburg (Germany), Fax + 49 941 944 6202, e-mail: gerd.schmitz@klinik.uni-regensburg.de

Received 31 January 2002; received after revision 11 March 2002; accepted 11 March 2002

Abstract. Studies in the past years have implicated multispan transmembrane transport molecules of the ATP binding cassette (ABC) transporter family in cellular lipid export processes. The prototypic ABC transporter ABCA1 has recently been demonstrated to act as a major facilitator of cellular cholesterol and phospholipid export. Moreover, the transporter ABCA4 (ABCR) plays a pivotal role in retinaldehyde processing, and ABCA3 has recently implicated in lung surfactant processing. These pioneering observations have directed considerable attention to the A subfamily of ABC proteins. ABCA2 is the codefining member of the ABC A-transporter subclass. Although known for some time, it was not until recently that its complete molecular structure was established. Unlike other ABC A-subfamily members, ABCA2 is predominantly expressed in the brain and neural tissues. The unique expression profile together with available structural data suggest roles for this largest known ABC protein in neural transmembrane lipid export.

Key words. ABC transporter; ABCA2; macrophage; cholesterol; neural lipid transport; neuropathy; myelination.

Introduction

ATP binding cassette (ABC) transporters constitute a supergene family of multispan transmembrane proteins that are evolutionarily highly conserved across species. These molecules mediate the energy-dependent unidirectional transmembrane transport of a multitude of specific hydrophilic and xenobiotic substrates [1–3]. Typically, ABC transporters are composed of two tandemly linked functional units, each consisting of an ABC and a complex transmembrane domain. Alternatively, they can form dimers which consist of two half-size transporters that are encoded by distinct genes [4]. Within the cell, ABC transporters localize to the plasma membrane and a variety of subcellular compartments, including peroxisomes, the Golgi complex, endoplasmic reticulum, lamellar bodies and intracellular secretory vesicles. Substrates that are transported by ABC molecules include lipids, peptides, amino acids, carbohydrates, vitamins, ions, glucuronide and glutathione conjugates, and xenobiotics [1, 4, 5]. Available information supports the view that ABC transporters form multiunit complexes upon activation as shown for SUR1, which is functionally associated with the K^+ channel KIR6.1 and a potassium-sensitive Ca^{2+}-channel [6, 7]. The transporter CFTR binds with syntaxin 1 A, a member of the syntaxin family of membrane fusion regulators [8], a sodium channel [9] and with the endocytic adaptor complex AP-2 [10]. Moreover, it was shown that CFTR physically interacts with the Na^+/H^+ exchanger regulatory factor (NHERF) through PDZ domains, a family of conserved protein-interaction modules [11], suggesting that PDZ-dependent domain-specific protein-protein interactions may be critical for the function of ABC proteins. It is thus conceivable that ABC transporters, as integral components of membrane multi-

* Corresponding author.

unit complexes, are not necessarily restricted to serve one defined function but are likely involved in a broad spectrum of biologic activities, depending on the specific assembly of the functional complex and the biologic activities of their interaction partners.

The ABC A subfamily of transporters

Based on their structural homologies, ABC transporters can be subdivided into seven distinct subclasses, denoted ABC A–G [3]. To date, a total of 12 genes have been assigned to the ABC A subclass. The complete coding regions and genomic structures have been determined for six transporters of this subfamily. Beside the prototypic members ABCA1 [12, 13] and ABCA2 [12, 14–16], these include ABCA3 [17], ABCR (ABCA4) [18], ABCA6 [19] and ABCA7 [20], respectively (table 1). Among these ABC A molecules, ABCA6 exhibits strikingly fewer sequence similarities with the transporters ABCA1 – ABCA4, and ABCA7, respectively, which share significant amino acid homologies and are dispersed in the genome (table 1). ABCA6 together with the novel transporter ABCA9 [21; W. E. Kaminski et al., unpublished] and most likely other as yet uncharacterized ABC A transporters may constitute a distinct subgroup within the ABC A subfamily [unpublished] whose members appear to localize to a common region on chromosome 17q24 [3].

Structural features of ABCA2

cDNA and genomic structure

ABCA2 (previously designated ABC2) was originally codiscovered with ABCA1 (ABC1) in 1994 from embryonic mouse brain by Luciani and co-workers [12]. The cloned murine ABCA2 complementary DNA (cDNA) predicted a 1472 amino acid polypeptide bearing all features of a full-size ABC transporter. However, cloning of rat ABCA2 revealed a full-length cDNA encoding a 2434-amino acid molecule suggesting that a significant part of the originally reported murine sequence at the 5′ end was missing [16]. Recent cloning of ABCA2 cDNA from human macrophages in our laboratory provided evidence for the existence of an open reading frame coding for a protein of 2436 amino acids [14]. These results, which are in agreement with a more recent study that also reports an open reading frame of 7308 bp [15], indicate that ABCA2 is the as yet largest member of the human ABC transporter family (table 1) (for a current synopsis see http://nutrigene.4t.com/humanabc.htm).

Work from our laboratory revealed that the human ABCA2 gene comprises 48 exons which are localized within a genomic region of only 21 kb [14]. This contrasts strikingly with the considerably larger genomic sizes reported for other human ABC A transporters [19, 21–24] (table 1) and also the gene sizes of full-size transporters of other known ABC subfamilies that range from 74 kb (MDR3) to 250 kb (CFTR) [25, 26]. The ABCA2 gene is thus unique in that it not only encodes the largest ABC molecule but also possesses the most condensed gene structure among all known human full-size transporters.

Domain organization of ABCA2

The calculated model of ABCA2 predicts a polypeptide that features 12 transmembrane segments which can be subgrouped into two major transmembrane domains, each consisting of 6 transmembrane regions that are followed by nucleotide binding folds (NBFs) (fig. 1). This configuration is similar to that predicted for ABCA1 [12], ABCR [18] and ABCA7 [20] and conforms to the structural features reported for other full-size ABC transporters. Based on recent topological data obtained for ABCA1 [27] and ABCR [28], one can postulate an analogous steric model for ABCA2. According to such a hypothetical view, ABCA2 features two large extracellular domains (domains I and IV). The largest predicted extra-

Table 1. Structural features of ABCA2 and other cloned transporters of the human ABC A subclass.

