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ABC A-subclass proteins: Gatekeepers of cellular phospho- and sphingolipid transport

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1. ABSTRACT

During the past years, available evidence suggests that members of a novel family of structurally highly related multispan proteins, designated ABC A-subclass transporters, exert critical functions in the control of cellular lipid transport processes. Loss-of-function scenarios, thus far, have revealed pivotal roles of individual ABC A-transporters in specialized lipid secretory pathways of the cell including HDL biogenesis (ABCA1), lung surfactant production (ABCA3), retinal integrity (ABCA4/ABCR) and skin lipid barrier formation (ABCA12). Although the specific transporter activities of many members of this novel protein family have not yet been established in detail, available evidence indicates that ABC A-subclass transporters function as key components of highly specialized cellular phospho- and sphingolipid export machineries in major physiologic systems.

2. INTRODUCTION

Multispan molecules of the class of ATP-binding cassette (ABC) transporters constitute an evolutionarily highly conserved superfamily of proteins. They are integral components of the molecular machinery of living cells ranging from prokaryotes to eukaryotes (1). The defining feature of all ABC transporters is that they use the energy of ATP hydrolysis to translocate specific substrates across cell membranes. A great diversity of compounds are transported by ABC molecules including lipids, amino acids, carbohydrates, vitamins, ions, glucuronide conjugates and xenobiotics, respectively (1, 2). In eukaryotes, ABC transporters are localized in the plasma membrane and in the membranes of cell organelles such as the endoplasmic reticulum, the Golgi, the endo-lysosomal compartment, peroxisomes and also mitochondria. During the past few years, a total of 48 transcriptionally active

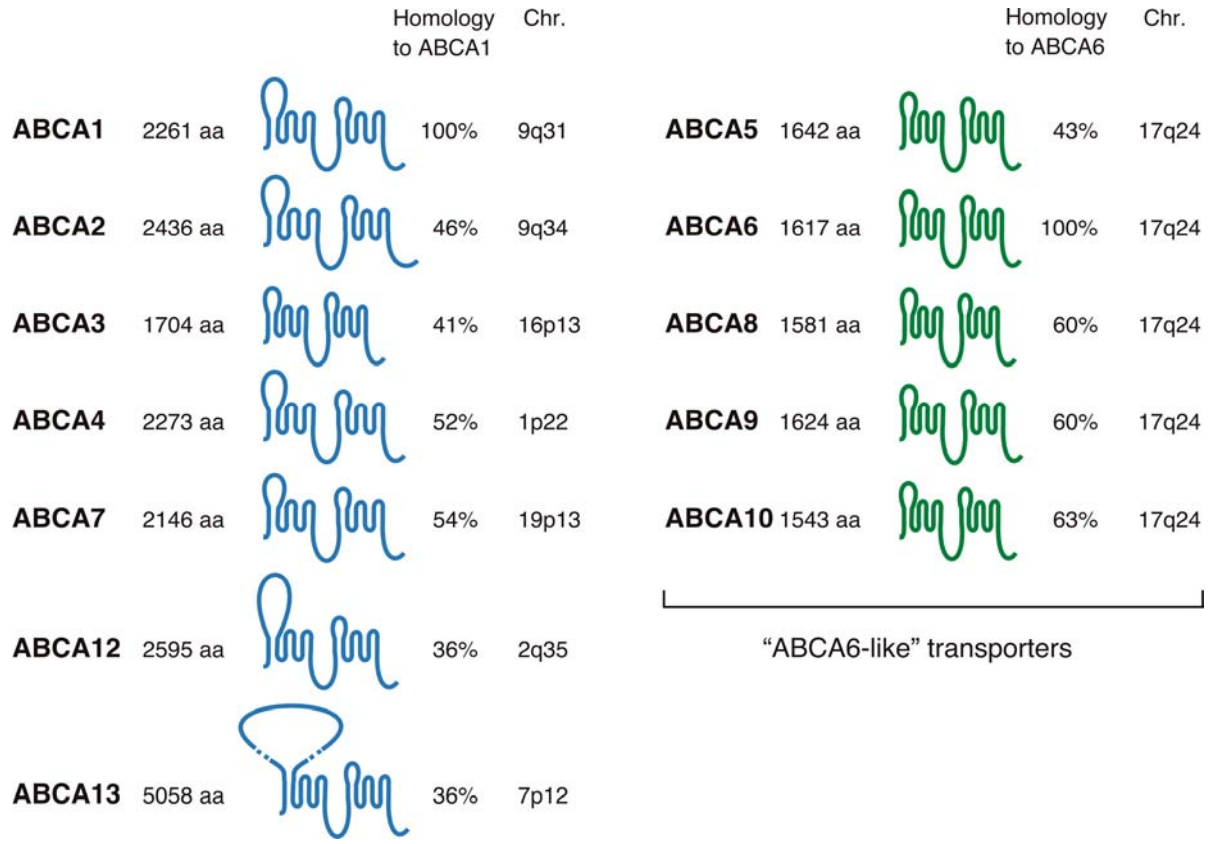

Figure 1. Structural features of ABC A-subclass transporters. Chromsomal localizations refer to the UCSC Genome browser (http://genome.ucsc.edu). Gene sizes were assessed on the basis of the respective complete cDNAs using the UCSC Genome browser. Note the absence of a functional "ABCA11" gene.

ABC transporter genes have been identified within the human genome that code for functional proteins. Based on their structural homologies, these have been divided into 7 subclasses referred to as ABC A-G (1, 3). Functional ABC transporters share a common molecular design. So-called "full-size" transporters are composed of two tandemly linked functional subunits (4), each consisting of a nucleotide binding fold (NBF) and a multispan transmembrane domain complex. Alternatively, they are made up of two half-size transporter components, which are encoded by one and the same or two distinct genes, respectively (5).

The fascinating gene family of ABC transporters has been the subject of extensive reviews contributed by outstanding experts in the field (for ref. see (1, 6, 7)). In this article, we focus on the biology of the A-subclass of ABC molecules and provide a current synopsis of the various implications of this emerging group of proteins in cellular lipid transport.

3. BIOLOGICAL FEATURES OF ABC A-SUBCLASS TRANSPORTERS

ABCA1 and ABCA2 are the defining members of the ABC A-subfamily. Both genes were co-identified by Luciani et al. (8) in the mouse (initially referred to as ABC1 and ABC2, respectively). Throughout the past decade 10 additional functional genes were discovered. Based on their common structural features they were categorized into a distinct subgroup, designated the ABC A-subfamily (1). Structurally, all ABC A-transporters are full-size transporters and range from 1543 - 5058 amino acids in size (Figure 1). With the exception of ABCA4, which appears to be expressed in significant quantities only in the retina, ABC A-transporters are generally expressed in a broad spectrum of human tissues (3). Like other full-size ABC transporters they are characterized by two NBF with conserved Walker A and B motifs, a signature sequence and two transmembrane domains, each typically composed of six membrane-spanning segments (5) (Figure 2). The evolutionarily highly conserved Walker A and B motifs bind ATP required for substrate transport, whereas the biological significance of the ABC signature, which is positioned between the Walker A and B sequences, is still unknown. Its strong conservation in evolution points to an important role in transporter function. Evidence from numerous mutation studies indicates that the specificity for the transported substrates is largely determined by the transmembrane domains and the cytoplasmic loops (9).

