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Sterols and sphingolipids: Dynamic duo or partners in crime?

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Abstract

One manner in which eukaryotic cells respond to their environments is by optimizing the composition and proportions of sterols and sphingolipids in membranes. The physical association of the planar ring of sterols with the acyl chains of phospholipids, particularly sphingolipids, produces membrane micro-heterogeneity that is exploited to coordinate several crucial pathways. We hypothesize that these lipid molecules play an integrated role in human disease; when one of the partners is mis-regulated, pathology frequently ensues. Sterols and sphingolipid levels are not coordinated by the action of a single master regulator, however the cross talk between their metabolic pathways is considerable. We describe our perspectives on the key components of synthesis, catabolism and transport of these lipid partners with an emphasis on evolutionarily conserved reactions that produce disease states when defective.

Keywords

yeast; ergosterol; cholesterol; ceramide; complex sphingolipids; membrane rafts

1. Introduction

Sterols and sphingolipids are evolutionarily conserved lipid molecules that collaborate to maintain the integrity of eukaryotic cellular membranes. These lipid complexes are asymmetrically distributed throughout the membrane, a phenomenon often explained by an “umbrella model”, which posits that cholesterol segregates into regions of membranes with strongly hydrated large head groups, like those found in sphingolipids. In this way, the sterol rings are effectively shielded from the aqueous environment. Moreover, the packing of cholesterol with saturated acyl chains of sphingolipids is entropically more favorable than with unsaturated acyl chains [1]. The coalescence of these lipids into membrane microdomains (rafts), likely functions to enhance the physical structure of the membrane as well as generate foci that integrate pathways such as lipid influx and efflux, protein trafficking, signal transduction, and even viral entry (reviewed, [2]).

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Despite the integral role ascribed to rafts in various biological processes, their existence is controversial (reviewed, [3,4]). This is partly because the majority of membrane microdomains are too small to resolve via conventional microscopy and have poorly defined morphology, unlike lipid bound organelles or even caveolae. As such the burden of proof for their existence has relied on the biochemical attributes of these complexes. The most accepted working definition of rafts is insolubility in cold non-ionic detergents, followed by flotation on sucrose-density gradients. Even this definition has provoked controversy as treatment of membranes with detergents can create artificial domains. However, it is widely accepted that the presence of both cholesterol and sphingomyelin in the plasma membrane is essential; removal of raft cholesterol with β -methylcyclodextrin or hydrolysis of membrane sphingolipids with sphingomyelinase, leads to dissociation and inactivation of most lipid micro-domain proteins [5,6].

Cholesterol and sphingolipids and the complexes they form are clearly beneficial to the cell. They represent a “dynamic duo” that withstands and responds to the environmental challenges that cells encounter. However they are also “partners in crime” in that an imbalance in either lipid invariably results in a disease pathology. In addition to monogenic disorders such as Niemann-Pick diseases, it is likely that the coordinated homeostasis of these lipids underlies many polygenic disorders such as atherosclerosis and neurodegeneration. The premise of this review is that the co-affinity of these molecules is essential for membrane function and is subject to multiple layers of regulation. We present our perspectives on sterol and sphingolipid homeostasis with particular emphasis on processes that partner these molecules.

2. Sterol and sphingolipid synthesis

The asymmetrical allocation of sterols to the inner and outer layer of cellular membranes arises from the biochemical affinity of sterol for ceramides and its derivatives. This affinity may arise as early as the ER, the point of synthesis of sterols and ceramides. Accordingly, much of the ensuing membrane micro-heterogeneity reflects the coordinated synthesis of these molecules. As described below (Fig. 1), these pathways are not controlled by the action of a single master regulator. Instead, it is apparently the cross talk between the pathways that facilitates their coordination.

2.1. Sterol biosynthesis and its regulation

Although cholesterol biosynthesis occurs in all mammalian cells, this capacity is greatest in liver, adrenal cortex, and reproductive tissues. The synthesis of cholesterol is a multi-step process (Fig. 1) involving nearly 30 enzymes. The rate-limiting step of this pathway HMG-CoA reductase catalyses the formation of mevalonate and is also the target of cholesterol lowering pharmaceuticals such as the statins. Mevalonate is then subjected to a series of phosphorylations and a decarboxylation reaction to produce isopentenyl pyrophosphate (IPP), an activated isoprenoid molecule. IPP is subsequently converted to squalene, which undergoes cyclization to produce lanosterol. Ultimately, lanosterol is converted to cholesterol through 19 additional reactions.

Cholesterol metabolism is transcriptionally regulated by sterol regulatory element binding proteins (SREBPs), a group of master regulators that control the feedback regulation of synthesis and uptake of many lipids, at many steps [7]. Cleavage of the membrane associated SREBP protein by two proteases in the Golgi complex releases a soluble amino-terminal transcription factor domain, which is then translocated to the nucleus to activate transcription of target genes [8]. Numerous accessory proteins (SREBP cleavage activating protein SCAP and INSIGs 1 or 2) and proteases (S1p and S2p) mediate the sensing of membrane composition and fluidity and the subsequent translocation and activation of the transcription factor (reviewed [7]). This process is repressed when cellular sterol concentrations peak; recent

studies elegantly describe the manner in which ER cholesterol concentrations greater than 5 mol % are sufficient to block ER exit of SREBP and the cholesterol sensor, SCAP [9]. Further fine-tuning of cholesterol biosynthesis via post-translational regulation of HMG-CoA reductase is achieved through INSIG1 dependent proteasomal degradation, which also responds to cholesterol levels in the ER [10,11].

2.2 De-novo sphingolipid biosynthesis and its regulation

Sphingolipids consist of a signature sphingoid long-chain base backbone modified by the addition of a fatty acid molecule (at C-2 to produce ceramide) and the subsequent addition of a hydrophilic head group to the OH group at C-1 to yield a variety of complex sphingolipids (e.g. glycosphingolipids, sphingomyelin, inositol-P-ceramide etc.). These reactions span two organelles and involve at least 11 enzymes (Fig. 1). Eight are housed in the ER and 3 in the Golgi, with active sites facing the cytoplasm or the lumen. The various biosynthetic intermediates must therefore be shuttled within, across and between the bilayer of several different organelles.

The first and rate-limiting step of the sphingolipid biosynthetic pathway is conserved from yeast through humans (Fig. 1) to produce 3-ketosphinganine (3-KS) by condensation of serine and palmitoyl CoA at the cytosolic surface of the ER. The reaction is catalyzed by serine palmitoyl transferase (SPT1, in mammals), comprising at least 3 subunits, SPTLC1, SPTLC2, and SPTLC3 [12,13]. SPTLC3 exhibits 68% sequence similarity to SPTLC2 and has similar enzymatic activity, but a different tissue expression pattern. In most tissues the SPTLC2:SPTLC1 mRNA expression ratio remains relatively constant, whereas the ratio of SPTLC3:SPTLC1 varies from 2:1 in colon to 1500:1 in placenta. In *Saccharomyces cerevisiae* (budding yeast), an accessory protein encoded by the *TSC3* gene likely acts as a regulatory component of the two-component SPT complex encoded by the *LCB1* and *LCB2* genes [14]. Whether the varied composition of the SPT complex in mammalian cells has regulatory consequences remains to be determined but is an intriguing possibility [15].