Transporter	ABCA2	ABCA1	ABCA3	ABCR (ABCA4)	ABCA6	ABCA7
Chromosome	9q34	9q22–q31	16p13.3	1p22.3–22.2	17q24	19p13.3
Gene size (kb)	21	149	?	100	62	32
Exons	48	50	?	50	38	46
Coding region (bp)	7308	6783	5115	6819	4851	6438
Amino acids	2436	2261	1704	2273	1617	2146
Amino acid homology with ABCA2 (%)	100	50	43	40	32	44
Reference	14, 15	13, 22	17	18, 24	19	20, 100

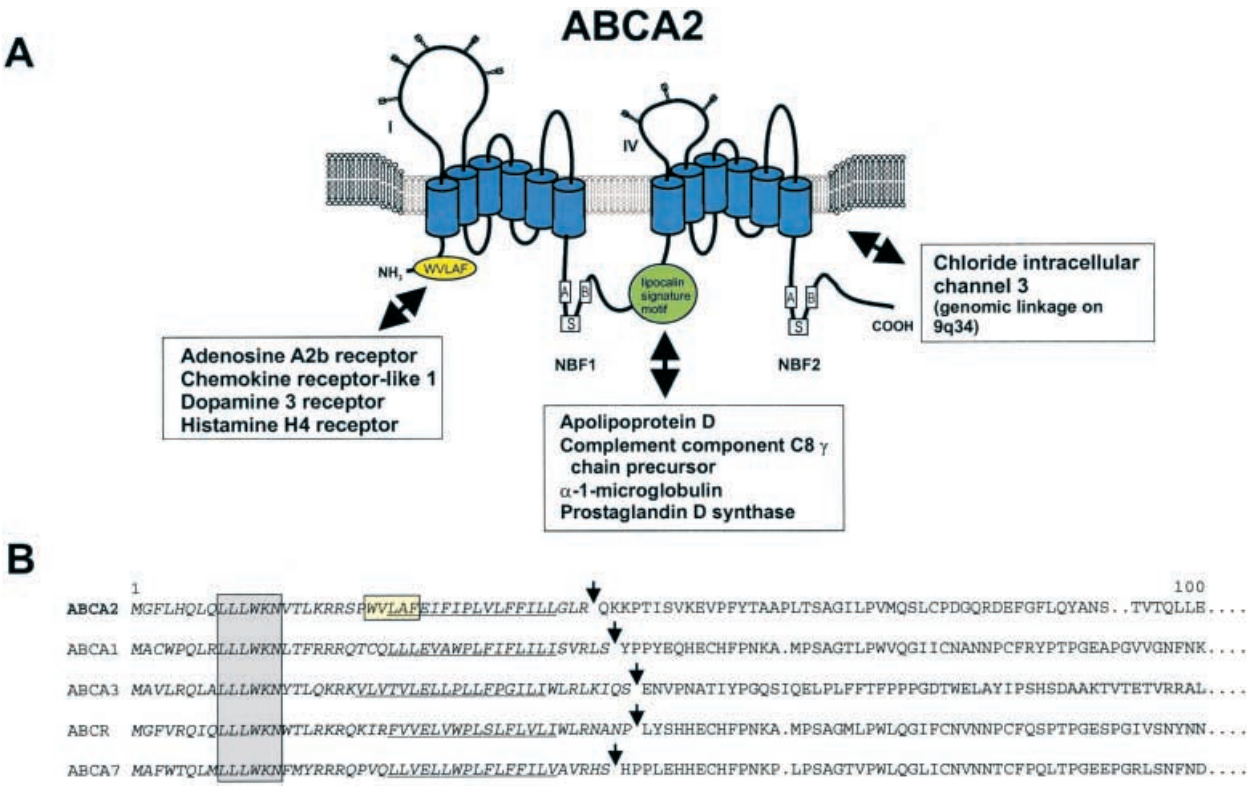

Figure 1. (*A*) Polypeptide structure and putative functional domains of ABCA2. Effector proteins with structural homologies to and potential functional associations with ABCA2 are highlighted in boxes. Candidate glycosylation sites are shown for the two large extracellular domains (I, IV). NBF, nucleotide binding folds; A, B, Walker A and B motifs; S, signature sequence. (*B*) Amino acid sequence of the human ABCA2 N-terminus aligned with highly homologous members of the ABC A subfamily (in order of overall amino acid homology with ABCA2). The N-terminal LLLWKN-heptapeptide present in all five ABC A transporters (gray shaded box) and the ABCA2-specific WVLAF sequence (yellow box) are shown. The putative leader sequences are italicized, and their predicted cleavage sites highlighted by arrows. The predicted first transmembrane domains are underlined. Amino acids are shown in the single letter code, gaps due to optimal alignment are represented by dots. For the prediction of signal peptide cleavage sites and transmembrane domains, the SignalP software and TMpred algorithm were used [30, 103].

cellular domain, which is localized close to the N-terminus, consists of ~650 amino acids (domain I), whereas the second largest extracellular region (domain IV) comprises 300 residues (fig. 1). A similar configuration has been proposed for ABCA1 and recently supported by experimental data [29]. Importantly, this study provides evidence that the N-terminal 45 amino acids of ABCA1 are cleaved off in the mature protein. By analogy, it is thus possible that the same holds true for ABCA2. In fact, the SignalP program [30] predicts a signal peptide for ABCA2 with a cleavage site between residues 45 and 46. Whether this peptide sequence is indeed clipped off in mature ABCA2 remains to be established in further studies.

A heptapeptide motif ('LLLWKN', aa 9–14) close to the ABCA2 N-terminus deserves specific attention because it is present in all as yet cloned ABC A transporters with the exception of ABCA6 and ABCA9, respectively [unpublished observations] (fig. 1B). Database search revealed no homology to any known consensus sequences. As suggested by the presence of a potential cleavage site between residues 43 and 44, it is likely that the LLLWKN sequence is part of the cleaved-off leader peptide sequence which controls the intracellular transport of ABCA2 and other ABC A family members to organelles or the plasma membrane. This is supported by the fact that next to ABCA2 and ABCA1 potential leader peptide cleavage sites are also identifiable at corresponding regions in the transporters ABCA7, ABCR and ABCA3, respectively (fig. 1B). In addition to the LLLWKN consensus sequence, the ABCA2 N-terminal region contains a sequence ('WVLAF'), which is absent from other ABC A subfamily members (fig. 1). Intriguingly, we found this motif in several G-protein-coupled receptor molecules, including the adenosine A2b receptor, the chemokine receptor-like 1, the histamine H4 receptor and the dopamine 3 receptor, respectively. In the D3 receptor, which is highly expressed in the limbic system, the WVLAF sequence is located in the center of a domain referred to as G-protein-coupled receptor family

2 profile. Presently, the role of the WVLAF motif is unknown; however, given the likely involvement of ABCA2 in neural development and integrity (see below), it is tempting to speculate that ABCA2 and the postsynaptic D3 receptor [31] may interact with common interaction partners and thus be functionally interlinked (fig. 1).

Immunoblot analyses from rat brain using a rabbit polyclonal antiserum revealed that mature ABCA2 migrates as a single band of ~260-kDa size [16, 32]. This is in line with another study reporting a band in the same size range after transfection of HEK 293 cells with a human ABCA2 construct [15]. Based on the assumption that mature ABCA2 comprises 2436 amino acids, one would expect that this transporter is glycosylated. In fact, 21 potential glycosylation sites are identifiable in human ABCA2 [15]. However, further work is required to identify the authentic glycosylation sites and their biologic significance for ABCA2 function.

