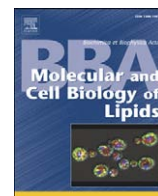




Contents lists available at ScienceDirect

## Biochimica et Biophysica Acta

journal homepage: [www.elsevier.com/locate/bbalip](http://www.elsevier.com/locate/bbalip)

## Review

## Mechanisms and consequences of impaired lipid trafficking in Niemann–Pick type C1-deficient mammalian cells

Barbara Karten<sup>a</sup>, Kyle B. Peake<sup>b</sup>, Jean E. Vance<sup>b,\*</sup><sup>a</sup> Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada<sup>b</sup> Department of Medicine and Group on the Molecular and Cell Biology of Lipids, University of Alberta, Edmonton, AB, Canada

## ARTICLE INFO

## Article history:

Received 24 December 2008

Accepted 20 January 2009

Available online xxx

## Keywords:

Cholesterol

Glycosphingolipid

Neuron

Glia

Neurodegeneration

Brain

Liver

## ABSTRACT

Niemann–Pick C disease is a fatal progressive neurodegenerative disorder caused in 95% of cases by mutations in the *NPC1* gene; the remaining 5% of cases result from mutations in the *NPC2* gene. The major biochemical manifestation of NPC1 deficiency is an abnormal sequestration of lipids, including cholesterol and glycosphingolipids, in late endosomes/lysosomes (LE/L) of all cells. In this review, we summarize the current knowledge of the NPC1 protein in mammalian cells with particular focus on how defects in NPC1 alter lipid trafficking and neuronal functions. NPC1 is a protein of LE/L and is predicted to contain thirteen transmembrane domains, five of which constitute a sterol-sensing domain. The precise function of NPC1, and the mechanism by which NPC1 and NPC2 (both cholesterol binding proteins) act together to promote the movement of cholesterol and other lipids out of the LE/L, have not yet been established. Recent evidence suggests that the sequestration of cholesterol in LE/L of cells of the brain (neurons and glial cells) contributes to the widespread death and dysfunction of neurons in the brain. Potential therapies include treatments that promote the removal of cholesterol and glycosphingolipids from LE/L. Currently, the most promising approach for extending life-span and improving the quality of life for NPC patients is a combination of several treatments each of which individually modestly slows disease progression.

© 2009 Elsevier B.V. All rights reserved.

## 1. Introduction to Niemann–Pick type C (NPC) disease

Niemann–Pick type C (NPC) disease is a fatal, progressive neurodegenerative disorder that is inherited in an autosomal recessive manner. Over 260 mutations in the *NPC1* gene have been identified in humans. The incidence of NPC disease is ~1/150,000 live births [1]. The family of Niemann–Pick diseases, in which the lysosomal storage of sphingomyelin was originally shown to be a common feature, was first reported by Drs. Albert Niemann (1880–1921) and Ludwig Pick (1868–1944). These two German physicians described the pathology of these diseases and recognized that they were distinct from another glycosphingolipid (GSL) storage disorder, Gaucher disease. In 1958 Crocker proposed that these sphingomyelin storage diseases could be classified into three groups (A, B and C) according to their clinical manifestations [2]. Brady subsequently established that Niemann–

Pick disease A and B are caused by reduced activity of lysosomal (acid) sphingomyelinase [3].

The pioneering work of Pentchev et al. on a strain of mice in which unesterified cholesterol accumulated dramatically in tissues when the mice were fed a high cholesterol diet, established that NPC disease is caused by a defect in a gene other than the acid sphingomyelinase gene [4]; these mice were later shown to be a model for NPC disease [5]. Subsequent investigations in fibroblasts from NPC patients and Chinese hamster ovary (CHO) cell mutants indicated that the fundamental problem in NPC disease was a defect in the export of lipids, particularly cholesterol and GSLs, from lysosomes. As a result of this sequestration of cholesterol, the availability of unesterified cholesterol for esterification by acyl-CoA:cholesterol acyltransferase is reduced and cholesterol-mediated homeostatic responses in the endoplasmic reticulum (ER) are impaired [6–9]. In addition, acid sphingomyelinase activity is reduced by up to 80% in NPC1-deficient fibroblasts as a consequence of excessive lipid storage [10,11]. In 1997, a positional cloning approach identified the defective human gene in NPC disease patients as the *NPC1* gene that resides on chromosome 18q11; the corresponding cDNA complemented the NPC phenotype in mutant cells [12,13]. More recent studies have revealed that not all cases of NPC disease are caused by mutations in the *NPC1* gene but that ~5% of cases result from mutations in the *NPC2* gene that maps to chromosome 14q24.3 [14] (*NPC2* will be discussed in detail in an accompanying review in this issue).

**Abbreviations:** ABC, ATP-binding cassette; apo, apolipoprotein; BDNF, brain-derived neurotrophic factor; CHO, Chinese hamster ovary; CNS, central nervous system; ER, endoplasmic reticulum; GSL, glycosphingolipid; LAMP, lysosome-associated membrane protein; LBPA, lyso-bis-phosphatidic acid; LDL, low density lipoprotein; LE/L, late endosomes/lysosomes; NFT, neurofibrillary tangles; NPC, Niemann–Pick type C; LXR, liver X receptor; SCAP, sterol response element binding protein cleavage activating protein; TNF $\alpha$ , tumor necrosis factor- $\alpha$

\* Corresponding author. 328 HMRC, University of Alberta, Edmonton, Canada, AB T6G 2S2. Tel.: +1 780 492 7250; fax: +1 780 492 3383.

E-mail address: [jean.vance@ualberta.ca](mailto:jean.vance@ualberta.ca) (J.E. Vance).

The symptoms of NPC disease – progressive neurodegeneration, often accompanied by hepatosplenomegaly – usually become evident in early childhood but disease progression and age of onset vary among patients. Since the NPC1 and NPC2 proteins appear to be expressed in all tissues, the reason why the nervous system is so severely affected in NPC disease is not clear. In this review we shall summarize current knowledge of the NPC1 protein in mammalian cells with particular focus on how defects in NPC1 affect lipid trafficking and impair functions of the nervous system.

## 2. Models used for studying NPC deficiency

The majority of *in vitro* studies on NPC disease have been performed on human skin fibroblasts [7,15] and CHO cell mutants [7–9]. In addition, treatment of mammalian cells with the amphiphilic amine U18666A [3-β-(2-diethylaminoethoxy) androst-5-en-17-one], which inhibits NPC1 function, has been used to mimic the NPC phenotype. Although U18666A induces lipid accumulation in LE/L, caution needs to be exercised in its use since this agent is quite toxic and has other effects in the cell such as inhibition of cholesterol synthesis.

The most commonly used animal model for studies on NPC1 deficiency is the Balbc/Nctr-Npc1<sup>m1N/J</sup> mouse in which a spontaneous null mutation arose in the *Npc1* gene [4,5]. The phenotype of this mouse closely resembles that of NPC1-deficient humans. Nevertheless, differences have been observed in disease progression within colonies of these mice as a consequence of different strain backgrounds and genetic drift [16]. NPC2-hypomorphic mice, whose tissues contain only ~0.4% of residual NPC2 protein, have also been generated [17]. The progression of the disease, neurodegenerative characteristics and lipid storage phenotype are essentially identical in *Npc1*<sup>-/-</sup> and NPC2-deficient mice, as well as in mice deficient in both NPC1 and NPC2 [17]. Thus, it appears that NPC1 and NPC2 are non-redundant and act in concert to transfer lipids out of LE/L.

A colony of NPC1-deficient cats has also been established [18–20]. This genetic model arose from a spontaneous missense mutation in the NPC1 protein (C955S). NPC1-deficient cats have a reduced lifespan and exhibit neurological problems and liver disease very similar to those in NPC1-deficient humans [20]. NPC deficiency has also been studied in non-mammalian models such as *Drosophila* [21] and *Saccharomyces cerevisiae* [22,23].

## 3. Cellular biology and lipid storage in NPC1-deficient cells

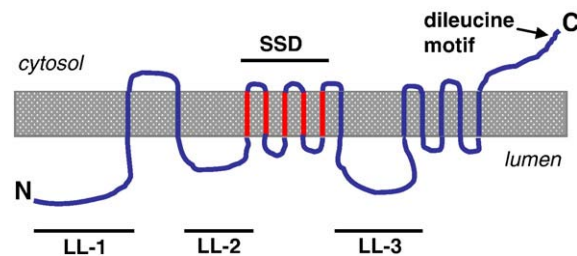
### 3.1. Cellular location of NPC1 and NPC2 proteins

NPC1 is an integral membrane protein that is primarily associated with both the intraluminal vesicles and limiting membranes of multi-vesicular late endosomes [24–27,28,29]. NPC1 also transiently cycles through the *trans*-Golgi network [24,30]. NPC1 is ubiquitously expressed in all tissues of mice and humans that have been examined, with highest expression in the liver [31] and its expression appears to be modulated by sterol metabolism. Levels of NPC1 mRNA in fibroblasts were decreased by 22- or 25-hydroxycholesterol. When fibroblasts were cultured in cholesterol-depleted medium or when cholesterol synthesis was inhibited, NPC1 promoter activity and NPC1 mRNA abundance were increased [32–34].

NPC2 was originally identified as the cholesterol-binding protein, HE1 [35], which is a major secreted protein of the human epididymis. NPC2 is a soluble protein within the lumen of lysosomes to which it is targeted by binding to the mannose-6-phosphate receptor [14].

### 3.2. Structure and lipid-binding properties of the NPC proteins

The human NPC1 protein is an integral membrane protein that consists of 1278 amino acids and contains 13 putative transmembrane



**Fig. 1.** Predicted topological model of the NPC1 protein. Human NPC1 is a late endosomal/lysosomal protein that consists of 1278 amino acids (aa) and contains 13 putative transmembrane helices. The following structural domains have been identified: luminal loop-1 (LL-1) (aa 25–264); luminal loop-2 (LL-2) (aa 371–615); a putative sterol-sensing domain (SSD) (aa 616–791); a cysteine-rich loop, luminal loop-3 (LL-3) (aa 855–1098), and a lysosomal targeting motif (LLNF) at the C-terminus.

domains that are separated by three large glycosylated luminal loops (Fig. 1) [13]. Other features of the protein are a cysteine-rich loop, a di-leucine lysosomal targeting motif and a leucine zipper motif that suggests that NPC1 interacts with other proteins.

An important feature of NPC1 is that five of its thirteen transmembrane domains constitute a sterol-sensing domain similar to that in 3-hydroxy-3-methylglutaryl-CoA reductase, sterol regulatory element-binding protein cleavage activating protein (SCAP), NPC1-like protein-1 [a protein required for cholesterol absorption by the intestine [36]] and Patched (a regulatory protein in the Hedgehog signaling pathway). The sterol-sensing domain of SCAP binds cholesterol [37]. The sterol-sensing domains of SCAP and 3-hydroxy-3-methylglutaryl-CoA reductase also bind to the Insig proteins that regulate cholesterol homeostasis in the ER [38]. In contrast, the sterol-sensing domains of NPC1, NPC1-like protein-1 and Patched do not bind Insigs [39,40]. Mutations in the sterol-sensing domain or the cysteine-rich domain of NPC1 are common in NPC disease patients and cause severe disease phenotypes. The P692S mutant of NPC1 (mutation in the sterol-sensing domain) has reduced ability to deliver LDL-derived cholesterol to the plasma membrane and ER [41]. This mutant protein also exhibits markedly reduced binding of a photoactivatable analog of cholesterol (7,7-azocholesterol), suggesting that an intact sterol-sensing domain is required for the binding of cholesterol to NPC1 [40]. These studies did not, however, establish whether the sterol binds directly to the sterol-sensing domain or whether this domain is only indirectly involved in the binding of sterol to NPC1 [40]; nor was the sterol specificity of binding to NPC1 determined.

Despite knowledge of the structure of the NPC1 protein, and the well-established demonstration that NPC1 is required for the movement of cholesterol out of late endosomes/lysosomes (LE/L), the precise function of NPC1 has remained elusive. To address this shortcoming, the NPC1 protein was recently purified 14,000-fold from rabbit liver and was shown to bind not only to cholesterol, but also to oxysterols such as 25- and 24-hydroxycholesterol, in a saturable fashion [42]. Interestingly, the affinity of purified NPC1 for cholesterol is at least an order of magnitude lower than for 25-hydroxycholesterol [42]. In addition, oxysterols compete for binding of several fluorescent sterol analogs to purified recombinant human NPC1 [43]. In other studies, a photoactivatable analog of cholesterol bound to NPC1 in intact cells and this binding was prevented by mutations in the sterol-sensing domain [40]. A sterol binding site on NPC1 was localized to a 240-amino acid segment in luminal loop-1 (Fig. 1), the sequence of which is highly conserved in vertebrate and yeast orthologs of NPC1. Luminal loop-1 of NPC1 was expressed in cultured cells and purified as a dimer that bound one sterol molecule/dimer [44]. Mutation of glutamine-79 (Q79A) of loop-1 completely abolished the binding of 25-hydroxycholesterol and reduced the binding of cholesterol by 40%; in addition, the binding of both cholesterol and 25-hydroxycholesterol to the purified full-length Q79A mutant of detergent-solubilized NPC1

was completely eliminated. Thus, glutamine-79 is important for the binding of NPC1 to sterols. Unexpectedly, however, when the Q79A mutant of NPC1 was expressed in NPC1-deficient fibroblasts, normal cholesterol transport out of LE/L was restored [44]. Thus, the sterol-binding site on luminal loop-1 is not essential for NPC1 function in fibroblasts. It is possible that this site constitutes an oxysterol regulatory site rather than a cholesterol binding site that is required for transport. These studies did not completely resolve the issue of whether or not luminal loop-1 of NPC1 contains the sole sterol-binding site.

