HHS Public Access

Author manuscript

Annu Rev Genomics Hum Genet. Author manuscript; available in PMC 2018 December 13.

Published in final edited form as:

Annu Rev Genomics Hum Genet. 2014 ; 15: 173–194. doi:10.1146/annurev-genom-091212-153412.

Disorders of cholesterol metabolism and their unanticipated convergent mechanisms of disease

Frances M. Platt[#*], **Christopher Wassif**[#*,**], **Alexandria Colaco**[*], **Andrea Dardis**[***], **Emyr Lloyd-Evans**[****], **Bruno Bembi**[***], and **D. Forbes Porter**[**]

[*]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK

[**]*Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.

[***]University Hospital "Santa Maria della Misericordia" Udine, Italy.

[****]School of Biosciences, Cardiff University, Museum Avenue, Cardiff, CF10 3AX, UK.

[#] These authors contributed equally to this work.

The cell biology of cholesterol

The evolution and functions of sterols

Sterols are a group of primarily unsaturated solid steroid alcohols found in the membranes of all eukaryotic cells. Phylogenetically distinct organisms synthesize characteristic sterols; for example, cholesterol is the predominant sterol found in terrestrial vertebrates, ergosterol in fungi, and phytosterols in plants. The evolution of sterols has been the subject of much debate as they are absent from prokaryotes (although rare exceptions have been documented (63)), but are ubiquitously expressed in eukaryotes (81). One hypothesis to explain this phylogenic divide is that sterol evolution was driven by the increase in atmospheric oxygen levels, which coincided with the prokaryote / eukaryote transition (16; 34). Consistent with this idea, sterols are totally dependent on oxygen for their biosynthesis (81) as the biosynthesis of one molecule of cholesterol consumes 11 molecules of O_2, where as 12 are needed for ergosterol biosynthesis (105; 116). Sterols have also been proposed to have the capacity to serve as oxygen sensors in yeast (22; 49), which may have been another selective pressure that drove their evolution. For the remainder of this article we will focus on the mammalian sterol cholesterol, reviewing the complexities associated with cholesterol-related inherited metabolic disorders and their novel unanticipated inter-relationships.

Cholesterol

Cholesterol was first isolated from gallstones and has been intensively studied since its discovery in 1789 in France (35). It is a highly regulated amphipathic lipid (36) that plays important roles in a variety of homeostatic systems (25; 35; 50; 73; 87; 104; 114). Cholesterol is essential for the normal growth and development of mammals and in membranes, where it regulates membrane fluidity and is a key constituent of lipid rafts (94;

Address Correspondence to: frances.platt@pharm.ox.ac.uk and fdporter@mail.nih.gov.

102). These dynamic signalling platforms are implicated in a number of cellular processes but their physiological relevance is still the subject of debate (65; 78; 102). Cholesterol is also essential for myelin formation (56; 127) and in developmental signalling via the hedgehog (HH) pathway (10; 104) in which active HH proteins are covalently modified with cholesterol, and sterols also play multiple other roles in this signalling pathway (10; 104). There is also growing evidence that sterols and sphingolipids are co-regulated, although the details of the underlying mechanisms and biological significance of this remain to be fully elucidated (40; 41).

In addition to functions in membrane biology, cholesterol and it's biosynthetic intermediates also serve as key metabolic precursor for the synthesis of corticosteroids, vitamin D, bile acids (106) and steroid hormones including neurosteroids (Fig. 1) (5; 26; 120). These in turn interact with nuclear receptors (e.g. FXR, PXR and VDR nuclear receptors for bile acids and ER, PR, AR, GR and MR the steroid nuclear receptors) thereby regulating other aspects of cell function (141). With the exception of the liver and steroidogenic tissues, mammalian cells do not metabolise cholesterol but instead modulate cholesterol content in their membranes by regulating cholesterol biosynthesis, cholesterol uptake and cholesterol export from the cell via ABC transporters (90).

In view of the complex regulation and diverse functions attributable to sterols it is perhaps not surprising that inherited defects in genes involved in cholesterol metabolism lead to a number of particularly severe and complex human diseases (99). Before discussing these diseases, aspects of cholesterol homeostasis will be reviewed to provide a framework for understanding the consequences of cholesterol metabolism/transport defects in human disease.

Sources of cholesterol

Most mammalian cells can acquire cholesterol from two independent sources. The first is *de novo* biosynthesis by a multi-enzyme catalysed pathway (Fig. 1). The second is from the uptake of exogenously derived cholesterol associated with plasma low-density lipoprotein (LDL) from the circulation (Fig. 2A). The balance between these two pathways depends on cell type and the availability of LDL-derived cholesterol. The *de novo* biosynthetic pathway can be manipulated pharmacologically using statins, which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Fig. 1). There are inherited diseases associated with defects in both pathways (*de novo* synthesis and exogenous uptake/intracellular trafficking) and we will briefly review what is known about each pathway to provide the context for understanding the unexpected links between the different diseases involving defects in cholesterol homeostasis.

Cholesterol biosynthesis

All nucleated cells have the capacity to generate cholesterol *de novo,* with the liver being the most significant source. Cholesterol biosynthesis occurs in two distinct stages, the pre- and post-squalene pathways (81). The pre-squalene pathway contributes to sterol and isoprenoid synthesis, whereas the post-squalene pathway is essential for cholesterol and vitamin D biosynthesis (Fig. 1). Cholesterol contains 27 carbons that are all derived from acetyl coA

(81). The first step in the pathway involves the condensation of three acetates to form the six-carbon intermediate, mevalonate. The next stage of the pathway converts mevalonate to activated isoprenes, these are then polymerised to form a 30 carbon linear molecule, squalene. Squalene then cyclises to form the classical four ring steroid nucleus and the final generation of cholesterol involves oxidation reactions and methyl group modifications (81). The cholesterol biosynthetic pathway is detailed schematically in Fig. 1.

LDL-derived cholesterol

LDL-derived cholesterol originates primarily from dietary sources via the liver. The major route by which exogenously derived cholesterol is taken up into cells is through the low-density lipoprotein receptor (LDL) receptor (Fig. 2A). Additional receptors that can mediate the uptake of modified LDL include scavenger receptors (75; 115), but for the purposes of this review we will focus exclusively on the LDL receptor pathway. The significance of the LDL receptor (LDLR) was first appreciated through the pioneering work of Goldstein and Brown who were studying familial hypercholesterolemia (FH). Carl Muller originally described FH in 1938 (77) as an autosomal dominant trait with affected individuals exhibiting high levels of cholesterol in their blood that resulted in myocardial infarctions at a relatively early age. Two forms of the disease were later described by Khachadurian, the severe homozygous form and the milder heterozygous form (57). Goldstein and Brown showed the existence of the LDL receptor (37) and found that it was internalised by a clathrin-dependent mechanism (1; 2). They also discovered that the cholesterol taken up via this route mediates key regulatory functions, including feedback inhibition of cholesterol biosynthesis (11). A defect in the gene encoding the LDL receptor was subsequently shown to be the cause of FH (139). This is an excellent example of how the study of a rare inherited metabolic disease identified a fundamental cellular pathway and furthermore provided the conceptual framework for the concept of receptor-mediated endocytosis (1). LDL-derived cholesterol undergoes a complex intracellular trafficking itinerary, which facilitates its utilisation by the cell. Remarkably, the details of how cholesterol traffics within cells and in particular how it leaves the lysosome still remains the subject of investigation.

Cholesterol trafficking

Most cells express cell surface LDLR that binds apolipoprotein B (ApoB) proteins in the phospholipid layer of LDL particles (11; 139). It can also recognise apolipoprotein E (ApoE) in chylomicron remnants and VLDL remnants (115). The cholesterol rich particles bound by LDLR are internalised via clathrin-coated vesicles (2). As the endosomes acidify, LDL dissociated from its receptor and LDLR recycles back to the plasma membrane for re-utilisation (115). When free LDL reaches the LE/Lys compartments cholesterol esters are hydrolysed by the action of acid lipase (115). The free cholesterol generated is then available for transport to other sites in the cell e.g. plasma membrane, mitochondria and the ER. There is evidence that the Rab11 GTPase is involved in vesicular transport of cholesterol to the PM and MLN64 facilitates cholesterol movement to steroidogenic mitochondria (15). However, how cholesterol reaches the ER remains unclear. One possibility is that it involves direct transfer via lysosome:ER contact sites. Another possibility is cholesterol is effluxed from lysosomes by the action of a cholesterol transporter to an as yet unidentified sterol transfer protein located in the cytosol or on the cytosolic face of the limiting membrane of the

lysosome. The potential role of a cholesterol transport pathway gained support based on two lysosomal storage diseases (LSDs), Niemann-Pick Disease, type C1 (NPC1) and Niemann-Pick Disease, type C2 (NPC2)(125). They both involve the storage of multiple lipids (cholesterol and sphingolipids) in peripheral tissues and the brain, with cholesterol a prominent storage lipid in non-CNS tissues such as the liver (125). NPC1 is a multi-membrane spanning protein localised to the limiting membrane of the LE/Lys (14), whereas NPC2 is a soluble mannose-6-phosphate targeted soluble cholesterol binding protein found in the lysosol (80) and also had previously been found at high levels in epididymal fluid where it was first described (termed HE1 in that context) (64). One hypothesis that has been proposed is that NPC2 transfers cholesterol to NPC1, which then through an unknown mechanism facilitates its egress from the lysosome (52). Deficiency of NPC1 or NPC2 causes accumulation (storage) of unesterified cholesterol in Le/Lys, preventing its delivery to the ER and subsequent esterification. This in turn, leads to impaired regulation of cholesterol homeostatic genes including LDLR and HMG-CoA reductase and impairs oxysterol generation (33). NPC disease therefore paradoxically has features of storage and deficiency (129).