Expression of ABCA2 in tissues

ABCA2 messenger RNA (mRNA) is predominantly expressed in the brain; however, ABCA2 transcripts are also found in kidney, liver, uterus, thymus and heart [12, 15, 16]. In addition, a multitude of tumor cells has been reported to express ABCA2 transcripts. These include cell lines derived from the skin, central nervous system (CNS), lung, intestine, breast and the hematopoietic system [15]. We could demonstrate ABCA2 expression in peripheral blood monocytes and during monocyte differentiation into macrophages [14, 33]. Moreover, human ABCA2 mRNA expression is induced during cholesterol import into macrophages, indicating that ABCA2 is a cholesterol-responsive gene, which raises the possibility that ABCA2 may be directly or indirectly involved in cholesterol transport in these cells [14].

Analysis of the putative ABCA2 promoter region revealed multiple potential binding sites for transcription factors with roles in the differentiation and activation of myeloid and neural cells [14]. For example, we identified binding sites for Ets-2, a transactivator that has been implicated in macrophage activation [34] and several target sites for SP1, a transcription factor which is typically expressed in hematopoietic cells [35]. Interestingly, it has been shown that SP1 can modulate the promoter activity of the cholesterol-responsive transporter ABCA1, the closest homolog of ABCA2 [36]. Moreover, target sites for SP1 are also present in the cholesterol-sensitive half-size transporter ABCG1 [36]. It is thus conceivable that SP1 is a critical determinant of ABCA2 promoter activity which may in part account for the observed sterol-responsive regulation of ABCA2 in human macrophages. Unlike ABCA1 and ABCG1, however, ABCA2 appears not to have canonical target sites for the nuclear receptor LXR, which has been implicated in sterol-responsive gene regulation [37].

The predominant expression of ABCA2 in neural tissues may have a structural basis in the presence of a variety of 'neurotropic' consensus elements in the ABCA2 promoter region. For example, we identified a series of potential binding sites for the transcription factors NF-1, ETF, EGR2 and the Wilms' tumor gene product WT1 within the ABCA2 upstream sequence. The transcription factor NF-1 is highly expressed in the neocortex [38] and has been implicated in the regulation of olfactory neuron gene expression [39]. WT1 has recently been proposed to play an important role in the differentiation of nerve cells [40]. The expression of the transcription factor ETF is strictly regulated during embryonal development and is limited to tissues such as the hindbrain, strongly suggesting that it is involved in gene regulation during neural development [41]. The Cys2-His2 zinc-finger transcription factor early growth response 2 gene (EGR2) [42], also referred to as Krox20, is believed to play a role in the regulation of peripheral nervous system myelination. This is supported 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 [43]. Moreover, mutations in the human EGR2 gene have recently been associated with inherited peripheral neuropathies, including congenital hypomyelinating neuropathy, Charcot-Marie-Tooth type 1 disease and Dejerine-Sottas syndrome [44, 45]. In addition, the ABCA2 promoter contains several early growth response 3 gene (EGR3)-like binding elements [46]. In this context, it is of note that defective EGR3 has been associated with sensory ataxia and muscle spindle agenesis in mice. These data emphasize the involvement of EGR transcription factors in disorders characterized by neurologic dysfunction [47].

Potential interaction partners of ABCA2

Besides the sterol-dependent regulation of ABCA2 and the identification of promoter elements that are associated with sterol response, the presence of a lipocalin signature motif (GQGSRKLDGGWLKV) at amino acid position 1424–1437 supports a role for ABCA2 in lipid transport [15]. Lipocalins constitute a phylogenetically conserved group of proteins which bind and transport small hydrophobic molecules, including sterols, lipids, bilins and retinoids [48–50]. These molecules share limited regions of sequence homology and a common tertiary structure architecture which is characterized by an eight-stranded antiparallel β barrel that forms a binding pocket for small hydrophobic molecules [50]. To date, more than 40 proteins containing a lipocalin signature motif have been characterized. In the human system, mol-

ecules that belong to the lipocalin family include, among others, apolipoprotein D, prostaglandin D synthase, complement component C8 γ-chain and α-1-microglobulin. Apolipoprotein D (apoD) is a member of the family of bilin-binding protein, which was initially identified as a constituent of plasma high-density lipoproteins [51]. It functions as a transport protein for small hydrophobic molecules, including sterols, steroid hormones and arachidonic acid [52, 53]. Importantly, apoD is expressed in peripheral and neural tissues [54–56] and upregulated in neural injury, neuronal degeneration and regeneration. For example, increased levels of apoD have been found in Alzheimer disease [57] and in Niemann-Pick C deficient mice, a cholesterol trafficking disease with progressive neurodegeneration [58]. Although available information suggests an involvement of apoD in the pathogenesis of neurologic disorders, the physiological role of apoD within the brain is unknown. Interestingly, a recent study has shown that apoD is expressed in human oligodendrocyte precursor cells [59], suggesting a role for apoD in the transport of sterols and small hydrophobic molecules in the cortical glial cells. Moreover, the finding that apoD associates with preβ – high-density lipoprotein (HDL) particles provides a potential interlink between HDL metabolism and this apolipoprotein [60].

Prostaglandin D synthase is a major brain-derived protein component of cerebrospinal fluid [61]. Notably, meningeal macrophages have been shown to express prostaglandin D synthase [62]. In these cells, prostaglandin D was detected in lysosomes, which may be indicative of an uptake of the protein from the cerebrospinal fluid. In addition, prostaglandin D synthase was found to be expressed in oligodendrocytes [62, 63].

The complement component C8 γ-chain, another protein carrying a lipocalin signature motif, is located on 9q34.3 [64]. Notably, two other genes from the lipocalin family, α-1-microglobulin and α-1-acid-glycoprotein (orosomucoid) map to the same area, a locus to which prostaglandin D synthase has also been assigned [65]. It remains to be established whether the genomic clustering of these lipocalin proteins and ABCA2 in the 9q34 is reflective of functional associations.

Another molecule that is potentially functionally associated with ABCA2 comes from genomic information. In an effort to explore the genomic microenvironment on chromosome 9q34, in which ABCA2 is positioned, we searched human genome project draft sequences for the presence of neighboring genes. Surprisingly, we found that the gene coding for the chloride intracellular channel 3 (CLIC3) [66] is located immediately downstream of ABCA2. The positioning of genes in tandem arrays or gene clusters suggests their functional linkage and a common ancestry which has been proposed to originate from primordial gene duplication events [67]. The CLIC gene family has been implicated in chloride ion transport within various subcellular compartments. A recent study reported association of the mitogen-activated protein (MAP) kinase ERK7 and CLIC3 using a yeast two-hybrid approach, and epitope-tagging experiments indicated that CLIC3 is localized predominantly in the nucleus [66]. These data suggest a role for CLIC3 in the regulation of cell proliferation; however, the definitive biologic function of this gene has as yet not been elucidated. Given the highly dissimilar gene structures and predicted peptide sequences of ABCA2 and CLIC3, it is unlikely that both genes arose from a duplication event. Little information is currently available on the expression of CLIC3 in tissues. It appears not to be expressed in significant quantities in the brain. High expression, however, has been reported in placenta and heart, and to a lesser degree in the liver [66], tissues that also express ABCA2 [12, 15, 16]. It can thus not be ruled out that a functional and regulatory association exists between CLIC3 and ABCA2 in these tissues. It will be challenging to investigate this intriguing possibility.

