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Cholesterol in Niemann–Pick Type C disease

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Abstract

Niemann-Pick Type C (NPC) disease is associated with accumulation of cholesterol and other lipids in late endosomes/lysosomes in virtually every organ; however, neurodegeneration represents the fatal cause for the disease. Genetic analysis has identified loss-of-function mutations in NPC1 and NPC2 genes as the molecular triggers for the disease. Although the precise function of these proteins has not yet been clarified, recent research suggests that they orchestrate cholesterol efflux from late endosomes/lysosomes. NPC protein deficits result in impairment in intracellular cholesterol trafficking and dysregulation of cholesterol biosynthesis. Disruption of cholesterol homeostasis is also associated with deregulation of autophagic activity and early-onset neuroinflammation, which may contribute to the pathogenesis of NPC disease. This chapter reviews recent achievements in the investigation of disruption of cholesterol homeostasis-induced neurodegeneration in NPC disease, and provides new insight for developing a potential therapeutic strategy for this disorder.

Keywords

Autophagy; Cholesterol; Cyclodextrin; Endosome; Inflammation; Lysosome; Neuronal death

11.1 Introduction

Niemann–Pick disease type C (NPC) is a severe neurovisceral lysosomal lipid storage disorder first described by Niemann in 1914 (Niemann, 1914), and further characterized by Pick in 1933 (Pick, 1933). NPC disease is rare with a prevalence of 1:150,000 in the general population. The associated loss-of-function mutations in NPC1 (accounting for 95% of the cases) or NPC2 (accounting for the remaining 5%) genes were identified as the genetic cause of this disease in 1997 and 2000, respectively (Carstea et al., 1997; Naureckiene et al., 2000). Clinical manifestations of NPC include vertical gaze palsy, ataxia, dystonia, dementia, cognitive impairment, and seizures with hepatosplenomegaly in early childhood; progressive neurological function defects are considered the cause of death that often occurs in teenage years (Fink et al., 1989). Pathologic features in NPC brain include neuronal loss, especially in the cerebellum, axonal spheroids, meganeurite formation (Higashi et al., 1993), and ectopic neurites (*reviewed by* Walkley & Suzuki, 2004). The hallmark of NPC at the cellular level is accumulation of cholesterol and other lipids in late endosome/lysosomes.

How disruption of cholesterol metabolism contributes to NPC neuropathology remains largely unknown and currently no effective therapy is available for this disease. Extensive investigations have focused on characterization of NPC protein functions and the links between NPC loss-of-function and cholesterol storage, however the mechanism underlying NPC pathogenesis still remains to be further elucidated. Interest in studying NPC disease was markedly increased after a link between NPC disease and Alzheimer's disease was discovered (Love et al., 1995; Ohm et al., 2003). NPC and Alzheimer's disease exhibit several similarities, including endosomal/lysosomal abnormalities, cholesterol imbalance, neurofibrillary tangle formation, deregulation of the phosphatidylinositol-3 kinase signalling cascade, and glial-mediated neuroinflammation (Auer et al., 1995; Baudry et al., 2003; Bi & Liao, 2007; Bi et al., 2005; Distl et al., 2003; German et al., 2002; Liao et al., 2007; Lynch & Bi, 2003; Suzuki et al., 1995). In addition, amyloid-beta peptide deposition was also evident in brains of NPC patients with ApoE epsilon4 homozygosity (Saito et al., 2002) (*see also* Chapter 2). Similarly, accumulation of beta-C-terminal fragments of amyloid precursor proteins was found in brains of a mouse model for NPC (Burns et al., 2003). Recently, it has been reported that brains of some NPC patients also contain aberrant alpha-synuclein accumulation and Lewy bodies (Saito et al., 2004), which inspires the proposal to include NPC as a subclass of "Lewy body diseases" (Hardy et al., 2009). New methodological and technological developments have also greatly improved our understanding of the functions of cholesterol and lipoproteins in brain. This review focuses on disruption of cholesterol homeostasis, especially cholesterol intracellular trafficking-induced neurodegeneration in NPC disease.