Reduction of 3-KS yields dihydrosphingosine, which is subsequently acylated to form dihydroceramide. Dihydroceramide is then desaturated to produce ceramide by a family of enzymes encoded by the longevity assurance genes (*Lass*), first identified in budding yeast [16]. In mammals, the *Lass* isoforms have unique tissue distributions that reflect the incorporation of a particular acyl CoA into dihydroceramide [15]. The production of ceramide is another key point in the pathway; ceramide serves as a precursor for complex sphingolipids and is itself a signaling molecule in various cellular processes, including the induction of apoptosis. Ceramide is subsequently transported to the Golgi where it meets with varying fates, as different head groups are added to produce different classes of complex sphingolipids [17].

Unlike cholesterol biosynthesis, regulation of *de novo* sphingolipid biosynthesis for the most part remains enigmatic; it does not appear to require a single class of master transcriptional regulators, or respond solely to cellular sphingolipid concentrations. Surprisingly, it has been shown that an increase of one or more sphingolipid does not reflect an increase in enzyme levels. This finding has been further corroborated by microarray analysis across a wide variety of conditions and cells, where transcriptional regulation was absent for key enzymes in the sphingolipid metabolic pathway [14]. The weight of evidence indicates substrate availability (*i.e.* serine and fatty acids) as a major determinant of flux through the sphingolipid pathway. SPT is sensitive to fluctuations of intracellular concentrations of these molecules [18]. A recent study in yeast showed that heat stress results in serine uptake from the media and transport to the ER, where it is utilized for *de novo* synthesis of ceramide. Sphingoid bases generated in this pathway mediate specific responses to heat stress including regulation of nutrient permeases, cytoskeletal changes, cell cycle arrest, and RNA translation [19]. Interestingly,

depleting the medium of serine or inhibiting fatty acid synthesis prohibited *de novo* sphingolipid synthesis in response to heat shock [14].

2.3. Co-regulation of sterol and sphingolipid anabolic pathways

The metabolism of these lipid molecules is clearly co-regulated, as demonstrated by the striking impact of sphingolipid storage disorders on cholesterol synthesis. This coordination arises indirectly via the SREBP master regulator [20]. In sphingolipid storage disorders, excess sphingolipids in the lysosome sequester cholesterol away from the ER. Consequently, SREBP is activated, thus commencing a futile cycle of uptake and entrapment. Similarly, a decrease in cellular sphingomyelin via the administration of myriocin, results in a reduction in SREBP that, in turn, correlates with decreased plasma cholesterol and triglyceride [21]. By contrast, ceramide, unlike sphingomyelin has an inverse relationship with SREBP, in that elevated intracellular ceramide concentrations correlate with decreased SREBP activity, independent of cellular cholesterol status [21]. Ceramide also down-regulates cholesterol synthesis by promoting the phosphorylation and therefore inactivation of HMG CoA reductase.

In insects, the primary regulators of SREBP activity are derivatives of palmitate and the SPT pathway, particularly phosphatidylethanolamine [22]. *Drosophila* cells with mutations in SPT fail to elevate mSREBP or SRE-mediated gene transcription in response to sterol depletion; this is overcome if cells are supplemented with intermediates of sphingolipid synthesis. Similarly, inhibition of SPT or ceramide synthase in wild type insect cells blocks SRE mediated gene transcription independent of sterol levels. In a corollary experiment, cells provided with precursors, or intermediates of sphingolipid synthesis exhibited an increase in SREBP activity and SRE mediated gene transcription [23]. This correlation appears to be conserved; in budding yeast, sterol biosynthesis and sphingolipid hydroxylation are closely coordinated, although the mechanism is ambiguous [24].

3. Sterol and sphingolipid catabolism

Numerous, precisely regulated pathways have evolved to modify cholesterol and sphingolipids and thus protect cells from the aberrant accumulation of either molecule. In situations where these detoxification events go awry, for example in lysosomal storage disorders, the outcome is frequently lethal [25].

3.1. Esterification of sterols

The esterification and storage of free cholesterol as cytosolic lipid droplets represents a critical lipid detoxification reaction. Two distinct isoforms of acyl-coenzyme A:cholesterol acyl-transferases (ACATs) catalyze the formation of cholesteryl ester (CE) from a long chain fatty acyl-CoA and free cholesterol. ACAT1 [26] and ACAT2 [27-29] are located primarily in the ER with a small portion of ACAT1 observed near the trans-Golgi network and the endocytosis recycling compartment [30]. In humans, ACAT1 is expressed in most tissues while ACAT2 is mainly expressed in intestine [31,32]. The tissue distributions of murine ACATs are similar to the human enzymes except that ACAT2 is the major isoform in mouse liver [33]. Substrate availability (*i.e.* sterol) allosterically regulates the reaction, thus linking membrane status with detoxification. When esterification is awry, ER safeguards such as the unfolded protein response are initiated, although ultimately the cell succumbs to apoptosis [34].

3.2. Hydroxylation of cholesterol and synthesis of bile acids

Bile acid biosynthesis represents a key catabolic and non-reversible departure point of sterols from the animal cell. Approximately 50% of *de novo* synthesized cholesterol is converted to bile acids daily in the adult liver. Although approximately 20-30 grams of the bile acids

synthesized are secreted into the intestine, 90% of the excreted bile acids are reabsorbed via active transport in the ileum and recycled back to the liver.

The synthesis of bile acids occurs primarily via a classical (neutral), liver specific pathway accounting for ~75% of the bile acid pool [35]. When the liver is diseased, an alternative (acidic) pathway may predominate, whereby side-chain oxidation of cholesterol in extra-hepatic locations precedes steroid ring modification, yielding acidic intermediate metabolites. The two pathways are initiated by the formation of 7 α -hydroxycholesterol or 27 α -hydroxycholesterol respectively; the former catalyzed by cholesterol 7 α -hydroxylase (encoded by the CYP7A1 gene) and the latter by 27 α -hydroxylase (encoded by CYP27A1). 7 α -hydroxycholesterol and 27 α -hydroxycholesterol undergo further modification (including alteration of the sterol ring, oxidation, shortening of the side chain, and ultimately conjugation) to produce the most abundant primary bile acids in humans, cholic acid and chenodeoxycholic acid. Prior to transport out of the hepatocytes, most bile acids are conjugated to either taurine (in the mouse) or glycine (in humans), thereby increasing water solubility and preventing passive re-absorption once secreted into the small intestine. These conjugated bile acids are then carried from the liver via the bile ducts to the gallbladder, where they are stored for future use. Ultimately, they are secreted into the intestine, where they emulsify dietary lipids. In the gut the glycine or taurine residues are removed and the bile acids are either excreted (a small fraction) or reabsorbed by the gut and returned to the liver.