ABCA2 localizes to the lysosomal compartment of oligodendrocytes

A recent study by Zhou and co-workers [32] provided evidence that in rat brain ABCA2 is predominantly expressed in the white matter but also in the gray matter, and ABCA2 expression appears to be restricted to oligodendrocytes. The latter represent the glial cells that synthesize myelin. ABCA2 was detectable in oligodendrocyte only in the cell body but not in myelin sheaths. Importantly, it was shown that ABCA2 is associated with lysosomes, suggesting a role for this transporter in the endo-lysosomal compartment [32]. This membrane system is enriched in highly glycosylated transmembrane proteins. Lysosomal membrane proteins are translocated along the secretory pathway and reach lysosomes indirectly via the cell surface and endocytosis. Alternatively, proteins can exit the trans-Golgi network in clathrin-coated vesicles for direct delivery to endosomes and lysosomes [68]. Sorting from the Golgi or the plasma membrane into the endosomal system is a tightly regulated process that is mediated by signals encoded by the short cytosolic domain of these proteins. Consistent with the lysosomal localization of ABCA2, a cluster of lysosomal targeting motifs is identifiable at the ABCA2 C-terminal region [unpublished]. These recognition sites, which conform to the consensus YXXΦ (Y is a tyrosine, X any amino acid and Φ is a bulky hydrophobic side chain such as leucine, isoleucine, phenylalanine, methionine or valine), have been shown to mediate targeting of integral membrane proteins to the endo-lysosomal system [69–71]. In addition, two di-leucine (or isoleucine) motifs, which represent the second class of lysosomal and

endosomal targeting signals [68], are localized close to the ABCA2 C-terminus. It is noteworthy, however, that both YXXΦ and di-leucine motifs are also found in many other ABC transporters which are not known to localize to the endo-lysosomal compartment. Further studies are required to determine to which extent these signal sequences are implicated in the lysosomal targeting of ABCA2.

Besides ABCA2, another ABC transporter with unknown function, the half-size transporter ABCB9, has recently also been shown to localize to the lysosomal compartment [72]. Interestingly, ABCB9, like ABCA2, is highly expressed in brain and the spinal cord, raising the question whether these transporter molecules have roles in a common functional endo-lysosomal transport complex in neural tissues. Another potential functional interplay may exist between ABCA2 and apoD in oligodendrocytes. This is based on the findings that both molecules are not only expressed in oligodendrocytes [32, 59] but also share a lipocalin domain. Because both proteins potentially interact with small hydrophobic substrates via their lipocalin signature sequence, this raises the attractive possibility that they may cooperate synergistically in the transmembrane transport of lipid compounds in oligodendrocytes (fig. 1).

ABC transporters and human disease: a role for ABCA2?

In the past 2 years, evidence has been provided that a number of hereditary disorders affecting cellular lipid transport processes are caused by genetic defects in ABC transporters (table 2). For example, it was shown that mutations in the ABC transporter genes MRP2, MDR3 and BSEP account for Dubin-Johnson syndrome and subforms of progressive familial intrahepatic cholestases (PFICs) [73–75]. Another member of the MRP family, ABCC6 (MRP6), has recently been causatively linked to pseudoxanthoma elasticum, a disease presenting with skin abnormalities, visual defects and cardiovascular manifestations [76–78]. Moreover, mutations in the ALDP gene have been shown to cause the neurodegenerative disorder adrenoleukodystrophy, which is characterized by an impaired peroxisomal β-oxidation of very long chain fatty acids [79]. An important link between ABC transporters and the cellular cholesterol uptake machinery was recently established by studies demonstrating that mutations in the genes for ABCG5 and ABCG8 are the underlying cause of β-sitosterolemia [80, 81]. This rare autosomal recessive disorder is characterized by hyperabsorption of sterols, hypercholesterolemia, decreased biliary excretion of dietary sterols and premature coronary atherosclerosis [82].

Within the ABCA subfamily, two members have recently received considerable attention based on studies showing causative linkage to human disease (table 2). Work from our laboratory and others has demonstrated that ABCA1 is a key regulator of systemic lipoprotein metabolism, which is dysfunctional in genetic high-density lipoprotein (HDL)-deficiency syndromes [83–86]. At the cellular level ABCA1 appears to function as a facilitator of choline phospholipid and cholesterol export rather than a *bona fide* transporter [87]. Mutations in the transporter ABCR, which is predominantly expressed in the retina, have been associated with a group of degenerative retinopathies including Stargardt disease [18, 88], retinitis pigmentosa [89, 90], cone-rod dystrophy [89] and age-related macular degeneration [91], respectively.

By analogy, it can thus be expected that ABCA2 is also causatively involved in the pathogenesis of monogenetic human disorders. Of particular interest in this context are clinical entities that have been assigned to 9q34 and/or are associated with neurologic phenotypes. Attractive

Table 2. ABC transporters involved in the transmembrane transport of cellular lipids or lipophilic compounds that are causatively linked to human diseases.

ABC lipid transporter	Associated disease	Physiologic substrate	References
ABCA1 (ABC1)	genetic HDL deficiency syndromes	cholesterol, phospholipids	83–86
ABCA2 (ABC2)	?	?	
ABCA4 (ABCR)	Stargardt disease	retinaldehyde	18
ABCA4 (ABCR)	retinitis pigmentosa	retinaldehyde	89, 90
ABCA4 (ABCR)	cone-rod dystrophy	retinaldehyde	89
ABCA4 (ABCR)	age-related macular degeneration	retinaldehyde	91
ABCB4 (MDR3)	PFIC3	PC	74
ABCB11 (BSEP)	PFIC2	bile acids	75
ABCC2 (cMOAT)	Dubin-Johnson syndrome	glutathione conjugates, LT C_4	73, 102
ABCC6 (MRP6)	Pseudoxanthoma elasticum	?	76–78
ABCD2 (ALDR)	adrenoleukodystrophy	VLCF	79
ABCG5	β-sitosterolemia	sterols	80, 81
ABCG8	β-itosterolemia	sterols	80

PFIC, progressive familial intrahepatic cholestasis; PC, phosphatidycholine; LT, leukotriene; VLCF very long chain fatty acids.

candidates that meet these criteria include amyotrophic lateral sclerosis 4 (ALS4) (OMIM 602433), the lethal congenital contraction syndrome (OMIM 253310) and recessive spinocerebellar ataxia (OMIM 606002). Experiments are currently under way to address the question whether these hereditary diseases are causatively linked to structural abnormalities in the human ABCA2 gene.

Another clue that ABCA2 may be implicated in human disease comes from a report demonstrating amplification of the ABCA2 gene in estramustine-resistant human ovarian carcinoma SKEM cells [92]. Estramustine is a potent chemotherapeutic agent, which structurally represents a conjugate of estradiol and nor-nitrogen [93]. These findings, which highlight the genomic plasticity of the ABCA2 locus under conditions of cytotoxic drug stress, suggest that ABCA2 is potentially involved in multidrug resistance in tumor therapy. Gene multiplication is a well-known mechanism conveying multidrug resistance to cells which has also been reported for other ABC transporters, including Bcrp1/Mxr/Abcp, MRP and MDR2 [94–96]. At present, it remains to be established whether ABCA2-mediated resistance to estramustine is of clinical relevance in tumor therapy.