Reverse cholesterol transport

Cholesterol can leave cells by a process termed reverse cholesterol transport (88). ABC transporters are the key players in this process, particularly the ubiquitously expressed ABCA1 (118). Inherited defects in ABCA1 lead to Tangier disease (84) (discussed in detail below). When ABCA1 is knocked out in mice virtually no HDL is detectable in the circulation, illustrating that the function of ABCA1 is a pre-requisite for HDL formation and maintenance of circulating HDL levels (39). Tangier patients are also characterised by low plasma HDL levels (47; 58). The primary apolipoprotein of HDL is ApoA-1 and when this binds to ABCA1 it triggers cholesterol and phospholipid efflux via a poorly understood mechanism (118). These lipids are then transferred to ApoA-I to form discoidal HDL particles (Figure 2A). ABCG1 and ABCG4 then transfer additional cholesterol to nascent HDL and also to other acceptors (114; 126). Lecithin:cholesterol acyl transferase (LCAT) is an enzyme found in plasma and this esterifies cholesterol leading to the maturation to globular HDL particles (114). ABCA1 traffics between the late endocytic system and the plasma membrane and has been implicated to play a role in late endocytic trafficking. (82; 83).

Cholesterol Homeostasis: the role of “active” cholesterol

An unanswered question is how cells sense cholesterol levels and how they regulate cholesterol levels in their membranes. New evidence has recently emerged which focuses attention on “active” or “free” cholesterol. It has been known for many years that plasma membrane sterols complex with polar lipids, such as sphingolipids (6; 87). Once the binding capacity of the polar lipids is saturated the excess uncomplexed cholesterol is in an “active” or “free” state (114). These active molecules are dispersed in the membrane and have an increased ability to spontaneously escape the membrane or be chemically modified. This suggests that in this active state, cholesterol becomes exposed from the membrane and thus accessible to acceptor proteins (114). Experiments that increase PM cholesterol levels revealed enhanced transfer of cholesterol to other membranes or increased extractability by

β-cyclodextrins. This then suggests the intriguing possibility that “basal” cholesterol levels in cells are equivalent to the total binding capacity of cholesterol in a given membrane, such that there is minimal active cholesterol. If excess active cholesterol is present it will equilibrate down a concentration gradient leading to changes in homeostatic gene expression. The point of this model is that it is not total cholesterol levels that are sensed, just the fraction above the binding capacity of the membrane lipids. In support of this model is experimental evidence that has been recently reviewed (114). It has also been proposed that in reverse cholesterol transport the function of the ABC transporters, such as ABCA1, is to increase exposure of cholesterol at the PM (not fully efflux it) to facilitate collisional transfer to protein acceptors (122). This is supported by the observation that addition of ceramide to membranes displaces cholesterol and stimulates ABCA1 mediated cholesterol efflux, where as the reverse is true if sphingomyelin levels are elevated (114).

Cholesterol metabolism in the brain

As diseases of cholesterol metabolism frequently present with central nervous system (CNS) pathology it is important to consider some of the unique aspects of brain cholesterol metabolism. Unesterified cholesterol is an important component of the plasma membrane of all cells, but is present at particularly high levels in cells of the brain. It is a major component of compact myelin, which is a specialised form of the plasma membrane of oligodendrocytes. Brain cholesterol accounts for some 23% of the sterol content of the mammalian body, despite the brain being only about 2% of total body weight (25). It is distributed between myelin and the plasma membranes of neurons and glia. The main barrier to movement of metabolites into or out of the CNS is the blood-brain barrier. Based upon a variety of experimental approaches, in multiple species, there is no evidence of cholesterol moving from the plasma into the CNS, even during development when sterol levels in the brain undergo dramatic expansion (25). Instead, cholesterol is synthesised locally within the brain and, most importantly, the differential rates of synthesis mirror rates of sterol accumulation in regions of the brain measured (25). Although neurons can synthesise cholesterol they require a significant additional source of cholesterol for their function that is derived primarily from glial cells (25; 43; 72; 93). Although the details of cholesterol metabolism in the brain remains relatively poorly understood, it is, however, known that cholesterol egress from the CNS into the plasma does occur, but only in the form of 24-hydroxy cholesterol (25).

Disorders of cholesterol biosynthesis

Amongst the approximately 7000 inborn errors of metabolism are a family of diseases that result from defects in genes involved in sterol metabolism. We will focus on disorders of post-squalene cholesterol biosynthesis. All of these disorders are rare in nature, with Smith-Lemli-Optiz syndrome (SLOS) being the most common disorder with an average incidence of 1 in 50,000. In 1993 SLOS was recognized as the prototypic cholesterol biosynthesis disorder (53; 119). Many of these syndromes have corresponding mouse models; some are spontaneous mutants while others have been generated by genetic manipulation. Interestingly, all these disorders, although distinct, have overlapping phenotypes suggesting some common pathological mechanisms. We will provide a brief clinical description here

for each disease, but we refer the reader to Porter and Herman (99) who recently reviewed this topic in considerable detail.

Antley-Bixier Syndrome

Antley-Bixier Syndrome (ABS, OMIM #207410) falls in a grey zone, as there is debate as to whether pathology of this disorder is due to impaired cholesterol synthesis or impaired steroidogenesis. The gene mutated in this disorder is a cytochrome P450 oxidoreductase (*POR*). The protein acts as an electron donor to numerous P450 enzymes. One of these enzymes is the cholesterogenic C14 lanosterol demethylase. Dysregulation of this enzyme leads to the storage of 4,4-Dimethylcholesta-8(9),14-dien-3β-ol, and 4,4-Dimethylcholesta-8(9),14,24-trien-3β-ol. ABS patients present with severe craniofacial abnormalities, skeletal defects including radiohumoral synostosis, and frequently ambiguous genitalia (99).

Hydrops-Ectopic Calcification-Moth-eaten skeletal dysplasia

Hydrops-Ectopic Calcification-Moth-eaten skeletal dysplasia (HEM dysplasia OMIM #215140), also referred to as Greenberg dysplasia is a lethal skeletal dysplasia that is a potential disorder of cholesterol biosynthesis although there is debate as to whether this is a laminopathy due the dual role of lamin B receptor (LBR) (130). Initially HEM dysplasia was noted to have trace elevations of 14-diene-3β-ol, and cholesta-8(9),14,24-trien-3β-ol. (Fig. 1). The accumulation of these sterols indicated a defect at the level of the sterol delta 14 reducatse. Interestingly, within this region of the pathway this is the only enzymatic step in which redundancy exists i.e. the sterol delta 14-reductase ((TM7SF2,SR-1) and the lamin B receptor (LBR). The first case was identified as a homozygous mutation in *LBR* (134). A subsequent study identified 8 additional cases of HEM dysplasia(89).

Congenital Hemidysplasia with Ichthyosiform nevus and Limb Defects syndrome (CHILD) and CK syndrome

Congenital Hemidysplasia with Ichthyosiform nevus and Limb Defects syndrome (CHILD, OMIM #308050) and CK syndrome are two distinct yet related disorders and are commonly referred to as NSDHL-Related Disorders. Both disorders are caused by mutations in the NADH steroid dehydrogenase-like gene (*NSDHL*), while CHILD syndrome presents, as an X-linked male lethal disorder CK syndrome. NSDHL is part of a three-enzyme complex responsible for the C-4 demethalization of the sterol ring structure. Biochemically elevated levels of 4,4-dimethylcholesta-8-en-3β-ol and 4,4-dimethylcholesta-8,24-en-3β-ol (Fig. 1) have been reported with the highest aberrant sterol concentrations detected in cultured fibroblast from the affected skin lesions grown in lipoprotein deficient media. The traditional clinical presentation of CHILD syndrome is the presence of unilateral skin lesions and ipsilateral limb reductions (45). A second member of the C-4 demethalization complex, sterol C4 methyloxidase (SC4MOL) has been found disrupted in an autosomal recessive fashion in one human patient to date. The patient presented with low serum cholesterol and elevated levels of 4α-methyl-5α−cholest-8(9)-en-3β-ol; dihydrolanosterol; 4α-methyl-5a-cholest-7(8)-en-3β-ol; 4,4′-dimethyl-5α-cholesta-8(9)-en-3β-ol; 4,4′-dimethyl-5α-cholesta-8(9)-en-3β-ol; and 4,4′-dimethyl-5α-cholesta-8(9),24-dien-3β-ol all consistent with impairment of this complex. Clinical presentation began at the age of two as

ichthyosiform erythroderma around her umbilicus that has progressed to cover her whole body, sparing her palms (44).

Conradi-Hunermann syndrome

Conradi-Hunermann syndrome (CDPX2, OMIM #302960) is a second X-linked disorder that was thought to be male lethal as well although there are scattered reports of affected males presenting with mutations that preserve some enzymatic function (74). CDPX2 is caused by mutations in the gene encoding the Emopamil Binding Protein (EBP). This gene, while named for its ability to bind Emopamil, a drug developed as a calcium channel blocker, is in fact the sterol Δ7-Δ8 isomerase. As would be expected, mutations in this gene lead to the increase levels of cholesta-8(9)-en-3β-ol and Zymosterol (Fig 1). Clinical manifestations of this disorder in females involve predominantly the skin and skeleton. The skeletal findings include rhizomelic shortening, epiphyseal stippling, short stature, and scoliosis, while the skin phenotype presents as hyper or hypo pigmentation, dry scaly skin that frequently follows the lines of X-inactivation.

Lathosterolosis

Lathosterolosis (OMIM #607330) is an autosomal recessive disorder caused by mutations in the sterol 5-desaturase (SC5D) gene. SC5D is responsible for conversion of lathosterol to 7-dehydrocholesterol (Fig. 1) accomplished by desaturating the bond between C5 and C6 of the B-ring of cholesterol generating a double bond (Fig. 1). To date only 4 patients have been reported in the literature. The first case was originally misdiagnosed as a case of atypical SLOS with nonneuronal mucolipidosis (12). Subsequent biochemical and molecular testing revealed elevated levels of lathosterol and a homozygous missense mutation p.Y46S confirming the correct diagnosis of lathosterosis (59). Two patients were siblings and the forth was just reported (12; 46). All 4 patients presented with multiple malformations, several of which are also present in Smith-Lemli-Opitz syndrome (SLOS, see below) such as ptosis, congenital cataracts, anteverted nares, micronathia, postaxial polydactyly, ambiguous genitalia, cutaneous toe syndactyly, and cognitive impairment (46). Interestingly, cultured fibroblasts from all three patients under certain growth conditions developed lamellar lysosomal inclusions, similar to those seen in NPC disease. Targeted disruption of the *Sc5d* gene in mice generated a mouse model of this disease (59). Consistent with the human disorder, these mice had elevated levels of lathosterol, craniofacial malformations, postaxial polydactyly, and fibroblasts derived from these mice mimic the lamellar lysosomal inclusions seen in the human lines (59). There is no specific therapy for this disease.