These pathways generate a vast array of bile acids, whose functions vary from solubilization and absorption of a broad range of lipophilic molecules in the intestine to intracellular signaling. Bile acids are potent signaling molecules, acting as ligands for G protein coupled receptors (specifically TGR5) and nuclear hormone receptors (*e.g.* the farnesoid $\times$ receptor; FXR). Consequently, bile acids exert regulatory effects on many lipid homeostatic processes, including their own synthesis and enterohepatic recirculation as well as triglyceride, cholesterol, and glucose metabolism.

3.3. Sphingolipid hydrolysis

Catabolism of sphingolipids serves to prevent accumulation, but importantly generates and recycles intermediates and metabolites such as sphingosine (and thus sphingosine-1-phosphate) and ceramide. These are potent signaling molecules that mediate a variety of cellular processes including differentiation and apoptosis, and as such their levels are closely monitored and controlled.

Sphingomyelinases (SMases) mediate the hydrolysis of sphingomyelin to ceramide and phosphocholine. This family of hydrolases is classified into three groups according to pH optimum (alkaline, acid, and neutral) and then further sub-categorized according to their primary structure, localization, and cation dependence [36]. The acid SMase (A-SMase) was the first member of this enzyme family to be characterized. It consists of two spatially and functionally distinct isoforms, secretory and lysosomal, which are both encoded by the *Smpd1* gene. Although both forms require zinc for their activity, the lysosomal form is tightly bound to zinc and the secretory form requires exogenous zinc [36].

Acid Sphingomyelinases play a pivotal role in the cellular stress response. A-SMase knockout mice have impaired ceramide generation and are protected from a variety of stress stimuli, including Fas ligand, lipopolysaccharide (LPS), and photo-cytotoxicity. Although these genetic models have been instrumental in suggesting the overall molecular function of A-SMase, they do not identify the individual roles of the isoforms. Although both isoforms share a common precursor and function, they likely hydrolyze distinct pools of sphingomyelin [37].

Lysosomal A-SMase localizes to the lumen of endosomes, phagosomes and lysosomes as well as to the outer leaflet of the plasma membrane. Stress conditions cause the lysosomal A-SMase to translocate from its intracellular location to the outer leaflet of the plasma membrane, which is rich in sphingomyelin. The hydrolysis of sphingomyelin at the plasma membrane results in a relatively slow reorganization of membranes and the formation of ceramide enriched microdomains. Although this acute activation and relocation of lysosomal A-SMase in response to cellular stress has been seen in many cell types, the precise relationship between A-SMase, SM hydrolysis to ceramide at the PM, and lipid rafts has yet to be elucidated [37].

The secretory A-SMase hydrolyzes lipoprotein-bound sphingomyelin forming a ceramide - enriched low-density lipoprotein with an increased propensity for aggregation. Aggregation of these atherogenic lipoproteins promotes their subendothelial retention and engulfment by arterial wall macrophages that over time become macrophage foam cells. Mice lacking A-SMase in an atherosclerosis-prone genetic background had no change in serum cholesterol or circulating LDL, relative to control mice, but showed decreased focal atheroma formation [37].

Neutral sphingomyelinases (N-SMases) have an optimum pH of 7.4 and require Mg^{2+} ions for activity. Two mammalian isoforms, N-SMase1 and N-SMase2, exhibit significant sequence conservation with the catalytic region of bacterial and yeast N-SMases suggesting a common catalytic mechanism [38]. Interestingly, although N-SMase1 displays sphingomyelinase activity *in vitro*, nSMase1 knockout mice showed no lipid storage disease or abnormalities in SM metabolism. Furthermore, cells overexpressing N-SMase1 did not exhibit any marked changes in sphingomyelin metabolism, but surprisingly showed an increase in the metabolism of 1-*O*-alkyl-lyso-phosphatidylcholine (lyso-platelet activating factor). The ambiguity of the *in vivo* role of N-SMase1 was further complicated by its localization to the ER, which is suboptimal for a protein involved in SM hydrolysis [38].

By contrast, cells over-expressing N-SMase2 displayed lower SM and higher ceramide levels, suggesting a more conventional role for this isoform in sphingolipid metabolism. Further analysis of ceramide composition in such cells showed that the enzyme primarily increased levels of very long chain ceramides, which correlated with a decrease in the level of C_{24:0}- and C_{24:1}-SM species. Importantly, down-regulation of N-SMase2 using siRNA prevented confluence-induced increases in levels of C_{24:0} and C_{24:1} ceramides. N-SMase2 is activated by anionic phospholipids, especially phosphatidylserine, cardiolipin and phosphatidylglycerol, suggesting the presence of an anionic phospholipid binding domain, similar to yeast N-SMases such as Isc1p [38].

Alkaline SMase (alk-SMase) exhibits an optimum pH of 8.5 and shares 30% sequence similarity with members of the nucleotide pyrophosphatase/phosphodiesterase (NPP) family of ecto-enzymes [39]. Alk-SMase localizes to endosome-like structures near the plasma membrane of the intestinal tract, in particular the duodenum and colon, where its activity is low, and in the jejunum, where its activity is high. The mid jejunum is the primary site of dietary SM hydrolysis. For optimum activity, alk-SMase requires bile salts with maximal activation at the critical micelle concentrations of each bile salt. Although all bile salts have a stimulatory effect, the degree of activation varies greatly among bile salt species. Taurocholate (TC) and taurochenodeoxycholate (TCDC) exhibit a 20-fold more potent effect on alk-SMase than other bile salts [39].

3.4. Coordinated catabolism of sterols and sphingolipids

The mechanism by which sphingolipid and cholesterol catabolism are co-regulated is virtually unknown, although it is clear that perturbing the homeostasis of either lipid has profound effects on the other's metabolism. For example, sphingolipid hydrolysis has been shown to impact

cholesterol metabolism, specifically the synthesis and uptake of CE. Incubation of cells with a bacterial SMase markedly increased cholesterol esterification without expanding cellular cholesterol pools [40]. Interestingly, sphingosine derived from ceramide has been shown to inhibit cholesterol esterification by ACAT [41].