Phylogenetic analysis suggests that all ABC A-subclass genes have evolved from a primordial ancestor gene and were subsequently dispersed across the entire

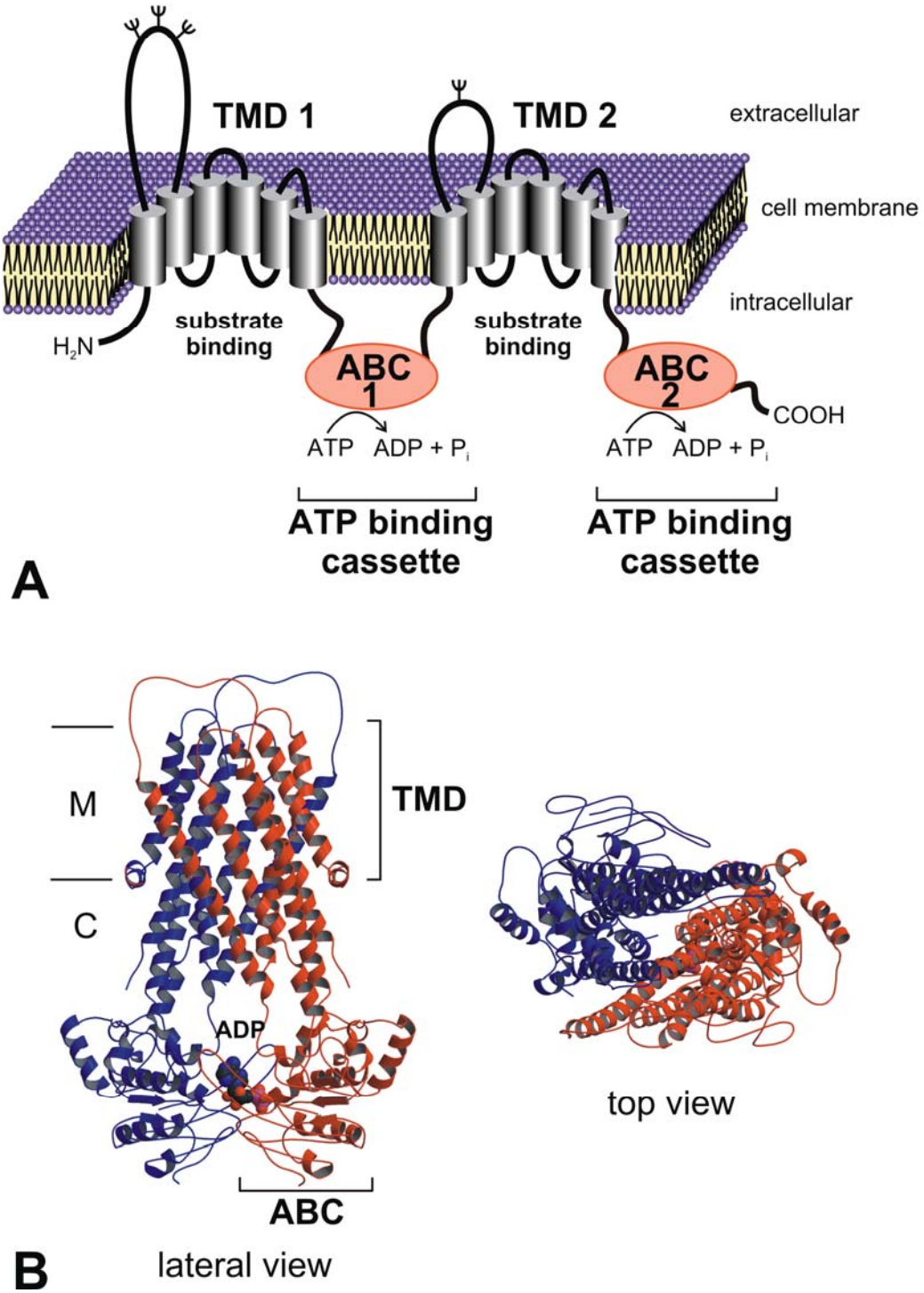

Figure 2. A. Molecular architecture of ABC A-subclass transporters representatively shown for ABCA1. Each of these large molecules is typically composed of two ATP binding cassettes (ABC1, ABC2) and two transmembrane domains (TMD1, TMD2) which consist of six membrane-spanning alpha-helices (gray shaded barrels). The ABC bind and hydrolyze ATP required for substrate translocation, whereas the transmembrane domains and the cytoplasmic loops are associated with substrate recognition. psi, putative N-linked glycosylation sites. B. Crystallographic structure model of the ABC Transporter MsbA with bound ADP. Both TMD are embedded in the cell membrane (M, lateral view), whereas the ABC are located on the cytosolic side (C) and provide the binding site for ATP/ADP (gray, red and blue). The respective models were generated with the *Molscript* and *Raster3D* software based on the published structure dataset 1z2r.pdb.

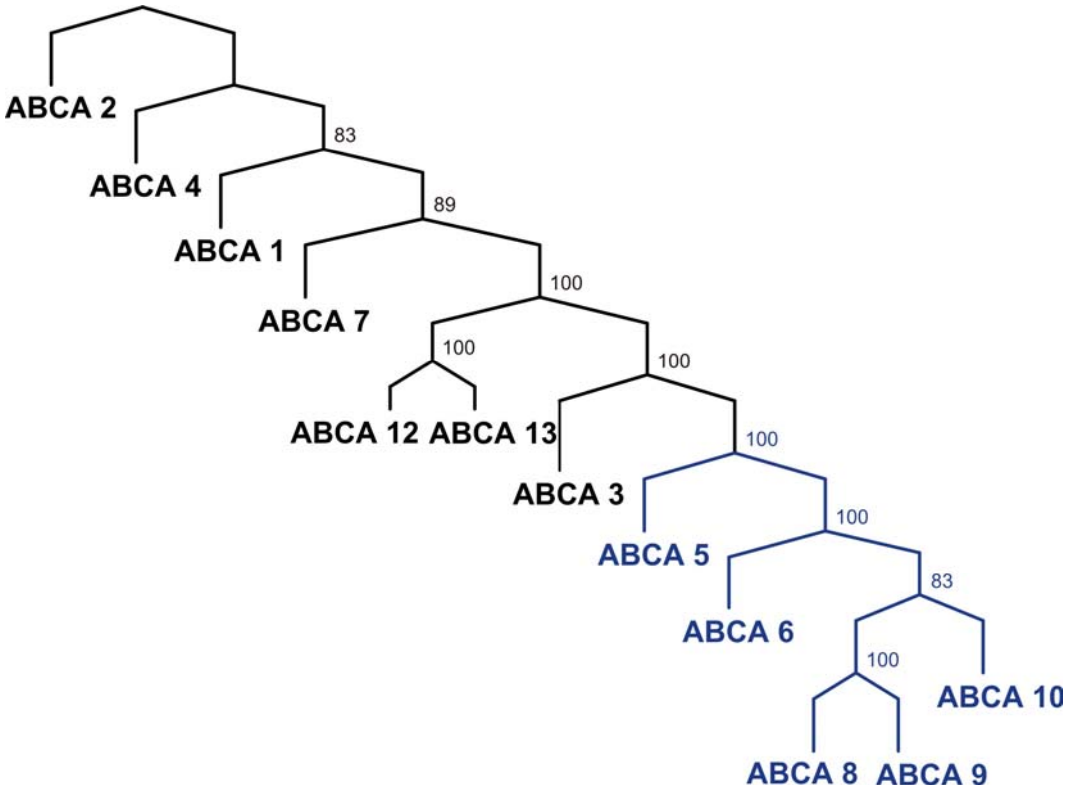

Figure 3. Phylogenetic consensus tree of human ABC A-transporter genes. Full length amino acid sequences were aligned using CLUSTALX and phylogenetic analysis was performed utilizing the PHYLIP software (http://evolution.genetics.washington.edu/phylip.html). Bootstrap values (%) out of 100 iterations are indicated at each branch point. The highly homologous subgroup of ABCA6-like transporters is shown in blue color.

genome (Figure 3). On the genomic level, five highly homologous ABC A-transporter genes (ABCA5, ABCA6, ABCA8, ABCA9, ABCA10; referred to as "ABCA6-like transporters") are unique in that they are clustered within a single locus on chromosome 17q (1). These striking features strongly suggest that they arose evolutionarily more recently from a common ancestral gene as a result of multiple gene duplication events (10, 11).