Desmosterolosis

Desmosterolosis is another rare autosomal recessive disorder of cholesterol biosynthesis caused by mutations in the sterol 24-reductase (DHCR24) gene (19). It is believed that this side chain reduction can occur at many places within the Kandutsch-Russell cholesterol biosynthesis pathway (Fig. 1). These mutations lead to an increase in desmosterol levels in all tissues. Three cases of desmosterolosis have been published to date, all with divergent clinical presentations (3; 19; 29; 110; 135). DHCR24 was initially cloned as Selective Alzheimer disease indicator-1 (seladin-1). DHCR24/seladin-1 has two distinct roles in the cell. First in the endoplasmic reticulum (ER) as a member of the cholesterol biosynthetic

machinery, and second in the cytoplasm and nucleus where it serves as a hydrogen peroxide scavenger, protecting the cell from oxidative stress (60). More patients will need to be identified to determine if mutations in one region of the gene result in one phenotype versus the other. Pharmacological models of desmosterolosis have been generated using either U18666A or triparanol (7; 30). Interestingly, varying the dose of U18666A generates models of 2 distinct and yet potentially related disorders, desmosterolosis and NPC. The phenotype of the desmosterolosis mouse model however, does not replicate that of the *Npc1*$^{-/-}$ mouse model. As with lathosterolosis there is currently no specific therapy for this disease.

Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz syndrome (SLOS, OMIM #270400) is the prototypic disorder of cholesterol biosynthesis first described in 1963 by Smith, Lemli and Opitz (112). SLOS is biochemically characterized by the abnormal accumulation of 7-dehydrocholesterol (7DHC) (Fig. 1) (53). The causative gene defect is in 7-dehydrocholesterol reductase (*DHCR7*) which functions to reduce 7DHC to generate cholesterol in the final step of the Kandutsch-Russell biosynthetic pathway (98) (Fig. 1). Three groups independently cloned *DHCR7* in 1998 and mutations within the gene were proven to be the molecular basis for SLOS (28; 131; 136) (119). SLOS is more common than lathosterolosis and desmosterolosis with an incidence estimated to be between 1 in 25,000 to 1 in 60,000 (98). The carrier rate for this disease is high in the general population but does not lead to the high frequency of cases predicted based on this carrier frequency (86). This is because, at the severe end of the spectrum, SLOS can be a lethal disorder with major congenital anomalies and may indeed account for a significant number of miscarriages in the general population (86). However, mild cases combine minor physical stigmata with behavioral and learning disabilities. Typical physical manifestations include second and third toe syndactyly, microcephaly, micrognathia, cleft palate, polydactyly, cardiac malformations, pyloric stenosis, and genital malformation (4; 85; 101). Advancements in clinical management of this disorder has increase the life expectancy of these patients, however, there is currently little data to predict what additional clinical issues may arise later in the lives of these patients.

Currently, the only treatment for SLOS is dietary cholesterol supplementation (117). While there is anecdotal evidence that cholesterol supplementation benefits growth through improving overall general health of individuals with SLOS and reducing serum levels of 7-DHC, cholesterol therapy has significant limitations (98). 7DHC level elevation persists, which is not without consequence. 7DHC may have toxic effects and has been shown to substitute for cholesterol within various membranes (38) and processes, such as oxysterol production, bile acid formation (79), membrane raft formation and steroid production (71; 111) One significant limitation of cholesterol therapy is the inability of cholesterol to cross the blood-brain barrier in any appreciable amounts (24). Treating the brain in SLOS is of paramount importance as SLOS individuals present with a myriad of behavioural and learning deficits, including autistic characteristics (23; 32).

Three mouse models of SLOS have been generated to date. The first two models are null mutations, one deleting exons 3 to 5 (133) and the second deleting exons 4 to 8 (128). Both of these models recapitulate the biochemical phenotype of the SLOS patients presenting

with elevated levels of 7DHC and decreased levels of cholesterol in all tissue and serum (20). These effects are most prominent in the CNS due to the closure of the blood-brain barrier to cholesterol early on in embryonic development (70). The peripheral organs do not reach as high a level of dehydrocholesterol (combination of 7DHC and 8DHC) to cholesterol ratio as a result of some maternal cholesterol being supplied by the yolk sac and additional small amounts of cholesterol transported across the placenta (54). Both models die within twenty-four hours of birth. The mice present with cleft palate, an abnormal suck-swallow response cause by impairment of the NMDA receptors, intrauterine grow retardation and reduced mobility (128; 133). The third mouse model is a hypomorphic "knock-in" mouse model (20) where the human T93M mutation has been replicated in the mouse *Dhcr7* gene. At birth these mice also mimic the human syndrome with elevated levels of dehydrocholesterol with mild reduction in cholesterol. These mice are viable and reproduce. Phenotypically they present with two-three-toe syndactyly, the most common physical finding in SLOS, as well as minor growth retardation, and approximately one third of the mice develop ventricular dilatation by three months of age (20).

Disorder of Cholesterol Trafficking

Niemann-Pick Disease, type C

Niemann-Pick Disease, type C (NPC) is an autosomal recessive lysosomal storage disease, a feature of which is cholesterol mistrafficking (125). Free cholesterol is "stored" in Le/Lys with minimal escape of cholesterol from the acidic compartment to the ER. In addition to defects in cholesterol transport, several other lipid species are stored, including glycosphingolipids (GSLs), sphingomyelin (SM) and spingosine (generated from ceramide catabolism in LE/Lys) (124). In addition to this biochemical complexity, NPC disease is unusual in that it is caused by mutations in two independent genes, *NPC1* or *NPC2* (14; 80). NPC disease occurs at a combined frequency of 1:120,000 live births with about 95% of cases resulting from mutations in the *NPC1* gene (125). Apart from a small group of patients who die within the first months of life from hepatic or pulmonary failure, most patients present with neonatal cholestatic jaundice, which usually resolves spontaneously. Hepatosplenomegaly occurs in some cases. Relentless neurodegeneration then dominates the clinical course of the disease leading to cerebellar ataxia, dysarthria, dysphagia, dementia and premature death, typically around the end of the second decade of life (51). Juvenile and adult onset variants also occur (125).

The *NPC1* gene encodes a thirteen trans-membrane spanning protein of the limiting membrane of late endosomes/lysosomes whereas NPC2 is a soluble lysosomal cholesterol binding protein(138). At the cell biological/biochemical level two key features of this disease make it unique: 1) it has a complex profile of lipid storage 2) late endosome:lysosome fusion is profoundly impaired (123). This disorder has therefore brought to light a previously unknown cell biological pathway, the NPC pathway. As mentioned above, because unesterified cholesterol is stored in NPC disease and NPC1 and NPC2 bind and exchange cholesterol, it has led to the prevailing view that the primary function of this pathway is to facilitate cholesterol egress from the lysosome to the ER. However, an alternative hypothesis is that NPC1 functions in lysosomal sphingosine

transport (68). When NPC1 is inactivated in healthy cells the first metabolite to accumulate is sphingosine (68). Sphingosine is generated in the lysosome from the catabolism of ceramide via the action of acid ceramidase. It is protonated at acidic pH and requires a transporter to leave the lysosome. The sphingosine derived from sphingolipid catabolism in the lysosome either enters the sphingolipid salvage pathway or becomes phosphorylated to generate sphingosine-1-phosphate (S1P), a key pro-survival signaling molecule (42). There is evidence in the NPC1 mouse and in NPC patients suggesting that S1P dependent cell lineages, such as NK cells, are altered in this disease (113).

Sphingosine storage has another profound effect on cells, which is to directly or indirectly cause a defect in the filling of the acidic compartment with calcium (68). The lysosome is a regulated calcium store that is uniquely mobilized via the endogenous second messenger NAADP (18; 76). Fusion and vesicular trafficking in the endocytic pathway are calcium dependent processes and the calcium is derived from the lysosomal compartment itself (68). Failure to release sufficient calcium in NPC disease leads to a block in trafficking/fusion essential for the functioning of the endosomal/lysosomal system, causing the secondary storage of cholesterol, GSLs and sphingomyelin (68). This study of the temporal relationship of the multiple metabolites stored in NPC suggests cholesterol is a secondary storage metabolite in NPC and is not central to triggering the pathogenic cascade (68; 69).

Disorder of reverse cholesterol transport

Tangier disease

Frederickson reported the first case of Tangier disease in 1961 where he examined two siblings from Tangier Island located in the Chesapeake Bay in the USA (31). Both patients had typical symptoms now associated with the disease: orange-colored tonsils, enlarged spleen, liver, and lymph nodes, and decreased high-density lipoprotein (HDL) cholesterol levels (31; 100). While orange coloured tonsils have been described as the presenting symptom in almost all children with Tangier disease, peripheral neuropathy is a common presenting symptom in adults with Tangier disease (100). Tangier disease has been reported in approximately 100 patients worldwide (100) and is caused by mutations in the ATP binding cassette transporter protein 1 (ABCA1) (103; 107). Patients have little to no circulating HDL and accumulate cholesterol leading to the formation of foam cells, an early marker of atherosclerosis, and patients develop cardiovascular disease later in life (100; 109).