Sphingomyelin has also been shown to inhibit the uptake of CE from HDL via scavenger receptor class B type 1. Incorporation of SM into recombinant high density lipoprotein (rHDL) that contain labeled CE resulted in approximately 50% inhibition of the selective uptake of CE in SR-BI-transfected Chinese Hamster Ovary (CHO) cells, hepatocytes (HepG2), and adrenocortical cells (Y1BS1). This inhibition was completely reversed by treatment of rHDL with SMase [41].

4. Subcellular Lipid Transport

Lipid movement between organelles is a major component of eukaryotic membrane homeostasis. The PM contains most of the cellular sterols and sphingolipids while the ER is sterol/sphingolipid poor despite being the primary site of their biosynthesis. Transport of these lipids is not random as the cell maintains a precise allocation that is essential for cell viability. As such multiple vesicular and non-vesicular transport pathways have evolved to move these lipids throughout the cell (Fig. 2).

4.1 Sterol transport

The cholesterol pool of the plasma membrane is mobile and continually recycles with intracellular pools (Fig. 2A). Nascent cholesterol is rapidly transported to the PM by an ATP-dependent mechanism [42] that is only partially accounted for by the protein secretion pathway, as treatment with Brefeldin A to collapse the Golgi does not abrogate movement. Similarly, vesicular and non-vesicular pathways are involved in the retrograde transport of cholesterol. Pharmacological reagents that disrupt the cytoskeleton, acidic compartments, and intermediate filaments of cells inhibit cholesterol movement from PM to ER, consistent with the involvement of vesicular pathways [42]. However, energy depletion only modestly affects the movement of cholesterol from the PM [43] indicating a significant non-vesicular component. Membrane juxtapositions may be sufficient for diffusion or protein-mediated transport, however the molecular mechanisms of these processes remain obscure.

In the search for subcellular cholesterol carriers, several candidates have been presented. The steroidogenic acute regulatory protein (StAR) is clearly required to deliver cholesterol to the inner membrane of mitochondria for steroid hormone production [44]. Mutations in human StAR gene cause congenital lipoid adrenal hyperplasia, which is characterized by defective steroid synthesis in newborns. Sixteen human proteins have been identified that possess a lipid-binding domain in common with StAR, the StAR-related lipid transfer (START) domain [45,46]. One family member, MLN64, is a membrane protein localized to late endosomes consistent with a role in cholesterol mobilization in this organelle. Indeed, expression of a truncated form of MLN64 lacking the START domain led to cholesterol accumulation in lysosomes [47]. Another member of this family, StarD4 responds to cellular cholesterol levels via SREBP mediated transcription [48] and thus is also a candidate cholesterol transfer protein.

Caveolins, were first identified as coat proteins found in specific plasma membrane invaginations. Subsequently they were also found in the Golgi, the ER, vesicles, the surface of lipid droplets, and in the cytosol [49-51]. Three caveolin family members (Caveolin-1, -2 and -3) have been characterized, one of which (Caveolin-1) binds to cholesterol [52] and mediates bidirectional vesicular and non-vesicular cholesterol transport from ER to PM [53, 54]. Over-expression of Caveolin-1 leads to increased transfer of intracellular cholesterol to PM, increased efflux to HDL, and up-regulation of cholesterol biosynthesis. However, the role

of caveolins in intracellular cholesterol trafficking is clearly dispensable, since they are not expressed in all cell types.

Sterol carrier protein 2 (SCP-2) is a non-specific lipid transfer protein that binds to cholesterol, fatty acids, fatty acyl-CoA, and phospholipids *in vitro* and is localized to cytosol, peroxisomes, mitochondria and ER. Its diverse localization suggests a general role in lipid movement between a variety of organelles. SCP-2 selectively enhances cholesterol transfer from caveolae/lipid rafts and facilitates retention of cholesterol in many cultured cell systems [55,56]. Consistent with a general role in lipid transfer, the targeted deletion of the SCP2 gene in mice resulted in defective peroxisomal β -oxidation of fatty acids [57], as well as impaired biliary cholesterol secretion when the animals received a cholesterol enriched lithogenic diet [58].

4.2 Sphingolipid transport

The synthesis of SM and complex sphingolipids begins at the cytosolic surface of the ER and is completed at the trans-Golgi, suggesting the presence of an ER to Golgi transport mechanism (Fig. 2B). Genetic complementation of a mutant CHO cell line that was defective in SM metabolism identified ceramide transfer protein (CERT) as essential for the transport of ceramide from the ER to the Golgi complex. Analysis of CERT revealed a Golgi-associating pleckstrin homology (PH) domain, an ER-targeting FFAT motif (two phenylalanines in an acidic tract) followed by a START family domain that in this case specifically bound ceramide.

Interestingly, the down-regulation of CERT in various cell lines had no effect on the synthesis of complex glycosphingolipids (GSLs). As such, it was believed that the generation of GSLs from glucosylceramide (GlcCer) occurred independent of a transfer protein. In actuality, GlcCer enters both a retrograde and anterograde transport pathway, mediated by the four-phosphate adaptor protein, FAPP2, a protein that binds to phosphatidylinositol-4-phosphate (PtdIns4P) and ARF1 (via its PH domain) [59,60]. FAPP2 is now recognized as an essential component of the GSL synthetic machinery. It mediates non-vesicular transport of the common GSL precursor GlcCer from its site of synthesis at the early Golgi to post Golgi compartments, and the surface of the cell. Counter-intuitively, GlcCer is also transferred by FAPP2 back to the ER, where it re-enters the vesicular transport pathways for movement from ER to Golgi where most of the enzymes involved in complex GSLs assembly are located. This circuitous route may actually confer regulation upon the transport process by linking GlcCer to Arf1 and the signaling lipid, phosphatidylinositol 4-phosphate.

4.3. Mutual transport of sterols and sphingolipids

Sterol and sphingolipid homeostasis is strikingly conserved throughout evolution. In some cases, the major players (Tables 1 and 2) are shared between these classes of lipids to the extent that they may even be co-transported (Fig. 2A and B). This provides the means for significant cross talk between these pathways and likely represents a key regulatory event in the homeostasis of multiple lipid classes.

4.3.1 Oxysterol binding proteins—Oxysterol binding protein (OSBP) and OSBP-related proteins (ORPs; characterized by an OSBP-related domain ORD) constitute a large conserved family of proteins (twelve human ORP family members, seven yeast OSBP homologue (OSHs) [61,62]). Besides the core ORD, many ORPs also contain pleckstrin homology (PH) domains, transmembrane regions, ER-targeting FFAT motifs, GOLD (Golgi dynamics) domains and ankyrin repeats [63]. Structural studies and *in vitro* binding assays have shown that OSBPs, such as Osh4p can bind and transport oxysterols, cholesterol, and ergosterol [64].