Valuable insights into the physiologic functions of ABC A-transporters as regulators of lipid export processes has come from human monogenetic diseases that have been causatively linked to members of the ABC A-subfamily (Table 1). These include familial HDL-deficiency (Tangier disease), caused by mutations in ABCA1, neonatal surfactant deficiency (ABCA3), several forms of retinal dystrophies (ABCA4) and two types of hereditary keratinization disorders. Currently available information suggests that the members of the ABC A-subfamily have specialized in the transmembrane transport of specific phospholipid and sphingolipid compounds throughout evolution in distinct physiologic systems. Bearing in mind that our knowledge of the function of ABC A-subclass transporters is still fairly incomplete, three groups of ABC A-transporters can currently be distinguished based on their known or postulated substrate specificities: (i) Transporters that mediate the translocation of glycero-phospholipid compounds (ABCA1, ABCA3, ABCA4, ABCA7), (ii) transporters that are implicated in the transport of ceramide backbone lipids (ABCA7, ABCA12), and (iii) transporters with unknown substrate specificity or function (ABCA6-like transporters, ABCA2, ABCA13). Known glycero-phospholipid substrates of ABC A-proteins include phosphatidylcholine (ABCA1, ABCA3), phosphatidylserine (ABCA7), and N-retinylidene phosphatidylethanolamine (ABCA4), respectively. Within the group of sphingolipids, ceramide (ABCA7, ABCA12) and sphingomyelin (ABCA3) have been associated with ABC A-transporter function. Another important facet of ABC A-transporter biology is the fact that individual members of this protein family appear to mediate the co-transport of free cholesterol along with their *bona fide* substrates through as yet unknown ATPase independent mechanisms.

4. ABCA1 AND HDL BIOGENESIS

ABCA1 is the prototypic member of the A-subclass of ABC proteins. It is expressed in a variety of human tissues with highest expression levels in placenta, liver, lung, adrenal glands and fetal organs (12). The observation that *ABCA1* expression is upregulated by cholesterol influx and suppressed by HDL_3 mediated cholesterol efflux in human macrophages was the initial clue pointing to the involvement of ABCA1 in cellular HDL metabolism (12). Subsequent studies showed that cholesterol-responsive regulation is a feature of most ABC

Table 1. Association of ABC A-transporters with monogenetic and complex diseases

Gene	OMIM entry	*Monogenetic disorder*	Complex disease
ABCA1	600046	*Familial HDL-deficiency* *Tangier disease*	Atherosclerosis Alzheimer's disease
ABCA2		*?*	Alzheimer's disease?
ABCA3	601615	*Neonatal surfactant deficiency*	Pediatric interstitial lung disease
ABCA4 (ABCR)	601691	*Stargardt disease 1 (Fundus flavimaculatus)* *Retinitis pigmentosa 19* *Cone rod dystrophy 3*	Age-related macular degeneration
ABCA7		*?*	Sjogren's syndrome?
ABCA12	607800	*Lamellar ichthyosis type 2* *Harlequin ichthyosis*	?

A-subfamily members. After the identification of ABCA1 by Luciani and colleagues in 1994 (8), only little was known about its molecular function for quite some time. The breakthrough came when we and others discovered that mutations in the human *ABCA1* gene cause familial high-density lipoprotein (HDL)-deficiency syndromes (Tangier disease, TD) which identified ABCA1 as a key regulator of HDL metabolism (13-15). TD is a rare autosomal recessive disorder of lipid metabolism characterized by almost complete absence of plasma HDL and the accumulation of cholesteryl esters in the cells of the reticulo-endothelial system leading to splenomegaly and enlargement of tonsils and lymph nodes (16). Consistent with the finding that mutations in *ABCA1* cause HDL deficiency in humans, we found that mice lacking functional ABCA1 exhibit plasma lipid alterations that are concordant with those in TD (17). Subsequent murine experiments demonstrated that selective overexpression of *Abca1* in the liver increases HDL plasma cholesterol levels (18, 19). In keeping with this, reverse experiments using conditional gene targeting revealed that deletion of hepatic *Abca1* in mice leads to a dramatic reduction in plasma HDL cholesterol (20). These elegant *in vivo* studies not only document that the liver is the predominant source of plasma HDL but also demonstrate the pivotal role of ABCA1 in hepatic HDL biogenesis. Beside these observations in the liver, recent evidence indicates that the intestine is also an important source of the circulating HDL pool and intestinal HDL formation critically depends on intact ABCA1 function (21).

To date, more than 70 mutations within the human *ABCA1* gene have been reported (for a current synopsis see The Human Gene Mutation Database, http://www.hgmd.cf.ac.uk). Analysis of the topological distribution of known mutations within the ABCA1 protein product reveals that a significant portion of the mutations within the *ABCA1* gene occurs in three clusters. Two of these mutation hot spots are localized at the N-terminal (codons 138-222) and C-terminal ends (codons 527-578), respectively, of the first (large) extracellular domain (Figure 2 A). A third one is present immediately downstream of the first transmembrane domain (codons 823-877). These observations highlight the critical role of the N-terminal large extracellular domain for ABCA1 function. Given the fact that TD is a very rare disease, it is uncertain to what extent allelic variants in the *ABCA1* gene affect HDL levels in the general population. A number of studies have reported polymorphisms in the coding and non-coding regions of ABCA1 that are associated with increased risk for cardiovascular disease (22-25). In particular, a recent study in a large Caucasian cohort suggests that at least 10% of individuals with low HDL cholesterol are heterozygous for mutations in *ABCA1* and that both mutations and allelic variants in this gene contribute to HDL levels in the general population (26). On the other hand, an Arg219Lys dimorphism in the N-terminal large extracellular domain appears to exert atheroprotective effects (24). Presently, however, it remains unclear whether single allelic variants in the *ABCA1* gene are indeed useful risk predictors in cardiovascular disease.

On the subcellular level, ABCA1 is localized in the plasma membrane and its surface expression is upregulated in macrophages by cholesterol loading (17). In the plasma membrane, ABCA1 is associated with a Lubrol-detergent resistant raft subfraction (27). ABCA1 is also present in the Golgi compartment and in lysosomes (17, 28, 29) which supports the view that it is a mobile molecule that may shuttle between the plasma membrane, the Golgi and the lysosomal compartment (Figure 4).