11.2 NPC Proteins and Intracellular Cholesterol Transport

Human NPC1 protein contains 1278 amino acids and 13 putative transmembrane domains (Davies & Ioannou, 2000). To-date more than 200 mutations that induce NPC phenotype have been identified in the *NPC1* gene (Runz et al., 2008). NPC1 proteins have been localized in late endosomes of various cell types using different methods (Berger et al., 2007; Chikh et al., 2004; Higgins et al., 1999; Neufeld et al., 1999; Urano et al., 2008; Zhang et al., 2003). Biochemical and structural analyses have indicated that the protein contains a sterol sensing domain, homologous to the sterol sensing domain found in other key proteins in cholesterol homeostasis such as morphogen receptor Patched, 3-hydroxy-3-methylglutaryl coenzyme A reductase, SREBP cleavage activating protein, and Niemann-Pick C1-like 1 (Carstea et al., 1997; Davies et al., 2000a, b; Loftus et al., 1997; Scott et al., 2004), which is located between the third and seventh transmembrane domains (Davies & Ioannou, 2000; Millard et al., 2005); the sterol sensing domain is essential for NPC1 binding of cholesterol as demonstrated by using a photoactivatable cholesterol analog (Ohgami et al., 2004). The sterol-sensing domain is also critically involved in regulation of NPC1 protein stability (Ohsaki et al., 2006), as well as its late endosomal targeting (Scott et al., 2004). Besides the sterol-sensing domain, the N-terminal domain (amino acids 25–264) also exhibits high affinity binding for cholesterol and side-chain oxysterols in vitro (Infante et al., 2008a); however, NPC1 proteins with mutations in this region affecting sterol binding still rescue NPC1- deficient cells (Infante et al., 2008b), suggesting that the binding function of

this domain is not essential. Recently, it has been shown that this region may interact with NPC2 to facilitate cholesterol efflux from late endosome/lysosomes (Infante et al., 2008c).

Human NPC2 protein (also termed HE1) is a small soluble protein, which contains 132 amino acids (Kirchhoff et al., 1996; Okamura et al., 1999). Eighteen mutations in NPC2 gene have been identified (Runz et al., 2008). Structural and biochemical studies have shown that NPC2 has a hydrophobic ligand binding pocket (Friedland et al., 2003) and binds cholesterol with a 1:1 stoichiometry (Xu et al., 2007) and a high affinity ($K_d = 30\text{--}50\text{ nM}$) (Ko et al., 2003). Cholesterol binding is essential for NPC2 function since mutant NPC2 proteins that lack high affinity cholesterol binding also fail to rescue NPC2-null cells (Ko et al., 2003). Currently, it is generally accepted that NPC2 is mainly present in the lysosomal lumen (Naureckiene et al., 2000; Willenborg et al., 2005). Although the two proteins are very different structurally, recessive inheritance of either one leads to NPC disease with almost indistinguishable phenotypes (Vance, 2006; Vanier & Millat, 2004), suggesting that the two proteins must function in a closely related fashion. This notion has been further confirmed by a direct comparison study of mice with *Npc1*, *Npc2*, or *Npc1/Npc2* double deficiency (Sleat et al., 2004). However, exactly how the two proteins participate in cholesterol efflux from late endosomes/lysosomes remains an open question. Using fluorescence-labelled NPC2 a recent study showed that NPC2 was able to transfer cholesterol to vesicular membranes (Cheruku et al., 2006), possibly by direct NPC2-membrane interaction (Xu et al., 2008). Using in vitro assays, Infante et al. (2008c) showed that a bidirectional transfer of cholesterol occurs between liposomes and either NPC1 or NPC2, although that mediated by NPC1 is much slower compared to NPC2. However, in the presence of NPC2, the bidirectional transfer is enhanced over 100 fold. These data suggest a model in which NPC1 and NPC2 may bind cholesterol sequentially and promote its egress from late endosomes/lysosomes.