In addition to their role as sterol carriers, OSBPs may mediate cross-talk between sphingolipids and cholesterol. Activation and translocation of OSBP via oxysterols has been shown to recruit

CERT into an active transport pathway from the ER to the Golgi, thereby promoting sphingomyelin synthesis. Ridgway *et al.* stimulated SM synthesis threefold in CHO-K1 cells by treating with 25 hydroxycholesterol, an event augmented by the overexpression of OSBP [65] and inhibited by the expression of a mutant OSBP retained in the ER [66].

Functional studies of OSBP have revealed that the associations of OSBP with a resident ER protein VAP (vesicle associated membrane protein-associated protein) and with PtdIns-4-P at the Golgi are both required for CERT recruitment to the Golgi and subsequent SM synthesis. Furthermore, the Golgi localization of CERT in the absence of OSBP-VAP interactions is insufficient to increase SM synthesis and thus ceramide and SM synthesis are reduced in cells with OSBP or CERT knockdowns treated with 25OH-cholesterol [67].

Conversely at the Golgi, it is not the shared interaction of OSBP and CERT with PtdIns-4-P, but the sequential binding of these two transporters that enhance SM synthesis. It has been suggested that recruitment of ARF1 by the PH domain of OSBP at the Golgi could stimulate PtdIns-4-k-III β and subsequent increased PtdIns-4-P synthesis for recruitment of CERT. Collectively these data suggests a mechanism for integrating cholesterol and SM metabolism mediated by OSBP and CERT [68]. Interestingly, CERT was originally identified based on hypersensitivity of LY-A cells to the cholesterol-absorbing agent methyl- β -cyclodextrin [69].

4.3.2. ARV1, a transporter of sterols and sphingolipids—The ACAT-related enzymes (*ARE1* and *ARE2*) of budding yeast are 49 percent identical to each other and exhibit 23 percent identity to human ACAT1 [70,71]. Deletion of *ARE1* and *ARE2* produces a viable yeast cell with no detectable esterification activity [72]. The deletion of *ARV1* (*ARE2* Required for Viability 1) in this context is inviable, probably due to accumulation of unesterified sterols in ER at the expense of plasma membrane sterol pools [73]. Mutations in *ARV1* were also independently isolated in a genetic screen for mutants defective in sphingolipid biosynthesis, suggesting a more general role for this protein in lipid transport [74]. Lower steady state levels of complex sphingolipids, with an accumulation of hydroxylated ceramides were indicative of a defect in ceramide export from the ER in *arv1 Δ* mutant cells [74]. The coordinated regulation of sterols with other lipids makes it difficult to distinguish whether these defects are secondary to sterol transport defects or reflect the Arv1-mediated transport of multiple lipid species besides sterols. To add to the confusion of what are secondary versus primary activities of this protein, Arv1p is also required for the maturation of GPI-anchored proteins in the ER [75].

4.3.3. Lipid flux through membranes by ATP-binding cassette transporters—ATP binding cassette (ABC) transporters (a superfamily of 48 integral membrane proteins in humans and 31 in yeast) harness the energy of ATP hydrolysis to transport a diverse set of hydrophobic molecules across membranes. ABC transporters are typically composed of two nucleotide binding domains and two transmembrane domains. The nucleotide binding domains contain characteristic motifs (Walker A and Walker B) and the ABC linker region, which are the hallmarks of this protein superfamily.

To date, approximately 20 members of this protein superfamily have been implicated in lipid transport (Table 2). Mutations in ABCA1 are the underlying molecular defect in Tangier disease, highlighting its role as a key regulator of HDL metabolism and cholesterol flux. ABCA1 function and regulation is not restricted to cholesterol, but extends to phospholipids and sphingolipids as well. ABCA1 was found to translocate phospholipids and expression levels were impacted by several sphingolipids. For example, an increase in intracellular concentration of ceramide enhanced expression of ABCA1 while increased cellular GSL levels promoted cholesterol accumulation by impairing ABCA1 function. This was further substantiated by the observation that fibroblasts derived from patients with genetic GSL storage

diseases (Fabry disease, Sandhoff disease, and GM1 gangliosidosis) had impaired apoAI-mediated cholesterol efflux [76].

Genetic and functional assays have revealed the seminal role that ABCA1 and its relatives play in lipid metabolism. These transporters are pivotal members of the cellular cholesterol, phospholipid, and sphingolipid transport machinery. Other members of this subclass to have been implicated in lipid transport include ABCA3 (required for the transfer of phospholipids into the lamellar body, a prerequisite for alveolar type 2 cell surfactant production [77]), ABCA4 (a retinylidene-phosphatidylethanolamine transporter in the photoreceptor cells of the retina [78]), and ABCA12 (involved in the keratinocyte lipid export system [79]).

Several members of the ABC-G subclass of half transporters (comprised of one nucleotide binding domain and one transmembrane domain) have also been implicated in sterol transport across the plasma membrane. In some yeast species, ABC-G family members such as *AUS1* encode proteins essential for sterol uptake and anaerobic growth, a state that induces sterol and fatty acid auxotrophy [80]. In mammals, the majority of this sub-family is required for sterol efflux. Two tandemly aligned genes, *ABCG5* and *ABCG8*, encode proteins that promote sterol efflux into bile and the intestinal lumen and act as obligate partners in the transport reaction [81]. *ABCG1* also mediates sterol efflux to HDL particles and is also the first member of this superfamily to transport sphingomyelin. *ABCG1* effluxes several species of sphingomyelin and phosphatidylcholine [82]. Furthermore, overexpression of CERT in CHO-K1 cells produced an increased efflux of cholesterol and SM mediated by *ABCG1* [83].

4.3.4. Lipid transport in endosomes and lysosomes—Subcellular pools of sterols and sphingolipids arise from the convergence of endogenous and exogenous sources. In cells lacking the genes defective in Niemann Pick type C (NP-C) disease, exogenous sterols and PM sphingolipids accumulate in the lysosome, while the circuits traveled by ER-derived lipids are unaffected. The former process is likely an ancient one; the NPC1 gene is impressively well conserved throughout eukaryotic evolution [84]. Strikingly, expression of the yeast NP-C-related gene 1 (*NCR1*), a sequence ortholog of human NPC1, fully restores lipid transport to CHO NPC1 mutant cells [85].