ABCA2 in the context of other ABC A subclass transporters

All currently known ABC transporters of the A subfamily are expressed in monocytes, and many of them are regulated during cholesterol influx or efflux in human macrophages [33]. In particular, upregulation during sterol import has been demonstrated for ABCA1 [13], ABCA2 [14] and ABCA7 [20], respectively, which share highest structural homology among the group of ABC A subclass members. ABCA1 has been shown to function as a facilitator of cholesterol and choline phospholipid transport [86, 87], and most recent experiments from our laboratory indicate that ABCA7 exports or facilitates the export of ceramide and phosphatidylserine from the cell [D. Kielar et al., unpublished]. However, the question which physiological substrates are translocated by ABCA2 still remains to be resolved. Conceptually, it is possible that ABCA1, ABCA2 and ABCA7 serve nonredundant or, alternatively, (at least) partially overlapping functions in the transport of lipophilic substances in macrophages. In such a context, it is possible that ABCA2, like ABCA1, is involved in the translocation of cholesterol in these cells. This view is consistent with the fact that large quantities of cholesterol are present in the myelin sheath of oligodendrocytes [97]. The myelin sheath represents an extension of the plasma membrane of the oligodendrocyte; however, it has a lipid and protein composition that differs considerably from the rest of the plasma membrane. Its predominant lipids are cholesterol and two glycosphingolipids, galactocerebroside, also designated galactosylceramide, and its sulphated derivative galactosulphocerebroside [98]. Unlike typical glycerophospholipids, such as phosphatidylcholine, which are based on glycerol, galactocerebroside and galactosulphocerebroside are based on a long-chain sphingosine. Considering this, it is tempting to speculate that ABCA2 is involved in the translocation of cholesterol and galactocerebroside complexes to the oligodendrocyte plasma membrane and thus may play a critical role in the development and the maintenance of the integrity of myelin sheaths.

Moreover, a recent report suggests that the formation of cholesterol- and galactosylceramide-rich membrane domains may be critical for the assembly of myelin in oligodendrocytes [98]. This raises the intriguing possibility that ABCA2 may be associated with these ‘myelin rafts’. Further studies are required to address this fascinating issue. In this context it is noteworthy that an association of ABCA1 and a Lubrol-resistant raft subfraction has been established [W. Drobnik et al., unpublished].

Another feature that ABCA1, ABCA2 and also ABCA7 have in common is the fact that each of these genes is physically linked to a structurally unrelated gene within their genomic microenvironment. In the case of ABCA1, two genes designated NIPSNAP3 and NIPSNAP4, respectively, are located immediately downstream of the ABCA1 gene [99]. ABCA7, which is located on chromosome 19q13, is tandemly linked with the minor histocompatibility antigen HA-1 [100], and in an analogous structural configuration, the chloride intracellular channel 3 (CLIC3) is localized immediately downstream of ABCA2 on chromosome 9q34 (see above). These findings pose the question whether and in which way the structural genomic linkage of an ABC transporter and its intimately linked genomic neighbor have a reflection in functional interdependencies. Especially, in the interpretation of gene amplification events, as they have been observed for ABCA2 in estramustine treated tumor cells [92], the aspect of physical coupling of adjacent genes deserves specific attention.

Conclusion

Significant evidence has recently accumulated to support the concept that ABC transporters serve critical physiologic functions in cellular transmembrane transport. Whereas the ABC A subfamily transporters ABCA1 and ABCR have been in the center of attention for quite some time, we are currently only beginning to understand the biologic role of ABCA2. Although there is only limited information available to date, existing data suggest roles for this ABC transporter in myelination and the maintenance of membrane integrity in neural cells such as

oligodendrocytes. More work is required to determine the precise role ABCA2 plays in neural cell transmembrane lipid transport and to identify functional interaction partners of this ABC transporter. In particular, the identification of the physiologic substrates that are translocated by ABCA2 will significantly further our understanding of the biologic function of this largest known ABC transporter. Another aspect that needs to be addressed in the future is the question whether and to what extent ABCA2 is involved in the pathogenesis of diseases, in particular, neurodegenerative disorders. Finally, it will be most challenging to characterize in detail the molecular mechanisms by which ABCA2 and also other ABC A subfamily transporters translocate substrates across membranes barriers. The recent identification of the first crystal structure of a complete ABC transporter, the bacterial half-size transporter MsbA [101], is an important milestone in structural biology that will set the stage to gain more insight into the workings of ABC proteins.