The human NPC2 protein is a soluble, highly glycosylated protein of 151 amino acids (131 amino acids after cleavage of the signal sequence). NPC2 binds cholesterol, but not oxysterols, with micromolar affinity [45,46] and also binds fatty acids, but with lower affinity [47]. *In vitro* lipid transport studies using a fluorescence dequenching assay demonstrated that NPC2 rapidly transfers cholesterol between phospholipid vesicles and that this transfer is enhanced by the LE/L-specific phospholipid lyso-*bis*-phosphatidic acid (LBPA) the levels of which are markedly increased in NPC1- and NPC2-deficient cells [45]. The transfer of cholesterol occurs via a collisional mechanism that involves direct interaction of NPC2 with acceptor membranes. An X-ray crystal structure of bovine NPC2 without bound ligand revealed a series of hydrophobic cavities that could accommodate a cholesterol molecule [48]. Mutant versions of NPC2 (with the mutations F66A, V96F and Y100A that reside within the presumed cholesterol-binding pocket) do not bind cholesterol [47].

Thus, it is clear that both NPC1 and NPC2 are cholesterol-binding proteins of LE/L. The mechanism by which NPC1 and NPC2 act together in exporting cholesterol from LE/L is not yet clear. The observation that the binding of cholesterol to NPC1 is abolished by sub-micromolar concentrations of detergent suggests that cholesterol prefers to partition into micelles rather than bind to luminal loop-1 [42,44]. Moreover, NPC2 binds and releases cholesterol very rapidly and accelerates by ~100-fold the transfer of cholesterol between liposomes and NPC1 [45,46]. On the other hand, NPC1 transfers cholesterol only slowly to NPC1 or NPC2. Nevertheless, the binding of a fluorescent analog of cholesterol to NPC1 does not require the presence of NPC2 [40]. Two potential mechanisms have been proposed for the sequential action of NPC1 and NPC2 [49]. In one model, cholesterol that has accumulated in membranes of intra-luminal vesicles of LE/L binds to NPC1 located in these membranes. Subsequently, soluble NPC2 acquires cholesterol from NPC1 and transfers it to the limiting membrane of LE/L, from where the cholesterol is distributed to other cellular membranes by an unknown mechanism. In a second proposed model, NPC2 removes cholesterol from the intraluminal vesicles of LE/L, then delivers the cholesterol to NPC1 in the limiting membrane of LE/L. Both of these models require the participation of at least one additional protein, currently unidentified, which would facilitate the export of cholesterol from the limiting membrane of LE/L to a sink outside the LE/L.

An alternative function that was proposed for NPC1 was as a eukaryotic member of the RND permease family of transport proteins that, when expressed in bacteria, transported fatty acids [50]. However, Liscum et al. demonstrated that the flux of fatty acids through NPC1-deficient LE/L is normal [51].

### 3.3. Impaired lipid trafficking in NPC1-deficient cells

The most striking biochemical feature of NPC1-deficient cells is an excessive storage of unesterified cholesterol [6,7,10,15,52], sphingomyelin, GSLs including glucosylceramide, lactosylceramide and complex gangliosides (mainly GM2 and GM3) [53,54], sphingosine [55] and other lipids such as the lysosome-specific phospholipid, LBPA [56].

The itinerary of intracellular cholesterol transport has been reviewed in some detail [57,58]. The sequestration of unesterified cholesterol in LE/L of NPC-deficient cells is frequently visualized by

staining the cells with filipin, a polyene antibiotic that specifically binds unesterified, but not esterified, cholesterol. Another agent that is used to visualize the distribution of cellular cholesterol is BC-theta, a biotinylated, membrane-impermeable cholesterol-binding probe that can be detected by fluorophore-conjugated streptavidin [59].

In NPC1-deficient fibroblasts the storage of lipids in LE/L becomes pronounced only when the cells have been loaded with low density lipoproteins (LDLs) [6,7,10,15,52]. LDLs are taken up by the LDL receptor via endocytosis and enter the LE/L system where the LDL-cholesteryl esters are hydrolyzed to unesterified cholesterol. In normal fibroblasts the cholesterol is then distributed to other organelle membranes, particularly the plasma membrane where the majority of cellular cholesterol resides. LDL-derived cholesterol that exits the LE/L is also transported to the ER where cholesterol regulates cholesterol homeostatic mechanisms [60], including the synthesis and esterification of cholesterol, and LDL receptor synthesis.

In NPC1-deficient cells, the trafficking of LDL-derived cholesterol is profoundly altered. Although the endocytic uptake of LDL and the hydrolysis of LDL-derived cholesteryl esters to unesterified cholesterol in LE/L are normal in NPC1-deficient cells, the exit of unesterified cholesterol from LE/L is impaired. Consequently, cholesterol accumulates, along with other lipids, in LE/L. In addition, the movement of LDL-derived cholesterol to the ER is delayed, resulting in altered regulation of cholesterol homeostasis in the ER. Thus, despite higher-than-normal amounts of cellular cholesterol, the rate of cholesterol synthesis and production of LDL receptors are enhanced, and the rate of cholesterol esterification is diminished [6,7,10,15,52]. Furthermore, the amount of LDL-derived cholesterol that reaches the plasma membrane from LE/L is reduced [61]. In contrast, the intracellular transport of cholesterol delivered from exogenously-supplied high density lipoproteins is unaffected by NPC1 deficiency, presumably because this source of cholesterol does not enter LE/L via endocytosis [62,63]. Moreover, the transport of endogenously-synthesized cholesterol to the plasma membrane is not significantly reduced in NPC1-deficient fibroblasts [61]; following re-endocytosis from the plasma membrane, this source of cholesterol accumulates in LE/L much more slowly than does LDL-derived cholesterol [64]. The mechanisms by which cholesterol is transported from LE/L to the ER, plasma membrane and the endocytic recycling compartment have not yet been defined at the molecular level [65]. Recently, a vesicular cholesterol transport pathway from late endosomes to the *trans*-Golgi and ER that involved a SNARE complex was described [66]. It is likely that non-vesicular pathways, such as transport via juxtaposition between donor and acceptor membranes, or transport via soluble carrier proteins such as proteins of the oxysterol binding protein family [reviewed in [67]] play major roles in cholesterol transport out of LE/L.

LBPA is another lipid that accumulates in LE/L in NPC1-deficient cells. LBPA is found mainly in the internal membranes of the multi-vesicular storage bodies that are produced in response to loss of NPC1 function. Interestingly, some other lysosomal lipid storage diseases are also characterized by increased levels of LBPA. For example in the mouse model of the human neurodegenerative disease neuronal ceroid lipofuscinosis, LBPA levels in the brain are 20-fold higher than in wild-type mice [68]. LBPA appears to regulate endosomal levels of cholesterol [56] and is itself regulated by Alix/AIP1, a LBPA-interacting protein involved in sorting components into multi-vesicular endosomes [69]. Decreased expression of Alix reduces LBPA levels and decreases the number of luminal vesicles in late endosomes. In contrast, addition of LBPA to Alix knock-down cells restores the luminal vesicles and increases the late endosomal content of cholesterol. It is not known if the accumulation of LBPA *per se* contributes to the neurodegeneration in NPC disease.

Sphingosine is normally a quantitatively minor lipid that is toxic to cells and accumulates in LE/L of NPC1-deficient cells. This storage appears to alter calcium homeostasis. For example, the amount of

calcium in LE/L was reduced by NPC1 deficiency, and a typical NPC phenotype was induced by chelation of calcium in wild-type cells [55]. Moreover, treatment of NPC1-deficient cells with thapsigargin which releases calcium from the ER and thus elevates the level of cytosolic calcium, led to mobilization of GSL and cholesterol, but not sphingosine, from LE/L.

The question of whether cholesterol or GSL is the primary offending lipid whose accumulation leads to neurological problems remains under debate. Moreover, the issue of which lipid accumulates first in NPC1-deficient cells, and which lipids accumulate secondarily, has not been completely resolved. Although the general consensus in the field is that impaired cholesterol trafficking is the initial defect in NPC1-deficient cells, some observations had suggested that mutations in NPC1 or NPC2 compromised GSL metabolism/transport directly. For example, the accumulation of endogenously-made, as well as exogenously-supplied, GSLs can induce cholesterol storage in LE/L [70]. In addition, levels of GSLs, particularly GM2 and GM3 gangliosides, are markedly elevated in brains of adult NPC1-deficient mice whereas cholesterol is not [53,71]. However, NPC1 deficiency in the brain causes an extensive loss of myelin which is highly enriched in cholesterol. Consequently, any accumulation of cholesterol in neurons and other cells in NPC1-deficient brains would be masked by the loss of myelin [72,73].

Importantly, NPC1 [40,42–44] and NPC2 [45] bind sterols but not GSLs, suggesting that these proteins are directly involved in sterol transport and that other lipids (GSLs and sphingomyelin) accumulate in LE/L secondarily as a consequence of the defect in cholesterol trafficking. Indeed, cholesterol does accumulate in brains of very young mice and the storage of cholesterol precedes the onset of neurological symptoms [59,74]. Staining of brain slices with filipin [53,74–76] or BC-theta [59] revealed that the intracellular sequestration of cholesterol occurs throughout the brain. Consistent with these observations, compartmented cultures of sympathetic neurons isolated from one-day-old *Npc1*<sup>-/-</sup> mice [77] and hippocampal neurons from embryonic *Npc1*<sup>-/-</sup> mice (Fig. 2) revealed extensive intracellular cholesterol sequestration in LE/L of cell bodies even when the neurons were cultured in the absence of serum. Nevertheless, the total amount of cholesterol in the neurons was not significantly increased by NPC1 deficiency [77] although the distribution of cholesterol in the neurons was altered such that cholesterol (mass) accumulated in cell bodies whereas distal axons were correspondingly deficient in cholesterol (mass) [77]. In accordance with these findings, the anterograde transport of endogenously-synthesized cholesterol from cell bodies to

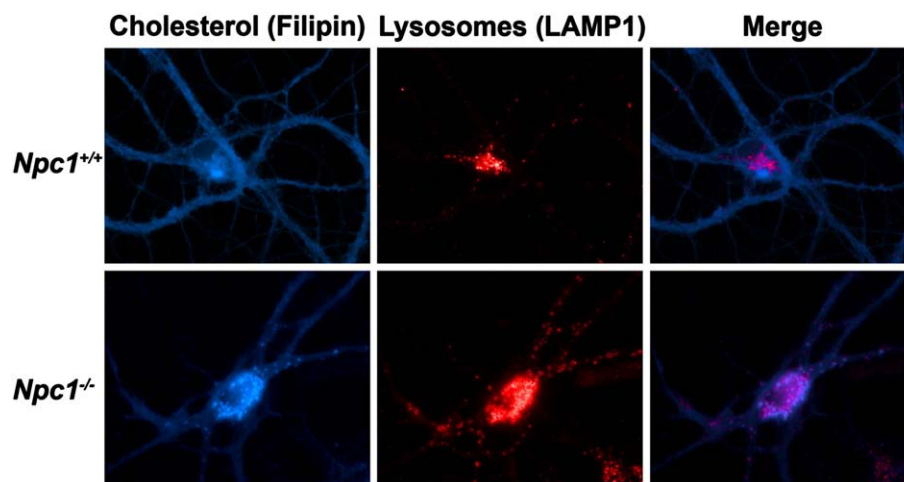
distal axons of *Npc1*<sup>-/-</sup> neurons was compromised [78]. Furthermore, LDL-cholesterol that was provided to cell bodies was taken up and transported normally into distal axons of *Npc1*<sup>-/-</sup> neurons, but this source of cholesterol did not restore a normal rate of axonal extension to *Npc1*<sup>-/-</sup> neurons in which cholesterol synthesis had been inhibited [78]. Since axons perform crucial functions in neurons, such as signaling and synaptic transmission, a reduced cholesterol content of axonal plasma membranes is likely to have severe consequences.

The majority of cells in the central nervous system (CNS) are not neurons but glial cells (astrocytes, oligodendrocytes and microglia). Cholesterol is sequestered not only in NPC1-deficient neurons but also in LE/L of glial cells (primarily astrocytes) isolated from the cerebellum of *Npc1*<sup>-/-</sup> mice [79]. The distribution of cholesterol is similarly altered in microglia isolated from NPC1-deficient mouse brains (K. Peake and J.E. Vance, unpublished data). Thus, it appears that in all NPC1-deficient cells, including cells of the CNS (neurons, astrocytes and microglia), the trafficking of cholesterol out of LE/L is markedly compromised.

### 3.4. Impaired vesicle transport in NPC1-deficient cells

Loss of functional NPC1 profoundly influences vesicular transport. Trafficking defects have been observed in both early and late endosomes. The normally rapid tubulovesicular movement of late endosomes [80], and the trafficking of mannose-6-phosphate receptors, are retarded [56]. In addition, the movement of LDL-derived cholesterol through the Golgi of NPC1-deficient fibroblasts is reduced [81].

Rab proteins perform multiple roles in vesicle trafficking events. Thus, the trafficking of the transferrin receptor and GSLs out of early endosomes, a process that depends on Rab4, was investigated in NPC1-deficient human fibroblasts. The Rab4-dependent intracellular trafficking of this cargo was dramatically slowed by the presence of high levels of endosomal cholesterol [82]. Another Rab, Rab9, is a late endosome-specific, small GTPase that participates in vesicle trafficking between LE/L and the *trans*-Golgi network. The level of Rab9 is increased by 80% in NPC1-deficient fibroblasts due to decreased degradation of the protein, and Rab9 is sequestered in LE/L of NPC1-deficient cells, resulting in disrupted trafficking of mannose-6-phosphate receptors [83]. Remarkably, the accumulation of cholesterol in NPC1-deficient cells was attenuated by over-expression of Rab9 or Rab7 [84,85]. Moreover, when the amount of Rab9 was increased by protein transduction in NPC1-deficient human fibroblasts and mouse cortical neurons, lipid storage was reduced [86]. Similar results were



**Fig. 2.** Cholesterol accumulation in NPC1-deficient neurons. Hippocampal neurons isolated from embryonic wild-type (*Npc1*<sup>+/+</sup>) and NPC1-deficient (*Npc1*<sup>-/-</sup>) mice were cultured for 20 days in serum-free medium, then fixed and stained with filipin (blue, cholesterol) and rat anti-LAMP1 antibodies followed by Texas Red-conjugated anti-rat IgG (red, LE/L). Images were acquired with a Nikon TE2000 epifluorescence microscope equipped with a CCD camera and a 63× oil-immersion objective at filter settings of 387/11 nm and 579/34 nm for excitation and 447/60 nm and 624/40 nm for emission.

obtained when Rab4, Rab7 or Rab8 were over-expressed in cultured fibroblasts, and when Rab9 was over-expressed in mouse neurons [82,86,87]. These observations suggested that Rab9 might be able to bypass the requirement for NPC1/2. Thus, an *in vivo* study was undertaken in which *Npc1*<sup>-/-</sup> mice were crossed with Rab9 transgenic (over-expressing) mice. Over-expression (30-fold) of Rab9 increased the life-span of *Npc1*<sup>-/-</sup> mice by up to 22%, reduced the storage of gangliosides and delayed the onset of disease symptoms [88].