11.3 Cholesterol Accumulation in Niemann-Pick Type C Disease

The essential role of cholesterol in maintaining functional integrity of virtually all types of cell has gained tremendous attention. Cholesterol homeostasis is critical for normal function of the central nervous system (CNS), which is particularly rich in cholesterol. Although the human brain comprises only 2% of the body mass, it contains about 25% of the total body unesterified cholesterol (Dietschy & Turley, 2001). In contrast to other peripheral tissues that obtain cholesterol from both de novo synthesis within the cells and uptake of cholesterol-containing lipoprotein particles from serum, nearly all cholesterol supply in the CNS comes from in situ synthesis (Turley et al., 1996). Previous studies have shown that during early development neurons rely heavily on de novo cholesterol synthesis (Jurevics et al., 1997; Turley et al., 1996), whereas uptake of exogenous cholesterol provided by glia may be critical for mature neurons later (Cruz & Chang, 2000; Pitas et al., 1987; Weisgraber et al., 1994). Dysfunction of either de novo synthesis or uptake of exogenous cholesterol can lead to disruption of cholesterol homeostasis in neurons.

Cholesterol esterification impairment in NPC disease was first revealed in fibroblasts cultured from NPC patients, which distinguished NPC disease from other lysosomal storage diseases (Pentchev et al., 1985; Vanier et al., 1988). Subsequent research found that not only

unesterified cholesterol, but also gangliosides GM2 and GM3, and bis-monoacylglycerol phosphate accumulated in late endosomes/lysosomes (Kobayashi et al., 1999; Liscum & Munn, 1999; Sokol et al., 1988; te Vrugte et al., 2004; Watanabe et al., 1998; Zervas et al., 2001b). The discovery that lipids other than cholesterol also accumulated in late endosomes/lysosomes has led to the debate over whether aberrant trafficking of cholesterol or of other lipids is the primary cause of the NPC phenotype (te Vrugte et al., 2004; Zervas et al., 2001b). It was suggested that cholesterol accumulation was ganglioside-dependent since depletion of the ganglioside-related enzyme GM2/GD2 synthase in NPC-deficient neurons diminished cholesterol accumulation (Gondre-Lewis et al., 2003). However, a more recent study failed to reproduce these results; Li et al reported that deprivation of either GM2/GD2 or GM3 did not reduce cholesterol accumulation or pathology in *Npc1*^{-/-} mice (Li et al., 2008). In fact, the lifespan was shortened by these manipulations (Li et al., 2008). Another recent study suggested that sphingosine storage was an initiating factor that caused altered calcium homeostasis in lysosomes, leading to the secondary accumulation of sphingolipids and cholesterol (Lloyd-Evans et al., 2008). The caveat for this hypothesis is that both NPC1 and NPC2 have been shown to directly bind cholesterol, and not sphingosine. Therefore, to argue that sphingosine storage is the initiating event, some additional mechanism is needed. In this regard, one recent study reported that mutation in the sterol-sensing domain of a yeast NPC-related protein led to subcellular sphingolipid redistribution (Malathi et al., 2004). Whether this holds true in mammals remains to be determined.

Besides abnormal late endosomes/lysosomes (Zervas et al., 2001a), early endosomes were also reported to be substantially enlarged and to contain high levels of the lysosomal hydrolase cathepsin D in Purkinje cells and microglia in brain tissues of NPC patients (Jin et al., 2004). Furthermore, our previous research has shown that cathepsin D immunoreactivity was increased not only in microglia, but also in neurons in *Npc1*^{-/-} mice (Liao et al., 2007, 2009), a murine model of NPC disease. The phenotype in these mice is almost identical to that in humans except that only hyperphosphorylation of tau, but not neurofibrillary tangles, has been observed in the mutant mouse brain (German et al., 2001). These observations suggest that mutations in NPC1 gene impair functions in both early and late endocytic pathways; whether disruption of early endosomes is induced by accumulation of cholesterol in late endosomes/lysosomes or is an independent deficit needs further study.