The NPC1 gene product, a protein with a sterol-sensing domain and 13 transmembrane domains, facilitates export of free cholesterol from late endosomes and lysosomes either by direct binding of cholesterol as demonstrated for a soluble sub-domain of NPC1 or alternatively by transport not of cholesterol but some other accompanying hydrophobic molecule [86-88]. NPC1 could be involved in sphingolipid transport with concomitant cholesterol movement because of the high affinity between these molecules [85]. Mutations in the NPC2 gene also cause NP-C disease, due to defects in a soluble lysosomal protein with clear cholesterol binding capability [89]. The precise role of NPC2 in sterol and sphingolipid homeostasis is unknown. However, NPC2 is also an ancient protein; expression of the yeast ortholog of the human NPC2, also suppresses lesions in human NPC2 mutant fibroblasts [90]. NPC1 and NPC2 double knockout mice have the same phenotype as single NPC1 or NPC2 null mice [91] suggesting the proteins work cooperatively in the same pathway. Surprisingly, sterol binding by NPC1 is accomplished by an NH₂-terminal “soluble” domain of the protein, unique to this gene family [92]. Subsequently, crystal structures of both apo- and ligand-associated forms of this domain [93] and of NPC2 [94] were resolved and prompted a model of cholesterol transfer in this compartment of the cell. The proposed transfer model rests upon the manner in which cholesterol is oppositely orientated in NPC 2 versus NPC1(NTD), such that the 3 β -hydroxyl is buried and isoctyl side chain exposed in the latter molecule and vice versa in NPC2.

NPC1L1, a mammalian paralog of NPC1 [95], has been implicated in cholesterol absorption by several criteria. Unlike the NPC1 gene, expression of NPC1L1 is more restricted; transcripts

are most abundant in the liver and intestine. NPC1L1-null mice are resistant to diet-induced hypercholesterolemia [96-98] and to the effect of ezetimide on cholesterol absorption in the intestine, prompting the hypothesis that NPC1L1 is the target of this sterol absorption inhibitor [99,100]. As for NPC1, the precise substrate for NPC1L1 is uncertain, in that sphingolipids and cholesterol accumulate in NPC1L1 deficient cells [97].

5. Sterol and sphingolipid pathobiology

5.1. Lysosomal lipid storage disorders

Lysosomal lipid storage disorders arise from a panoply of gene defects resulting in accumulation of sterols and/or sphingolipids (see [101] for review). Indeed, there are few if any instances where the trafficking of these molecules can be disassociated [102]. Of particular relevance to this review are the Niemann-Pick diseases; these are autosomal recessive metabolic disorders that are typified by the accumulation of cholesterol and sphingolipids in various organs including the spleen, liver, and brain. Niemann Pick Type A and B are both caused by mutations in *SMPD1*, which result in dysfunctional A-SMase activity. As a result, cellular sphingomyelin levels are elevated by approximately 70%. Although types A and B both stem from the same enzymatic deficiency, they exhibit contrasting clinical symptoms and prognoses. Niemann Pick Type A patients typically present within the first 6 months of life with enlarged livers and spleens and progressive neurodegeneration that ultimately leads to death within 3 years. Conversely, Niemann Pick Type B patients have little or no neurodegeneration and frequently survive into adulthood.

Homozygous A-SMase knockout mouse models also display progressive lipid (sphingomyelin along with ganglioside and cholesterol) storage in reticuloendothelial organs and the brain. Macrophages derived from A-SMase knockout mice showed a significant decrease in cholesterol efflux (approximately 60-70 %) and cholesteryl ester (30-50%) formation. Furthermore, aberrant cholesterol trafficking was evident as filipin staining showed bright perinuclear staining, some of which coincided with late endosomes/lysosomes.

Niemann Pick Type B patients also exhibit decreased HDL-C levels, despite normal phospholipids and cholesterol efflux to the mature HDL particle. Previous studies had established that sphingomyelin inhibits the unfolding of ApoA-I in discoidal and spherical reconstituted HDL and impairs the LCAT reaction. Lee *et al.* observed that both nascent HDL particles and mature HDL particles isolated from NP-B patient plasma had a significant increase (~50–100%) in sphingomyelin content and a decrease in LCAT-mediated cholesterol esterification.

Niemann-Pick type C patients exhibit progressive central nervous system degeneration and visceral storage of cholesterol, sphingomyelin, and glycosphingolipids. Typical NPC patients show symptoms at early ages and live only into teenage years. The disease arises from mutations in the NPC1 or NPC2 gene [86], which direct the traffic of LDL derived cholesterol from late endosomes and lysosomes to the ER and the PM [103]. Heterozygous deficiency of NPC1 selectively blocks cholesterol trafficking to the ER and protects macrophages from cholesterol-induced apoptosis [104]. In mouse models, NPC1 heterozygosity confers resistance to lesional necrosis in atherosclerosis [104]. There is a significant discrepancy in this syndrome between action of the protein and loss of function- phenotype. In budding yeast for example, where the function of the orthologous genes have clearly been conserved, there is no growth defect in the null mutants. In mammalian cells the identity of the NPC1 substrate remains contentious, perhaps unknown. Moreover, it is not clear how lipid accumulation in the lysosome causes neuronal demise, especially in the cerebellum.

5.2. Atherosclerotic vascular disease

Atherosclerotic cardiovascular disease remains the leading cause of morbidity and mortality in Western societies. Many risk factors for atherosclerosis have been identified, some of which are reversible, including hypercholesterolemia, hypertriglyceridemia, hypertension, and high plasma homocysteine concentration. Most prominent amongst these is an elevated level of plasma cholesterol. Serum LDL elevation and modification, such as oxidation, glycation, and aldehyde-conjugation confers unregulated uptake through scavenger receptors (mainly SR-AI/II and CD36). Consequently, cholesteryl esters accumulate to form macrophage-derived foam cells, the hallmark of the fatty streak. In the later stages of atherosclerosis, foam cells accumulate free cholesterol possibly due to compromised free cholesterol efflux and ACAT1 activity. Foam cells then undergo FC-induced apoptosis or necrosis and release their lipid contents [105]. The accumulation of extracellular lipids, cell debris and a mixture of leukocytes may form a necrotic core of atherosclerotic plaque. At the advanced stage, plaques rupture and as a result, many thrombotic complications of atherosclerosis occur. Plaque rupture and thrombosis are responsible for approximately 50% of cases of acute coronary syndromes and myocardial infarction [106].

The reduction of plasma LDL cholesterol concentrations lowers the risk of heart attacks, strokes and all forms of atherosclerotic vascular disease. Currently, the most commonly used cholesterol lowering drugs are 'statins' which are potent inhibitors of HMG-CoA reductase and thus cholesterol biosynthesis. Decreased cholesterol biosynthesis is associated with the upregulation of LDLR due to activation of the SREBP pathways and thus elevated clearance of LDL from plasma. Decreasing dietary cholesterol and biliary cholesterol is another approach to manipulating body cholesterol. The inverse correlation between HDL and atherosclerosis suggest that HDL is athero-protective. HDL can protect macrophages from FC-induced cell death by reverse cholesterol transport but has been recalcitrant to therapeutic intervention.