#### 4. Consequences of NPC1-deficiency in the brain

##### 4.1. Cholesterol metabolism in the brain

Cholesterol is required for optimal functioning of membranes in all mammalian cells and appears to be of particular importance in the brain which contains 25% of the body's cholesterol but makes up only ~2% of body weight. Interest in the role of cholesterol in normal brain function and in neurodegenerative disorders has blossomed over the past few years because aberrant cholesterol metabolism has been implicated in several neurodegenerative disorders including NPC disease, Alzheimer disease, Smith–Lemli Opitz syndrome, and Huntington disease [89].

Since the blood brain barrier is impermeable to plasma lipoproteins, cholesterol cannot be delivered to the brain from LDLs [90] so that under normal conditions, all cholesterol in the brain is synthesized within the brain. Thus, the metabolism of cholesterol in the CNS is largely segregated from that in the periphery, except when the integrity of the blood-brain barrier has been compromised. The majority (70–80%) of cholesterol in the brain of adult animals resides in myelin, and the rate of cholesterol synthesis in the brain is highest during myelination. After myelination is complete, cholesterol synthesis continues slowly [90]. Astrocytes appear to be the main source of cholesterol in the adult CNS. These glial cells play a crucial role in delivering substrates to neurons and in modulating synaptic activity [91]. Cholesterol is secreted from glia (primarily astrocytes) in combination with apolipoprotein (apo) E in lipoprotein particles that are the size and density of plasma high density lipoproteins. These lipoproteins can be delivered to neurons and taken up by receptors of the LDL receptor superfamily [92–94]. The interaction between astrocytes and neurons is important for several neuronal functions. For example, apo E- and cholesterol-containing lipoproteins that are synthesized by astrocytes [95] increase synaptogenesis and synaptic strength [96,97], stimulate axonal growth [98], and protect neurons from apoptosis [99]. Although the mechanisms by which apo E is lipidated in the CNS are not fully understood, this process is likely to depend on ATP-binding cassette (ABC) transporters such as ABCA1, ABCG1 and ABCG4 [100].

Cholesterol has a remarkably long half-life of 4–6 months in the brain. Since cholesterol continues to be made, albeit at a low rate, in the adult brain [90], the maintenance of cholesterol homeostasis requires the export of excess cholesterol. At least 40% of cholesterol that exits the brain is converted to 24-hydroxycholesterol by CYP46A1 in a small subset of neurons in the cerebral cortex, hippocampus and thalamus [101,102]. The 24-hydroxycholesterol, which is able to cross the blood-brain barrier, enters the circulation and is excreted from the liver into bile. NPC1 deficiency did not alter mRNA or protein levels of cholesterol 24-hydroxylase in mouse brains [103], although deletion of cholesterol 24-hydroxylase in *Npc1*<sup>-/-</sup> mice reduced the rate of excretion of cholesterol from the brain. However, these double knock-out mice were phenotypically indistinguishable from *Npc1*<sup>-/-</sup> mice in terms of age of onset of neurological symptoms and life-span [103].

Alterations in cholesterol homeostasis in the brain would be expected to have profound consequences for neuronal function. Cholesterol regulates membrane fluidity and lateral membrane organization, and is a component of so-called “lipid rafts”, defined in the 2006 Keystone Symposium on Lipid Rafts and Cell Function as “small (10–200 nm), highly dynamic, sterol- and sphingolipid-enriched

domains that compartmentalize cellular processes” [104–106]. Although evidence that lipid rafts exist in living cells remains elusive [107], these proposed structures have been implicated in many aspects of nervous system function, including growth factor signaling, axon guidance, vesicular trafficking and synaptic transmission.

##### 4.2. NPC1 deficiency and neuronal function

Most studies on NPC1-deficient cells have utilized human fibroblasts or CHO cell mutants. However, in light of the debilitating progression of neurological symptoms in NPC disease it is important to understand the nature of the NPC defect in neurons and other cells of the brain. Studies on primary neurons from *Npc1*<sup>-/-</sup> mice are technically challenging and only a few groups have investigated NPC1 functions in these cells. In one of the first such studies, Henderson *et al.* reported that striatal neurons isolated from NPC1-deficient mice had reduced axon length and a reduced number of branch points, as well as impaired responsiveness to brain-derived neurotrophic factor (BDNF); BDNF failed to elicit normal autophosphorylation of its receptor, TrkB [108]. Since extraction of cholesterol from *Npc1*<sup>+/+</sup> neurons also reduced the phosphorylation of TrkB upon BDNF binding, it was proposed that NPC1-deficiency reduced the cholesterol-rich domains of the plasma membrane where the TrkB receptor resides [108]. In contrast, growth and morphology of NPC1-deficient mouse sympathetic neurons, as well as TrkA activation by nerve growth factor [77], are normal.

Functional defects are less obvious in the peripheral nervous system than in the CNS. However, the ability of NPC1-deficient mice to reutilize cholesterol liberated from myelin during peripheral nerve injury is impaired, leading to reduced growth and myelination of the regenerating axons [109].

Some symptoms of NPC disease, such as the occurrence of seizures, suggest that synaptic transmission is altered. In NPC1-deficient mice, brain area-specific changes in levels of neurotransmitters were observed. For example, GABA and glutamate were decreased in the cerebral cortex, whereas the cerebellum showed an increased level of glycine but no alteration in GABA or glutamate [110]. Proteomic analysis of NPC1-deficient murine cerebellum revealed a significant increase in GAT3 (the glial GABA transporter) in mice at 4 weeks of age, as well as increases in GAT3 (glial) and GAT1 (neuronal) and a decrease in the glial glutamate transporter, EAAT1, in 8-week-old mice [111]. This reciprocal change in GABAergic and glutamatergic systems indicates that the balance of inhibitory (GABA) and excitatory (glutamate) synaptic transmission is disturbed which might contribute to some of the observed neurological deficiencies. In support of this view, the *Drosophila* model for NPC disease, the *dnp1a* null mutant, showed intra-neuronal cholesterol accumulation, progressive neurodegeneration and severe defects in photoreceptor synaptic transmission [112].

The NPC1 protein is present in axons of sympathetic neurons and in isolated synaptosomes from mouse brains suggesting that NPC1 has a function in axons [113]. In NPC1-deficient synaptosomes some enlarged vesicular structures, either aberrant synaptic vesicles or endosomal structures, were observed. In addition, the protein composition of synaptic vesicles isolated from NPC1-deficient synaptosomes was altered [113]. Several electrophysiological parameters (resting membrane potential, neuronal input resistance and action potential amplitude) were measured in isolated cortical neurons from embryonic mice, and in cortical brain slices from older mice, but no significant differences were detected between *Npc1*<sup>+/+</sup> and *Npc1*<sup>-/-</sup> neurons in synaptic activity or growth morphology [114]. Nor were basal cytosolic calcium levels, or the return to baseline calcium levels following depolarization, altered by NPC1 deficiency. However, the NPC1-deficient neurons were more fragile than their wild-type counterparts, which led to a much lower success rate for patch clamping [114]. In contrast, Kavalali *et al.* observed increased

spontaneous synaptic vesicle release and decreased evoked post-synaptic responses in NPC1-deficient mouse hippocampal neurons and in wild-type neurons following treatment with cyclodextrin to deplete cholesterol [115]. In addition, synaptic transmission from NPC1-deficient neurons was normalized by the addition of cholesterol complexed to cyclodextrin [115]. These observations support the view that the pre-synaptic plasma membrane of NPC1-deficient neurons is relatively depleted of cholesterol and that cholesterol plays a crucial role in distinguishing between pools of synaptic vesicles used for spontaneous release and evoked release. Importantly, the apparent discrepancies among these studies suggest that culture conditions and neuronal sub-type might have a significant influence on synaptic function in NPC1-deficient neurons.

Most neurodegenerative diseases are characterized by impaired mitochondrial function, which in turn can contribute to neuronal dysfunction and death. Neurons have a particularly high energy requirement for maintenance of membrane potential and seem to be highly dependent on mitochondrial oxidative phosphorylation. Mitochondria isolated from NPC1-deficient brains had a lower rate of ATP generation than did mitochondria from wild-type brains [116]. Moreover, ATP levels in primary cortical neurons isolated from NPC1-deficient mice were lower than in control neurons, as were neurite length and number; the defects were normalized by addition of exogenous ATP [116]. These changes were ascribed to a possible build-up of cholesterol in mitochondrial outer and inner membranes. However, since it is usually assumed that ATP does not cross the plasma membrane, the latter effect might be due to ATP signaling rather than supplementation of energy.

#### 4.3. Neuron death and pathological changes in NPC1-deficient brains

In the brain, the most obvious consequence of NPC1-deficiency is the progressive loss of Purkinje cells in the cerebellum, which correlates with gait ataxia, dysarthria and dysphagia. In the BALB/c mouse model, which closely resembles late infantile human NPC disease [5], Purkinje cell death begins around 3 weeks of age and follows a consistent pattern wherein zebrin-II negative Purkinje cells are lost first, causing a “striped” pattern of neurodegeneration [117]. Within the Purkinje cell population, neurons in lobules IX and X survive the longest [118]. By 7 weeks of age, only 30% of Purkinje cells remain, and by 11 weeks Purkinje cell death is nearly complete [119]. Neuronal loss also occurs in the substantia nigra of the midbrain, the pons, certain areas of the brainstem and, to a lesser extent, in the thalamus and pre-frontal cortex [119–122]. The reason for the selective death of defined populations of neurons in NPC1-deficient brains is not clear. However, changes in activities and distribution of lysosomal enzyme are more pronounced in areas of the brain that undergo extensive neuron loss (cerebellum) than in relatively protected areas (hippocampus) (S. Kar, personal communication).

In light of the extensive neuron death in NPC1-deficient brains, it is surprising that the survival of cultured *Npc1*<sup>-/-</sup> mouse neurons is not compromised under normal culture conditions (B. Karten, K.B. Peake and J.E. Vance, unpublished observations). In addition, in most cases, only subtle differences in function have been observed between *Npc1*<sup>+/+</sup> and *Npc1*<sup>-/-</sup> neurons, despite the pronounced lipid storage that is typical of all NPC1-deficient cells. Nevertheless, many other factors that are not recapitulated in neuronal cultures are likely to influence neuronal functions *in vivo*, including demyelination, abnormal synaptic connections due to ectopic dendrites or degeneration of axonal termini, and cross-talk between glia and neurons. Furthermore, neuronal culture conditions are normally optimized to provide sufficient glucose, oxygen and antioxidants and a stable pH environment at all times. It will be important to understand why the survival of cultured NPC1-deficient neurons is nearly normal whereas extensive neuron death occurs in NPC1-deficient brains since this might indicate strategies for therapeutic intervention. Nevertheless, if

neuron death and dysfunction in NPC disease were an inherent property of neurons *per se*, one would expect that survival of primary *Npc1*<sup>-/-</sup> neurons would decrease after extended periods of culture.

The precise mode of neuronal death in NPC1-deficiency is not yet clear. The widespread occurrence of apoptosis in NPC1-deficient brains is indicated by an increased number of TUNEL-positive nuclei [123], increases in caspase 1 and 3 mRNA [124] and marked increases in tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and its downstream signaling molecules caspase 8, FADD, TNFRp55, TRADD, and RIP [119,123]. Consistent with these observations, TNF $\alpha$  signaling was identified as a key pathway leading to apoptosis of hepatocytes from mice in which NPC1 deficiency was induced in the liver [125]. Further evidence supporting a central role for apoptosis in the death of NPC1-deficient neurons is the finding that c-Abl, p73 and its phosphorylated form, as well as p73 target proteins, were increased in murine NPC1-deficient cerebellum [126]. Moreover, *in vivo* inhibition of the c-Abl pathway with weekly injections of imatinib mesylate delayed cerebellar neuron loss and improved motor function of NPC1-deficient mice [126]. However, the imatinib treatment modestly prolonged life-span of the mice, but only when the treatment was initiated at postnatal day 7, not at postnatal day 28. Activation of c-Abl/p73 by TNF $\alpha$  signaling has also been previously observed in mouse embryonic fibroblasts [127]. These findings support a role for TNF $\alpha$  signaling in neuronal death in NPC disease. On the other hand, neuronal over-expression of the anti-apoptotic protein Bcl-2 did not improve neuron survival or increase lifespan of *Npc1*<sup>-/-</sup> mice [128]. It is likely that several pro-apoptotic factors contribute to neuronal death in NPC1-deficiency, including lysosomal dysfunction, increased oxidative stress, ER stress and/or energy deprivation.

Neuronal death in NPC disease might also involve autophagy as well as apoptosis. Autophagy is the mechanism by which long-lived proteins and organelles are degraded by lysosomes. Cytoplasmic constituents are enclosed by a limiting membrane thereby forming autophagosomes that subsequently fuse with lysosomes, allowing the cargo to be digested by lysosomal enzymes [129–132]. A basal level of autophagy is crucial for neuron viability [133,134]. During starvation, autophagy promotes cell survival by digesting non-essential proteins and organelles for energy substrates, a process which is particularly important during development [135]. Autophagy is distinct from apoptosis and can occur in cells that are resistant to apoptosis [136].