Cholesterol accumulation was detected as early as postnatal day 9 in various brain regions in *Npc1*^{-/-} mice (Reid et al., 2004). In the cerebellum, although the morphology of Purkinje cells was normal at this age, cholesterol accumulation was already evident in cell bodies and dendritic arbors. In other brain areas, cholesterol accumulation was first observed in neuronal perikarya and at the base of axonal hillocks, especially in the thalamus (Reid et al., 2004). In later stages, cholesterol accumulation was also found in astrocytes (Mutka et al., 2004; Reid et al., 2004) and active microglia (Liao et al., 2009). Cholesterol accumulation in cell bodies, and to a smaller degree in axons, was observed in sympathetic neurons cultured from *Npc1*^{-/-} mice and maintained in serum-free medium for only one day (Karten et al., 2002). However, whether cholesterol accumulation occurs in embryonic brain tissues is still under debate. Interestingly, the percentage of *Npc1*^{-/-} pups bred from heterozygous parents is about 12% instead of the predicted 25% (Karten et al., 2002), implicating possible embryonic lethality in *Npc1*^{-/-} mice. At embryonic day 16, the percentage of homozygous

embryos is still 25%, which indicates that death takes place after E16 (Henderson et al., 2000). Additional research is needed to define the potential links between disruption of cholesterol homeostasis and embryonic death. Nevertheless, studies reviewed in this section have clearly shown that cholesterol accumulation occurs early in life in *Npc1*^{−/−} mice, although the mechanism for this early event remains obscure.

11.4 Suppression of Brain Cholesterol Synthesis in NPC Disease

A paradox in brain cholesterol metabolism in *Npc1*^{−/−} mice is that although cholesterol accumulation in neurons and glia is clearly evident, the total amount of brain cholesterol is not significantly increased, which is in contrary to what is found in other organs. A direct measurement study showed that the total amount of cholesterol in brain of newborn *Npc1*^{−/−} mice was more than that of wild-type mice, but gradually reduced with age (Xie et al., 2000). By 7-week postnatal, cholesterol levels were significantly reduced in midbrain, brainstem and spinal cord in *Npc1*^{−/−} mice and the reduction was paralleled with an increase in net cholesterol turnover (Xie et al., 2000). Further study from the same research group demonstrated that the synthesis rate of cholesterol was reduced while its excretion from brain was enhanced (Xie et al., 2003). Excretion was independent of the 24-hydroxycholesterol pathway that the brain normally uses to transfer excess cholesterol to plasma. Research from other groups supported the notion that cholesterol synthesis in *Npc1*-deficient mice was decreased. For instance, an in vitro study showed that cholesterol synthesis in *Npc1*-deficient astrocytes was reduced (Reid et al., 2003). Furthermore, the synthesis of neurosteroids, such as allopregnanolone (Griffin et al., 2004) and testosterone (Roff et al., 1993), was also decreased in *Npc1*^{−/−} mice. Using microarray analysis we found that mRNAs for several key proteins in the sterol biosynthesis pathway were significantly reduced (Liao et al., unpublished data). However, other studies indicated that there were no significant changes in cholesterol synthesis in *Npc1*^{−/−} mice (Karten et al., 2005; Reid et al., 2008). These controversial findings suggest that the impairment in cholesterol synthesis requires further investigation.

11.5 Impairment of Cholesterol Transport in NPC Disease

The cloning of NPC1 protein, and later of NPC2 protein, sped up the investigation of the mechanisms underlying pathogenesis in the disease. NPC1 protein is generally located in late endosomes (Higgins et al., 1999; Kobayashi et al., 1999). In situ hybridization study showed that in mouse brain, *Npc1* mRNA was detected in the majority of neurons in nearly all regions, but at significantly higher levels in cerebellum and in specific pontine nuclei; this regional specificity was established by postnatal day 7 (Prasad et al., 2000). The earliest neuronal expression of *Npc1* mRNA was detected at embryonic day 15 (Prasad et al., 2000). As discussed above, while the structure of the NPC1 protein is well characterized, little is known regarding its function in vivo. Several lines of evidence indicate that NPC1 may be involved in the trafficking of both LDL-derived and endogenously synthesized cholesterol from the endoplasmic reticulum to the trans-Golgi network (Higgins et al., 1999; Reid et al., 2003; see Scott & Ioannou, 2004 for a recent review).