Recent observations have shown that plasma sphingomyelin levels are also independent risk factors for coronary heart disease. The inhibition or deletion of SPT significantly decreases atherosclerotic lesions in apolipoprotein E knockout mice and inhibits cholesterol absorption [107,108]. Furthermore, inhibition of sphingolipid synthesis lowers plasma cholesterol and triglyceride mass by modulating SREBP-1. Concurrently, inhibition of sphingolipid synthesis also induced expression of apolipoprotein A1 and LCAT proteins to promote increased plasma concentrations of anti-atherogenic HDL. The beneficial effect exerted by inhibition of sphingolipid synthesis was morphologically reflected in a decreased formation of atherosclerotic lesions and macrophage accumulation. In contrast, increased sphingolipid synthesis correlates with increased atherogenic potential. Hepatic over-expression of sphingomyelin synthase in wild-type mice shifts plasma cholesterol and sphingomyelin concentration to the pro-atherogenic non-HDL lipoproteins [21].

Dietary SM significantly decreases cholesterol absorption in the intestine; the maximal inhibition occurs at an equal molar amount of SM and cholesterol. Sphingosine, a product of SM hydrolysis, may be responsible for this phenomenon, as it can form condensed lipid complexes with the α -face of cholesterol. Furthermore, this inhibition may occur via a NPC1L1-dependent transport of micellar cholesterol since the inhibitory effect of sphingosine on cholesterol absorption was far more pronounced in NPC1L1 expressing Caco-2 cells [39].

5.3. Alzheimer's disease

Alzheimer's disease (AD) is a polygenic neurodegenerative disorder that is characterized pathologically by amyloid- β (A β) plaques, neurofibrillary tangles, and neuronal cell loss. One of the strongest known risk factors for sporadic AD is the status of apolipoprotein E (ApoE), the major carrier of cholesterol in the CNS. Individuals carrying one or two copies of the ApoE

$\epsilon 4$ allele have a higher risk of developing the disease, compared to those carrying $\epsilon 3$ (the most common) or $\epsilon 2$ (which appears to be protective) alleles. Lipids not only serve as a risk factor for AD but also play an essential role in modulating the disease [109,110]. Data from both *in vivo* and *in vitro* studies have shown that there is a linear relationship between intracellular cholesterol levels and the production of A β peptides [111].

Recent research suggests that A β generation and the maintenance of lipid homeostasis are intrinsically linked as alterations in cellular lipid levels, trafficking, or organization in the protein–lipid bilayer have been associated with A β production. Specifically, the conservation of lipid rafts appears to be integral to the formation of A β peptides, since several of the proteins essential for A β generation, including the γ -secretase complex, β -secretase, and the β amyloid precursor protein (β -APP) are localized in a raft-cholesterol dependent manner [112]. Furthermore, β -APP exists in two pools, one associated with membrane rafts in which β -cleavage occurs and another outside of membrane rafts where α -cleavage (nonpathogenic) occurs, suggesting that raft localization favors the generation of the former [113]. These data suggest that the raft or non-raft membrane distribution of β -APP may in part determine its processing fate [110].

Cholesterol may not be the only culpable lipid in AD; defects in sphingolipid metabolism may also contribute to the generation of some of the pathological and clinical hallmarks. Microarray analysis of 17 brain regions from subjects with varying severity of AD revealed anomalous expression of various key enzymes involved in sphingolipid metabolism [114]. Early in disease progression (marked by mild dementia), enzymes that regulate *de novo* synthesis of ceramide were upregulated, whereas those enzymes regulating glycosphingolipid synthesis were down-regulated. Concomitantly, as expected, there was a significant increase in ceramide content during the early stages of AD. However, ceramide content eventually declines as cognitive impairment increases. Interestingly, during the period of advanced cognitive impairment there were changes in the expression of genes regulating ceramide catabolism [114]. Complex sphingolipids and derivatives of ceramide such as GM1 gangliosides have also been implicated in this syndrome [115].

5.4. Huntington's disease

Huntington's disease (HD) is a progressive and fatal neurological disorder that presents at midlife and is characterized by motor, psychiatric, and cognitive dysfunction. HD is caused by an expanded CAG repeat in the gene encoding the protein huntingtin, however the mechanism by which this mutation contributes to neurodegeneration remains elusive. Defects in lipid homeostasis likely contribute to the onset of HD [116]; microarray analysis of a transgenic mouse model of HD (the R6/2 mice) showed decreased levels of mRNA of SRE regulated genes such as HMG-CoA reductase, Cyp51, and 7 dehydroxycholesterol reductase just prior to the onset of motor symptoms [117]. Furthermore, total cholesterol content and the ability to synthesize cholesterol was markedly reduced when human fibroblasts derived from HD patients were cultured in lipoprotein deficient serum [118]. Enzymatic analysis of brains from HD mice were shown to have reduced total sterol mass (including free and esterified cholesterol). In addition, HD cells exhibited a decrease in SREBP nuclear translocation and activity.

In keeping with the theme of this review, defects in cholesterol metabolism in various models of HD are also accompanied by abnormal sphingolipid metabolism. Microarray data from the R6/2 mouse model of HD and from postmortem human caudate samples of HD patients, revealed a decrease in expression of the genes involved in glycosphingolipid synthesis, relative to disease-free controls. Specifically, there was a decreased accumulation of transcripts for glycosyl- and sialyl-transferases, the enzymes contributing to ganglioside synthesis [119].

6. Conclusions and perspectives

It is virtually impossible to separate the relevance of sterols and sphingolipid, both to cellular metabolism and human disease. This is, of course, not happenstance; evolution has perfected membranes of cells to be adaptive, and this can be readily achieved by modulating the sphingolipid:sterol ratio. Our mission in this survey was to highlight the striking physical, genetic and metabolic interactions of sterols with sphingolipids (Figs. 1 and 2). Somewhat surprisingly, there is no single master regulator; instead it is the cross-talk between the pathways that apparently optimizes the lipid proportions. As such, there are numerous points of intervention where genetic variation in such interactions may impact several lipidoses and future therapies will ideally accommodate the roles of both molecules in the pathways in question. For example, there are a significant number of NP-C disease kindreds in which disparate cholesterol and sphingolipid phenotypes occur between siblings, with variable age of onset and rate of progression [120]. This observation strongly suggests segregation of modifier alleles in such families. We believe many of these pathways have already been identified in genome-wide panning of yeast genetic and physical interactions. For example, in an unbiased survey of all known sterol and sphingolipid genes in yeast, we asked what genetic data indicates a sterol-sphingolipid interaction. The result is clear (Fig. 3), these pathways can be readily represented as a web of interactions, further exemplifying the manner in which variation in one component of either pathway will likely have feed-forward and feed-backward sequelae that are initially homeostatic but perhaps ultimately incompatible with life. The identification of these genes and their biochemical consequences might ultimately lead to new therapeutic interventions in many disorders, ranging from atherosclerosis to Alzheimer's disease.