Early efforts to examine the biologic role of ABCA1 revealed that it acts as a translocator of phospholipids and cholesterol between the inner and outer plasma membrane (30). This was based on experiments showing an increase in cholesterol and phospholipid export under conditions of forced ABCA1 expression and in ABCA1 deficient cells which characteristically display the reverse scenario (17). Further, overexpression experiments also suggested that ABCA1 may actively mediate intracellular cholesterol transport (31). The detailed molecular mechanisms that lead to the translocation of phospholipids and free cholesterol at the plasma membrane are presently poorly understood. Current evidence supports the view that the extracellular lipid acceptor apoA-I binds to ABCA1 and phospholipids and the resulting phospholipid-apoA-I complex induces passive cholesterol efflux from the cell as a consequence of conformational changes in ABCA1. This notion is based on the finding that phospholipid efflux from vascular smooth muscle cells to apoA-I is less sensitive to vanadate inhibition than cholesterol efflux, and medium containing apoA-I can lead to cholesterol efflux from vascular endothelial cells that are deficient in ABCA1 (32). In experiments by others it was shown that pretreatment of ABCA1-expressing HEK293 cells with cyclodextrin reduces cholesterol efflux to apoA-I without reducing phospholipid efflux, and medium containing apoA-I induces cholesterol efflux from cells lacking ABCA1 (33). These studies together with the demonstration that ABCA1 mediates phosphatidylserine translocase activity (34) have led to the view that the

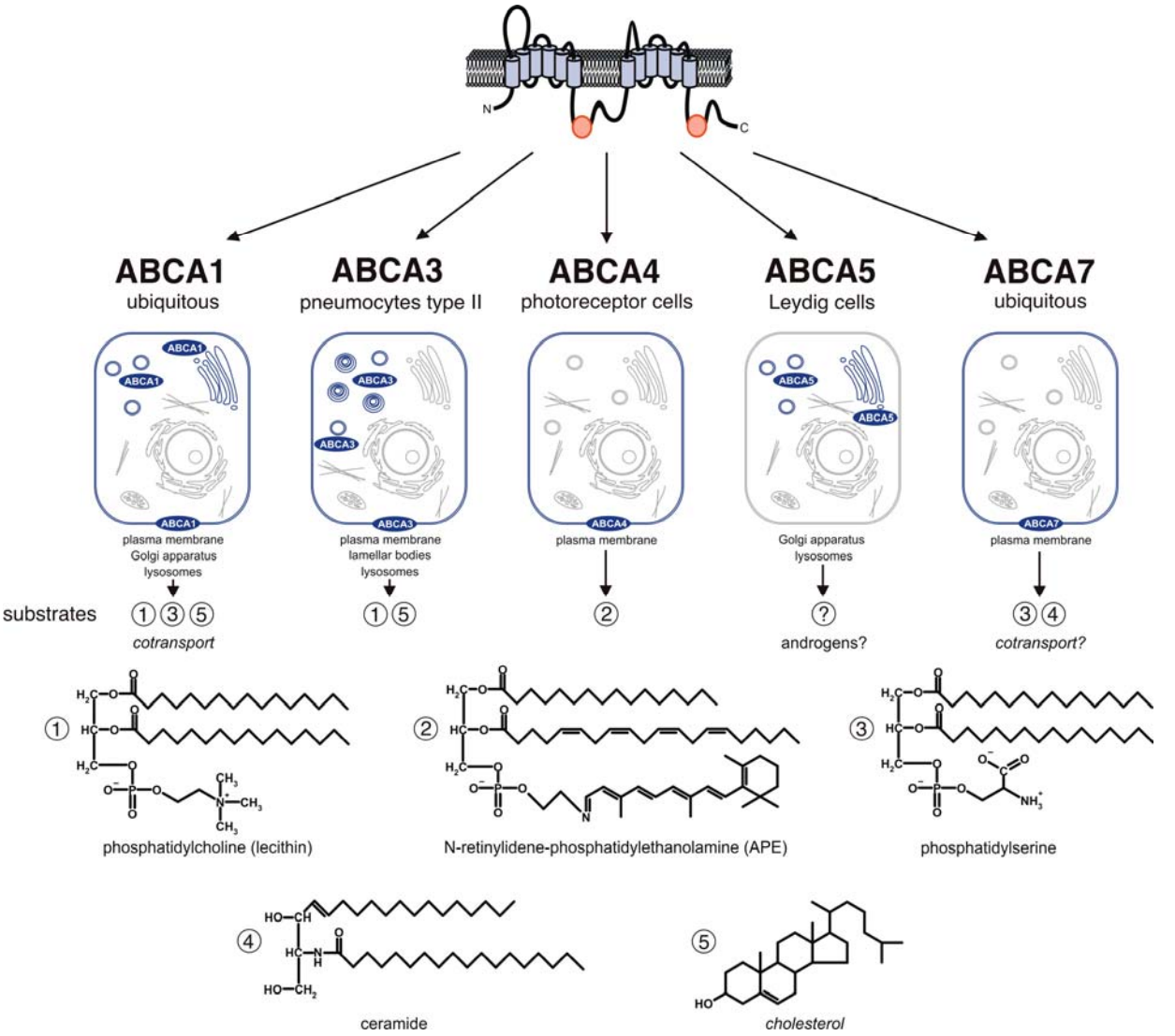

Figure 4. Tissue-specificity, subcellular localization and known or likely lipid substrates of human ABC A-subclass transporters. Encircled numbers refer to the lipid compounds detailed at the bottom.

ABCA1 dependent efflux of phospholipids and cholesterol occurs as a two-step process with the assembly of phospholipids onto acceptors being the primary activity of ABCA1, whereas cholesterol efflux represents a passive ABCA1 independent mechanism (32, 33). This two-stroke engine model has recently been challenged by the demonstration in the murine RAW264.7 macrophage cell line that ABCA1 can mediate concurrent phospholipid and cholesterol efflux to apolipoprotein A-I (35). More work is required to assess the validity of both models. In particular, the question as to whether free cholesterol can act as a direct substrate for ABCA1 still awaits clarification. Interestingly, recent experiments demonstrating highest ABCA1 ATPase activity in phosphatidylcholine containing liposomes suggest that this phospholipid may represent the preferential substrate of ABCA1 (36). It needs to be cautioned, however, whether and to which extent these findings, which are based on an artificial membrane environment, can be extrapolated to the physiological *in vivo* situation.

Efforts to elucidate the molecular function of ABCA1 also need to take into account that ABCA1, like other full-size ABC transporters, may exert its function as component of a membrane associated higher order functional complex. Our present knowledge on molecular interaction partners which potentially interfere with ABCA1 function is still rudimentary. For example, it has been shown that ABCA1 binds to the PDZ (*P*SD95-*D*iscs large-*Z*O1) domain protein beta-2-syntrophin which may couple ABCA1 to the F-actin cytoskeleton (37). Another syntrophin family member, alpha-1-syntrophin, also interacts with the C-terminal amino acids of ABCA1 and inhibits ABCA1 degradation (38). Finally, ABCA1 function appears to depend on binding to Fas-associated death domain protein (FADD), an adaptor molecule

involved in death receptor signal transduction suggesting a potential interlink between ABCA1 and apoptosis (39).