Brain cholesterol homeostasis is achieved through different mechanisms from those in other organs. In vivo, direct measurement of the uptake of low density lipoproteins (LDL) in different brain regions has indicated that cholesterol carried in LDL circulating in serum plays little or no role in the process of sterol acquisition during brain development or in cholesterol turnover in the mature central nervous system (Turley et al., 1996). In contrast, lipoproteins in brain transport exogenous cholesterol generated in glia to neurons. Several members of the LDL receptor family, including apolipoprotein E, A1, D, and J, are expressed in brains with apolipoprotein E and apolipoprotein J being the major apolipoproteins in CNS (Gong et al., 2002). Apolipoprotein E is mainly synthesized by astrocytes and microglia and to a small extent by neurons (Brecht et al., 2004). Extracellular cholesterol in the brain is transported mostly by apolipoprotein E (Boyles et al., 1985), and a small amount by apolipoprotein A1, apolipoprotein D, and apolipoprotein J (Patel et al., 1995). Expression of apolipoprotein D is increased in *Npc1*-deficient mice (Li et al., 2005; Ong et al., 2002; Suresh et al., 1998), although the exact function of apolipoprotein D in brain is not clear. Levels of apolipoprotein E mRNA (Li et al., 2005) and protein (Liao et al., unpublished data) are also increased in *Npc1*^{-/-} mice. However, using a functional assay, Karten and colleagues have shown that apolipoprotein E-containing lipoproteins generated by *Npc1*^{-/-} and *Npc1*^{+/+} glia were equally capable of stimulating axonal elongation (Karten et al., 2005). Furthermore, degeneration of neurons and glia in double *Npc1*^{-/-}/*LDLR*^{-/-} deficient mice was similar to that in *Npc1*^{-/-} mice, which indicates an *LDLR*-independent pathogenic process (German et al., 2001). On the other hand, neurons cultured from *Npc1*^{-/-} mice exhibited cholesterol accumulation in cell bodies, while distal axons had reduced cholesterol (Karten et al., 2002), suggesting impairment in intracellular cholesterol trafficking. Overall, although the precise role of lipoproteins in NPC disease needs to be further defined, these studies suggest that disruption of cholesterol transport, especially inside neurons, may play a critical role in NPC pathogenesis.

11.6 Cholesterol Accumulation-Associated Autophagy in NPC Disease

Although *NPC1* gene is expressed in all tissues, the nervous system manifestations of the disease are predominant and lethal. The reason why neurons are most vulnerable to *NPC1* deficiency remains unknown. Apoptosis was found in cortical neurons treated with a blocker of cholesterol transport, U18666A (Koh et al., 2006, 2007), in liver cells of *Npc1*^{-/-} mice (Beltroy et al., 2005), and in brains of NPC patients and *Npc1*^{-/-} mice (Wu et al., 2005). However, additional results support the notion that another type of programmed cell death, autophagic cell death, plays a critical role in neuronal death in NPC disease.

Autophagy or “self-eating” is an adaptation process conserved in cells from yeasts to mice and humans (Klionsky & Emr, 2000). As a house-keeping mechanism, autophagy engulfs fragments of damaged organelles and long-lived membrane proteins and transfers packaged cargos to lysosomes for degradation (Xie & Klionsky, 2007). Recent evidence indicates that autophagy is associated with neurodegeneration in Alzheimer’s disease (Nixon, 2007), Parkinson’s disease (Pan et al., 2008), and Huntington disease (Ravikumar et al., 2004). Research from our laboratory and others have also shown that autophagy activity is increased in *Npc1*^{-/-} mice (Ko et al., 2005; Liao et al., 2007; Pacheco et al., 2007). Levels of LC3 (microtubule-associated protein 1 light chain 3 protein)-II, a marker of autophagic