Autophagy has been observed in a range of neurodegenerative disorders including Alzheimer disease, Parkinson disease and Huntington disease. Electron microscopic examination of degenerating Purkinje cells in NPC1-null mice showed increased numbers of autophagic vacuoles [137–139]. The autophagy marker, microtubule-associated protein 1 light chain 3-II (LC3-II) [140], is increased in cerebellar and liver isolates from NPC1-null mice, as well as in fibroblasts from NPC patients [138,139]. Beclin-1, which promotes autophagy, is also increased, as is the degradation of long-lived proteins [138]. Moreover, cells treated with U18666A, and NPC2-deficient cells show similarly increased numbers of autophagic vesicles, LC3-II and Beclin-1 [138]. However, depletion of cellular cholesterol in human fibroblasts with methyl- $\beta$ -cyclodextrin, nystatin or mevastatin also induced autophagy [141], suggesting that altered cholesterol distribution in NPC1-deficient cells promotes autophagy as compensation for the trapping of cholesterol within LE/L. Autophagy-deficient mice show ataxia, tremor and premature death, as well as degeneration of Purkinje cells [142]. In addition, polyubiquitinated protein inclusion bodies are increased in autophagy-deficient mice [142] and NPC1-deficient cells [118,139,143]. Thus, controversy remains about whether autophagy leads to neurodegeneration or is an attempt to promote survival. To determine if autophagy is beneficial or harmful in NPC disease, experiments are required in which autophagy is inhibited in models of NPC deficiency.

The late endosomal/lysosomal sequestration of cholesterol and GSLs, including glucosylceramide, lactosylceramide and complex gangliosides (mainly GM2 and GM3), in NPC1-deficient brains

[71,144–147] is thought to be responsible not only for neuron death and axonal degeneration but also for some of the profound morphological changes that occur in human, feline and murine NPC1-deficient brains (reviewed in [148]). Swollen neuronal cell bodies, containing lamellar and multi-vesicular structures, have been detected throughout the CNS in NPC1-deficient mice and cats [75,148]. Moreover, abnormal lipid storage in NPC1-deficient brains is thought to induce the formation of “meganeurites” which are enlargements of axonal hillocks in neurons [148] and are probably caused by the aberrant movement of lipid-filled storage vesicles from cell bodies into proximal axons [147]. Similarly, in cultured NPC1-deficient neurons LAMP1-immunoreactive lysosomal structures, which normally are largely restricted to cell bodies, were detected in proximal neurites (B. Karten and J.E. Vance, unpublished observations and Fig. 2). The presence of these large storage vesicles in proximal axons might create a “roadblock” that would impair axonal trafficking. In NPC1-deficient cats and humans, and, to a lesser extent in NPC1-deficient mice, abnormal dendritogenesis, particularly around the swollen neurites, leads to formation of ectopic dendrites, which could contribute to altered synaptic connectivity [18,148]. Ectopic dendrite formation is likely caused by the accumulation of gangliosides because similar morphological changes are observed in other lysosomal ganglioside storage disorders [149].

Although much insight has been provided in the last few years into the links between impaired lipid homeostasis and morphological/functional changes in the brain in NPC disease, the mechanistic pathways that connect the loss of NPC1 function to neuronal dysfunction and the symptoms of NPC disease remain to be elucidated.

#### 4.4. Axonal transport and degeneration

In addition to classical apoptosis, where cell death is initiated in the cell body, axonal degeneration, that can lead to neuronal dysfunction and death, is also observed in NPC disease [148]. Degenerating axonal termini have been observed histochemically in many areas of NPC1-deficient brains, even in areas that do not show prominent cell death, such as the hippocampus [25]. Widespread formation of axonal spheroids in NPC1-deficient brains correlates with neuroaxonal dystrophy and degeneration of axons [18,147,148,150,151]. Histochemically, axonal spheroids in NPC1-deficient mouse brains stain for  $\beta$ -amyloid protein, ubiquitin, and phosphorylated and non-phosphorylated neurofilaments, and often also contain stranded organelles such as mitochondria [150,151]. The dying back of axons from the terminal in NPC disease strongly indicates impaired axonal transport. This conjecture is supported by studies in isolated neurons in which defects in retrograde signaling of BDNF [108] and impaired anterograde transport of cholesterol [78] were observed. On the other hand, axonal extension of cultured NPC1-deficient sympathetic neurons, a process which clearly requires active anterograde axonal transport, is normal [77].

In addition to axonal spheroids, neurofibrillary tangles (NFT) that are ultrastructurally identical to those seen in Alzheimer disease, were observed in human post-mortem brains from NPC patients as young as 10 years of age [76,152–154]; NPC1 disease is the only known condition in which tangle formation occurs at such an early age. The presence of NFT has been linked to impaired axonal transport, and often follows axonal spheroid formation [155]. The exact mechanism of tangle formation and the role of lipid/cholesterol homeostasis in this process are not clear although several observations indicate that defective cholesterol homeostasis is involved in NFT formation. First, neurons that have a high cholesterol content are more likely to contain NFT than are neurons with little cholesterol storage [76]. Second, cholesterol deficiency in cultured murine neurons leads to hyperphosphorylation of tau and microtubule degeneration [156,157]. Although NPC1-deficient mice do not form NFT [120], these mice show a tau phosphorylation pattern that is characteristic of the NFT that are seen

in human Alzheimer disease [74,158,159]. It should be noted that the lack of tangle formation by hyperphosphorylated tau is common to most rodents and has been ascribed to species differences in tau [160].

Candidate kinases that might phosphorylate tau include cyclin dependent kinases 2 and 5 (cdk2 and cdk5) [159,161]. The level of p25, a cdk5-activating cleavage fragment of p35, is increased in NPC1-deficient mouse brains suggesting a role for cdk5 in tau phosphorylation [159]. Moreover, *in vivo* inhibition of cdk5 by infusion of roscovitine directly into brains of NPC1-deficient mice reduced tau phosphorylation and axonal spheroid formation [162]. The authors hypothesized that the localization of calpain (which cleaves p35 to p25 and thereby indirectly activates cdk5) to cholesterol-rich “raft” domains provides a link between altered cholesterol homeostasis and tau phosphorylation [163]. However, deletion of p35 in NPC1-deficient mice did not reduce tau hyperphosphorylation despite a 78% reduction in cdk5 activity [164]. The involvement of cdk2 in tau phosphorylation remains to be elucidated.

#### 4.5. Involvement of glia in NPC disease: astrocytes and microglia

Neurons are not the only cells in the brain that are affected by loss of NPC1. Astrocytes [73,79] and microglia (K.B. Peake and J.E. Vance, unpublished observations) also accumulate lipids. Astrocytes are the major glial cells in the CNS and express high levels of NPC1 [165,166]. Activated astrocytes accumulate in NPC1-null mouse brains [73,120,167] although this accumulation might be secondary to microglial activation [168]. The level of glial fibrillary acidic protein (a marker of astrocytes) is increased in all brain areas, particularly in the cerebellum and thalamus, of NPC1-deficient mice as young as 3 weeks of age [73,79,123,169]. NPC1-deficient astrocytes also secrete increased amounts of interleukin-6; elimination of interleukin-6 modestly extended life-span of NPC1-null mice [170]. These data suggest that astrocytes might contribute to the disease process.

Microglia are the primary immunocompetent and phagocytic cells of the CNS (reviewed in [171]). Typically, microglia are the first cells that respond to injury, becoming rapidly activated as indicated by a change in morphology from a resting (ramified) state to a motile (ameboid) state [172,173]. Activated microglia can proliferate [174] and migrate to the site of injury [173,175] where they phagocytose dying cells and debris [176]. Activated microglia also secrete a variety of potentially cytotoxic molecules including glutamate [177,178], reactive oxygen species [179], nitric oxide [180], and pro-inflammatory cytokines [181,182], which might potentiate an inflammatory response. On the other hand, microglia also release anti-inflammatory cytokines that might be neuroprotective [183–185]. The type of response appears to depend on the stimulus acting upon the microglia. These wide ranging capabilities allow microglia to provide the first line of defense against a variety of insults in the CNS.

Although microglia clearly play a critical role in maintaining a healthy brain, there is increasing evidence that chronic activation or dysfunction of microglia contributes to cell death in several neurodegenerative disorders including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis and the lysosomal storage disorder, Sandhoff disease [186–189]. Minocycline, a tetracycline derivative that inhibits microglial activation [190] delayed disease progression in animal models of some of these disorders [191]. In mouse models of amyotrophic lateral sclerosis and Sandhoff disease, replacement of mutant microglia with wild-type microglia by bone marrow transplantation delayed the onset, and slowed the course, of the disease [189,192]. Furthermore, activation of microglia precedes the loss of neurons in Sandhoff disease [189]. Thus, microglial activation appears to be detrimental in these disorders and might directly contribute to the neurodegenerative process. In NPC1-deficient mouse brains, microglia contain intracellular inclusions [73,143] and show altered cholesterol distribution characteristic of other NPC1-deficient cells (K.B. Peake and J.E. Vance, unpublished observations). The thalamus and cerebellum of

NPC1-null mouse brains contain increased numbers of activated microglia, even in 2-week-old mice, prior to any detectable neurodegeneration. As the disease progresses, activated microglia appear throughout the brain [73,167,168]. In addition, levels of mRNAs encoding the pro-inflammatory cytokine TNF $\alpha$  and other genes of the TNF $\alpha$  pathway are increased in the cerebellum of NPC1-null mice [119,123,193], and interleukin-6 and toll-like receptor-4 are increased in microglia and other glial cells in the cerebellum [170].

None of these experiments, however, provides direct evidence that microglia cause neuronal death in NPC disease. Treatment of NPC1-null mice with a low dose of minocycline did not increase survival but inactivation of the microglia was not demonstrated [128]. Since minocycline is highly acidic and is not well tolerated by mice, it is possible that any beneficial effects of minocycline were masked by the stress induced by repeated injections (K.B. Peake and J.E. Vance, unpublished data).

In experiments designed to determine whether neuronal death in NPC disease is due to an inherent problem in the neurons or is caused by activation of glial cells, Ko et al. generated a chimeric mouse model in which various numbers of *Npc1*<sup>+/+</sup> and *Npc1*<sup>-/-</sup> cells were present in the brain. NPC1-expressing Purkinje cells survived even when surrounded by NPC1-null microglia, and NPC1-expressing microglia did not rescue NPC1-null Purkinje cells [137]. From these observations, the authors concluded that Purkinje cell death in NPC disease is a cell-autonomous process. These experiments did not, however, rule out the possibility that NPC1-null microglia cause the death of NPC1-null Purkinje cells. Furthermore, astrocyte-only expression of NPC1 in *Npc1*<sup>-/-</sup> mice, using the glial fibrillary acidic protein promoter, reduced cholesterol sequestration in the brain and tripled the life-span of *Npc1*<sup>-/-</sup> mice in the absence of increased Purkinje cell survival [194].

Thus, the role of glia in neuronal death in NPC disease remains controversial. Further work is required to elucidate the cause of microglial activation and to determine if microglial activation translates into neurotoxicity. Although microglia might secrete neurotoxic molecules, leading to neuronal death, it is also possible that since microglia normally secrete neuroprotective molecules and phagocytose cellular debris, these glial cells might contribute to the neurodegeneration through lack of trophic support and/or other beneficial functions.

## 5. NPC1-deficiency in other tissues

Although neurodegeneration is the most severe manifestation of NPC disease, other tissues including the liver, ovaries and lung also exhibit lipid storage and other abnormalities. A deficiency of neither NPC1 nor NPC2 inhibits intestinal sterol uptake or absorption [195], although the structurally-related NPC1-like-1 protein is required for sterol uptake by enterocytes [36].

Approximately 50% of infants with NPC disease suffer from hepatosplenomegaly and cholestasis [196,197]. Indeed, NPC disease is the second most common cause of neonatal cholestasis [198], and some infants die from liver disease by the age of 6 months [199]. NPC1-null mice have greatly increased (up to 15-fold) plasma levels of alanine aminotransferase and aspartate aminotransferase which are markers of liver disease [197]. The principal reason why the liver is more severely affected than many other tissues is that ~80% of LDL-derived cholesterol, and nearly all of the cholesterol in chylomicron remnants, is removed from the circulation by the liver [63,200]. Consequently, although the rate of receptor-mediated uptake of LDLs is not altered by NPC1 deficiency [63], this massive load of exogenously-supplied cholesterol becomes sequestered in the LE/L system of the liver. Thus, the cholesterol content of livers and hepatocytes isolated from *Npc1*<sup>-/-</sup> mice is 5- to 10-fold higher than in wild-type livers/hepatocytes [197,201]. In marked contrast to the situation in fibroblasts [6] and macrophages [202], however, the rate

of cholesterol esterification was increased several-fold in NPC1-deficient hepatocytes [201]. The rate of synthesis of cholesterol and phosphatidylcholine was also significantly increased, and the rate of secretion of very low density lipoproteins was doubled [201]. Another difference between lipid homeostasis in NPC1-deficient hepatocytes and fibroblasts/macrophages is that in hepatocytes the expression of ABCA1 and the efflux of cholesterol and phospholipids for formation of high density lipoproteins are increased by up to 8-fold [202]. In contrast, in fibroblasts and macrophages the expression of ABCA1 and lipidation of high density lipoproteins are reduced [203]. Clearly, NPC1 deficiency regulates lipid metabolism by distinct mechanisms in hepatocytes and other cell types such as fibroblasts and macrophages but the regulatory mechanisms involved have not yet been elucidated.

Livers of NPC1-null mice are characterized by inflammation, fibrosis and apoptosis of hepatocytes. Since TNF $\alpha$  has been implicated in pathways leading to both apoptosis and inflammation, Rimkunas et al. tested the hypothesis that TNF $\alpha$  plays a key role in liver disease in NPC-deficient mice [125]. In these studies, the expression of NPC1 was reduced by intraperitoneal injection of a specific anti-sense oligonucleotide [204] into mice that lacked TNF $\alpha$ . The liver disease (apoptosis and inflammation) caused by NPC1 deficiency was less pronounced than in mice that expressed TNF $\alpha$  [125].