The observation that ABCA1 is upregulated in human macrophages during sustained uptake of cholesterol (12, 17) and that it mediates cholesterol export from the cell early on suggested that ABCA1 is implicated in the pathogenesis of atherosclerosis. Macrophages play a pivotal role in the initiation and progression of lesions of atherosclerosis (40). In the nascent lesion, they transform into foam cells through the excessive accumulation of cholesteryl esters. Dysfunctional cholesterol homeostasis in macrophages and foam cells results in the breakdown of membrane integrity, cell death and ultimately leads to extracellular deposition of cholesteryl esters. Among patients with defective ABCA1 one subgroup develops premature atherosclerosis, whereas another one presents predominantly with splenomegaly (16) indicating differences in macrophage targeting to tissues in ABCA1 deficiency. Experiments from our laboratory utilizing chimeric LDLR -/- mice lacking ABCA1 in their circulating cells (LDLR-/-(ABCA1-/-)) indicated that the selective disruption of ABCA1 in macrophages has a significant impact on macrophage targeting into the vascular wall and lesion formation *in vivo*. LDLR-/- chimeras deficient in ABCA1 develop significantly larger (60%) and more advanced atherosclerotic lesions compared to chimeric LDLR-/- mice with functional ABCA1 in their hematopoietic cells (41). Importantly, targeted disruption of leukocyte ABCA1 function had no effect on plasma HDL cholesterol levels. Consistent with the findings in the LDLR-/-(ABCA1-/-) knockout model, an inverse approach – overexpression of ABCA1 in macrophages – results in reduced lesion progression in LDL-/- mice (42). These complex studies clearly show that macrophage ABCA1 alone can exert significant anti-atherosclerotic activity independently of plasma HDL levels. They also indicate that the absence of ABCA1 from leukocytes is sufficient to induce aberrant monocyte recruitment into the spleen identifying ABCA1 as a critical leukocyte factor in the control of monocyte targeting into tissues (41). The concept that macrophage ABCA1 functions as a potent anti-atherosclerotic factor is also supported by a study in apoE knockout mice, another mouse model of atherosclerosis (43). Again, a reciprocal experimental approach demonstrated that forced expression of human ABCA1 in these mice leads to the formation of smaller, less advanced lesions of atherosclerosis (44). Collectively, available *in vivo* data thus strongly suggest a critical role of ABCA1 in macrophage lipid homeostasis and document the pronounced anti-atherosclerotic activity of this ABC transporter.

Beside its well established role in HDL metabolism and atherogenesis, a series of recent findings suggests the implication of ABCA1 in brain lipid homeostasis. Studies in *Abca1* null mice which demonstrated markedly reduced apoE levels in the cortex (80% reduction) and the CSF (98% reduction) of these mice have indicated that ABCA1 is critical for the maintenance of normal apoE levels and lipidation in the central nervous system (45, 46). ApoE is the most abundant CNS apolipoprotein and it represents the only well documented risk factor for late-onset Alzheimer disease (AD) (47). AD is the most common cause of senile dementia and currently affects ~40% of the population over 80 years of age (48). Characteristic neuropathological hallmarks of AD include intraneuronal fibrillary tangles composed of hyperphosphorylated tau protein and amyloid deposits that are composed largely of beta-amyloid peptides, apoE, lipids, and other proteins that accumulate in the neural parenchyma and the cerebrovasculature (49). These important findings raised the question as to whether an interlink exists between ABCA1 and beta-amyloid deposition in the CNS and whether ABCA1 is involved in the pathogenesis of AD. Indeed, three recent studies using various murine AD models strongly support this concept. In APP23 transgenic mice carrying the human familial Swedish AD mutant in their neurons, deletion of *Abca1* leads to increased parenchymal and vascular amyloid deposition in the brain (50). This effect was also observed in transgenic mice which co-express a combination of mutations in the human amyloid precursor protein and the human *presenilin 1* gene (51). In line with this, disruption of the Abca1 gene in PDAPP transgenic mice, another mouse model for AD, results in increased beta-amyloid deposition in the hippocampus. Interestingly, these mice have a dramatic decrease in the level of soluble brain apoE and poorly lipidated apoE deposits co-localize with amyloid plaques (52). Together these data reveal an amyloidogenic mechanism which involves the generation and deposition of poorly lipidated apoE in the absence of functional ABCA1 suggesting roles of ABCA1 in the pathogenesis of AD. It will be a major challenge to assess in detail the mechanisms by which ABCA1 and beta-amyloid metabolism are interlinked in the CNS. In particular, the tantalizing question whether enhanced ABCA1 activity has a beneficial effect on the pathogenesis and progression of AD deserves serious attention.

5. ABCA3 AND LUNG SURFACTANT PRODUCTION

ABCA3, the third member of the ABC A-subfamily, is a 1704 amino acid glycoprotein of 190 kDa size (Figure 1) (53-55). It shows highest expression in the lung but *ABCA3* mRNA is also detected in other tissues including brain, heart and pancreas. In the lung, *ABCA3* expression is restricted to alveolar type II pneumocytes (56). Intracellularly, it localizes to the vesicle membranes of lysosomes and so-called "lamellar bodies" (57) (Figure 4). In these latter cell organelles, the lipid and protein constituents of lung surfactant are assembled before they are secreted into the alveolar space (58). Pulmonary surfactant is a mixture of lipids, primarily phospholipids, and proteins that is essential for normal breathing. It forms a lipid-rich monolayer that lines the alveolar surface of the lung and lowers surface tension at the air-liquid interface thus preventing collapse of the alveoli on expiration.

Beside its presence in lamellar bodies several additional observations pointed to the potential involvement of ABCA3 in surfactant metabolism. First, *ABCA3* mRNA expression was found to be markedly

upregulated during lung development in the rat, and mRNA levels peaked shortly before birth synchronously with the transcriptional induction of the surfactant-associated proteins. Secondly, dexamethasone treatment of human fetal lung explants results in a dramatic increase in *ABCA3* gene expression (59). Third, the *ABCA3* promoter contains indeed a functional glucocorticoid-responsive element (GRE). This is based on the demonstration that transcriptional activity of ABCA3 mediated by the GRE containing region is significantly increased in response to dexamethasone in the human pneumocyte type II cell line A549, and the fact that the glucocorticoid receptor binds to the GRE (60).

Compelling *in vivo* evidence documenting the essential role of ABCA3 in surfactant metabolism has been provided by a recent study which demonstrated that mutations in this ABC transporter gene cause neonatal surfactant deficiency (61). What is the precise molecular function of ABCA3 in the cascade of events that lead to lung surfactant production? A potential clue comes from the observation that patients with defective ABCA3 have abnormal lamellar bodies in their lung tissue. The lamellar bodies in these individuals are small in size and densely packed. Mechanistically, it is thus likely that ABCA3 mediates the translocation of lipid compounds that are critical for the proper formation of lamellar bodies and the subsequent secretion of surfactant into alveoli. Because phospholipids, in particular phosphatidylcholine, constitute the major fraction of surfactant lipids, it has been proposed that ABCA3 functions as a regulator of lamellar body phospholipid transport (55, 56, 59, 61). Indeed, recent evidence showed that targeted suppression of *ABCA3* expression significantly decreases the uptake of phosphatidylcholine by lamellar bodies (57). These experiments also revealed that inactivation of *ABCA3* leads to a generally reduced import of the surfactant lipids phosphatidylcholine, cholesterol and sphingomyelin, respectively, into type II alveolar cells suggesting a potential function of ABCA3 in cellular lipid trafficking processes involving these lipid compounds. Because human ABC transporters are known to act predominantly as components of the cellular export machinery, it is unlikely, however, that these findings represent regulatory events that may be directly related to the *bona fide* function of ABCA3.