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Abbreviations Used

ACAT	AcylCoA cholesterol acyltransferase
AD	Alzheimer's disease
ABC	ATP binding cassette
CERT	Ceramide Transport protein
CE	Cholesteryl ester
ER	Endoplasmic reticulum
FAPP2	Four-phosphate Adaptor Protein
GSLs	glycosphingolipids
HD	Huntington's disease
NP-C	Niemann Pick Type C
PM	Plasma membrane
SPT	serine palmitoyl transferase
SMase	Sphingomyelinase
A-SMase	Acid Sphingomyelinase
N-SMase	Neutral Sphingomyelinase
Alk--SMase	Alkaline Sphingomyelinase
SREBP	Sterol Regulatory Element Binding Protein

Figure 1. Sterol and sphingolipid biosynthetic pathways

Yeast (italics and in red) and mammalian (underlined) gene designations of key enzymes are shown. Negative regulation of SREBP by ceramide represents a pivotal point of crosstalk between the mammalian sterol and sphingolipid pathways. SREBP regulates the mevalonate pathways at many points, however, for simplicity, the rate limiting reactions are depicted as targets. There is no clear ortholog of SREBP in yeast, although an analogous role is performed by the *UPC2*-encoded transcription factor [80]. CoA, Coenzyme A; HMG, 3-hydroxy-3-methylglutaryl; GPP, Geranyl Pyrophosphate; FPP, Farnesyl Pyrophosphate; IPC, Inositol Phosphoceramide; MIPC, Mannose Inositol Phosphoceramide; MIP₂C, Mannose Di-Inositol Phospho-ceramide.

Figure 2. Cholesterol and sphingolipid homeostasis in mammalian cells

Cholesterol (panel A) is synthesized, esterified into cholesteryl ester (CE) and deposited in lipid droplets at the ER. Cholesterol can be mobilized back into free (FC) pool by ester hydrolysis. Cells take up cholesterol by LDL-receptor mediated LDL uptake or through diffusion of FC from lipoproteins to plasma membrane. Excess FC can be removed by efflux through passive diffusion from plasma membrane to acceptors or ABCA1 mediated FC transfer to apo AI. Beside these common pathways, there are also cell type specific pathways to handle cholesterol. In hepatocytes and enterocytes, cholesterol can be packaged into lipoproteins and secreted into the circulation. Cholesterol is also converted into steroid hormones or bile acids by steroidogenic cells or hepatocytes, respectively. Panel B depicts the cellular location of key

components of sphingolipid synthesis, maturation and hydrolysis. Both lipid classes activate and/or respond to transcriptional regulation via SREBP which itself must be translocated from the ER (triangle; precursor) to the Golgi (square; mature) in response to lipids to be activated and subsequently imported into the nucleus.

Figure 3. Genetic and physical interactions within the sterol and sphingolipid metabolome
 OSPREY v1.2 (<http://biodata.mshri.on.ca/osprey>) was used to generate interaction maps from data inventoried in the BioGRID (www.thebiogrid.org) between yeast genes with known roles in sterol metabolism and sphingolipid metabolism (sterol metabolism, $n = 35$; sphingolipid metabolism, $n = 26$; sterol and sphingolipid metabolism, $n = 1$, *Saccharomyces* Genome Database, www.yeastgenome.org). 18 sterol and 12 sphingolipid metabolism genes interact with at least one gene of the other metabolic class. Only interacting genes are shown in this figure.

Table 1

Subcellular lipid transporters, putative and proven. Members of the ATP-binding Cassette family of membrane transporters are the topic of Table 2.

Transporter	Protein Family	Lipid Transported	Disease State	Reference
NPC1	NPC	C, Oxysterols	NPC Type 1	[121,122]
NPC2	Der p 2	C	NPC Type 2	[122,123]
NPC1L1	NPC	C, SM	Resistance to dietary induced hypercholesterolemia	[124]
CERT	START/ORP	Cer	?	[125]
FAPP2		PtdIns4P, GlcCer	?	[60]
SCP2		C, PL, FA	?	[126]
MLN64	START	C	?	[47]
OSBP	START/ORP	Oxysterols	?	[68]
StarD4	START	C	?	[48]
StAR	START	C	Congenital lipoid adrenal hyperplasia	[127]
ARV1		C, Cer	?	[73,74]
Caveolin-1	Caveolin	C		[52]

Abbreviations: C, cholesterol; SM, sphingomyelin; Cer, ceramide; PL, phospholipid; PtdIns4P, phosphatidylinositol-4-phosphate; GlcCer, glucosylceramide; FA, fatty acids; Der p 2,; START,; ORP,

Table 2

Members of the ATP binding cassette super-family of membrane associated transporters involved in lipid movement. Abbreviations; C, cholesterol; Cer, ceramide; PL, phospholipids; PC, phosphatidylcholine; SM, sphingomyelin; GSL, glycosphingolipid; E ergosterol; Ret, retinaldehyde; PE, phosphatidylethanolamine; FA, fatty acids.

Transporter gene	Function	Lipids transported	Disease state/mutant phenotype	Ref.
ABCA1	Apo-A1 dependent formation of nascent HDL	C, PL	Tangiers Disease Familial HDL Deficiency	[79]
ABCA2	Unknown, correlated with C concentration in brain	SM, myelin	Alzheimer's Disease (putative)	[79,128]
ABCA3	Secrete pulmonary surfactant	C, SM, PC	Neonatal surfactant deficiency, Pediatric interstitial lung disease	[79]
ABCA4 (ABCR)	Retinal integrity	Ret, PE	Age-related macular degeneration, Stargardt disease 1, Retinitis pigmentosa 19,	[79]
ABCA6	Unknown, sterol regulated		?	[129]
ABCA7	Unknown	C, PL	?	[130]
ABCA9	Unknown, sterol regulated		?	[130,131]
ABCA10	Unknown, sterol regulated		?	[130,131]
ABCA12	Maintains the integrity of lamellar granules	C, Cer, FA	Lamellar ichthyosis type 2 Harlequin ichthyosis	[79]
ABCB1 (MDR1)	Multiple drug efflux (indirect effect on C)	PL, SM, GSL	Parkinsons	[128]
ABCB4 & ABCB11	Phosphatidylcholine Translocator	PC	Progressive Familial Intrahepatic Cholestasis	[128]
ABCG1	Promote cholesterol efflux to HDL-2 & HDL-3 particles	C, SM	Atherosclerosis	[132]
ABCG4	Promote cholesterol efflux to HDL-2 & HDL-3 particles	C	Atherosclerosis	[132]
ABCG5 & ABCG8	Secretion of plant sterols and cholesterol into bile	C, sitosterol	Sitosterolemia	[132]
<i>AUS1, PDR11</i>	Influx of sterol during anaerobiosis in yeast	Ergosterol?	Anaerobic inviability	[80]