## 6. Potential therapies for NPC disease

Currently, the main criterion used for identification of a compound or pathway that would be beneficial for NPC disease is the ability to reduce filipin staining (cholesterol sequestration) in NPC1-deficient fibroblasts. These measurements can be automated for high throughput screening of drug [205] or genetic interventions. However, it is not certain that an intervention that reduces cholesterol sequestration in fibroblasts will also do so in cells of the brain, such as neurons. Although it is likely that mobilization of cholesterol from LE/L will improve neuronal function, this assumption also needs to be confirmed. Furthermore, the extent to which cholesterol must be mobilized to achieve benefit has not been established. Importantly, any potential therapeutic compound must be able to cross the blood-brain barrier and act on cells in the CNS since defects in the brain are the major cause of death and disability in NPC disease.

Ideally, a therapy for NPC disease would prevent neuronal death and dysfunction and restore any functions lost prior to the time of diagnosis. These goals can likely be achieved only by gene replacement and/or replacement of lost and dysfunctional cells. Neither of these approaches is currently feasible. A gene such as *NPC1*, which encodes a ubiquitously expressed, integral membrane protein that is segregated in the CNS by the blood-brain barrier, is probably the most difficult target for gene replacement therapy. By the time that symptoms of NPC disease are noticeable, significant neurodegeneration has already occurred. Remarkably, even when 40% of Purkinje cells in *Npc1*<sup>-/-</sup> mice have died, almost no neurological symptoms are evident [137]. At that stage, improvement of disease symptoms would require cell replacement therapy (for example, stem cell therapy) which, although a promising approach, has not yet been used for consistently successful treatment of neurodegenerative diseases. Currently, therapeutic efforts are mainly directed towards treatment of symptoms, and as such can be expected to improve quality of life of NPC patients. However, there is an obvious need for effective ways to slow disease progression. Treatment strategies employed so far are as varied as the cellular pathologies [206]. In the following sections we shall summarize some of the drug and genetic interventions tested to date. If not stated otherwise, all these studies were performed in NPC1-deficient mice.

Independent of which lipids become trapped first in the endocytic pathway, and which accumulate secondarily, the excessive storage of any lipid in multi-vesicular bodies within neurons is bound to be

detrimental. Most intervention strategies for treatment of NPC disease are aimed at reducing the lipid storage and have been designed to target the sequestration of either GSLs or cholesterol in LE/L. It is important to bear in mind, however, that storage of a lipid in one compartment of the cell can lead to a deficiency of that lipid in other cellular locations, and to a general imbalance of lipid homeostasis, both of which might be detrimental.

### 6.1. Strategies for reducing GSL storage

Reduction of GSL storage has been approached both genetically and through substrate reduction therapy in analogy to other treatments of GSL storage disorders. Several mouse models have been generated in which *Npc1*<sup>-/-</sup> mice were crossed with mice defective in GSL synthesis. For example, targeted deletion of GalNAc transferase in *Npc1*<sup>-/-</sup> mice prevented GM2 ganglioside accumulation in the brain and slightly reduced cholesterol storage but did not improve Purkinje cell survival or extend the life-span of NPC1-deficient mice [207]. These observations suggested that the neuropathology was not caused by GM2 accumulation but more likely resulted from the accumulation of cholesterol, GM3, glucosylceramide, lactosylceramide and/or sphingosine, which were essentially unaltered by elimination of GalNAc transferase [207]. More recently, mice were generated that were unable to synthesize GM2/GD2 or GM3 complex gangliosides due to deletion of the *Galgt1* or *Siat9* genes, respectively. The cholesterol content and rate of cholesterol synthesis in brains of these mice were the same as in wild-type mice, indicating that cholesterol metabolism in the brain is independent of complex ganglioside metabolism [169]. When *Npc1*<sup>-/-</sup> mice were crossed with either the *Galgt1*<sup>-/-</sup> or *Siat9*<sup>-/-</sup> mice, cholesterol concentration and the rate of synthesis in the CNS were unchanged. However, life-span was significantly shorter than for *Npc1*<sup>-/-</sup> mice, presumably because some essential functions require GSLs [169]. These results support the concept that cholesterol sequestration plays a central role in NPC disease and demonstrate that ganglioside concentration does not determine the concentration of cholesterol in *Npc1*<sup>+/+</sup> and *Npc1*<sup>-/-</sup> mice.

Substrate reduction therapy has provided some benefit for treatment of NPC disease. Inhibition of glycolipid synthesis in NPC1-deficient mice at the step catalyzed by glucosylceramide synthase, using *N*-butyl deoxynojirimycin, showed modest benefit. The depletion of GSLs delayed the onset of symptoms and increased life-span by ~20% [147]. Since this drug had already been approved for treatment of Gaucher disease under the name miglustat, clinical trials were initiated for its use in a small number of NPC patients. Results to date indicate that treatment with miglustat caused a mild clinical improvement in the patients according to brain magnetic resonance spectroscopy [208]. Thus, prevention of GSL storage appears to provide a modest, beneficial effect on NPC disease pathogenesis.

### 6.2. Strategies for reducing cholesterol storage

Attempts to reduce cholesterol accumulation by methods commonly used to modulate peripheral cholesterol metabolism have not been successful either in reducing cholesterol sequestration in neurons or glial cells, or in alleviating the symptoms of NPC disease. For example, cholesterol-lowering drugs (probucol and nifedipine) reduced the unesterified cholesterol content of livers of NPC1-deficient mice, but did not decrease either cholesterol sequestration in the CNS or ameliorate the neurodegeneration [209]. In addition, treatment of NPC patients with statins (inhibitors of cholesterol synthesis) reduced plasma cholesterol but did not noticeably improve the neuropathology [210]. In other studies, intraperitoneal injection of squalstatin into 2-week-old mice reduced brain cholesterol biosynthesis and storage, as well as GM3 accumulation and infiltration by microglia [211]. However, neither the neuropathological status nor the life-span of these mice was reported, possibly because the treatment impaired myelination

which would likely have masked any beneficial effect. In other studies, cholesterol feeding of *Npc1*<sup>-/-</sup> mice increased the hepatic storage of cholesterol but did not adversely affect life-span [16]. In addition, breeding of *Npc1*<sup>-/-</sup> mice with LDL-receptor knock-out mice did not change cholesterol levels in the brain, improve the neuropathology, or increase the life-span of *Npc1*<sup>-/-</sup> mice [16,193,209]. When considering the implications of these experiments one must bear in mind that plasma lipoproteins do not enter the CNS. Thus, lowering the plasma cholesterol content is unlikely to lower the cholesterol content of the brain. Moreover, only the types of statins that can effectively cross the blood-brain barrier are likely to modulate cholesterol metabolism in the brain.

Another idea that was tested to remove excess cholesterol from the brain was to increase the expression of ABCA1, a membrane transporter that is required for the efficient efflux of cholesterol and phospholipids from cells. The expression of several ABC transporters, including ABCA1, ABCG1 and ABCG4, which mediate cholesterol efflux from glia and neurons in the CNS, is increased by the liver X receptor (LXR) [100,212–214]. In cultured NPC1-deficient fibroblasts ABCA1 expression was decreased and the ABCA1-dependent efflux of cholesterol was impaired [203,215]. It is likely that the diminished production of the endogenous oxysterol ligands for LXR was responsible for the decreased expression of NPC1 [216]. Treatment of *Npc1*<sup>-/-</sup> mice with a LXR agonist, T0901317, delayed the onset of neurological symptoms and decreased Purkinje cell loss, but did not increase survival although the effects of T0901317 treatment on brain lipid storage in *Npc1*<sup>-/-</sup> mice were not reported [217]. In addition, treatment of primary cultured glia from *Npc1*<sup>-/-</sup> mice with T0901317 alone, and in combination with apo A1, reduced cholesterol accumulation [79]. In contrast, filipin staining of cultured *Npc1*<sup>-/-</sup> hippocampal neurons was not significantly reduced following treatment with T0901317, with or without apo A1 (B. Karten, unpublished observation). In another study T0901317 increased cholesterol excretion from the brains of *Npc1*<sup>-/-</sup> mice, slightly reduced the number of cells with intracellular cholesterol accumulation, modestly prolonged life-span (from 90 to 101 days) and improved motor coordination [16,193]. Microglial morphology was also shifted from an activated state to a resting state and Purkinje cell loss was reduced [193]. The role of LXR and ABCA1 in NPC disease was also investigated by breeding *Npc1*<sup>-/-</sup> mice with ABCA1 knock-out mice. Sterol balance in the brain was not altered, nor was longevity increased [16,193]. However, the importance of LXR for cholesterol homeostasis in the CNS and in NPC disease was underscored by the finding that elimination of LXR $\beta$  in NPC1-deficient mice significantly reduced their life-span [193]. From a combination of these studies, it is unclear whether cholesterol mobilization is the primary mechanism that mediates the beneficial effects of LXR agonists in NPC1-deficient mice or whether the anti-inflammatory effects of LXR activation play a key role [193,218].

### 6.3. Other therapeutic strategies

Not all of the small molecules tested for treatment of NPC disease are directed towards reducing lipid storage in the brain. In 2004, Mellon et al. reported that neurosteroidogenesis is impaired in NPC1-deficient mice; levels of allopregnanolone and activities of most steroidogenic enzymes downstream of the P450<sub>scc</sub> complex (CYP11A1) that converts cholesterol to pregnenolone, were reduced [219,220]. In an *in vivo* study, a single subcutaneous injection of allopregnanolone into *Npc1*<sup>-/-</sup> mice at postnatal day 7 significantly extended the life-span of the mice from 67 days to 124 days, whereas injections later in life were ineffective [219]. The benefit of allopregnanolone treatment was also examined in *Npc1*<sup>-/-</sup> mice in combination with repeated treatments with a LXR agonist [217]; survival and the neuropathological phenotype were further improved. Moreover, allopregnanolone treatment of cultured NPC1-deficient

fibroblasts or SH-SY5Y neuroblastoma cells reduced the generation of reactive oxygen species and normalized the increased sensitivity to oxidative insult [221]. Allopregnanolone was initially proposed to act through the highly specific GABA<sub>A</sub> receptor [219,220]. However, this was found not to be the case since the enantiomeric form of this steroid, which does not activate the GABA<sub>A</sub> receptor, was also beneficial [217]. Moreover, the proposal that allopregnanolone acted via the PXR nuclear receptor, as suggested by Langmade et al. [217], is also unlikely because PXR expression is below the limit of detection in mouse brain [193].

Recently, the beneficial effect of allopregnanolone in NPC1-deficient mice has been questioned. In contrast to the original studies by Mellon et al., similar improvement in disease symptoms was achieved by treatment of larger groups of *Npc1*<sup>-/-</sup> mice with the vehicle alone (i.e. the cyclodextrin that carried the allopregnanolone) on postnatal day 7 [16]. The cyclodextrin treatment significantly increased Purkinje cell survival, and life-span was extended by 20–25% [16]. Since cyclodextrin is known for its ability to complex cholesterol and other sterols, the beneficial effects of this agent are likely to be due to the removal of cholesterol from cells of the brain.

Yet another proposed treatment for NPC disease was based on the observation that calcium homeostasis is altered by NPC1-deficiency as a result of sphingosine storage in LE/L [55]. The origin of the accumulating sphingosine was not addressed but this toxic lipid is likely to be generated from the breakdown of GSLs that accumulate in LE/L. The naturally-occurring compound curcumin, a component of the spice turmeric, was proposed to release calcium from the ER, elevate cytosolic calcium levels, and thus mobilize cholesterol from LE/L [55]. When *Npc1*<sup>-/-</sup> mice were treated with curcumin, life-span was modestly increased and the onset of neurological symptoms was delayed [55]. Since curcumin exhibits low toxicity, this agent was proposed as an appealing nutraceutical intervention for NPC disease. However, the bioavailability of curcumin is very low which limits its effectiveness [222]. Furthermore, the dose of curcumin given to the mice [55] (150 mg/kg/day) corresponds to a daily intake of ~10.5 g of curcumin (or 315 g turmeric) per day for an adult human [222], which is not feasible as a dietary intake for NPC patients. The development of new forms of curcumin with increased bioavailability, might make this a more useful treatment [223]. It is important to note, however, that the beneficial effect of curcumin in *Npc1*<sup>-/-</sup> mice cannot necessarily be ascribed to modulation of calcium homeostasis, since curcumin also has well-characterized anti-oxidant and anti-inflammatory effects [222].

A novel, possible therapeutic approach for the future for some patients with NPC disease is based on the recent observation that the NPC1 protein containing the most common mutation in human patients, I1061T, is functional if it can reach its correct localization in LE/L [224]. The vast majority of NPC1 carrying this mutation is normally degraded by ER-associated degradation [224]. Provision of molecular chaperones that would aid in the correct folding of the mutant NPC1 protein, and enable it to escape the ER quality control mechanism, might be a viable option for treatment of NPC patients with this mutation [225]. Development of such a chaperone molecule would require a new mouse model carrying the I1061T mutation in NPC1 since the null mutation in the commonly-used NPC1-deficient mice would not be amenable to chaperone therapy. The discovery of a molecular chaperone for NPC1 will likely involve a trial-and-error approach. Importantly, any molecule that will be useful for treatment of NPC patients must be able to cross the blood-brain barrier and not cause severe detrimental side effects. In addition, the therapeutic agent would most likely need to target both neurons and glia to be effective.

Thus, a combination of several treatments currently appears to be the most promising approach for extending the life-span and improving the quality of life of NPC patients since each of the treatments tested to date only modestly improves disease progression.