Notwithstanding these currently unresolved mechanistic complexities, data from loss- and gain-of-function studies strongly support the view that ABCA3 is a key player in the biogenesis of lamellar bodies. For example, silencing of *ABCA3* leads to the formation of abnormal lamellar bodies in fetal alveolar type II cells consistent with the histopathological abnormalities found in the lamellar bodies from individuals with defective ABCA3. On the other hand, ectopic expression of ABCA3 in human embryonic kidney cells (HEK-293) leads to the formation of lipid containing vesicular bodies reminiscent of lamellar bodies (55, 57). The concept that ABCA3 is a critical regulator of lamellar body metabolism raises the question as to the role of this ABC transporter in other tissues that are known to form lamellar bodies. Organs of interest include the brain, the heart, and the pancreas. In this context, *in vivo* experiments involving organ-specific gene targeting may help to address this important issue.

6. ABCA4 (ABCR) AND RETINAL INTEGRITY

ABCA4 (also known as ABCR) is a retinal-specific member of the family of ABC transporters. The protein is homologous to the bovine and *Xenopus* Rim proteins previously identified in the rim of the rod outer segment disks (62, 63). The mature ABCA4 polypeptide consists of 2273 amino acids and it is localized in the retina along the rims and incisures of both rod and cone photoreceptor outer segment disk membranes (62-65). *In vitro* reconstitution studies utilizing purified bovine ABCR suggest that retinoids, specifically retinal, are the substrate for ABCA4 (66). This is supported by ocular characterization of ABCR null mutant mice which display delayed dark adaptation and increased levels of all-*trans*-retinaldehyde and phosphatidylethanolamine in the outer segments following light exposure (67). Consistent with these findings, it is likely that ABCA4 acts as an outwardly directed flippase of the protonated complex of all-*trans*-retinal and phosphatidylethanolamine (*N*-retinylidindene-PE). This notion is in agreement with the recent observation that ABCA4 indeed binds the retinoid *N*-retinylidene-PE with high affinity (68). ABCA4 transporter activity may thus promote the recycling of *all-trans* retinal released from photobleached rhodopsin via the retinoid cycle. Consequently it has been suggested that such a mechanism may prevent from accumulation of toxic *all-trans* retinal derivatives in rods and cones, which results in apoptosis of the supporting retinal pigment epithelium cells and, ultimately, degeneration of the photoreceptors (66, 67, 69).

ABCA4 was the first ABC A-transporter that has been causatively linked to genetic disease (64, 65). Presently, several hundred sequence variations in the *ABCA4* gene have been documented (for a current synopsis see http://www.retina-international.org/sci-news/abcrmut.htm) and linked to four degenerative retinal diseases including Stargardt disease (STGD), cone-rod dystrophy type 3 (CRD3), retinitis pigmentosa type 19 (RP19) and age-related macular degeneration (AMD), respectively. STGD is an autosomal recessive macular dystrophy causing progressive impairment of central vision, which clinically manifests typically in childhood or young adulthood. Affected individuals display atrophic lesions in the macula, as well as characteristic yellowish flecks in the macular and perimacular region (70). Allikmets et al. provided the first report demonstrating that patients with STGD have mutations in their *ABCA4* gene (64). Subsequent extensive mutation analyses have confirmed that *ABCA4* is the gene underlying STGD and have initiated a characterization of the mutational spectrum (71-76).

ABCA4 mutations can cause a broad spectrum of retinal disease, with the clinical phenotype determined by the level of residual ABCA4 protein activity (residual activity model) (71, 73, 77, 78). For example, the

homozygous frameshift mutation 5917delG is associated with a relatively severe STGD phenotype and the truncating mutations Y362X and R1300X, respectively, are associated with milder clinical symptoms (74, 77). Although, no direct assay for the assessment of the transporter activity of ABCA4 is currently available, this view is supported by a series of indirect observations including human genetics studies that correlate age of onset and/or phenotypes conveyed by the selective combination of mutant *ABCA4* alleles (72, 79). Studies in *Abca4* knockouts, which demonstrate that these null mutant mice partially develop human retinal disease by manifesting the accumulation of lipofuscin, delayed dark adaptation and slow progression of photoreceptor degeneration also support an inverse correlation between ABCA4 activity and clinical phenotype (67, 69, 80, 81). Autosomal recessive retinitis pigmentosa 19 is the most severe retinal dystrophy caused by mutations in the *ABCA4* gene and is believed to be caused by complete loss of ABCA4 activity. Consistent with the residual activity model truncating or aberrant splicing mutations are predominantly found in families with RP19 (77, 78, 82).

AMD accounts for more than half of severe visual impairment in studied populations of European descent, with >1.5 million individuals affected in the USA (83). Initially, an association between AMD and heterozygous mutations in ABCA4 has indeed been established (65). This was supported by the observation that the frequency of two common ABCA4 variants, G1961E and D2177N, in 1200 patients with AMD is significantly higher (3.4%) than that of controls (0.95%) (84). However, other studies could not confirm these results (74, 85). More recent reports in both humans and mice suggest that ABCA4 is a dominant susceptibility locus for predisposition to AMD (80, 83). Bearing in mind the fact that 7% of individuals age >75 years are affected with late-stage AMD (86) and an estimated mutant *ABCA4*-heterozygote frequency of 2-3% in the general population (65), in detail characterization of the pathogenetic role of ABCA4 in AMD will be a major challenge for the future.

7. ABCA12 AND THE SKIN LIPID BARRIER

ABCA12 was recently discovered in human placenta (87). RT-PCR experiments revealed highest expression levels in skin, testis and fetal brain (87). The gene is composed of 53 exons and maps to chromosome 2q34-35. This very region harbors a locus for the keratinization disorder lamellar ichthyosis (88) and thus rendered ABCA12 a positional candidate for this hereditary skin disease. A recent study indeed provided evidence that mutations in the *ABCA12* gene cause lamellar ichthyosis type 2 (89). Lamellar ichthyosis type 2 is an autosomal recessive form of ichthyosis which is characterized by skin desquamation over the whole body with large, adherent, pigmented and dark scales. Two additional independent studies demonstrated that mutations in the *ABCA12* gene also cause “harlequin” ichthyosis, the most severe form of hereditary keratinization disorders (90, 91). These findings indicate that the nature and severity of mutations in the *ABCA12* gene can give rise to two clinical phenotypes which include a mild form manifesting as lamellar ichthyosis type 2 and a severe form presenting as harlequin ichthyosis.