The ABC transporters are the largest known membrane transport family, consisting of 49 members divided into seven subfamilies – A through G (48; 121). The membrane associated protein ABCA1, which is defective in Tangier Disease, regulates cellular cholesterol and phospholipid homeostasis by functioning as a cholesterol efflux pump (67; 108). ABCA1 mediates the transfer of lipids across the plasma membrane to apolipoproteins, apoA-1 in particular, to form HDL particles (58), hence the low levels of HDL in Tangier disease patients. In addition to regulating cholesterol efflux, ABCA1 has also been proposed to have anti-inflammatory functions (67). ABCA1 expression is regulated at multiple levels throughout the body, with highest protein expression found in the liver, brain, adrenal glands and macrophage foam cells. Interactions between apolipoproteins and ABCA1 activate

multiple signalling pathways, including the JAK/STAT, PKA and PKC pathways (67). The c-terminus of ABCA1 contains a PDZ domain responsible for mediating protein-protein interactions, in addition to a VFVNFA motif (13). Several mutations in the ABCA1 c-terminal domain have been identified in Tangier disease patients suggesting it has a crucial function. Deletion of the VFVNFA domain also results in diminished apoA-1 binding and lipid efflux (13). Tangier disease patients have structurally abnormal late endocytic vesicles in addition to impaired motility and trafficking, which is also observed in NPC disease patient cells (8). Previous studies have shown that while ABCA1 and NPC1 may interact in the cell, the NPC1 protein is not required for delivery of LE/LY cholesterol to ABCA1 to form HDL (8). Additionally, the ABCA1 transporter may convert pools of lipids, which otherwise might associate with NPC1, to pools that can associate with apoA-1 to form HDL particles(83).

The majority of NPC patients have low HDL-cholesterol, suggesting diminished ABCA1 activity (17). NPC is the first disease to have low HDL levels as a consequence of impaired ABCA1 regulation, rather than a mutated ABCA1 protein as seen in Tangier disease (17). It is likely that the sequestration of cholesterol in LE/Lys in NPC, which leads to impaired sterol-response gene expression, is responsible for a failure to up regulate ABCA1 despite the storage of cholesterol (8). Interestingly, cholesterol mobilisation by ABCA1 is critically dependent on NPC2 but not NPC1 function (9). There is currently no specific treatment for patients with Tangier disease.

Unexpected mechanistic links between NPC, SLOS and Tangier disease

As discussed above, SLOS, NPC and Tangier are three unique inherited disorders involving very different defects in cholesterol homeostasis. SLOS is the prototypic disorder of cholesterol biosynthesis, Tangier a reverse cholesterol transport disorder and NPC a disease involving defective cholesterol trafficking, associated with an acidic store calcium defect. However, a recent serendipitous discovery has highlighted an unanticipated mechanistic link connecting these three disparate diseases, notably the presence of perturbations in the NPC pathway in all three disorders. This discovery is shedding light on convergent pathogenic mechanisms and suggesting novel approaches to therapeutic interventions in these three severe human disorders.

NPC and SLOS

The first evidence of a potential mechanistic link between NPC and SLOS came from studies with SLOS fibroblasts. In principle, SLOS cells should be correctable with cholesterol replacement therapy, as this would bypass the genetic defect in the conversion of 7DHC to cholesterol (Fig. 1). However, when SLOS patient fibroblasts were cultured in lipid-depleted medium to induce *de novo* cholesterol synthesis and by the nature of the defect elevate 7DHC, these cells exhibited a significant cholesterol trafficking defect leading to accumulation of unesterified cholesterol in LE/Lys (132). Sequestration of intracellular cholesterol in the endolysosomal compartment would decrease bioavailability of cholesterol for other cellular functions in SLOS cells (132), a cellular phenotype that superficially mimics the fate of LDL-derived cholesterol in NPC disease cells. The question posed by this

study was did this superficial similarity between these two apparently unrelated disorders suggest mechanistic convergence? The cholesterol precursor 7DHC, while structurally very similar to cholesterol has a nonplaner B ring. Could, for example, the increased levels of 7DHC be inhibiting NPC1 or NPC2 function causing exogenous cholesterol to be mis-trafficked in SLOS? It is conceivable that 7-DHC could interfere with this process by acting as an inhibitor, analogous to U18666A, a drug that induces NPC cellular phenotypes (66). If this hypothesis is correct the cell biological and biochemical features of NPC disease cells should also be present in SLOS cells, in addition to the SLOS specific accumulation of 7DHC. We therefore investigated this possibility by analyzing the cellular and biochemical hallmarks of NPC disease in SLOS patient fibroblasts that spanned the phenotypic spectrum. We found that the accumulation of 7-DHC in SLOS led to lysosomal storage of cholesterol, sphingomyelin and multiple GSLs, all hallmarks of NPC disease (69)(Table 1 and Fig. 3). Furthermore, SLOS cells had the lysosomal calcium defect identified as a unique feature of NPC disease, induced by sphingosine storage (68). This was responsible for defective transport of cholesterol out of the endocytic system (***Wassif et al, In preparation***). These defects are all down-stream of 7-DHC accumulation and this combination of phenotypes had only previously been reported in NPC disease (68). These data suggest a potential interaction between 7-DHC and the NPC1 protein or NPC2 proteins. We found an inverse correlation between residual enzyme activity (*DHCR7*) and levels of GSLs and sphingosine storage (***Wassif et al, In preparation***). Indeed, sphingosine levels in SLOS patient CSF were elevated 2 fold compared to controls, as was replicated in the brain from the full null mouse model at embryonic day 18.5. GSL's were 2.5 fold higher in SLOS CSF compared to controls but did not correlate with severity or residual enzyme activity (***Wassif et al, In preparation***).

NPC and Tangier Disease

There are several published studies linking ABCA1 expression/function to the NPC disease pathway (8; 9). These findings are consistent with the general homeostatic network that exists to regulate cholesterol trafficking and cholesterol levels in cells. If one element is perturbed it will have an impact on other pathways in the regulatory network. However, a very recent and unanticipated finding has arisen that suggests a specific mechanistic link between NPC and Tangier disease. Interestingly, this link came to light not in the laboratory but from a clinical observation following a diagnostic error. A female patient presenting with thrombocytopenia, splenomegaly, and neurological symptoms that included dysphagia and gait ataxia, was misdiagnosed as Niemann-Pick Type C (NPC) and put on the current EU approved treatment for NPC, miglustat (92; 137). After 4 months of treatment with 300 mg/day of miglustat the patient demonstrated improvement with respect to neurological symptoms (***Sechi et al, In Preparation***). The patient was subsequently diagnosed with Tangier disease after molecular testing of NPC1 and NPC2 failed to support the NPC diagnosis. Although the neurological symptoms in Tangier disease can be relapsing and remitting, the correlation of symptoms with miglustat therapy was intriguing and suggested a potential mechanistic convergence between Tangier disease and Niemann-Pick Disease, type C. This potential convergence was further explored. NPC disease is characterised at the cellular level by storage of GSLs, fatty acids, cholesterol, sphingomyelin and sphingosine. NPC cells also have low levels of calcium in LE/Lys. All of these cellular hallmarks were

found in the Tangier disease patient cells *(Colaco et al, In Preparation)* (Table 1) suggesting that a consequence of the loss of function of ABCA1, through an as yet unknown mechanism, inhibits the NPC pathway. When gene expression levels of *NPC1* and *NPC2* were investigated in Tangier disease fibroblasts NPC2 was found to be upregulated two fold suggesting that its function may be in some way impaired in response to ABCA1 dysfunction ***(Colaco et al, In Preparation)***.

An expanded application of miglustat from NPC to SLOS and Tangier?

Miglustat is an imino sugar drug that inhibits glucosylceramide synthase, the enzyme, which catalyses the first step in GSL biosynthesis (95; 96). This orally available drug can therefore be used to pharmacologically inhibit the biosynthesis of GSLs for treating lysosomal storage diseases that store GSLs as primary or secondary metabolites (95). Miglustat was approved by the EMEA in 2002 and by the FDA in 2003 for treating type 1 Gaucher disease(21; 61). Gaucher disease results from an inherited defect in glucocerebrosidase leading to the storage of glucosylceramide in the lysosome. Miglustat reduces the number of GSL molecules synthesised by cells so fewer require degrading in the lysosome, allowing the rate of biosynthesis to better match the impaired rate of catabolism (62; 95). As the drug crosses the blood-brain barrier it also has the potential to treat CNS manifestations of LSDs involving GSL storage (27; 55; 97). Miglustat was tested in a mouse model of NPC1 disease and efficacy was demonstrated (140) and was subsequently approved by the EMA for treating NPC disease, following efficacy being demonstrated in an international clinical trial (92). Indeed it is now approved in most countries world-wide, with the exception of the USA (91).

Our findings that SLOS and Tangier disease involve secondary inhibition of the NPC pathway (Table 1) suggest that miglustat could be a potential therapy for these diseases, in addition to NPC. Images of wild type, NPC1, SLOS and Tangier disease patient fibroblasts are shown in Fig. 3 to illustrate cholesterol storage in the late endocytic compartment is a common feature of all three diseases. To date miglustat treatment of cultured SLOS cells caused a normalisation of cholesterol trafficking with cholesterol being delivered to the ER. In SLOS there are both fixed developmental abnormalities and functional deficits due to altered sterol membrane composition. Functional abnormalities in SLOS result from both a cholesterol deficiency and toxicity of elevated dehydrocholesterol levels. Current therapeutic approaches involve dietary provision of cholesterol and upregulation DHCR7 activity to increase endogenous cholesterol synthesis. Intracellular endolysosomal sequestration of cholesterol in SLOS will further compound the functional cholesterol deficiency and by limiting the intracellular bioavailability of cholesterol the NPC-like cellular phenotype may limit therapeutic efficacy of potential therapies. Miglustat treatment of SLOS may therefore provide correction of the NPC phenotypes allowing cholesterol trafficking to normalise, thus providing potentially more benefit from cholesterol supplementation. In addition, as miglustat crosses the blood-brain barrier it has the potential to improve cell biological defects associated with inhibition of the NPC pathway that may improve CNS function. Regarding Tangier disease, patient cells treated with miglustat showed biochemical and cell biological correction suggesting that partial correction of the defective NPC pathway may be of therapeutic benefit consistent with a case report suggesting that miglustat may have improved symptoms in a Tangier patient (*Colaco et al, In Preparation*).