1 Higgins C. F. (1992) ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. **8:** 67–113
2 Schmitz G., Kaminski W. E. and Orsó E. (2000) ABC transporters in cellular lipid trafficking. Curr. Opin. Lipidol. **11:** 493–501
3 Dean M., Hamon Y. and Chimini G. (2001) The human ATP-binding cassette (ABC) transporter superfamily. J. Lipid Res. **42:** 1007–1017
4 Klein I., Sarkadi B. and Varadi A. (1999) An inventory of the human ABC proteins. Biochim. Biophys. Acta **1461:** 237–262
5 Decottignies A. and Goffeau A. (1997) Complete inventory of the yeast ABC proteins. Nat. Genet. **15:** 137–145
6 Inagaki N., Gonoi T., Clement J. P. 4th, Namba N., Inazawa J., Gonzalez G. et al. (1995) Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. Science **270:** 1166–1170
7 Koriyama N., Kakei M., Nakazaki M., Yaekura K., Ichinari K., Gong Q. et al. (2000) PIP2 and ATP cooperatively prevent cytosolic Ca2+-induced modification of ATP-sensitive K+ channels in rat pancreatic beta-cells. Diabetes **49:** 1830–1839
8 Naren A. P., Quick M. W., Collawn J. F., Nelson D. J. and Kirk K. L. (1998) Syntaxin 1A inhibits CFTR chloride channels by means of domain-specific protein-protein interactions. Proc. Natl. Acad. Sci. USA **95:** 10972–10977
9 Hanrahan J. W., Mathews C. J., Grygorczyk R., Tabcharani J. A., Grzelczak Z., Chang X. B. et al. (1996) Regulation of the CFTR chloride channel from humans and sharks. J. Exp. Zool. **275:** 283–291
10 Weixel K. M. and Bradbury N. A. (2000) The carboxyl terminus of the cystic fibrosis transmembrane conductance regulator binds to AP-2 clathrin adaptors. J. Biol. Chem. **275:** 3655–3660
11 Raghuram V., Mak D.-O. D. and Foskett J. K. (2001). Regulation of cystic fibrosis transmembrane conductance regulator single-channel gating by bivalent PDZ-domain-mediated interaction. Proc. Natl. Acad. Sci. USA **98:** 1300–1305
12 Luciani M.-F., Denizot F., Savary S., Mattei M. G. and Chimini G. (1994) Cloning of two novel ABC transporters mapping on human chromosome 9. Genomics **21:** 150–159
13 Langmann T., Klucken J., Reil M., Liebisch G., Luciani M.-F., Chimini G. et al. (1999) Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. Biochem. Biophys. Res. Commun. **257:** 29–33
14 Kaminski W. E., Piehler A., Püllmann K., Porsch-Ozcurumez M., Duong C., Maa Bared G. et al. (2001) Complete coding sequence, promoter region and genomic structure of the human ABCA2 gene and evidence for sterol-dependent regulation in macrophages. Biochem. Biophys. Res. Commun. **281:** 249–258
15 Vulevic B., Chen Z., Boyd J. T., Davis W. Jr, Walsh E. S., Belinsky M. G. et al. (2001) Cloning and characterization of human adenosine 5′-triphosphate-binding cassette, sub-family A, transporter 2 (ABCA2). Cancer Res. **61:** 3339–3347
16 Zhao L. X., Zhou C. J., Tanaka A., Nakata M., Hirabayashi T., Amachi T. et al. (2000) Cloning, characterization and tissue distribution of the rat ATP-binding cassette (ABC) transporter ABC2/ABCA2. Biochem. J. **350 Pt 3:** 865–872
17 Connors T. D., Van Raay T. J., Petry L. R., Klinger K. W., Landes G. M. and Burn T. C. (1997) The cloning of a human ABC gene (ABC3) mapping to chromosome 16p13.3. Genomics **39:** 231–234
18 Allikmets R., Singh N., Sun H., Shroyer N. F., Hutchinson A., Chidambaram A. et al. (1997) A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat. Genet. **15:** 236–246
19 Kaminski W. E., Wenzel J. J., Piehler A., Langmann T. and Schmitz G. (2001) ABCA6, a novel a subclass ABC transporter. Biochem. Biophys. Res. Commun. **285:** 1295–301
20 Kaminski W. E., Orsó E., Diederich W., Klucken J., Drobnik W. and Schmitz G. (2000) Identification of a novel human sterol-sensitive ATP-binding cassette transporter (ABCA7). Biochem. Biophys. Res. Commun. **273:** 532–538
21 Reference removed in proof.
22 Remaley A. T., Rust S., Rosier M., Knapper C., Naudin L., Broccardo C. et al. (1999) Human ATP-binding cassette transporter 1 (ABC1): genomic organization and identification of the genetic defect in the original Tangier disease kindred. Proc. Natl. Acad. Sci. USA **96:** 12685–12690
23 Santamarina-Fojo S., Peterson K., Knapper C., Qiu Y., Freeman L., Cheng J. F. et al. (2000) Complete genomic sequence of the human ABCA1 gene: analysis of the human and mouse ATP-binding cassette A promoter. Proc. Natl. Acad. Sci. USA **97:** 7987–7992
24 Allikmets R., Wasserman W. W., Hutchinson A., Smallwood P., Nathans J., Rogan P. K. et al. (1998) Organization of the ABCR gene: analysis of promoter and splice junction sequences. Gene **215:** 111–122
25 Lincke C. R., Smit J. J., van der Velde-Koerts T. and Borst P. (1991) Structure of the human MDR3 gene and physical mapping of the human MDR locus. J. Biol. Chem. **266:** 5303–5310
26 Rommens J. M., Iannuzzi M. C., Kerem B., Drumm M. L., Melmer G., Dean M. et al. (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. Science **245:** 1059–1065
27 Bungert S., Molday L. L. and Molday R. S. (2001) Membrane topology of the ATP binding cassette transporter ABCR and its relationship to ABC1 and related ABCA transporters: identification of N-linked glycosylation sites. J. Biol. Chem. **276:** 23539–23546
28 Fitzgerald M. L., Mendez A. J., Moore K. J., Andersson L. P., Panjeton H. A. and Freeman M. W. (2001) ATP-binding cassette transporter A1 contains an NH2-terminal signal anchor sequence that translocates the protein's first hydrophilic domain to the exoplasmic space. J. Biol. Chem. **276:** 15137–15145
29 Tanaka A. R., Ikeda Y., Abe-Dohmae S., Arakawa R., Sadanami K., Kidera A. et al. (2001) Human ABCA1 contains a large amino-terminal extracellular domain homologous to an epitope of Sjogren's Syndrome. Biochem. Biophys. Res. Commun. **283:** 1019–1025

30 Nielsen H., Engelbrecht J., Brunak S. and von Heijne G. (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. **10:** 1–6

31 Sokoloff P., Giros B., Martres M. P., Bouthenet M. L. and Schwartz J. C. (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature **347:** 146–151

32 Zhou C., Zhao L., Inagaki N., Guan J., Nakajo S., Hirabayshi T. et al. (2001) Atp-binding cassette transporter ABC2/ABCA2 in the rat brain: a novel mammalian lysosome-associated membrane protein and a specific marker for oligodendrocytes but not for myelin sheaths. J. Neurosci. **21:** 849–857

33 Klucken J., Büchler C., Orsó E., Kaminski W. E., Porsch-Özcürümez M., Liebisch G. et al. (2000) ABCG1 (ABC8), the human homolog of the *Drosophila* white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc. Natl. Acad. Sci. USA **97:** 817–822

34 Tenen D. G., Hromas R., Licht J. D. and Zhang D. E. (1997) Transcription factors, normal myeloid development, and leukemia. Blood **90:** 489–519

35 Saffer J. D., Jackson S. P. and Annarella M. B. (1991) Developmental expression of Sp1 in the mouse. Mol. Cell. Biol. **11:** 2189–2199

36 Porsch-Ozcürümez M., Langmann T., Heimerl S., Borsukova H., Kaminski W. E., Drobnik W. et al. (2001) The zinc finger protein 202 (ZNF202) is a transcriptional repressor of ATP binding cassette transporter A1(ABCA1) and ABCG1 gene expression and a modulator of cellular lipid efflux. J. Biol. Chem. **276:** 12427–12433

37 Costet P., Luo Y., Wang N. and Tall A. R. (2000) Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. J. Biol. Chem. **275:** 28240–28245

38 Chaudhry A. Z., Lyons G. E. and Gronostajski R. M. (1997) Expression patterns of the four nuclear factor I genes during mouse embryogenesis indicate a potential role in development. Dev. Dyn. **208:** 313–325

39 Baumeister H., Gronostajski R. M., Lyons G. E. and Margolis F. L. (1999) Identification of NFI-binding sites and cloning of NFI-cDNAs suggest a regulatory role for NFI transcription factors in olfactory neuron gene expression. Mol. Brain Res. **72:** 65–79

40 Liu X. W., Gong L. J., Guo L. Y., Katagiri Y., Jiang H., Wang Z. Y. et al. (2000) The Wilms' tumor gene product WT1 mediates the down-regulation of the rat epidermal growth factor receptor by nerve growth factor in PC12 cells. J. Biol. Chem. **276:** 5068–5073

41 Yasunami M., Suzuki K., Houtani T., Sugimoto T. and Ohkubo H. (1995) Molecular characterization of cDNA encoding a novel protein related to transcriptional enhancer factor-1 from neural precursor cells. J. Biol. Chem. **270:** 18649–18654