activation (Kabeya et al., 2000; Klionsky et al., 2008; Tanida et al., 2005), are increased in brain of *Npc1*^{−/−} mice (Liao et al., 2007) and in fibroblasts with NPC1 deficiency (Pacheco et al., 2007). LC3-immunopositive granules were also labelled with filipin-stained cholesterol, suggesting that autophagy in NPC is closely associated with cholesterol accumulation (Liao et al., 2007). This notion is further supported by our recent finding that suppression of autophagy by treatment of mice with allopregnanolone, a neurosteroid that is deficient in brain of *Npc1*^{−/−} mice, was associated with reduction in cholesterol accumulation (Liao et al., 2009). Ultrastructural analysis with electron microscopy revealed the existence of classic double membrane vacuole-like structures in 6-weeks old *Npc1*^{−/−} mice (Fig. 11.1) (Liao et al., 2007). These results suggest an increase in autophagosomes in NPC. However, as the volume of autophagosomes depends on the dynamics of influx and efflux, whether this increase represents a net increase in autophagic activity or a efflux jam because of lysosomal dysfunction remains an open question (Bi & Liao, 2007).

The mechanism by which autophagic activity is elevated is largely unknown. It is generally agreed that amino acid starvation induces autophagic activity; whether lipid/cholesterol starvation also results in enhanced autophagic activity is not as certain. Depletion of cholesterol in human fibroblasts, by either acute chemical treatment or metabolic suppression of cholesterol synthesis, increased levels of LC3-II and LC3-II-immunopositive granules suggesting an increase in autophagic activity (Cheng et al., 2006). Electron microscopy examination revealed that autophagic vacuoles induced by cholesterol depletion were indistinguishable from that induced by amino acid starvation, which further supports the idea that cholesterol starvation can also initiate autophagy. More convincing evidence suggesting an increase in autophagic induction in NPC was obtained by Ishibashi and colleagues, who recently reported that cholesterol depletion by U18666A inhibited the formation of filipin-labeled LC3-immunopositive granules but promoted the formation of ring-shaped filipin-negative LC3-immunopositive structures (Ishibashi et al., 2009). However, the molecular basis for cholesterol depletion-induced autophagy remains elusive. Blocking intracellular cholesterol trafficking by U18666A in wild-type fibroblasts increased the expression of LC3 and the conversion of LC3-I to LC3-II, a process that was dependent on the Beclin-1 rather than the mTOR (mammalian target of rapamycin) signalling pathway (Pacheco et al., 2007), which may imply that cholesterol depletion-induced autophagy uses different molecular mechanisms from those induced by amino acid starvation. In reviewing the literature, it is clear that an increase in autophagosomes, possibly by enhanced initiation rather than decreased efflux, is associated with cholesterol accumulation in NPC disease. However, the underlying mechanism is not as clear.

11.7 Treatment Development for NPC Disease

Currently there is no effective treatment for NPC disease. Clinically, NPC patients are often placed on a cholesterol-lowering treatment, although the results are not very convincing. An animal study showed that introduction of functional *npc1* gene in *Npc1*^{−/−} mouse brain with a prion promoter prevented neurodegeneration, normalized lifespan, and corrected sterility (Loftus et al., 2002). Results from this study further emphasize the importance of neurodegeneration in NPC disease. Another recent study has shown that restoring *Npc1* function only in astrocytes triples *Npc1*^{−/−} mice lifespan, indicating that astrocytes play a