Concluding Remarks

Inborn errors of cholesterol metabolism have provided many fundamental insights into normal cholesterol homeostasis and cell biology over several decades. They have been viewed as discreet diseases with their own unique genetic, biochemical and cell biological consequences that are in turn responsible for the clinical spectrum of symptoms associated with each disease. What has been surprising is that at least three of these diseases; SLOS, NPC and Tangier, share a common pathological hallmark at the cellular level, namely inhibition of the NPC pathway. The precise mechanism causing inhibition of the NPC pathway in SLOS and Tangier remain to be fully elucidated. However, these findings are suggesting novel therapeutic approaches to treating SLOS and Tangier using drugs such as miglustat that modify the cell biology of NPC disease. Miglustat has already been "inadvertently" tested in one case of Tangier disease suggesting that this may represent a novel therapeutic approach for this currently untreatable disease. Clinical trials of miglustat in SLOS are currently being planned based on the convergent mechanism of pathogenesis shared with NPC and Tangier disease. It remains to be determined whether other human diseases also involve NPC pathway dysfunction. This is currently under investigation as it may pave the way for novel approaches to therapy for diseases that currently lack effective treatments using approved NPC disease therapeutics.

Acknowledgements

We thank Nick Platt for commenting on the manuscript. FMP is a Royal Society Wolfson Research Merit Award holder. The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7 2007 – 2013) under grant agreement n^{o} 289278 - "Sphingonet". (AC). This work was supported, in part by the intramural research program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development and a Bench to Bedside grant supported by the Office of Rare Diseases and NIH Clinical Center (FDP). CW is supported by the National Institutes of Health Oxford-Cambridge Scholars Program. ELE is an RCUK fellow.

References

1. Anderson RG, Brown MS, Goldstein JL. 1977 Role of the coated endocytic vesicle in the uptake of receptor-bound low density lipoprotein in human fibroblasts. Cell 10:351–64 [PubMed: 191195]
2. Anderson RG, Goldstein JL, Brown MS. 1976 Localization of low density lipoprotein receptors on plasma membrane of normal human fibroblasts and their absence in cells from a familial hypercholesterolemia homozygote. Proc Natl Acad Sci U S A 73:2434–8 [PubMed: 181751]
3. Andersson HC, Kratz L, Kelley R. 2002 Desmosterolosis presenting with multiple congenital anomalies and profound developmental delay. Am J Med Genet 113:315–9 [PubMed: 12457401]
4. Battaile KP, Steiner RD. 2000 Smith-Lemli-Opitz syndrome: the first malformation syndrome associated with defective cholesterol synthesis. Mol Genet Metab 71:154–62 [PubMed: 11001806]
5. Bhatti HN, Khera RA. 2012 Biological transformations of steroidal compounds: A review. Steroids 77:1267–90 [PubMed: 22910289]
6. Bjorkbom A, Rog T, Kaszuba K, Kurita M, Yamaguchi S, et al. 2010 Effect of sphingomyelin headgroup size on molecular properties and interactions with cholesterol. Biophysical journal 99:3300–8 [PubMed: 21081078]
7. Blohm TR, Stevens VL, Kariya T, Alig HN. 1970 Effects of clomiphene cis and trans isomers on sterol metabolism in the rat. Biochem Pharmacol 19:2231–41 [PubMed: 5520371]
8. Boadu E, Francis GA. 2006 The role of vesicular transport in ABCA1-dependent lipid efflux and its connection with NPC pathways. J Mol Med (Berl) 84:266–75 [PubMed: 16328207]

9. Boadu E, Nelson RC, Francis GA. 2012 ABCA1-dependent mobilization of lysosomal cholesterol requires functional Niemann-Pick C2 but not Niemann-Pick C1 protein. Biochim Biophys Acta 1821:396–404 [PubMed: 22179027]
10. Briscoe J, Therond PP. 2013 The mechanisms of Hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol 14:416–29 [PubMed: 23719536]
11. Brown MS, Goldstein JL. 1975 Regulation of the activity of the low density lipoprotein receptor in human fibroblasts. Cell 6:307–16 [PubMed: 212203]
12. Brunetti-Pierri N, Corso G, Rossi M, Ferrari P, Balli F, et al. 2002 Lathosterolosis, a novel multiple-malformation/mental retardation syndrome due to deficiency of 3beta-hydroxysteroid-delta5-desaturase. Am J Hum Genet 71:952–8 [PubMed: 12189593]
13. Brunham LR, Singaraja RR, Hayden MR. 2006 Variations on a gene: rare and common variants in ABCA1 and their impact on HDL cholesterol levels and atherosclerosis. Annual review of nutrition 26:105–29
14. Carstea ED, Morris JA, Coleman KG, Loftus SK, Zhang D, et al. 1997 Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. Science 277:228–31 [PubMed: 9211849]
15. Charman M, Kennedy BE, Osborne N, Karten B. 2010 MLN64 mediates egress of cholesterol from endosomes to mitochondria in the absence of functional Niemann-Pick Type C1 protein. J Lipid Res 51:1023–34 [PubMed: 19965586]
16. Chen LL, Wang GZ, Zhang HY. 2007 Sterol biosynthesis and prokaryotes-to-eukaryotes evolution. Biochem Biophys Res Commun 363:885–8 [PubMed: 17923113]
17. Choi HY, Karten B, Chan T, Vance JE, Greer WL, et al. 2003 Impaired ABCA1-dependent lipid efflux and hypoalphalipoproteinemia in human Niemann-Pick type C disease. J Biol Chem 278:32569–77 [PubMed: 12813037]
18. Churchill GC, Okada Y, Thomas JM, Genazzani AA, Patel S, Galione A. 2002 NAADP mobilizes Ca(2+) from reserve granules, lysosome-related organelles, in sea urchin eggs. Cell 111:703–8 [PubMed: 12464181]
19. Clayton P, Mills K, Keeling J, FitzPatrick D. 1996 Desmosterolosis: a new inborn error of cholesterol biosynthesis. Lancet 348:404
20. Correa-Cerro LS, Wassif CA, Kratz L, Miller GF, Munasinghe JP, et al. 2006 Development and characterization of a hypomorphic Smith-Lemli-Opitz syndrome mouse model and efficacy of simvastatin therapy. Hum Mol Genet 15:839–51 [PubMed: 16446309]
21. Cox T, Lachmann R, Hollak C, Aerts J, van Weely S, et al. 2000 Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. Lancet 355:1481–5 [PubMed: 10801168]
22. Davies BS, Rine J. 2006 A role for sterol levels in oxygen sensing in Saccharomyces cerevisiae. Genetics 174:191–201 [PubMed: 16783004]
23. Diaz-Stransky A, Tierney E. 2012 Cognitive and behavioral aspects of Smith-Lemli-Opitz syndrome. American journal of medical genetics. Part C, Seminars in medical genetics 160C:295–300
24. Dietschy JM, Turley SD. 2001 Cholesterol metabolism in the brain. Curr Opin Lipidol 12:105–12. [PubMed: 11264981]
25. Dietschy JM, Turley SD. 2004 Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. J Lipid Res 45:1375–97 [PubMed: 15254070]
26. Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, et al. 2009 Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. Frontiers in neuroendocrinology 30:259–301 [PubMed: 19505496]
27. Elliot-Smith E, Speak AO, Lloyd-Evans E, Smith DA, Spoel AC, et al. 2008 Beneficial effects of substrate reduction therapy in a mouse model of GM1 gangliosidosis. Mol Genet Metab 94:204–11 [PubMed: 18387328]
28. Fitzky BU, Witsch-Baumgartner M, Erdel M, Lee JN, Paik YK, et al. 1998 Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome. Proc Natl Acad Sci U S A 95:8181–6 [PubMed: 9653161]