42 Joseph L. J., Le Beau M. M., Jamieson G. A. Jr, Acharya S., Shows T. B., Rowley J. D. et al. (1988) Molecular cloning, sequencing, and mapping of EGR2, a human early growth response gene encoding a protein with 'zinc-binding finger' structure. Proc. Natl. Acad. Sci. USA **85:** 7164–7168

43 Schneider-Maunoury S., Topilko P., Seitandou T., Levi G., Cohen-Tannoudji M., Pournin S. et al. (1993) Disruption of Krox-20 results in alteration of rhombomeres 3 and 5 in the developing hindbrain. Cell **75:** 1199–1214

44 Warner L. E., Mancias P., Butler I. J., McDonald C. M., Keppen L., Koob K. G. et al. (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. Nat. Genet. **18:** 382–384

45 Timmerman V., De Jonghe P., Ceuterick C., De Vriendt E., Debrabandere S., Löfgren A. et al. (1999) Novel missense mutation in the early growth response 2 gene associated with a Dejerine-Sottas syndrome phenotype. Neurology **52:** 1827–1832

46 Patwardhan S., Gashler A., Siegel M. G., Chang L. C., Joseph L. J., Shows T. B. et al. (1991) EGR3, a novel member of the Egr family of genes encoding immediate-early transcription factors. Oncogene **6:** 917–928

47 Tourtellotte W. G. and Milbrandt J. (1998) Sensory ataxia and muscle spindle agenesis in mice lacking the transcription factor Egr3. Nat. Genet. **20:** 87–91

48 Pervaiz S. and Brew K. (1987) Homology and structure-function correlations between alpha 1-acid glycoprotein and serum retinol-binding protein and its relatives. FASEB J. **1:** 209–214

49 Igaraishi M., Nagata A., Toh H., Urade H. and Hayaishi, N. (1992) Structural organization of the gene for prostaglandin D synthase in the rat brain. Proc. Natl. Acad. Sci. USA **89:** 5376–5380

50 Flower D. R., North A. C. T. and Attwood T. K. (1993) Structure and sequence relationships in the lipocalins and related proteins. Protein Sci. **2:** 753–761

51 McConathy W. J. and Alaupovic P. (1973) Isolation and partial characterization of apolipoprotein D: a new protein moiety of the human plasma lipoprotein system. FEBS Lett. **37:** 178–182

52 Dilley W. G., Haagensen D. E., Cox C. E. and Wells S. A. (1990) Immunologic and steroid binding properties of the GCDFP-24 protein isolated from human breast gross cystic disease fluid. Breast Cancer Res. Treat. **16:** 253–260

53 Lea O. A. (1988) Binding properties of progesterone-binding Cyst protein, PBCP. Steroids **52:** 337–338

54 Boyles J. K., Notterpek L. M., Wardell M. R. and Rall S. C. Jr (1990) Identification, characterization, and tissue distribution of apolipoprotein D in the rat. J. Lipid Res. **31:** 2243–5622

55 Provost P. R., Weech P. K., Tremblay N. M., Marcel Y. L. and Rassart E. (1990) Molecular characterization and differential mRNA tissue distribution of rabbit apolipoprotein D. J. Lipid Res. **31:** 2057–2065

56 Seguin D., Desforges M. and Rassart E. (1995) Molecular characterization and differential mRNA tissue distribution of mouse apolipoprotein D. Mol. Brain Res. **30:** 242–250

57 Terrisse L., Poirier J., Bertrand P., Merched A., Visvikis S., Siest G. et al. (1998) Increased levels of apolipoprotein D in cerebrospinal fluid and hippocampus of Alzheimer's patients. J. Neurochem. **71:** 1643–1650

58 Suresh S., Yan Z., Patel R. C., Patel Y. C. and Patel S. C. (1998) Cellular cholesterol storage in the Niemann-Pick disease type C mouse is associated with increased expression and defective processing of apolipoprotein D. J. Neurochem. **70:** 242–251

59 Hu C. Y., Ong W. Y., Sundaram R. K., Chan C. and Patel S. C. (2001) Immunocytochemical localization of apolipoprotein D in oligodendrocyte precursor-like cells, perivascular cells, and pericytes in the human cerebral cortex. J. Neurocytol. **30:** 209–218

60 Böttcher A., Schlosser J., Kronenberg F., Dieplinger H., Knipping G., Lackner K. J. et al. (2000) Preparative free-solution isotachophoresis for separation of human plasma lipoproteins: apolipoprotein and lipid composition of HDL subfractions. J. Lipid Res. **41:** 905–915

61 Clausen J. (1961) Proteins in normal cerebrospinal fluid not found in serum. Proc. Soc. Exp. Biol. Med. **107:** 170–172

62 Beuckmann C. T., Lazarus M., Gerashchenko D., Mizoguchi A., Nomura S., Mohri I. et al. (2000) Cellular localization of lipocalin-type prostaglandin D synthase (beta-trace) in the central nervous system of the adult rat. J. Comp. Neurol. **428:** 62–78

63 Urade Y., Kitahama K., Ohishi H., Kaneko T., Mizuno N. and Hayaishi O. (1993) Dominant expression of mRNA for prostaglandin D synthase in leptomeninges, choroid plexus, and oligodendrocytes of the adult rat brain. Proc. Natl. Acad. Sci. USA **90:** 9070–9074

