Review

Bidirectional links between Alzheimer's disease and Niemann-Pick type C disease

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Abstract

Alzheimer's disease (AD) and Niemann–Pick type C (NPC) disease are progressive neurodegenerative diseases with very different epidemiology and etiology. AD is a common cause of dementia with a complex polyfactorial etiology, including both genetic and environmental risk factors, while NPC is a very rare autosomal recessive disease. However, the diseases share some disease-related molecular pathways, including abnormal cholesterol metabolism, and involvement of amyloid-β (Aβ) and tau pathology. Here we review recent studies on these pathological traits, focusing on studies of Aβ and tau pathology in NPC, and the importance of the NPC1 gene in AD. Further studies of similarities and differences between AD and NPC may be useful to increase the understanding of both these devastating neurological diseases.

Keywords

Alzheimer’s disease, amyloid-β, Niemann-Pick type C disease, NPC1, tau, Cholesterol, Lipids

Contents

Introduction

The cholesterol pathway has been repeatedly implicated in the pathogenesis of Alzheimer's disease (AD). Indeed in 1906 in addition to the two nowadays considered hallmark features of the disease, amyloid plaques and neurofibrillary tangles, Dr. Alois Alzheimer also described intracellular lipid deposits in the brain tissue of his demented patient Auguste Deter. But Alzheimer's initial identification of lipid changes as one of the significant features of the disorder was subsequently lost from view (Foley, 2010). In 1993 it was discovered that the apolipoprotein E type 4 allele (APOE-ε4) is genetically associated with the common late onset familial and sporadic forms of AD (Corder et al., 1993; Poirier et al., 1993; Rebeck et al., 1993). This variant of ApoE, the main protein carrier for cholesterol in the brain, remains a major risk factor for late onset AD and is the main reason for why lipid metabolism remains in focus of AD research (Holtzman et al., 2012). In addition to this finding, two retrospective epidemiologic studies reported that treatment with cholesterol-lowering statins is associated with reduced risk of AD (Jick et al., 2000; Wolozin et al., 2000). A number of following in vivo and in vitro studies showed that changes in cholesterol levels can have an effect on the processing of amyloid precursor protein (APP) and accumulation of amyloid-β (Aβ) peptide, the major constituent of AD plaques (reviewed in Burns and Rebeck, 2010). In guinea pigs and transgenic mouse models of AD (the latter are often based on APP transgenes with mutations that cause familial AD in humans), cholesterol-lowering statins lower brain Aβ levels, improve cerebrovascular function, and reduce neuroinflammation, favorable changes associated with neuroprotection (Fassbender et al., 2001; Tong et al., 2009; Yamada et al., 2000). In humans, the biochemical data have been less clear; statin treatment appears to stimulate non-amyloidogenic processing of amyloid precursor protein (APP), but the cerebrospinal fluid (CSF) levels of the 42 amino acid, plaque forming variant of amyloid-β (Aβ42) are largely unchanged (Hoglund et al., 2005). Also, a recent study using positron emission tomography (PET) imaging of fibrillar brain Aβ found associations between serum cholesterol levels and brain amyloidosis, but no statistical effects of concurrent statin treatment (Reed et al., 2014). While statins do not delay cognitive decline in patients with manifest AD (Feldman et al., 2010; Sano et al., 2011), their role in neuroprotection and the prevention of AD, supported by epidemiologic data (Jick et al., 2000; Wolozin et al., 2000), is still actively being investigated. Nevertheless, the molecular mechanism(s) that would explain how cholesterol metabolism modulates the pathogenesis of AD are largely unknown.

The link between cholesterol and Aβ was not only described in AD, but also in a rare inherited lysosomal storage disorder Niemann–Pick type C (NPC). NPC disease is characterized by extensive accumulation of cholesterol in liver, spleen and brain tissue and progressive neurodegeneration (reviewed in Vanier, 2010). Surprisingly, neurofibrillary

tangles and accumulation of Aβ peptides were also described. The intriguing reports of histopathological similarities between NPC and AD implicated NPC pathobiology as an innovative model to study the role of cholesterol in AD pathogenesis (Auer et al., 1995; Love et al., 1995). In this review we will discuss the molecular links between AD and NPC disease and will provide clues supporting that these two disorders are interrelated and that altered cholesterol homeostasis may represent a converging metabolic pathway which connects them.

Niemann-Pick type C disease - abnormal cholesterol metabolism and endocytic transport

NPC disease is a lipid storage disorder characterized by both visceral symptoms with dominating hepatosplenomegaly and progressive mental deterioration involving ataxia and dementia (Brady et al., 1989). The broad clinical spectrum ranges from a neonatal rapidly fatal disorder to an adult onset chronic neurodegenerative disease (reviewed in Vanier, 2010). NPC is an autosomal recessive inherited panethnic disease caused by mutations in NPC1 (95% of families) or less frequently in HE1, referred to as NPC2 gene (Carstea et al., 1997; Naureckiene et al., 2000). In addition to human NPC1 disease, feline and murine models of NPC disease are also known, both of which were discovered after occurring spontaneously in normal mouse and cat colonies (Loftus et al., 1997; Lowenthal et al., 1990; Morris et al., 1982; Somers et al., 1999).