critical role in NPC disease (Zhang et al., 2008). Substrate-reduction therapy, by using N-butyldeoxynojirimycin (Miglustat), an inhibitor of glycosphingolipid biosynthesis, has also shown promising results; it extended the average lifespan from 67 days to 89 days in the NPC mouse model (Zervas et al., 2001b). Supplementing the neurosteroid, allopregnanolone, by a single injection at postnatal day 7 has been shown to double *Npc1*^{-/-} mice lifespan (Griffin et al., 2004). Regarding the potential mechanisms of allopregnanolone treatment, *in vitro* experiments indicated that allopregnanolone-mediated Purkinje cell survival was blocked by the GABA_A receptor antagonist, bicuculline, suggesting that the effect of the drug might be mediated by GABA_A receptors (Griffin et al., 2004). However, this hypothesis has been challenged by the finding that ent-allopregnanolone, an allopregnanolone stereoisomer without GABA_A receptor agonist function, has identical effects as natural allopregnanolone, which strongly suggests the existence of GABA_A-independent mechanisms (Langmade et al., 2006). On the other hand, T0901317, a synthetic oxysterol ligand, acts in concert with allopregnanolone to promote survival and to delay the onset of neurological symptoms (Langmade et al., 2006). The effects of allopregnanolone and T0901317 correlate with their ability to activate the pregnane X receptor, suggesting a role for this receptor. However, other researchers have reported that there is no detectable pregnane X receptor activity in mouse cerebellum (Bookout et al., 2006; Gofflot et al., 2007; Repa et al., 2007). Liu et al. (2008) recently reported that administration of β -cyclodextrin, the vehicle used in the allopregnanolone studies, also rescued *Npc1*^{-/-} mice. This study has inspired a “compassionate use” of β -cyclodextrin to twin NPC patients, which was approved by the FDA (<http://www.addiandcassi.com>). However, results from our recent study showed that while combined allopregnanolone and cyclodextrin treatment markedly reduced cholesterol accumulation, autophagic/lysosomal dysfunction, microglia and astrocyte-mediated inflammation, and increased myelination in brain of *Npc1*^{-/-} mice at one month (Ahmad et al., 2005; Liao et al., 2009), cyclodextrin treatment alone only slightly reduced cholesterol accumulation and had little effect on other pathological features (Liao et al., 2009). These results raise caution regarding the clinical use of cyclodextrin in NPC.

11.8 Conclusions

Recent studies have indicated that disruption in cholesterol homeostasis plays an important role in several neurodegenerative diseases, including Alzheimer's disease (*see* Chapter 2) and NPC disease. In NPC, cholesterol accumulation occurs early and is closely associated with neurodegeneration. Although loss-of-function mutations in *NPC1* and *NPC2* genes have been identified as the genetic cause of this disorder, the precise mechanism by which NPC deficit leads to neuronal death remains elusive. Recent research has led to a better understanding of the roles of *NPC1* and *NPC2* in cholesterol flux through late endosomes/lysosomes, which may reveal new therapeutic strategies. Other pathological features such as neuroinflammation and autophagy are also linked to the development of the disease. Therefore, we speculate that multiple therapeutic strategies, including lipid transport improvement, inflammation suppression, and autophagy manipulation should be considered along with gene therapy to provide a comprehensive treatment of this disease.

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Abbreviations

GABA	gamma-Aminobutyric acid
GD2	disialoganglioside
LDL	low density lipoprotein
NPC	Niemann-Pick Type C

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Fig. 11.1.

Ultrastructure of Purkinje cells in *Npc1*^{-/-} and *Npc1*^{+/+} mice. **A** A Purkinje cell of a 6-week-old *Npc1*^{+/+} mice. Npc, nucleus of Purkinje cell. ER, endoplasmic reticulum; G, Golgi apparatus; L, lysosome; M, mitochondria. **B** A Purkinje cell of a 6-week-old *Npc1*^{-/-} mice. Numerous vacuoles (arrowheads) of different sizes with various levels of electron-dense materials are present in the cytoplasm. **C** Lysosome-like structures exist in Purkinje cells in *Npc1*^{+/+} mice. **D–F** Morphology of various membranous vacuoles. Some of them are with double membranes (arrowheads), whereas others have multilamellated structures (arrows). Scale bars = 2 μ m (A and B), 1 μ m (C–F). (Adapted from Liao et al., 2007)