64 Kaufman K. M., Snider J. V., Spurr N. K., Schwartz C. E. and Sodetz J. M. (1989) Chromosomal assignment of genes encoding the alpha, beta, and gamma subunits of human complement protein C8: identification of a close physical linkage between the alpha and the beta loci. Genomics **5:** 475–480
65 Chan P., Simon-Chazottes D., Mattei M. G., Guenet J. L. and Salier J. P. (1994) Comparative mapping of lipocalin genes in human and mouse: the four genes for complement C8 gamma chain, prostaglandin-D-synthase, oncogene-24p3, and progestagen-associated endometrial protein map to HSA9 and MMU2. Genomics **23:** 145–150
66 Qian Z., Okuhara D., Abe M. K. and Rosner M. R. (1999) Molecular cloning and characterization of a mitogen-activated protein kinase-associated intracellular chloride channel. J. Biol. Chem. **274:** 1621–1627
67 Graham G. J. (1995) Tandem genes and clustered genes. J. Theor. Biol. **175:** 71–87
68 Hunziker W. and Geuze H. J. (1996) Intracellular trafficking of lysosomal membrane proteins. Bioessays **18:** 379–389
69 Canfield W. M., Johnson K. F., Ye R. D., Gregory W. and Kornfeld S. (1991) Localization of the signal for rapid internalization of the bovine cation-independent mannose 6-phosphate/insulin-like growth factor-II receptor to amino acids 24–29 of the cytoplasmic tail. J. Biol. Chem. **266:** 5682–5688
70 Naim H. Y. and Roth M. G. (1994) Characteristics of the internalization signal in the Y543 influenza virus hemagglutinin suggest a model for recognition of internalization signals containing tyrosine. J. Biol. Chem. **269:** 3928–3933
71 Ohno H., Aguilar R. C., Yeh D., Taura D., Saito T. and Bonifacino J. S. (1998) The medium subunits of adaptor complexes recognize distinct but overlapping sets of tyrosine-based sorting signals. J. Biol. Chem. **273:** 25915–25921
72 Zhang F., Zhang W., Liu L., Fisher C. L., Hui D., Childs S., Dorovini-Zis K. et al. (2000) Characterization of ABCB9, an ATP binding cassette protein associated with lysosomes. J. Biol. Chem. **275:** 23287–23294
73 Wada M., Toh S., Taniguchi K., Nakamura T., Uchiumi T., Kohno K. et al. (1998) Mutations in the canalicular multispecific organic anion transporter (cMOAT) gene, a novel ABC transporter, in patients with hyperbilirubinemia II/Dubin-Johnson syndrome. Hum. Mol. Genet. **7:** 203–207
74 de Vree J. M., Jacquemin E., Sturm E., Cresteil D., Bosma P. J., Aten J. et al. (1998) Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc. Natl. Acad. Sci. USA **95:** 282–287
75 Strautnieks S. S., Bull L. N., Knisely A. S., Kocoshis S. A., Dahl N., Arnell H. et al. (1998) A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat. Genet. **20:** 233–238
76 Le Saux O., Urban Z., Tschuch C., Csiszar K., Bacchelli B., Quaglino D. et al. (2000) Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. Nat. Genet. **25:** 223–227
77 Bergen A. A., Plomp A. S., Schuurman E. J., Terry S., Breuning M., Dauwerse H. et al. (2000) Mutations in ABCC6 cause pseudoxanthoma elasticum. Nat. Genet. **25:** 228–231
78 Ringpfeil F., Lebwohl M. G., Christiano A. M. and Uitto J. (2000) *Pseudoxanthoma elasticum*: mutations in the MRP6 gene encoding a transmembrane ATP-binding cassette (ABC) transporter. Proc. Natl. Acad. Sci. USA **97:** 6001–6006
79 Mosser J., Douar A. M., Sarde C. O., Kioschis P., Feil R., Moser H. et al. (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. Nature **361:** 726–730
80 Berge K. E., Tian H., Graf G. A., Yu L., Grishin N. V., Schultz J. et al. (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science **290:** 1771–1775
81 Lee M. H., Lu K., Hazard S., Yu H., Shulenin S., Hidaka H. et al. (2001) Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat. Genet. **27:** 79–83
82 Bhattacharyya A. K. and Connor W. E. (1974) Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J. Clin. Invest. **53:** 1033–1043
83 Bodzioch M., Orsó E., Klucken J., Böttcher A., Diederich W., Drobnik W. et al. (1999) The ATP binding cassette transporter-1 gene (ABCA1) is mutated in familial HDL deficiency (Tangier disease). Nat. Genet. **22:** 347–351
84 Brooks-Wilson A., Marcil M., Clee S. M., Zhang L. H., Roomp K., van Dam M. et al. (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat. Genet. **22:** 336–345
85 Rust S., Rosier M., Funke H., Real J., Amoura Z., Piette J. C. et al. (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat. Genet. **22:** 352–355
86 Orsó E., Broccardo C., Kaminski W. E., Böttcher A., Liebisch G., Drobnik W. et al. (2000) Transport of lipids from Golgi to plasma membrane is defective in Tangier disease patients and Abc1 deficient mice. Nat. Genet. **24:** 192–196
87 Szakacs G., Langmann T., Ozvegy C., Orsó E., Schmitz G., Varadi A. and Sarkadi B. (2001) Characterization of the ATPase cycle of human ABCA1: implications for its function as a regulator rather than an active transporter. Biochem. Biophys. Res. Commun. **288:** 1258–1264
88 Sun H., Molday R. S. and Nathans J. (1999) Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. J. Biol. Chem. **274:** 8269–8281
89 Cremers F. P., van de Pol D. J., van Driel M., den Hollander A. I, van Haren F. J., Knoers N. V. et al. (1998) Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. Hum. Mol. Genet. **7:** 355–362
90 Martinez-Mir A., Paloma E., Allikmets R., Ayuso C., del Rio T., Dean M. et al. (1998) Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. Nat. Genet. **18:** 11–12
91 Allikmets R., Shroyer N. F., Singh N., Seddon J. M., Lewis R. A., Bernstein P. S. et al. (1997) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science **277:** 1805–1807
92 Laing N. M., Belinsky M. G., Kruh G. D., Bell D. W., Boyd J. T., Barone L. et al. (1998) Amplification of the ATP-binding cassette 2 transporter gene is functionally linked with enhanced efflux of estramustine in ovarian carcinoma cells. Cancer Res. **58:** 1332–1337
93 Tew K. D., Erickson L. C., White G., Wang A. L., Schein P. S. and Hartley-Asp B. (1983) Cytotoxicity of estramustine, a steroid-nitrogen mustard derivative, through non-DNA targets. Mol. Pharmacol. **24:** 324–328
94 Allen J. D., Brinkhuis R. F., Wijnholds J. and Schinkel A. H. (1999) The mouse Bcrp1/Mxr/Abcp gene: amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone or doxorubicin. Cancer Res. **59:** 4237–4241
95 O'Neill G. M., Peters G. B., Harvie R. M., MacKenzie H. B., Henness S. and Davey R. A. (1998) Amplification and expression of the ABC transporters ARA and MRP in a series of multidrug-resistant leukaemia cell sublines. Br. J. Cancer **77:** 2076–2080
96 Kirschner L. S. (1995) Amplification of the murine mdr2 gene and a reconsideration of the structure of the murine mdr gene locus. DNA Cell. Biol. **14:** 47–59
97 Stoffel W. and Bosio A. (1997) Myelin glycolipids and their functions. Curr. Opin. Neurobiol. **7:** 654–661

98 Simons M., Kramer E. M., Thiele C., Stoffel W. and Trotter J. (2000) Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. J. Cell Biol. **151:** 143–154
99 Qiu Y., Cavelier L., Chiu S., Yang X., Rubin E. and Cheng J. F. (2001) Human and mouse ABCA1 comparative sequencing and transgenesis studies revealing novel regulatory sequences. Genomics **73:** 66–76
100 Kaminski W. E., Piehler A. and Schmitz G. (2000) Genomic organization of the human cholesterol-responsive ABC transporter ABCA7: tandem linkage with the minor histocompatibility antigen HA-1 gene. Biochem. Biophys. Res. Commun. **278:** 782–789
101 Chang G. and Roth C. B. (2001) Structure of MsbA from *E. coli*: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. Science **293:** 1793–1800
102 Paulusma C. C., Bosma P. J., Zaman G. J., Bakker C. T., Otter M., Scheffer G. L. et al. (1996) Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. Science **271:** 1126–1128
103 Hofmann K. and Stoffel W. (1993) TMbase: a database of membrane spanning proteins segments. Biol. Chem. Hoppe-Seyler **374:** 166