During normal keratinocyte differentiation lipids accumulate in so-called lamellar granules (LG). They are subsequently extruded into the intercellular spaces where they undergo enzymatic processing to produce a lipid mixture consisting of ceramides, cholesterol and fatty acids (92). This lipid complex fills most of the intercellular space of the stratum corneum forming the water permeability barrier of the skin. Morphological studies revealed that ABCA12 is present in the upper epidermal layers of normal human skin and ultrastructural analyses demonstrated that it is exclusively present in LG of normal epidermal keratinocytes (91). LG are specialized lipid-rich organelles characteristically found in epidermal granular cells. ABCA12 containing LG were abundantly detected close to the cell membrane where they fused with the cell membrane to secrete their lipid content to the extracellular space of the stratum corneum. These observations clearly show expression of ABCA12 in keratinocytes during keratinization and they suggest its implication in the LG dependent lipid export pathway. Ultrastructurally, harlequin ichthyosis is characterized by abnormal or absent lamellar granules (93) indicating that the assembly of these lipid-rich organelles is perturbed in the absence of functional ABCA12. Importantly, cultured keratinocytes of harlequin ichthyosis patients exhibit a congested pattern of glucosylceramide, a major lipid component of LG, around the nuclei compared to healthy keratinocytes. Corrective *ABCA12* gene transfer into ABCA12 deficient keratinocytes increased the number of keratinocytes showing the diffuse glucoceramide staining present in healthy keratinocytes (91). These data together with the known colocalization of ABCA12 and LG strongly suggest that ABCA12 is implicated in intracellular translocation processes of sphingolipids that are essential for maintaining the integrity of lamellar granules. More work is required to identify the exact nature of the substrates that are translocated by ABCA12.

8. ABCA7 – A TRANSPORTER WITH ELUSIVE FUNCTION?

ABCA7 was originally discovered in human macrophages in which it is regulated during sustained cholesterol import and export (94). It exhibits striking protein sequence homology with ABCA1 (54%) and ABCR (49%), respectively (94, 95). ABCA7 mRNA is detectable in a variety of tissues with highest expression in peripheral leukocytes, platelets, thymus, spleen, and bone marrow (94, 96). ABCA7 expression has also been reported for keratinocytes (97). Because of its close structural relatedness with ABCA1 and its co-regulation with ABCA1 in response to cholesterol uptake and efflux in macrophages, ABCA7 has recently received considerable attention. However, the physiologic function of ABCA7 remains enigmatic. Overexpression experiments in human HeLa and HEK293 cells suggest that ABCA7 is implicated in the control of the export of ceramide, phosphatidylserine and choline backbone phospholipids, respectively, but not

of cholesterol (97, 98). This contrasts with a study demonstrating increased efflux of *both* cholesterol and phospholipids in ABCA7 overexpressing HEK293 cells (99). Current data on the subcellular localization of ABCA7 are equally inconclusive. Initial experiments in ABCA7 overexpressing HEK293 cells indicated its presence in the plasma membrane (98), whereas the same investigators found that in murine peritoneal macrophages ABCA7 is predominantly localized intracellularly but not at the plasma membrane (100). The situation is complicated by *in vivo* studies in *Abca7* null mice whose macrophages were found to have normal cholesterol and phospholipid efflux rates (101) precluding an indispensable function of Abca7 in the cholesterol/phospholipid export in the mouse. Unexpectedly, the *Abca7* knockout approach revealed a gender dependent reduction in visceral fat and serum cholesterol levels. At this point, it remains to be established whether these surprising findings represent a species-specific physiological feature of mice or whether they indeed reflect as yet unsuspected roles of ABCA7 in human lipid metabolism.

Another clue as to the molecular function of ABCA7 may come from its genomic microenvironment. We found that the *ABCA7* gene is intimately physically linked to the gene for the minor histocompatibility antigen HA-1 on chromosome 19p13.3. Both genes are arranged in a head-to-tail orientation with only 1.7 kb distance between the terminal exon of *ABCA7* and the first exon of the *HA-1* gene (102). This raises the intriguing possibility that due to common genomic structures functional and regulatory interdependencies exist between both genes. HA-1 is a cytosolic protein of 1165 amino acid size (103) and currently unknown function. A His168Arg dimorphism in the HA-1 amino acid sequence has been identified as the immunologic target for HA-1 specific cytotoxic T cells (104). Available information suggests that the ABCA7/HA-1 gene complex may be critically involved in autoimmune disease. This view is supported by the observation that both genes are expressed in macrophages and lymphocytes which represent the major effector cells in chronic inflammation. Moreover, computational proteomics revealed that a segment of app. 150 amino acids length within the first extracellular domain of ABCA7 is recognized by antisera from patients with Sjogren's syndrome ("Sjogren epitope") suggesting that ABCA7 is associated with this autoimmune disease. A recent study from our laboratory dismissed an association between allelic variants of the *ABCA7* gene and Sjogren's syndrome. However, we found that the 168His variant of the minor histocompatibility antigen HA-1 is associated with reduced risk of primary Sjogren's syndrome (105). It needs to be pointed out, however, that this finding on the genomic level does not categorically exclude the potential involvement of ABCA7 in Sjogren's syndrome. This notion is supported by a recent study reporting that ABCA7 is expressed in salivary glands of patients with Sjogren's syndrome (106). Efforts currently underway will help to dissect the role of the *ABCA7/HA-1* gene complex in Sjogren's syndrome.

9. ABCA2 - REQUIRED FOR NEURONAL INTEGRITY?

ABCA2 is a 2436 amino acid polypeptide with a molecular weight of ~270 kDa (Figure 1) (107, 108). Genomic analysis identified a total of 48 exons extending across a genomic region of only 21 kb on chromosome 9q34 (107). Recently, an additional 5' exon was identified encoding an alternative unique N-terminus with highest expression in lysosomal compartments of peripheral blood leukocytes (109). Expression profiling showed highest levels of ABCA2 mRNA levels in the brain, however, expression was also detected in a broad range of tissues including monocytes, macrophages, kidney, liver, thymus, heart, ovary, lung and various tumor cells (8, 107-111). Using a semiquantitative RT-PCR approach we demonstrated sterol sensitive regulation of the *ABCA2* gene in human macrophages suggesting a role for this transporter in monocyte/macrophage lipid metabolism (107). Analysis of the putative *ABCA2* promoter region revealed several potential binding sites for transcription factors with functions in myeloid and neural cell differentiation and activation (112). Luciferase reporter gene experiments showed the importance of two GC-rich regions and overlapping binding sites for the EGR-1 and Sp1 transcription factors in the control of *ABCA2* gene expression (113).

Although the *ABCA2* gene has been co-identified with ABCA1 more than a decade ago, its function is still ill-defined. Overexpression of a GFP-tagged ABCA2 chimeric protein revealed that ABCA2 localizes to the endolysosomal compartment and the Golgi apparatus (108). This is consistent with reports demonstrating the presence of ABCA2 in endo-lysosomal vesicles of rat oligodendrocytes and ABCA2 transfected HeLa cells (111, 114). Together, the high expression levels in the brain, the localization in oligodendrocytes and sterol sensitive regulation strongly suggest functions for ABCA2 in neural transmembrane lipid transport. Consistent with this, overexpression experiments demonstrate that ABCA2 expression levels influence the expression of a number of genes linked to Alzheimer's disease (AD) including amyloid beta precursor protein (chromosome 21q21.3) whose pathogenetic role in AD is well documented (115). Other authors noted an association between early onset AD and the synonymous SNP rs908832 in exon 14 of the *ABCA2* coding region (116). More extensive studies are required to establish whether such an association indeed exists.