29. FitzPatrick DR, Keeling JW, Evans MJ, Kan AE, Bell JE, et al. 1998 Clinical phenotype of desmosterolosis. Am J Med Genet 75:145–52 [PubMed: 9450875]
30. Fliesler SJ, Richards MJ, Miller C, Peachey NS, Cenedella RJ. 2000 Retinal structure and function in an animal model that replicates the biochemical hallmarks of desmosterolosis. Neurochem Res 25:685–94 [PubMed: 10905631]
31. Fredrickson DS. 1964 The Inheritance of High Density Lipoprotein Deficiency (Tangier Disease). J Clin Invest 43:228–36 [PubMed: 14162531]
32. Freeman KA, Eagle R, Merkens LS, Sikora D, Pettit-Kekel K, et al. 2013 Challenging behavior in Smith-Lemli-Opitz syndrome: initial test of biobehavioral influences. Cognitive and behavioral neurology : official journal of the Society for Behavioral and Cognitive Neurology 26:23–9 [PubMed: 23538569]
33. Frolov A, Zielinski SE, Crowley JR, Dudley-Rucker N, Schaffer JE, Ory DS. 2003 NPC1 and NPC2 regulate cellular cholesterol homeostasis through generation of low density lipoprotein cholesterol-derived oxysterols. J Biol Chem 278:25517–25 [PubMed: 12719428]
34. Galea AM, Brown AJ. 2009 Special relationship between sterols and oxygen: were sterols an adaptation to aerobic life? Free radical biology & medicine 47:880–9 [PubMed: 19559787]
35. Gibbons GF. 2002 From gallstones to genes: two hundred years of sterol research. A tribute to George J. Schroepfer Jr. Lipids 37:1153–62 [PubMed: 12617469]
36. Goedeke L, Fernandez-Hernando C. 2012 Regulation of cholesterol homeostasis. Cell Mol Life Sci 69:915–30 [PubMed: 22009455]
37. Goldstein JL, Brown MS. 1974 Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. J Biol Chem 249:5153–62 [PubMed: 4368448]
38. Gondre-Lewis MC, Petrache HI, Wassif CA, Harries D, Parsegian A, et al. 2006 Abnormal sterols in cholesterol-deficiency diseases cause secretory granule malformation and decreased membrane curvature. J Cell Sci 119:1876–85 [PubMed: 16636072]
39. Groen AK, Bloks VW, Bandsma RH, Ottenhoff R, Chimini G, Kuipers F. 2001 Hepatobiliary cholesterol transport is not impaired in Abca1-null mice lacking HDL. J Clin Invest 108:843–50 [PubMed: 11560953]
40. Guan XL, Souza CM, Pichler H, Dewhurst G, Schaad O, et al. 2009 Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. Mol Biol Cell 20:2083–95 [PubMed: 19225153]
41. Gulati S, Liu Y, Munkacsi AB, Wilcox L, Sturley SL. 2010 Sterols and sphingolipids: dynamic duo or partners in crime? Progress in lipid research 49:353–65 [PubMed: 20362613]
42. Hannun YA, Obeid LM. 2008 Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol 9:139–50 [PubMed: 18216770]
43. Hayashi H, Campenot RB, Vance DE, Vance JE. 2004 Glial lipoproteins stimulate axon growth of central nervous system neurons in compartmented cultures. J Biol Chem 279:14009–15 [PubMed: 14709547]
44. He M, Kratz LE, Michel JJ, Vallejo AN, Ferris L, et al. 2011 Mutations in the human SC4MOL gene encoding a methyl sterol oxidase cause psoriasiform dermatitis, microcephaly, and developmental delay. J Clin Invest 121:976–84 [PubMed: 21285510]
45. Herman GE. 2000 X-Linked dominant disorders of cholesterol biosynthesis in man and mouse. Biochim Biophys Acta 1529:357–73 [PubMed: 11111102]
46. Ho AC, Fung CW, Siu TS, Ma OC, Lam CW, et al. 2013 Lathosterolosis: A Disorder of Cholesterol Biosynthesis Resembling Smith-Lemli-Opitz Syndrome. JIMD reports
47. Hoffman HN, Fredrickson DS. 1965 Tangier disease (familial high density lipoprotein deficiency). Clinical and genetic features in two adults. Am J Med 39:582–93 [PubMed: 5831900]
48. Holland KA, Holland IB. 2005 Adventures with ABC-proteins: highly conserved ATP-dependent transporters. Acta Microbiol Immunol Hung 52:309–22 [PubMed: 16400872]
49. Hughes AL, Todd BL, Espenshade PJ. 2005 SREBP pathway responds to sterols and functions as an oxygen sensor in fission yeast. Cell 120:831–42 [PubMed: 15797383]
50. Huynh KK, Gershenzon E, Grinstein S. 2008 Cholesterol accumulation by macrophages impairs phagosome maturation. J Biol Chem 283:35745–55 [PubMed: 18955491]

51. Imrie J, Dasgupta S, Besley GT, Harris C, Heptinstall L, et al. 2007 The natural history of Niemann-Pick disease type C in the UK. J Inherit Metab Dis 30:51–9 [PubMed: 17160617]
52. Infante RE, Wang ML, Radhakrishnan A, Kwon HJ, Brown MS, Goldstein JL. 2008 NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid bilayers, a step in cholesterol egress from lysosomes. Proc Natl Acad Sci U S A 105:15287–92 [PubMed: 18772377]
53. Irons M, Elias ER, Salen G, Tint GS, Batta AK. 1993 Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. Lancet 341:1414
54. Jenkins KT, Merkens LS, Tubb MR, Myatt L, Davidson WS, et al. 2008 Enhanced placental cholesterol efflux by fetal HDL in Smith-Lemli-Opitz syndrome. Mol Genet Metab 94:240–7 [PubMed: 18346920]
55. Jeyakumar M, Butters TD, Cortina-Borja M, Hunnam V, Proia RL, et al. 1999 Delayed symptom onset and increased life expectancy in Sandhoff disease mice treated with N-butyldeoxynojirimycin. Proc Natl Acad Sci U S A 96:6388–93 [PubMed: 10339597]
56. Jurevics H, Morell P. 1995 Cholesterol for synthesis of myelin is made locally, not imported into brain. J Neurochem 64:895–901 [PubMed: 7830084]
57. Khachadurian AK. 1964 The Inheritance of Essential Familial Hypercholesterolemia. Am J Med 37:402–7 [PubMed: 14209286]
58. Kolovou GD, Mikhailidis DP, Anagnostopoulou KK, Daskalopoulou SS, Cokkinos DV. 2006 Tangier disease four decades of research: a reflection of the importance of HDL. Curr Med Chem 13:771–82 [PubMed: 16611066]
59. Krakowiak PA, Wassif CA, Kratz L, Cozma D, Kovarova M, et al. 2003 Lathosterolosis: an inborn error of human and murine cholesterol synthesis due to lathosterol 5-desaturase deficiency. Hum Mol Genet 12:1631–41 [PubMed: 12812989]
60. Kuehnle K, Crameri A, Kalin RE, Luciani P, Benvenuti S, et al. 2008 Prosurvival effect of DHCR24/Seladin-1 in acute and chronic responses to oxidative stress. Mol Cell Biol 28:539–50 [PubMed: 17984220]
61. Lachmann RH. 2003 Miglustat. Oxford GlycoSciences/Actelion. Curr Opin Investig Drugs 4:472–9
62. Lachmann RH. 2006 Miglustat: substrate reduction therapy for glycosphingolipid lysosomal storage disorders. Drugs Today (Barc) 42:29–38
63. Lamb DC, Jackson CJ, Warrilow AG, Manning NJ, Kelly DE, Kelly SL. 2007 Lanosterol biosynthesis in the prokaryote Methylococcus capsulatus: insight into the evolution of sterol biosynthesis. Molecular biology and evolution 24:1714–21 [PubMed: 17567593]
64. Legare C, Thabet M, Gatti JL, Sullivan R. 2006 HE1/NPC2 status in human reproductive tract and ejaculated spermatozoa: consequence of vasectomy. Molecular human reproduction 12:461–8 [PubMed: 16772431]
65. Lemaire-Ewing S, Lagrost L, Neel D. 2012 Lipid rafts: a signalling platform linking lipoprotein metabolism to atherogenesis. Atherosclerosis 221:303–10 [PubMed: 22071358]
66. Liscum L, Faust JR. 1989 The intracellular transport of low density lipoprotein-derived cholesterol is inhibited in Chinese hamster ovary cells cultured with 3-beta-[2-(diethylamino)ethoxy]androst-5-en-17-one. J Biol Chem 264:11796–806 [PubMed: 2745416]
67. Liu Y, Tang C. 2012 Regulation of ABCA1 functions by signaling pathways. Biochim Biophys Acta 1821:522–9 [PubMed: 21920460]
68. Lloyd-Evans E, Morgan AJ, He X, Smith DA, Elliot-Smith E, et al. 2008 Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. Nat Med 14:1247–55 [PubMed: 18953351]
69. Lloyd-Evans E, Platt FM. 2010 Lipids on trial: the search for the offending metabolite in Niemann-Pick type C disease. Traffic 11:419–28 [PubMed: 20059748]
70. Lossinsky AS, Shivers RR. 2004 Structural pathways for macromolecular and cellular transport across the blood-brain barrier during inflammatory conditions. Review. Histology and histopathology 19:535–64 [PubMed: 15024715]
71. Marcos J, Guo LW, Wilson WK, Porter FD, Shackleton C. 2004 The implications of 7-dehydrosterol-7-reductase deficiency (Smith-Lemli-Opitz syndrome) to neurosteroid production. Steroids 69:51–60 [PubMed: 14715377]

72. Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, et al. 2001 CNS synaptogenesis promoted by glia-derived cholesterol. Science 294:1354–7 [PubMed: 11701931]
73. McLean KJ, Hans M, Munro AW. 2012 Cholesterol, an essential molecule: diverse roles involving cytochrome P450 enzymes. Biochem Soc Trans 40:587–93 [PubMed: 22616871]
74. Milunsky JM, Maher TA, Metzenberg AB. 2003 Molecular, biochemical, and phenotypic analysis of a hemizygous male with a severe atypical phenotype for X-linked dominant Conradi-Hunermann-Happle syndrome and a mutation in EBP. Am J Med Genet A 116A:249–54 [PubMed: 12503101]
75. Mineo C, Shaul PW. 2012 Functions of scavenger receptor class B, type I in atherosclerosis. Curr Opin Lipidol 23:487–93 [PubMed: 22907331]
76. Morgan AJ, Platt FM, Lloyd-Evans E, Galione A. 2011 Molecular mechanisms of endolysosomal Ca2+ signalling in health and disease. Biochem J 439:349–74 [PubMed: 21992097]
77. Muller C 1938 Xanthomata, hypercholesterolemia, angina pectoris. Acta Med Scand. 89:75–84
78. Munro S 2003 Lipid rafts: elusive or illusive? Cell 115:377–88 [PubMed: 14622593]
79. Natowicz MR, Evans JE. 1994 Abnormal bile acids in the Smith-Lemli-Opitz syndrome. Am J Med Genet 50:364–7 [PubMed: 8209917]
80. Naureckiene S, Sleat DE, Lackland H, Fensom A, Vanier MT, et al. 2000 Identification of HE1 as the second gene of Niemann-Pick C disease. Science 290:2298–301 [PubMed: 11125141]
81. Nes WD. 2011 Biosynthesis of cholesterol and other sterols. Chem Rev 111:6423–51 [PubMed: 21902244]
82. Neufeld EB, Remaley AT, Demosky SJ, Stonik JA, Cooney AM, et al. 2001 Cellular localization and trafficking of the human ABCA1 transporter. J Biol Chem 276:27584–90 [PubMed: 11349133]
83. Neufeld EB, Stonik JA, Demosky SJ, Jr., Knapper CL, Combs CA, et al. 2004 The ABCA1 transporter modulates late endocytic trafficking: insights from the correction of the genetic defect in Tangier disease. J Biol Chem 279:15571–8 [PubMed: 14747463]
84. Nofer JR, Remaley AT. 2005 Tangier disease: still more questions than answers. Cell Mol Life Sci 62:2150–60 [PubMed: 16235041]
85. Nowaczyk MJ, Tan M, Hamid JS, Allanson JE. 2012 Smith-Lemli-Opitz syndrome: Objective assessment of facial phenotype. Am J Med Genet A 158A:1020–8 [PubMed: 22438180]
86. Nowaczyk MJ, Waye JS, Douketis JD. 2006 DHCR7 mutation carrier rates and prevalence of the RSH/Smith-Lemli-Opitz syndrome: where are the patients? Am J Med Genet A 140:2057–62 [PubMed: 16906538]
87. Ohvo-Rekila H, Ramstedt B, Leppimaki P, Slotte JP. 2002 Cholesterol interactions with phospholipids in membranes. Progress in lipid research 41:66–97 [PubMed: 11694269]
88. Ono K 2012 Current concept of reverse cholesterol transport and novel strategy for atheroprotection. Journal of cardiology 60:339–43 [PubMed: 23063661]
89. Oosterwijk JC, Mansour S, van Noort G, Waterham HR, Hall CM, Hennekam RC. 2003 Congenital abnormalities reported in Pelger-Huet homozygosity as compared to Greenberg/HEM dysplasia: highly variable expression of allelic phenotypes. J Med Genet 40:937–41 [PubMed: 14684694]
90. Oram JF. 2002 Molecular basis of cholesterol homeostasis: lessons from Tangier disease and ABCA1. Trends Mol Med 8:168–73 [PubMed: 11927274]
91. Patterson MC, Hendriksz CJ, Walterfang M, Sedel F, Vanier MT, Wijburg F. 2012 Recommendations for the diagnosis and management of Niemann-Pick disease type C: An update. Mol Genet Metab 106:330–44 [PubMed: 22572546]
92. Patterson MC, Vecchio D, Prady H, Abel L, Wraith JE. 2007 Miglustat for treatment of Niemann-Pick C disease: a randomised controlled study. Lancet neurology 6:765–72 [PubMed: 17689147]
93. Pfrieger FW. 2003 Outsourcing in the brain: do neurons depend on cholesterol delivery by astrocytes? Bioessays 25:72–8 [PubMed: 12508285]
94. Pike LJ. 2004 Lipid rafts: heterogeneity on the high seas. Biochem J 378:281–92 [PubMed: 14662007]
95. Platt FM, Jeyakumar M. 2008 Substrate reduction therapy. Acta Paediatr Suppl 97:88–93

96. Platt FM, Neises GR, Dwek RA, Butters TD. 1994 N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. J Biol Chem 269:8362–5 [PubMed: 8132559]

97. Platt FM, Neises GR, Reinkensmeier G, Townsend MJ, Perry VH, et al. 1997 Prevention of lysosomal storage in Tay-Sachs mice treated with N-butyldeoxynojirimycin. Science 276:428–31 [PubMed: 9103204]

98. Porter FD. 2008 Smith-Lemli-Opitz syndrome: pathogenesis, diagnosis and management. Eur J Hum Genet 16:535–41 [PubMed: 18285838]

99. Porter FD, Herman GE. 2011 Malformation syndromes caused by disorders of cholesterol synthesis. J Lipid Res 52:6–34 [PubMed: 20929975]

100. Puntoni M, Sbrana F, Bigazzi F, Sampietro T. 2012 Tangier disease: epidemiology, pathophysiology, and management. American journal of cardiovascular drugs : drugs, devices, and other interventions 12:303–11

101. Quelin C, Loget P, Verloes A, Bazin A, Bessieres B, et al. 2012 Phenotypic spectrum of fetal Smith-Lemli-Opitz syndrome. European journal of medical genetics 55:81–90 [PubMed: 22226660]

102. Reeves VL, Thomas CM, Smart EJ. 2012 Lipid rafts, caveolae and GPI-linked proteins. Adv Exp Med Biol 729:3–13 [PubMed: 22411310]

103. Remaley AT, Rust S, Rosier M, Knapper C, Naudin L, et al. 1999 Human ATP-binding cassette transporter 1 (ABC1): genomic organization and identification of the genetic defect in the original Tangier disease kindred. Proc Natl Acad Sci U S A 96:12685–90 [PubMed: 10535983]

104. Riobo NA. 2012 Cholesterol and its derivatives in Sonic Hedgehog signaling and cancer. Current opinion in pharmacology

105. Rosenfeld E, Beauvoit B, Blondin B, Salmon JM. 2003 Oxygen consumption by anaerobic Saccharomyces cerevisiae under enological conditions: effect on fermentation kinetics. Applied and environmental microbiology 69:113–21 [PubMed: 12513985]

106. Russell DW. 2009 Fifty years of advances in bile acid synthesis and metabolism. J Lipid Res 50 Suppl:S120–5 [PubMed: 18815433]

107. Rust S, Rosier M, Funke H, Real J, Amoura Z, et al. 1999 Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 22:352–5 [PubMed: 10431238]

108. Sahoo D, Trischuk TC, Chan T, Drover VA, Ho S, et al. 2004 ABCA1-dependent lipid efflux to apolipoprotein A-I mediates HDL particle formation and decreases VLDL secretion from murine hepatocytes. J Lipid Res 45:1122–31 [PubMed: 14993246]

109. Sampietro T, Puntoni M, Bigazzi F, Pennato B, Sbrana F, et al. 2009 Images in cardiovascular medicine. Tangier disease in severely progressive coronary and peripheral artery disease. Circulation 119:2741–2 [PubMed: 19470903]

110. Schaaf CP, Koster J, Katsonis P, Kratz L, Shchelochkov OA, et al. 2011 Desmosterolosis-phenotypic and molecular characterization of a third case and review of the literature. Am J Med Genet A 155A:1597–604 [PubMed: 21671375]

111. Shackleton CH. 2012 Role of a disordered steroid metabolome in the elucidation of sterol and steroid biosynthesis. Lipids 47:1–12 [PubMed: 21874273]

112. Smith DW, Lemli L, Opitz JM. 1964 A Newly Recognized Syndrome of Multiple Congenital Anomalies. J Pediatr 64:210–7 [PubMed: 14119520]

113. Speak AO, Te Vruchte D, Davis LC, Morgan AJ, Smith DA, et al. 2013 Altered distribution and function of natural killer cells in murine and human Niemann-Pick disease type C1. Blood

114. Steck TL, Lange Y. 2010 Cell cholesterol homeostasis: mediation by active cholesterol. Trends Cell Biol 20:680–7 [PubMed: 20843692]

115. Strickland DK, Gonias SL, Argraves WS. 2002 Diverse roles for the LDL receptor family. Trends in endocrinology and metabolism: TEM 13:66–74 [PubMed: 11854021]

116. Summons RE, Bradley AS, Jahnke LL, Waldbauer JR. 2006 Steroids, triterpenoids and molecular oxygen. Philos Trans R Soc Lond B Biol Sci 361:951–68 [PubMed: 16754609]

117. Svoboda MD, Christie JM, Eroglu Y, Freeman KA, Steiner RD. 2012 Treatment of Smith-Lemli-Opitz syndrome and other sterol disorders. American journal of medical genetics. Part C, Seminars in medical genetics 160C:285–94

118. Takahashi K, Kimura Y, Nagata K, Yamamoto A, Matsuo M, Ueda K. 2005 ABC proteins: key molecules for lipid homeostasis. Medical molecular morphology 38:2–12 [PubMed: 16158173]
119. Tint GS, Irons M, Elias ER, Batta AK, Frieden R, et al. 1994 Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. N Engl J Med 330:107–13 [PubMed: 8259166]
120. Tsutsui K 2012 Neurosteroid biosynthesis and action during cerebellar development. Cerebellum 11:414–5 [PubMed: 22198872]
121. Tusnady GE, Sarkadi B, Simon I, Varadi A. 2006 Membrane topology of human ABC proteins. FEBS Lett 580:1017–22 [PubMed: 16337630]
122. van Meer G, Halter D, Sprong H, Somerharju P, Egmond MR. 2006 ABC lipid transporters: extruders, flippases, or flopless activators? FEBS Lett 580:1171–7 [PubMed: 16376334]
123. Vanier MT. Niemann-Pick disease type C. Orphanet J Rare Dis 5:16 [PubMed: 20525256]
124. Vanier MT. 1999 Lipid changes in Niemann-Pick disease type C brain: personal experience and review of the literature. Neurochem Res 24:481–9. [PubMed: 10227680]
125. Vanier MT. 2010 Niemann-Pick disease type C. Orphanet J Rare Dis 5:16 [PubMed: 20525256]
126. Vaughan AM, Oram JF. 2006 ABCA1 and ABCG1 or ABCG4 act sequentially to remove cellular cholesterol and generate cholesterol-rich HDL. J Lipid Res 47:2433–43 [PubMed: 16902247]
127. Verheijen MH, Camargo N, Verdier V, Nadra K, de Preux Charles AS, et al. 2009 SCAP is required for timely and proper myelin membrane synthesis. Proc Natl Acad Sci U S A 106:21383–8 [PubMed: 19948958]
128. Waage-Baudet H, Lauder JM, Dehart DB, Kluckman K, Hiller S, et al. 2003 Abnormal serotonergic development in a mouse model for the Smith-Lemli-Opitz syndrome: implications for autism. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 21:451–9 [PubMed: 14659996]
129. Walkley SU. 2009 Pathogenic cascades in lysosomal disease-Why so complex? J Inherit Metab Dis 32:181–9 [PubMed: 19130290]
130. Wassif CA, Brownson KE, Sterner AL, Forlino A, Zerfas PM, et al. 2007 HEM dysplasia and ichthyosis are likely laminopathies and not due to 3beta-hydroxysterol Delta14-reductase deficiency. Hum Mol Genet 16:1176–87 [PubMed: 17403717]
131. Wassif CA, Maslen C, Kachilele-Linjewile S, Lin D, Linck LM, et al. 1998 Mutations in the human sterol delta7-reductase gene at 11q12–13 cause Smith-Lemli-Opitz syndrome. Am J Hum Genet 63:55–62 [PubMed: 9634533]
132. Wassif CA, Vied D, Tsokos M, Connor WE, Steiner RD, Porter FD. 2002 Cholesterol storage defect in RSH/Smith-Lemli-Opitz syndrome fibroblasts. Mol Genet Metab 75:325–34 [PubMed: 12051964]
133. Wassif CA, Zhu P, Kratz L, Krakowiak PA, Battaile KP, et al. 2001 Biochemical, phenotypic and neurophysiological characterization of a genetic mouse model of RSH/Smith--Lemli--Opitz syndrome. Hum Mol Genet 10:555–64 [PubMed: 11230174]
134. Waterham HR, Koster J, Mooyer P, Noort Gv G, Kelley RI, et al. 2003 Autosomal recessive HEM/Greenberg skeletal dysplasia is caused by 3 beta-hydroxysterol delta 14-reductase deficiency due to mutations in the lamin B receptor gene. Am J Hum Genet 72:1013–7 [PubMed: 12618959]
135. Waterham HR, Koster J, Romeijn GJ, Hennekam RC, Vreken P, et al. 2001 Mutations in the 3beta-hydroxysterol Delta24-reductase gene cause desmosterolosis, an autosomal recessive disorder of cholesterol biosynthesis. Am J Hum Genet 69:685–94 [PubMed: 11519011]
136. Waterham HR, Wijburg FA, Hennekam RC, Vreken P, Poll-The BT, et al. 1998 Smith-Lemli-Opitz syndrome is caused by mutations in the 7-dehydrocholesterol reductase gene. Am J Hum Genet 63:329–38 [PubMed: 9683613]
137. Wraith JE, Imrie J. 2009 New therapies in the management of Niemann-Pick type C disease: clinical utility of miglustat. Therapeutics and clinical risk management 5:877–87 [PubMed: 19956552]
138. Xu S, Benoff B, Liou HL, Lobel P, Stock AM. 2007 Structural basis of sterol binding by NPC2, a lysosomal protein deficient in Niemann-Pick type C2 disease. J Biol Chem 282:23525–31 [PubMed: 17573352]

139. Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, et al. 1984 The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell 39:27–38 [PubMed: 6091915]

140. Zervas M, Somers KL, Thrall MA, Walkley SU. 2001 Critical role for glycosphingolipids in Niemann-Pick disease type C. Curr Biol 11:1283–7 [PubMed: 11525744]

141. Zhu R, Ou Z, Ruan X, Gong J. 2012 Role of liver X receptors in cholesterol efflux and inflammatory signaling (review). Molecular medicine reports 5:895–900 [PubMed: 22267249]

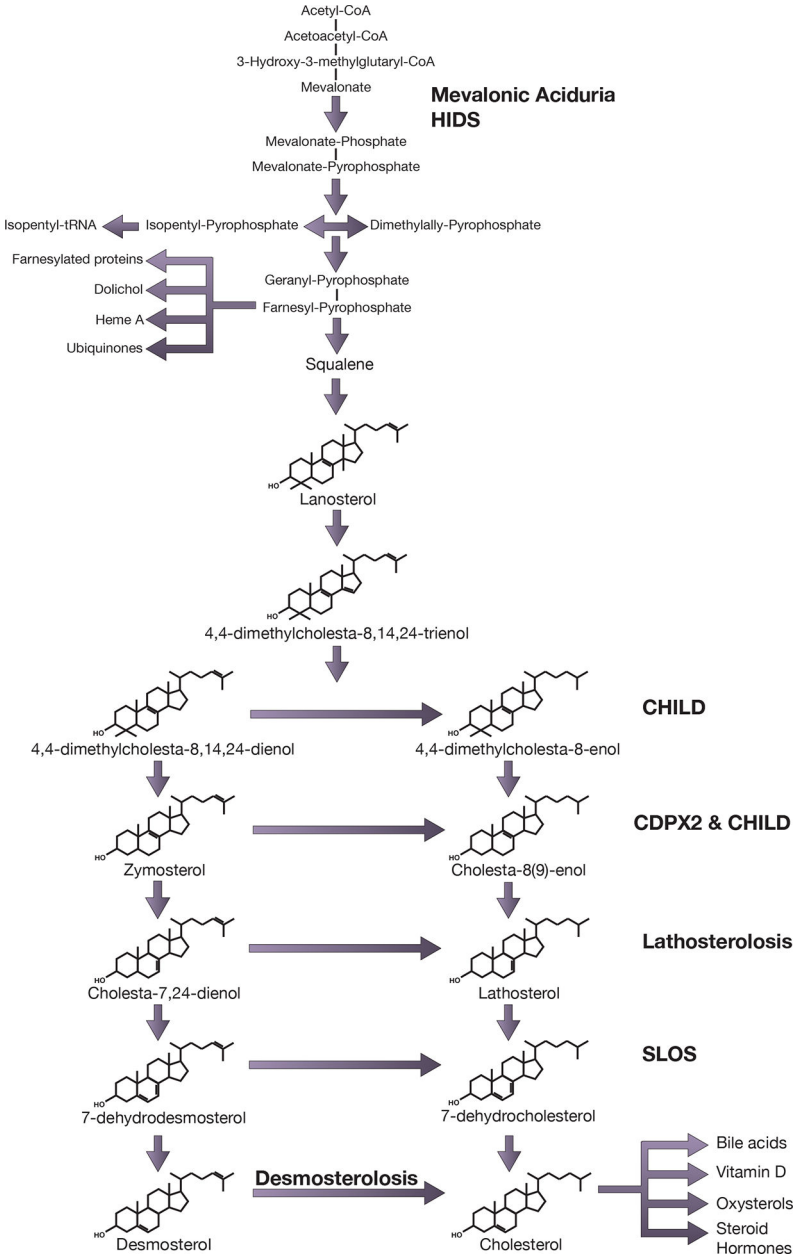

Figure 1:
Schematic depicting cholesterol biosynthesis and associated diseases.

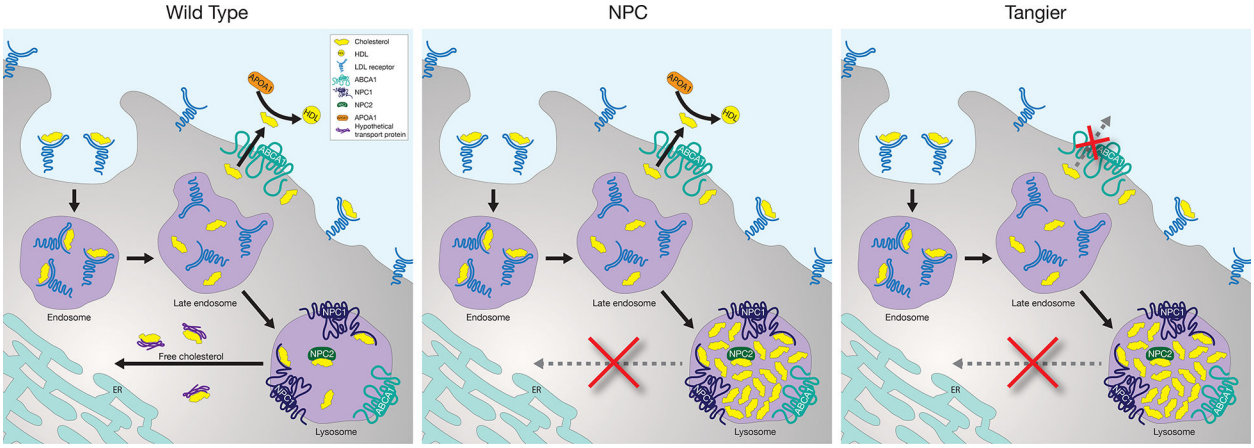

Figure 2:

Cartoon summarising the cellular consequences of NPC, SLOS and Tangier disease on cholesterol homeostasis and common involvement of the NPC pathway. Panel a) WT cell, b) NPC cell and SLOS and c) Tangier disease cell.

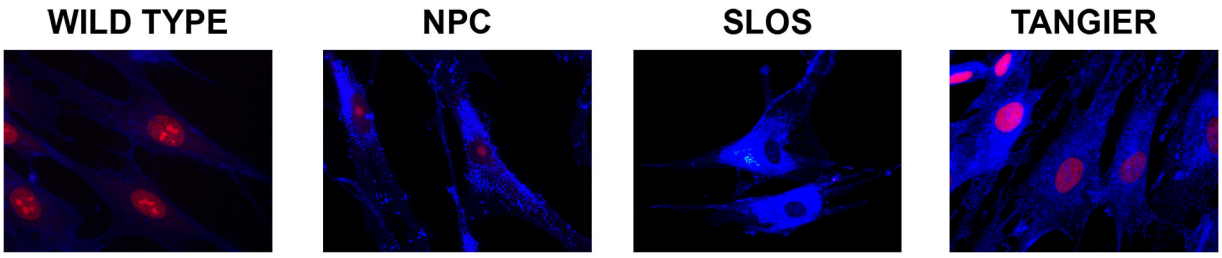

Figure 3.

Cholesterol storage (filipin staining) in late endocytic compartment is a common feature observed in NPC, SLOS and Tangier patient fibroblasts relative to a healthy control cells. Filipin (blue), nuclear strain (red).

Table 1:

Biochemical and cell biological phenotypes shared between NPC, SLOS and Tangier disease.

Cellular Phenotypes	NPC	SLOS	Tangier
Glycosphingolipid storage	+	+	+
Cholesterol storage	+	+	+
Sphingomyelin storage	+	+	+
Sphingosine storage	+	+	+
Sphingolipid mistrafficking	+	+	+
Reduced acidic store calcium levels	+	+	+
Response to miglustat	+ In clinical use, EMA approved)	+ Corrects SLOS cells	+ Corrects Tangier cells and improved symptoms in one patient