## References

- [1] M.T. Vanier, G. Millat, *Clin. Genet.* 64 (2003) 269–281.
- [2] A.C. Crocker, S. Farber, *Medicine (Baltimore)* 37 (1958) 1–95.
- [3] R.O. Brady, J.N. Kanfer, M.B. Mock, D.S. Fredrickson, *Proc. Natl. Acad. Sci. U. S. A.* 55 (1966) 366–369.
- [4] P.G. Pentchev, A.D. Boothe, H.S. Kruth, H. Weintraub, J. Stivers, R.O. Brady, *J. Biol. Chem.* 259 (1984) 5784–5791.
- [5] S.K. Loftus, J.A. Morris, E.D. Carstea, J.Z. Gu, C. Cummings, A. Brown, J. Ellison, K. Ohno, M.A. Rosenfeld, D.A. Tagle, P.G. Pentchev, W.J. Pavan, *Science* 277 (1997) 232–235.
- [6] L. Liscum, J.R. Faust, *J. Biol. Chem.* 262 (1987) 17002–17008.
- [7] L. Liscum, R.M. Ruggiero, J.R. Faust, *J. Cell Biol.* 108 (1989) 1625–1636.
- [8] K.M. Cadigan, M., D.M. Spillane, T.-Y. Chang, *J. Cell Biol.* 110 (1990) 295–308.
- [9] N.K. Dahl, K.L. Reed, M.A. Daunais, J.R. Faust, L. Liscum, *J. Biol. Chem.* 267 (1992) 4889–4896.
- [10] P.G. Pentchev, M.E. Comly, H.S. Kruth, T. Tokoro, J. Butler, J. Sokol, M. Filling-Katz, J.M. Quirk, D.C. Marshall, S. Patel, et al., *FASEB J.* 1 (1987) 40–45.
- [11] J.W. Reagan, M.L. Hubbert, G.S. Shelness, *J. Biol. Chem.* 275 (2000) 38104–38110.
- [12] E.D. Carstea, M.H. Polymeropoulos, C.C. Parker, S.D. Detera-Wadleigh, R.R. O'Neill, M.C. Patterson, E. Goldin, H. Xiao, R.E. Straub, M.T. Vanier, et al., *Proc. Natl. Acad. Sci. U. S. A.* 90 (1993) 2002–2004.
- [13] E.D. Carstea, J.A. Morris, K.G. Coleman, S.K. Loftus, D. Zhang, C. Cummings, J. Gu, M.A. Rosenfeld, W.J. Pavan, D.B. Krizman, J. Nagle, M.H. Polymeropoulos, S.L. Sturley, Y.A. Ioannou, M.E. Higgins, M. Comly, A. Cooney, A. Brown, C.R. Kanetski, E.J. Blanchette-Mackie, N.K. Dwyer, E.B. Neufeld, T.Y. Chang, L. Liscum, J.F. Strauss 3rd, K. Ohno, M. Zeigler, R. Carmi, J. Sokol, D. Markie, R.R. O'Neill, O.P. van Diggelen, M. Elleder, M.C. Patterson, R.O. Brady, M.T. Vanier, P.G. Pentchev, D.A. Tagle, *Science* 277 (1997) 228–231.
- [14] S. Naureckiene, D.E. Sleat, H. Lackland, A. Fensom, M.T. Vanier, R. Wattiaux, M. Jadot, P. Lobel, *Science* 290 (2000) 2298–2301.
- [15] J. Sokol, E.J. Blanchette-Mackie, H.S. Kruth, N.K. Dwyer, L.M. Amende, J.D. Butler, E. Robinson, S. Patel, R.O. Brady, M.E. Comly, M.T. Vanier, P.G. Pentchev, *J. Biol. Chem.* 263 (1988) 3411–3417.
- [16] B. Liu, H. Li, J.J. Repa, S.D. Turley, J.M. Dietsch, *J. Lipid Res.* 49 (2008) 663–669.
- [17] D.E. Sleat, J.A. Wiseman, M. El-Banna, S.M. Price, L. Verot, M.M. Shen, G.S. Tint, M.T. Vanier, S.U. Walkley, P. Lobel, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 5886–5891.
- [18] P.A. March, M.A. Thrall, D.E. Brown, T.W. Mitchell, A.C. Lowenthal, S.U. Walkley, *Acta Neuropathol. (Berl)* 94 (1997) 164–172.
- [19] K.L. Somers, D.E. Brown, R. Fulton, P.C. Schultheiss, D. Hamar, M.O. Smith, R. Allison, H.E. Connally, C. Just, T.W. Mitchell, D.A. Wenger, M.A. Thrall, *J. Inher. Metab. Dis.* 24 (2001) 427–436.
- [20] C.H. Vite, W. Ding, C. Bryan, P. O'Donnell, K. Cullen, D. Aleman, M.E. Haskins, T. Van Winkle, *Pediatr. Res.* 64 (2008) 544–549.
- [21] X. Huang, K. Suyama, J. Buchanan, A.J. Zhu, M.P. Scott, *Development* 132 (2005) 5115–5124.
- [22] K. Malathi, K. Higaki, A.H. Tinkelenberg, D.A. Balderes, D. Almanzar-Paramio, L.J. Wilcox, N. Erdeniz, F. Redican, M. Padamsee, Y. Liu, S. Khan, F. Alcantara, E.D. Carstea, J.A. Morris, S.L. Sturley, *J. Cell Biol.* 164 (2004) 547–556.
- [23] H. Yang, *Trends Cell Biol.* 16 (2006) 427–432.
- [24] E.B. Neufeld, M. Wastney, S. Patel, S. Suresh, A.M. Cooney, N.K. Dwyer, C.F. Roff, K. Ohno, J.A. Morris, E.D. Carstea, J.P. Incardona, J.F. Strauss, M.T. Vanier, M.C. Patterson, R.O. Brady, P.G. Pntchev, E.J. Blanchette-Mackie, *J. Biol. Chem.* 274 (1999) 9627–9635.
- [25] S.C. Patel, S. Suresh, U. Kumar, C.Y. Hu, A. Cooney, E.J. Blanchette-Mackie, E.B. Neufeld, R.C. Patel, R.O. Brady, Y.C. Patel, P.G. Pentchev, W.Y. Ong, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 1657–1662.
- [26] M.E. Higgins, J.P. Davies, F.W. Chen, Y.A. Ioannou, *Mol. Gen. Metab.* 68 (1999) 1–13.
- [27] H. Watari, E.J. Blanchette-Mackie, N.K. Dwyer, G. Sun, J.M. Glick, S. Patel, E.B. Neufeld, P.G. Pentchev, J.F. Strauss, *Exp. Cell Res.* 255 (2000) 56–66.
- [28] W.S. Garver, R.A. Heidenreich, R.P. Erickson, M.A. Thomas, J.M. Wilson, *J. Lipid Res.* 41 (2000) 673–687.
- [29] A. Frolov, K. Srivastava, D. Daphna-Iken, L.M. Traub, J.E. Schaffer, D.S. Ory, *J. Biol. Chem.* 276 (2001) 46414–46421.
- [30] M. Zhang, N.K. Dwyer, E.B. Neufeld, D.C. Love, A. Cooney, M. Comly, S. Patel, H. Watari, J.F. Strauss, P.G. Pentchev, J.A. Hanover, E.J. Blanchette-Mackie, *J. Biol. Chem.* 276 (2001) 3417–3425.
- [31] W.S. Garver, C. Xie, J.J. Repa, S.D. Turley, J.M. Dietsch, *J. Lipid Res.* 46 (2005) 1745–1754.
- [32] H. Watari, E.J. Blanchette-Mackie, N.K. Dwyer, M. Watari, C.G. Burd, S. Patel, P.G. Pentchev, J.F. Strauss 3rd, *Exp. Cell Res.* 259 (2000) 247–256.
- [33] W.S. Garver, D. Jelinek, G.A. Francis, B.D. Murphy, *J. Lipid Res.* 49 (2008) 1090–1102.
- [34] N. Gevry, K. Schoonjans, F. Guay, B.D. Murphy, *J. Lipid Res.* 49 (2008) 1024–1033.
- [35] N. Okamura, S. Kiuchi, M. Tamba, T. Kashima, S. Hiramoto, T. Baba, F. Dacheux, J.L. Dacheux, Y. Sugita, Y.Z. Jin, *Biochim. Biophys. Acta* 1438 (1999) 377–387.
- [36] S.W. Altmann, H.R. Davis Jr., L.J. Zhu, X. Yao, L.M. Hoos, G. Tetzloff, S.P. Iyer, M. Maguire, A. Golovko, M. Zeng, L. Wang, N. Murgolo, M.P. Graziano, *Science* 303 (2004) 1201–1204.
- [37] A. Nohturfft, M.S. Brown, J.L. Goldstein, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 12848–12853.
- [38] J.D. Horton, J.L. Goldstein, M.S. Brown, *J. Clin. Invest.* 109 (2002) 1125–1131.
- [39] D.C. Ko, M.D. Gordon, J.Y. Jin, M.P. Scott, *Mol. Biol. Cell* 12 (2001) 601–614.
- [40] N. Ohgami, D.C. Ko, M. Thomas, M.P. Scott, C.C. Chang, T.Y. Chang, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 12473–12478.