10. THE ABCA6-LIKE GROUP OF TRANSPORTERS (ABCA5, ABCA10, ABCA6, ABCA9, ABCA8)

Five highly homologous members of the human ABC A-subfamily form a compact gene cluster on chromosome 17q24.2-3 which is composed of the genes for *ABCA5*, *ABCA10*, *ABCA6*, *ABCA9* and *ABCA8* (in that order). *ABCA8* was the first ABCA6-like transporter that was discovered in the human genome (103). Subsequently, *ABCA6*, *ABCA9*, *ABCA5* and *ABCA10*, respectively, were identified in our laboratory and by others during the past few years (10, 117-119). Because the human *ABCA6* gene was the first to be characterized in completeness (117), the members of the ABC A-transporter pentacluster have been referred to as "ABCA6-like transporters" (10). The genomic clustering of the ABCA6-like transporters is

unique among all human ABC transporters. Based on the common chromosomal location of the ABCA6-like transporters and their strikingly high overall peptide sequence identities (42 - 72%), it has been proposed that they have arisen by multiple duplications of a primordial gene (1, 10). Phylogenetic analysis suggests that *ABCA5* is the oldest gene and thus likely represents the ancestral ABCA6-like transporter gene from which the others have evolved. With the exception of ABCA10, all ABCA6-like transporters are found in the rodent genome suggesting that the initial duplication events have taken place before the divergence of the rodent and primate lineages.

The defining member ABCA6, a polypeptide of 1617 amino acids, bears the structural features of full-size transporters, however, like all other ABCA6-like transporters, it is significantly smaller than ABC A-transporters outside this subgroup (Figure 1). ABCA6-like transporters generally show a broad tissue-specific distribution with individual quantitative expression profiles.

The biological significance of the group of ABCA6-like transporters remains to be defined. Our observation that the genes for *ABCA6*, *ABCA9* and *ABCA10* are regulated by cholesterol in human macrophages points to their potential involvement in lipid transport processes in these cells. Of note, ABCA6-like transporters display regulatory responses that are consistently reciprocal to those of ABCA1, ABCA2, and ABCA7, respectively (10, 117, 119) raising the possibility that in macrophages both transporter groups may serve functions in antagonistic pathways. Recent knockout experiments demonstrate that ABCA5 is localized in the endolysosomal compartment and Abca5 deficient mice develop dilatative cardiomyopathy leading to fatal cardiac failure (120). *Abca5* null mice also exhibit exophthalmos and an abnormal thyroid gland. The abnormalities in cardiomyocytes and follicular cells indicate that Abca5 serves critical functions in the endolysosomal system. Another facet of Abca5 biology may be the male reproductive system. Experiments in the rat show strikingly high expression of Abca5 mRNA in interstitial Leydig cells of the testis. Considering this, it is tempting to speculate that Abca5 may be implicated in transport processes involving male sex hormones and their precursors (121). Consequently, this raises the question as to whether ABCA5 is a regulator of cellular steroid transport.

11. ABCA13

ABCA13 stands out among all human ABC A-transporters in that it is by far the largest ABC transporter among all human ABC proteins with a molecular mass of app. 576 kDa (122). The human *ABCA13* gene is located on chromosome 7p12.3. The full-length cDNA, which was cloned from lung, contains an open reading frame of 15195 bp. Five additional alternatively spliced mRNA variants have been identified which code for truncated isoforms of the canonical ABCA13 protein (122). The structural hallmark of the ABCA13 polypeptide is the large N-terminal extracellular loop which consists of more than 3500 aa. This hydrophilic domain is conserved in the mouse, but no significant homology to any other protein domain could be documented so far. ABCA13 displays highest amino acid sequence identity with ABCA12 (38%), ABCA1 (36%) and ABCA7 (36%), respectively. In normal tissues, highest mRNA expression of the ABCA13 full-length mRNA was found in human trachea, testis and bone marrow. Expression profiling in tumor cell lines showed highest mRNA levels in leukemia, brain and prostate tumor cell lines, respectively (122). Presently, the molecular function of ABCA13 awaits elucidation. *ABCA13* shares a common genomic region on chromosome 7p12.3 with the locus linked to the T-cell tumor invasion and metastasis (122) and several potential binding sites for transcription factors associated with hematopoiesis of the myeloid and lymphoid lineages have been identified in the murine *Abca13* promoter (123). It is thus possible that ABCA13 is implicated in lipid translocation events during white blood cell formation or inflammation.

12. PERSPECTIVE

Although discovered only recently, major advances in our understanding of the biological significance of ABC A-transporters have been made during the past few years. Our current knowledge supports the concept that the members of this protein subfamily serve key functions in a diversity of physiological systems. This feature sets them apart from other ABC subfamilies such as the multidrug resistance transporters of the ABC-B and ABC-C subfamilies, respectively, whose members appear to exert their functions in similar physiologic contexts. Recent experimental data allow us to begin to understand what may be common functional features of ABC A-transporters beyond their structural relatedness. One of the emerging characteristics is that they appear to act as translocators of phospholipids and sphingolipids in specialized cellular secretory pathways. The thus far identified specific phospholipids and sphingolipids export machineries which are controlled by ABC A-transporters include the liver-intestine-macrophage HDL biosynthesis machinery (ABCA1), the pneumocyte II surfactant production system (ABCA3), the cone-rod photoreceptor complex (ABCA4) and the keratinocyte lipid export system (ABCA12). Given this, it can be expected that the remaining eight ABC A-subclass members may also play critical roles in major physiologic systems. It will be a challenging task to assess their implications in physiology and disease.

Another still unresolved issue is the question as to how ABC A-subclass transporters mediate the translocation of their lipid substrates on the molecular level. This task is complicated by an increasing body of evidence suggesting that ABC transporters do not act alone but rather function as components of molecular multi-unit complexes. As exemplified for ABCA1, such a transporter complex that controls the cellular export of phospholipids and cholesterol may include alpha-1-syntrophin, beta-2-syntrophin and FADD (37-39). To understand the workings of a defined ABC A-transporter it will thus be pivotal to elucidate any possible physical interaction with other proteins. Given the generally broad range of tissues that

express ABC A-transporters, it is also likely that tissue-specific interaction partners may define a much larger spectrum of biologic activities for these large transmembrane molecules than suggested by global loss- or gain-of-function approaches. Conditional gene targeting will be a most powerful tool to uncover thus far "cryptic" tissue-specific roles of ABC A-transporters. The usefulness of such approaches is convincingly evidenced by recent studies which revealed the importance of macrophage ABCA1 in atherogenesis and the critical involvement of ABCA1 in intestinal HDL biosynthesis (21, 41).

Although available evidence strongly supports roles of ABC A-subclass transporters in lipid export pathways only, their implication in cellular lipid transport may be more complex. This is suggested by the demonstration that both ABCA1 and ABCA5 are localized, among others, in the endolysosomal compartment raising the possibility that individual ABC A-transporters may be implicated in endocytic lipid transport processes. Consistent with such a scenario, it has been shown that the expression of most ABCA6-like transporters is inversely regulated during cholesterol import into macrophages to that of ABCA1 (10, 117, 119). It thus cannot be ruled out that individual members of the ABCA A-subclass may in part exert antagonistic functions in cellular lipid transport pathways.

The emerging concept that ABC A-transporters are pivotal regulators of cellular lipid transport in major physiological systems with pleiotropic roles in human disease has broad implications for clinical medicine. In this context, it needs to be emphasized that a steadily growing body of evidence suggests that ABC A-transporters play important roles in the pathogenesis of complex disorders. Given the fact that these include diseases with exceedingly high incidence rates in Western populations such as atherosclerosis, Alzheimer disease and age-related macular disease, it will be a most rewarding challenge for the future to characterize in detail the workings of this fascinating group of proteins in cellular lipid traffic.

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