- [41] E.E. Millard, S.E. Gale, N. Dudley, J. Zhang, J.E. Schaffer, D.S. Ory, *J. Biol. Chem.* 280 (2005) 28581–28590.
- [42] R.E. Infante, L. Abi-Mosleh, A. Radhakrishnan, J.D. Dale, M.S. Brown, J.L. Goldstein, *J. Biol. Chem.* 283 (2008) 1052–1063.
- [43] R. Liu, P. Lu, J.W. Chu, F.J. Sharom, *J. Biol. Chem.* 284 (2009) 1840–1852.
- [44] R.E. Infante, A. Radhakrishnan, L. Abi-Mosleh, L.N. Kinch, M.L. Wang, N.V. Grishin, J.L. Goldstein, M.S. Brown, *J. Biol. Chem.* 283 (2008) 1064–1075.
- [45] S.R. Cheruku, Z. Xu, R. Dutia, P. Lobel, J. Storch, *J. Biol. Chem.* 281 (2006) 31594–31604.
- [46] Z. Xu, W. Farver, S. Kodukula, J. Storch, *Biochemistry* 47 (2008) 11134–11143.
- [47] D.C. Ko, J. Binkley, A. Sidow, M.P. Scott, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 2518–2525.
- [48] N. Friedland, H.L. Liou, P. Lobel, A.M. Stock, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 2512–2517.
- [49] R.E. Infante, M.L. Wang, A. Radhakrishnan, H.J. Kwon, M.S. Brown, J.L. Goldstein, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 15287–15292.
- [50] J.P. Davies, F.W. Chen, Y.A. Ioannou, *Science* 290 (2000) 2295–2298.
- [51] J. Passaggio, L. Liscum, *J. Biol. Chem.* 280 (2005) 10333–10339.
- [52] P.G. Pentchev, M.E. Comly, H.S. Kruth, M.T. Vanier, D.A. Wenger, S. Patel, R.O. Brady, *Proc. Natl. Acad. Sci. U. S. A.* 82 (1985) 8247–8251.
- [53] M. Zervas, K. Dobrenis, S.U. Walkley, *J. Neuropathol. Exp. Neurol.* 60 (2001) 49–64.
- [54] D. te Vrugte, E. Lloyd-Evans, R.J. Veldman, D.C. Neville, R.A. Dwek, F.M. Platt, W.J. van Blitterswijk, D.J. Sillence, *J. Biol. Chem.* 279 (2004) 26167–26175.
- [55] E. Lloyd-Evans, A.J. Morgan, X. He, D.A. Smith, E. Elliot-Smith, D.J. Sillence, G.C. Churchill, E.H. Schuchman, A. Galione, F.M. Platt, *Nat. Med.* 14 (2008) 1247–1255.
- [56] T. Kobayashi, M.H. Beuchat, M. Lindsay, S. Frias, R.D. Palmiter, H. Sakuraba, R.G. Parton, J. Gruenberg, *Nat. Cell Biol.* 1 (1999) 113–118.
- [57] S. Mukherjee, F.R. Maxfield, *Biochim. Biophys. Acta* 1685 (2004) 28–37.
- [58] T.Y. Chang, C.C. Chang, N. Ohgami, Y. Yamauchi, *Annu. Rev. Cell Dev. Biol.* 22 (2006) 129–157.
- [59] P.C. Reid, N. Sakashita, S. Sugii, Y. Ohno-Iwashita, Y. Shimada, W.F. Hickey, T.Y. Chang, *J. Lipid Res.* 45 (2004) 582–591.
- [60] J.L. Goldstein, R.A. DeBose-Boyd, M.S. Brown, *Cell* 124 (2006) 35–46.
- [61] K.M. Wojtanik, L. Liscum, *J. Biol. Chem.* 278 (2003) 14850–14856.
- [62] R.D. Shamburek, P.G. Pentchev, L.A. Zech, J. Blanchette-Mackie, E.D. Carstea, J.M. VandenBroek, P.S. Cooper, E.B. Neufeld, R.D. Phair, H.B. Brewer Jr., R.O. Brady, C.C. Schwartz, *J. Lipid Res.* 38 (1997) 2422–2435.
- [63] C. Xie, S.D. Turley, J.M. Dietschy, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 11992–11997.
- [64] J.C. Cruz, T.-Y. Chang, *J. Biol. Chem.* 275 (2000) 41309–41316.
- [65] M. Hao, F.R. Maxfield, *J. Biol. Chem.* 275 (2000) 15279–15286.
- [66] Y. Urano, H. Watanabe, S.R. Murphy, Y. Shibuya, Y. Geng, A.A. Peden, C.C. Chang, T.Y. Chang, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 16513–16518.
- [67] V.M. Olkkonen, T.P. Levine, *Biochem. Cell Biol.* 82 (2004) 87–98.
- [68] S. Jabs, A. Quitsch, R. Kakela, B. Koch, J. Tyynela, H. Brade, M. Glatzel, S. Walkley, P. Saftig, M.T. Vanier, T. Braulke, J. Neurochem. 106 (2008) 1415–1425.
- [69] J. Chevallier, Z. Chamoun, G. Jiang, G. Prestwich, N. Sakai, S. Matile, R.G. Parton, *J. Gruenberg, J. Biol. Chem.* 283 (2008) 27871–27880.
- [70] V. Puri, J.R. Jefferson, R.D. Singh, C.L. Wheatley, D.L. Marks, R.E. Pagano, *J. Biol. Chem.* 278 (2003) 20961–20970.
- [71] M.T. Vanier, *Neurochem. Res.* 24 (1999) 481–489.
- [72] C. Xie, D.K. Burns, S.D. Turley, J.M. Dietschy, *J. Neuropathol. Exp. Neurol.* 59 (2000) 1106–1117.
- [73] D.C. German, C.L. Liang, T. Song, U. Yazdani, C. Xie, J.M. Dietschy, *Neuroscience* 109 (2002) 437–450.
- [74] S. Treiber-Held, R. Distl, V. Meske, F. Albert, T.G. Ohm, *J. Pathol.* 200 (2003) 95–103.
- [75] A. Yamada, M. Saji, Y. Ukita, Y. Shinoda, M. Taniguchi, K. Higaki, H. Ninomiya, K. Ohno, *Brain Dev.* 23 (2001) 288–297.
- [76] R. Distl, S. Treiber-Held, F. Albert, V. Meske, K. Harzer, T.G. Ohm, *J. Pathol.* 200 (2003) 104–111.
- [77] B. Karten, D.E. Vance, R.B. Campenot, J.E. Vance, *J. Neurochem.* 83 (2002) 1154–1163.
- [78] B. Karten, D.E. Vance, R.B. Campenot, J.E. Vance, *J. Biol. Chem.* 278 (2003) 4168–4175.
- [79] B. Karten, H. Hayashi, G.A. Francis, R.B. Campenot, D.E. Vance, J.E. Vance, *Biochem. J.* 387 (2005) 779–788.
- [80] M. Zhang, N.K. Dwyer, D.C. Love, A. Cooney, M. Comly, E. Neufeld, P.G. Pentchev, E.J. Blanchette-Mackie, J.A. Hanover, *Proc. Natl. Acad. Sci. U. S. A.* 98 (2001) 4466–4471.
- [81] R.A. Coxey, P.G. Pentchev, G. Campbell, E.J. Blanchette-Mackie, *J. Lipid Res.* 34 (1993) 1165–1176.
- [82] A. Choudhury, D.K. Sharma, D.L. Marks, R.E. Pagano, *Mol. Biol. Cell* 15 (2004) 4500–4511.
- [83] I.G. Ganley, S.R. Pfeffer, *J. Biol. Chem.* 281 (2006) 17890–17899.
- [84] A. Choudhury, M. Dominguez, V. Puri, D.K. Sharma, K. Narita, C.L. Wheatley, D.L. Marks, R.E. Pagano, *J. Clin. Invest.* 109 (2002) 1541–1550.
- [85] M. Walter, J.P. Davies, Y.A. Ioannou, *J. Lipid Res.* 44 (2003) 243–253.
- [86] K. Narita, A. Choudhury, K. Dobrenis, D.K. Sharma, E.L. Holicky, D.L. Marks, S.U. Walkley, R.E. Pagano, *FASEB J.* 19 (2005) 1558–1560.
- [87] M.D. Linder, R.L. Uronen, M. Holtta-Vuori, P. van der Sluijs, J. Peranen, E. Ikonen, *Mol. Biol. Cell* 18 (2007) 47–56.
- [88] T. Kaptzan, S.A. West, E.L. Holicky, C.L. Wheatley, D.L. Marks, T. Wang, K.B. Peake, J. Vance, S.U. Walkley, R.E. Pagano, *Am. J. Pathol.* 174 (2009) 14–20.
- [89] M. Valenza, D. Rigamonti, D. Goffredo, C. Zuccato, S. Fenu, L. Jamot, A. Strand, A. Tarditi, B. Woodman, M. Racchi, C. Mariotti, S. Di Donato, A. Corsini, G. Bates, R. Pruss, J.M. Olson, S. Sipione, M. Tartari, E. Cattaneo, *J. Neurosci.* 25 (2005) 9932–9939.
- [90] J.M. Dietschy, S.D. Turley, *J. Lipid Res.* 45 (2004) 1375–1397.
- [91] F.W. Pfrieger, *BioEssays* 25 (2003) 486–490.
- [92] J.K. Boyles, C.D. Zoellner, L.J. Anderson, L.M. Kosik, R.E. Pitas, K.H. Weisgraber, D.Y. Hui, R.W. Mahley, G.-H.P. J., I.M. J., E.M. Shooter, *J. Clin. Invest.* 83 (1989) 1015–1031.
- [93] E.I. Posse de Chaves, A.E. Rusiñol, D.E. Vance, R.B. Campenot, J.E. Vance, *J. Biol. Chem.* 272 (1997) 30766–30773.
- [94] E.I. Posse de Chaves, D.E. Vance, R.B. Campenot, R.S. Kiss, J.E. Vance, *J. Biol. Chem.* 275 (2000) 19883–19890.
- [95] R.W. Mahley, *Science* 240 (1988) 622–630.
- [96] D.H. Mauch, K. Nagler, S. Schumacher, C. Goritz, E.C. Muller, A. Otto, F.W. Pfrieger, *Science* 294 (2001) 1354–1357.
- [97] C. Goritz, D.H. Mauch, F.W. Pfrieger, *Mol. Cell. Neurosci.* 29 (2005) 190–201.
- [98] H. Hayashi, R.B. Campenot, D.E. Vance, J.E. Vance, *J. Biol. Chem.* 279 (2004) 14009–14015.
- [99] H.H. Hayashi, R.B. Campenot, D.E. Vance, J.E. Vance, *J. Neurosci.* 27 (2007) 1933–1941.
- [100] P.T. Tarr, P.A. Edwards, *J. Lipid Res.* 49 (2008) 169–182.
- [101] E.G. Lund, J.M. Guileyardo, D.W. Russell, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 7238–7243.
- [102] E.G. Lund, C. Xie, T. Kotti, S.D. Turley, J.M. Dietschy, D.W. Russell, *J. Biol. Chem.* 278 (2003) 22980–22988.
- [103] C. Xie, E.G. Lund, S.D. Turley, D.W. Russell, J.M. Dietschy, *J. Lipid Res.* 44 (2003) 1780–1789.
- [104] J.F. Hancock, *Nat. Rev., Mol. Cell Biol.* 7 (2006) 456–462.
- [105] L.J. Pike, *J. Lipid Res.* 47 (2006) 1597–1598.
- [106] G. Schmitz, M. Grandl, *Curr. Opin. Clin. Nutr. Metab. Care* 11 (2008) 106–112.
- [107] S. Munro, *Cell* 115 (2003) 377–388.
- [108] L.P. Henderson, L. Lin, A. Prasad, C.A. Paul, T.Y. Chang, R.A. Maue, *J. Biol. Chem.* 275 (2000) 20179–20187.
- [109] J.T. Goodrum, P.G. Pentchev, *J. Neurosci. Res.* 49 (1997) 389–392.
- [110] G. Yadid, I. Sotnik-Barkai, C. Tornatore, B. Baker-Cairns, J. Harvey-White, P.G. Pentchev, E. Goldin, *Brain Res.* 799 (1998) 250–256.
- [111] K. Byun, J. Kim, S.Y. Cho, B. Hutchinson, S.R. Yang, K.S. Kang, M. Cho, K. Hwang, M. Michikawa, Y.W. Jeon, Y.K. Paik, B. Lee, *Proteomics* 6 (2006) 1230–1236.
- [112] S.E. Phillips, E.A. Woodruff 3rd, P. Liang, M. Patten, K. Broadie, *J. Neurosci.* 28 (2008) 6569–6582.
- [113] B. Karten, R.B. Campenot, D.E. Vance, J.E. Vance, *J. Lipid Res.* 47 (2006) 504–514.
- [114] R.A. Deisz, V. Meske, S. Treiber-Held, F. Albert, T.G. Ohm, *Neuroscience* 130 (2005) 867–873.
- [115] C.R. Wasser, M. Ertunc, X. Liu, E.T. Kavalali, *J. Physiol.* 579 (2007) 413–429.
- [116] W. Yu, J.S. Gong, M. Ko, W.S. Garver, K. Yanagisawa, M. Michikawa, *J. Biol. Chem.* 280 (2005) 11731–11739.
- [117] J.R. Sarna, M. Larouche, H. Marzban, R.V. Sillitoe, D.E. Rancourt, R. Hawkes, *J. Comp. Neurol.* 456 (2003) 279–291.
- [118] Y. Higashi, S. Murayama, P.G. Pentchev, K. Suzuki, *Acta Neuropathol. (Berl)* 85 (1993) 175–184.
- [119] H. Li, J.J. Repa, M.A. Valasek, E.P. Beltroy, S.D. Turley, D.C. German, J.M. Dietschy, *J. Neuropathol. Exp. Neurol.* 64 (2005) 323–333.
- [120] D.C. German, E.M. Quintero, C.L. Liang, B. Ng, S. Punia, C. Xie, J.M. Dietschy, *J. Comp. Neurol.* 433 (2001) 415–425.
- [121] W.Y. Ong, U. Kumar, R.C. Switzer, A. Sidhu, G. Suresh, C.Y. Hu, S.C. Patel, *Exp. Brain Res.* 141 (2001) 218–231.
- [122] Z. Luan, Y. Saito, H. Miyata, E. Ohama, H. Ninomiya, K. Ohno, *J. Neurol. Sci.* 268 (2008) 108–116.
- [123] Y.P. Wu, H. Mizukami, J. Matsuda, Y. Saito, R.L. Proia, K. Suzuki, *Mol. Genet. Metab.* 84 (2005) 9–17.
- [124] M. Agassandian, J. Zhou, L.A. Tephly, A.J. Ryan, A.B. Carter, R.K. Mallampalli, *J. Biol. Chem.* 280 (2005) 21577–21587.
- [125] V.M. Rimmunas, M.J. Graham, R.M. Crooke, L. Liscum, *J. Lipid Res.* 50 (2009) 327–333.
- [126] A.R. Alvarez, A. Klein, J. Castro, G.I. Cancino, J. Amigo, M. Mosqueira, L.M. Vargas, L.F. Yevenes, F.C. Bronfman, S. Zanlungo, *FASEB J.* 22 (2008) 3617–3627.
- [127] B.N. Chau, T.T. Chen, Y.Y. Wan, J. DeGregori, J.Y. Wang, *Mol. Cell. Biol.* 24 (2004) 4438–4447.
- [128] R.P. Erickson, O. Bernard, *J. Neurosci. Res.* 68 (2002) 738–744.
- [129] A.U. Arstila, B.F. Trump, *Am. J. Pathol.* 53 (1968) 687–733.
- [130] W.A. Dunn Jr., *J. Cell Biol.* 110 (1990) 1935–1945.
- [131] D.J. Klionsky, S.D. Emr, *Science* 290 (2000) 1717–1721.
- [132] C.T. Chu, *J. Neuropathol. Exp. Neurol.* 65 (2006) 423–432.
- [133] M. Komatsu, S. Waguri, T. Chiba, S. Murata, J. Iwata, I. Tanida, T. Ueno, M. Koike, Y. Uchiyama, E. Kominami, K. Tanaka, *Nature* 441 (2006) 880–884.
- [134] T. Hara, K. Nakamura, M. Matsui, A. Yamamoto, Y. Nakahara, R. Suzuki-Migishima, M. Yokoyama, K. Mishima, I. Saito, H. Okano, N. Mizushima, *Nature* 441 (2006) 885–889.
- [135] A. Kuma, M. Hatano, M. Matsui, A. Yamamoto, H. Nakaya, T. Yoshimori, Y. Ohsumi, T. Tokuhisa, N. Mizushima, *Nature* 432 (2004) 1032–1036.
- [136] S. Shimizu, T. Kanaseki, N. Mizushima, T. Mizuta, S. Arakawa-Kobayashi, C.B. Thompson, Y. Tsujimoto, *Nat. Cell Biol.* 6 (2004) 1221–1228.
- [137] D.C. Ko, L. Milenkovic, S.M. Beier, H. Manuel, J. Buchanan, M.P. Scott, *PLoS Genet.* 1 (2005) 81–95.
- [138] C.D. Pacheco, R. Kunkel, A.P. Lieberman, *Hum. Mol. Genet.* 16 (2007) 1495–1503.
- [139] G. Liao, Y. Yao, J. Liu, Z. Yu, S. Cheung, A. Xie, X. Liang, X. Bi, *Am. J. Pathol.* 171 (2007) 962–975.
- [140] Y. Kabeya, N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, T. Yoshimori, *EMBO J.* 19 (2000) 5720–5728.

- [141] J. Cheng, Y. Ohsaki, K. Tauchi-Sato, A. Fujita, T. Fujimoto, *Biochem. Biophys. Res. Commun.* 351 (2006) 246–252.
- [142] M. Komatsu, S. Waguri, M. Koike, Y.S. Sou, T. Ueno, T. Hara, N. Mizushima, J. Iwata, J. Ezaki, S. Murata, J. Hamazaki, Y. Nishito, S. Iemura, T. Natsume, T. Yanagawa, J. Uwayama, E. Warabi, H. Yoshida, T. Ishii, A. Kobayashi, M. Yamamoto, Z. Yue, Y. Uchiyama, E. Kominami, K. Tanaka, *Cell* 131 (2007) 1149–1163.
- [143] H. Suzuki, T. Sakiyama, N. Harada, M. Abe, M. Tadokoro, *Pediatr. Int.* 45 (2003) 1–4.
- [144] M. Elleder, A. Jirasek, F. Smid, J. Ledvinova, G.T. Besley, M. Stopekova, *Virchows Arch., A Pathol. Anat. Histopathol.* 402 (1984) 307–317.
- [145] Y. Sugimoto, H. Ninomiya, Y. Ohsaki, K. Higaki, J.P. Davies, Y.A. Ioannou, K. Ohno, *Proc. Natl. Acad. Sci. U. S. A.* 98 (2001) 12391–12396.
- [146] M. Taniguchi, Y. Shinoda, H. Ninomiya, M.T. Vanier, K. Ohno, *Brain Dev.* 23 (2001) 414–421.
- [147] M. Zervas, K.L. Somers, M.A. Thrall, S.U. Walkley, *Curr. Biol.* 11 (2001) 1283–1287.
- [148] S.U. Walkley, K. Suzuki, *Biochim. Biophys. Acta* 1685 (2004) 48–62.
- [149] S.U. Walkley, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358 (2003) 893–904.
- [150] B. Bu, J. Li, P. Davies, I. Vincent, *J. Neurosci.* 22 (2002) 6515–6525.
- [151] S. Ohara, Y. Ukita, H. Ninomiya, K. Ohno, *Exp. Neurol.* 187 (2004) 289–298.
- [152] S. Love, L.R. Bridges, C.P. Case, *Brain* 118 (Pt 1) (1995) 119–129.
- [153] K. Suzuki, C.C. Parker, P.G. Pentchev, D. Katz, B. Ghetti, A.N. D'Agostino, E.D. Carstea, *Acta Neuropathol. (Berl)* 89 (1995) 227–238.
- [154] I.A. Auer, M.L. Schmidt, V.M. Lee, B. Curry, K. Suzuki, R.W. Shin, P.G. Pentchev, E.D. Carstea, J.Q. Trojanowski, *Acta Neuropathol. (Berl)* 90 (1995) 547–551.
- [155] K. Leroy, A. Bretteville, K. Schindowski, E. Gillissen, M. Authélet, R. De Decker, Z. Yilmaz, L. Buee, J.P. Brion, *Am. J. Pathol.* 171 (2007) 976–992.
- [156] Q.W. Fan, W. Yu, T. Senda, K. Yanagisawa, M. Michikawa, *J. Neurochem.* 76 (2001) 391–400.
- [157] V. Meske, F. Albert, D. Richter, J. Schwarze, T.G. Ohm, *Eur. J. Neurosci.* 17 (2003) 93–102.
- [158] N. Sawamura, J.S. Gong, W.S. Garver, R.A. Heidenreich, H. Ninomiya, K. Ohno, K. Yanagisawa, M. Michikawa, *J. Biol. Chem.* 276 (2001) 10314–10319.
- [159] B. Bu, H. Klunemann, K. Suzuki, J. Li, T. Bird, L.W. Jin, I. Vincent, *Neurobiol. Dis.* 11 (2002) 285–297.
- [160] J.C. Dodart, C. Mathis, K.R. Bales, S.M. Paul, *Genes Brain Behav.* 1 (2002) 142–155.
- [161] J.C. Cruz, L.H. Tsai, *Curr. Opin. Neurobiol.* 14 (2004) 390–394.
- [162] M. Zhang, J. Li, P. Chakrabarty, B. Bu, I. Vincent, *Am. J. Pathol.* 165 (2004) 843–853.
- [163] S.W. Altmann, H.R. Davis Jr., X. Yao, M. Laverty, D.S. Compton, L.J. Zhu, J.H. Crona, M.A. Caplen, L.M. Hoos, G. Tetzloff, T. Priestley, D.A. Burnett, C.D. Strader, M.P. Graziano, *Biochim. Biophys. Acta* 1580 (2002) 77–93.
- [164] J.L. Hallows, R.E. Iosif, R.D. Biasell, I. Vincent, *J. Neurosci.* 26 (2006) 2738–2744.
- [165] S.C. Patel, S. Suresh, U. Kumar, C.Y. Hu, A. Cooney, E.J. Blanchette-Mackie, E.B. Neufeld, R.C. Patel, R.O. Brady, Y.C. Patel, P.G. Pentchev, W.-Y. Ong, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 1657–1662.
- [166] C.Y. Hu, W.Y. Ong, S.C. Patel, *J. Neurocytol.* 29 (2000) 765–773.
- [167] J.S. Bae, S. Furuya, S.J. Ahn, S.J. Yi, Y. Hirabayashi, H.K. Jin, *Neurosci. Lett.* 381 (2005) 234–236.
- [168] M. Baudry, Y. Yao, D. Simmons, J. Liu, X. Bi, *Exp. Neurol.* 184 (2003) 887–903.
- [169] H. Li, S.D. Turley, B. Liu, J.J. Repa, J.M. Dietschy, *J. Lipid Res.* 49 (2008) 1816–1828.
- [170] M. Suzuki, Y. Sugimoto, Y. Ohsaki, M. Ueno, S. Kato, Y. Kitamura, H. Hosokawa, J.P. Davies, Y.A. Ioannou, M.T. Vanier, K. Ohno, H. Ninomiya, *J. Neurosci.* 27 (2007) 1879–1891.
- [171] G.W. Kreutzberg, *Trends Neurosci.* 19 (1996) 312–318.
- [172] N. Stence, M. Waite, M.E. Dailey, *Glia* 33 (2001) 256–266.
- [173] F.Y. Tanga, V. Raghavendra, J.A. DeLeo, *Neurochem. Int.* 45 (2004) 397–407.
- [174] M.B. Graeber, W.J. Streit, G.W. Kreutzberg, *J. Neurosci. Res.* 21 (1988) 18–24.
- [175] E. McGlade-McCulloh, A.M. Morrissey, F. Norona, K.J. Muller, *Proc. Natl. Acad. Sci. U. S. A.* 86 (1989) 1093–1097.
- [176] D. Giulian, J. Chen, J.E. Ingeman, J.K. George, M. Noponen, *J. Neurosci.* 9 (1989) 4416–4429.
- [177] M. Patrizio, G. Levi, *Neurosci. Lett.* 178 (1994) 184–189.
- [178] C.S. Lobsiger, D.W. Cleveland, *Nat. Neurosci.* 10 (2007) 1355–1360.
- [179] C.A. Colton, D.L. Gilbert, *FEBS Lett.* 223 (1987) 284–288.
- [180] C.C. Chao, S. Hu, T.W. Molitor, E.G. Shaskan, P.K. Peterson, *J. Immunol.* 149 (1992) 2736–2741.
- [181] M. Buttini, K. Appel, A. Sauter, P.J. Gebicke-Haerter, H.W. Boddeke, *Neuroscience* 71 (1996) 1–16.
- [182] Y. Nakamura, Q.S. Si, K. Kataoka, *Neurosci. Res.* 35 (1999) 95–100.
- [183] D. Lindholm, E. Castren, R. Kiefer, F. Zafra, H. Thoenen, *J. Cell Biol.* 117 (1992) 395–400.
- [184] T. Mizuno, M. Sawada, T. Marunouchi, A. Suzumura, *Biochem. Biophys. Res. Commun.* 205 (1994) 1907–1915.
- [185] K. Nakajima, S. Honda, Y. Tohyama, Y. Imai, S. Kohsaka, T. Kurihara, *J. Neurosci. Res.* 65 (2001) 322–331.
- [186] P.L. McGeer, S. Itagaki, B.E. Boyes, E.G. McGeer, *Neurology* 38 (1988) 1285–1291.
- [187] T. Kawamata, H. Akiyama, T. Yamada, P.L. McGeer, *Am. J. Pathol.* 140 (1992) 691–707.
- [188] P.L. McGeer, M. Schulzer, E.G. McGeer, *Neurology* 47 (1996) 425–432.
- [189] R. Wada, C.J. Tiffit, R.L. Proia, *Proc. Natl. Acad. Sci. U. S. A.* 97 (2000) 10954–10959.
- [190] J. Yrjanheikki, R. Keinanen, M. Pellikka, T. Hokfelt, J. Koistinaho, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 15769–15774.
- [191] Y. Du, Z. Ma, S. Lin, R.C. Dodel, F. Gao, K.R. Bales, L.C. Triarhou, E. Chernet, K.W. Perry, D.L. Nelson, S. Luecke, L.A. Phebus, F.P. Bymaster, S.M. Paul, *Proc. Natl. Acad. Sci. U. S. A.* 98 (2001) 14669–14674.
- [192] D.R. Beers, J.S. Henkel, Q. Xiao, W. Zhao, J. Wang, A.A. Yen, L. Siklos, S.R. Mckercher, S.H. Appel, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 16021–16026.
- [193] J.J. Repa, H. Li, T.C. Frank-Cannon, M.A. Valasek, S.D. Turley, M.G. Tansley, J.M. Dietschy, *J. Neurosci.* 27 (2007) 14470–14480.
- [194] M. Zhang, D. Strnatka, C. Donohue, J.L. Hallows, I. Vincent, R.P. Erickson, *J. Neurosci. Res.* 86 (2008) 2848–2856.
- [195] S.S. Dixit, D.E. Sleat, A.M. Stock, P. Lobel, *Biochem. J.* 408 (2007) 1–5.
- [196] J.C. Rutledge, *Pediatr. Pathol.* 9 (1989) 779–784.
- [197] E.P. Beltroy, J.A. Richardson, J.D. Horton, S.D. Turley, J.M. Dietschy, *Hepatology* 42 (2005) 886–893.
- [198] S. Reif, Z. Spirer, G. Messer, M. Baratz, B. Bembli, Y. Bujanover, *Clin. Pediatr. (Phila)* 33 (1994) 628–630.
- [199] D.A. Kelly, B. Portmann, A.P. Mowat, S. Sherlock, B.D. Lake, *J. Pediatr.* 123 (1993) 242–247.
- [200] Y. Osono, L.A. Woollett, J. Herz, J.M. Dietschy, *J. Clin. Invest.* 95 (1995) 1124–1132.
- [201] A. Kulinski, J.E. Vance, *J. Biol. Chem.* 282 (2007) 1627–1637.
- [202] M.D. Wang, V. Franklin, M. Sundaram, R.S. Kiss, K. Ho, M. Gallant, Y.L. Marcel, *J. Biol. Chem.* 282 (2007) 22525–22533.
- [203] H.Y. Choi, B. Karten, T. Chan, J.E. Vance, W.L. Greer, R.A. Heidenreich, W.S. Garver, G.A. Francis, *J. Biol. Chem.* 278 (2003).
- [204] V.M. Rimmunas, M.J. Graham, R.M. Croke, L. Liscum, *Hepatology* 47 (2008) 1504–1512.
- [205] N.H. Pipalia, A. Huang, H. Ralph, M. Rujoi, F.R. Maxfield, *J. Lipid Res.* 47 (2006) 284–301.
- [206] M.C. Patterson, F. Platt, *Biochim. Biophys. Acta* 1685 (2004) 77–82.
- [207] Y. Liu, Y.-P. Wu, R. Wada, E.B. Neufeld, K.A. Mullin, A.C. Howard, P.G. Pentchev, M.T. Vanier, K. Suzuki, R.L. Proia, *Hum. Mol. Genet.* 9 (2000) 1087–1092.
- [208] M.C. Patterson, D. Vecchio, H. Prady, L. Abel, J.E. Wraith, *Lancet Neurol.* 6 (2007) 765–772.
- [209] R.P. Erickson, W.S. Garver, F. Camargo, G.S. Hossain, R.A. Heidenreich, *J. Inherit. Metab. Dis.* 23 (2000) 54–62.
- [210] M.C. Patterson, A.M. Di Bisceglie, J.J. Higgins, R.B. Abel, R. Schiffmann, C.C. Parker, C.E. Argoff, R.P. Grewal, K. Yu, P.G. Pentchev, et al., *Neurology* 43 (1993) 61–64.
- [211] P.C. Reid, S. Lin, M.T. Vanier, Y. Ohno-Iwashita, H.J. Harwood Jr., W.F. Hickey, C.C. Chang, T.Y. Chang, *J. Neurosci. Methods* 168 (2008) 15–25.
- [212] V. Hirsch-Reinshagen, S. Zhou, B.L. Burgess, L. Bernier, S.A. McIsaac, J.Y. Chan, G.H. Tansley, J.S. Cohn, M.R. Hayden, C.L. Wellington, *J. Biol. Chem.* 279 (2004) 41197–41207.
- [213] S.E. Wahrle, H. Jiang, M. Parsadanian, J. Legleiter, X. Han, J.D. Fryer, T. Kowalewski, D.M. Holtzman, *J. Biol. Chem.* 279 (2004) 40987–40993.
- [214] B. Karten, R.B. Campenot, D.E. Vance, J.E. Vance, *J. Biol. Chem.* 281 (2006) 4049–4057.
- [215] E. Boadu, H.Y. Choi, D.W. Lee, E.I. Waddington, T. Chan, B. Asztalos, J.E. Vance, A. Chan, G. Castro, G.A. Francis, *J. Biol. Chem.* (2006).
- [216] A. Frolov, S.E. Zielinski, J.R. Crowley, N. Dudley-Rucker, J.E. Schaffer, D.S. Ory, *J. Biol. Chem.* 278 (2003) 25517–25525.
- [217] S.J. Langmade, S.E. Gale, A. Frolov, I. Mohri, K. Suzuki, S.H. Mellon, S.U. Walkley, D.F. Covey, J.E. Schaffer, D.S. Ory, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 13807–13812.
- [218] S.J. Bensingler, M.N. Bradley, S.B. Joseph, N. Zelcer, E.M. Janssen, M.A. Hausner, R. Shih, J.S. Parks, P.A. Edwards, B.D. Jamieson, P. Tontonoz, *Cell* 134 (2008) 97–111.
- [219] L.D. Griffin, W. Gong, L. Verot, S.H. Mellon, *Nat. Med.* 10 (2004) 704–711.
- [220] S. Mellon, W. Gong, L.D. Griffin, *Endocr. Res.* 30 (2004) 727–735.
- [221] S. Zampieri, S.H. Mellon, T.D. Butters, M. Nevyjel, D.F. Covey, B. Bembli, A. Dardis, *J. Cell. Mol. Med.* (in press).
- [222] A.S. Strimpakos, R.A. Sharma, *Antioxid. Redox Signal.* 10 (2008) 511–545.
- [223] P. Anand, S.G. Thomas, A.B. Kunnammakara, C. Sundaram, K.B. Harikumar, B. Sung, S.T. Tharakan, K. Misra, I.K. Priyadarisni, K.N. Rajasekharan, B.B. Aggarwal, *Biochem. Pharmacol.* 76 (2008) 1590–1611.
- [224] M.E. Gelsthorpe, N. Baumann, E. Millard, S.E. Gale, S.J. Langmade, J.E. Schaffer, D.S. Ory, *J. Biol. Chem.* 283 (2008) 8229–8236.
- [225] W.E. Balch, R.I. Morimoto, A. Dillin, J.W. Kelly, *Science* 319 (2008) 916–919.