NPC1/2 genes do not encode lysosomal enzymes unlike other genes involved in lysosomal storage diseases. NPC1 and NPC2 are ubiquitously expressed lysosomal proteins with cholesterol-binding domains (Carstea et al., 1997; Okamura et al., 1999). Although the exact functions of these proteins are still unclear, the 1278-amino acid multi-spanning NPC1 membrane protein and 151-amino acid soluble NPC2 protein are thought to work in tandem in egress of lipoprotein-derived cholesterol from lysosomes (Infante et al., 2008). At the cellular level, the most prominent feature of the NPC disease is lysosomal sequestration of low density lipoprotein (LDL)-derived cholesterol, resulting in downstream effects on cholesterol homeostasis. LDLs enter the cell via receptor-mediated endocytosis and are consequently delivered to late endosomes/lysosomes, where they are hydrolyzed, so that free cholesterol is released. In normal cells, this cholesterol is transported rapidly out of late endosomes/lysosomes to the plasma membrane and the endoplasmic reticulum (ER). In NPC cells, the cholesterol does not exit the endocytic pathway but accumulates within lysosomes which can be detected by filipin staining, indicating that the cholesterol is in a free, unesterified form. Due to this sequestration of free cholesterol in late endosomes/lysosomes, the concentration of free cholesterol in the ER is lowered below a

certain threshold leading to increase in cholesterol synthesis and decrease in cholesterol esterification (Pentchev et al., 1987). Indeed, the survey of a large number of human Niemann–Pick disorders revealed that a defect in cholesterol esterification was a unique and consistent abnormality found in all examined cultured fibroblasts from NPC patients (Pentchev et al., 1985). This discovery led to the view that NPC disease was primarily a cholesterol lipidosis, further splitting type C disease from types A and B in which sphingomyelin was the primary storage material. Nevertheless, lipid storage in NPC is highly complex and is not restricted to cholesterol. Deposition of sphingolipids, glycolipids and bis(monoacylglycerol) phosphate (BMP) in late endosomes/lysosomes of NPC cells also occurs (Kobayashi et al., 1999; Pentchev et al., 1980). Chemical analysis of lipid extracts from tissues has suggested a general accumulation of lipids with no predominating species in the spleen and liver while in the brain homogenate, the dominant forms were GM2 and GM3 gangliosides, with only limited apparent abnormalities of cholesterol (Pentchev et al., 1980). It should be noted, however, that this study was based on whole tissue extraction and could not address whether there were specific changes in certain cell types. When examining tissues from murine, feline and human NPC with immunocytochemistry and filipin histochemistry, the accumulation of unesterified cholesterol and GM2 was shown in late endosomes/lysosomes in neurons of the cerebral cortex, cerebellum, and hippocampus (Zervas et al., 2001). In parallel with the elevation of free cholesterol levels in neuronal somata, NPC brain is characterized by notable demyelination (Xie et al., 2000). Thus, the observed apparent lack of cholesterol accumulation in brain homogenates can be due to the loss of myelin which is rich in cholesterol. Lipid inclusions are detected not only in neurons but also in microglia/macrophages, astrocytes and oligodendrocytes (German et al., 2002). As the disease progresses, neuronal death becomes overt, affecting more specifically certain regions, particularly Purkinje cells of the cerebellum, but the basis of this selective neuronal vulnerability is still unknown.

How neurons degenerate and what causes neurodegeneration are not well defined in NPC. The restricted expression of a wild-type NPC1 gene in the central nervous system (CNS) was shown to be sufficient for rescuing the neurodegeneration and early fatality of NPC1-deficient mice (Loftus et al., 2002). This highlights the primary importance of the CNS pathology in this disorder, and points to the neurodegeneration as a primary event rather than a secondary effect of visceral defects. Relative importance of the abnormal accumulations of cholesterol, glycosphingolipids and/or other lipid species in the neuropathogenesis of NPC disease is a source of considerable debate and has been extensively studied (reviewed in Lloyd-Evans and Platt, 2010). As cholesterol has also been proposed to play an important role in the pathophysiology of AD, the most common form of

neurodegeneration, these studies and others have not only brought cholesterol to the forefront of AD research, but also stimulated additional interest in NPC disease. Finally, although toxicity from abnormal accumulations of cholesterol and gangliosides in late endosomes/lysosomes upon NPC1 dysfunction has been implicated (Aqul et al., 2011), further evidence has emphasized a more general defect in the trafficking of endocytic cargo leading to aberrant sorting and processing of proteins involved in the pathogenesis of other neurodegenerative disorders like AD.

Alzheimer's disease - the role of lipid metabolism and endocytic pathway in cerebral β-amyloidosis

While NPC disease is a rare disorder with an estimated incidence of 1/150,000 live births (Vanier and Millat, 2003), AD is the most common cause of dementia with a marked prevalence of 30 million people worldwide, a number that is expected to quadruple in 40 years (Ferri et al., 2005). In the last 20 years, extensive research on mechanisms behind AD has resulted in a wealth of data exploring the potential underlying processes, particularly with regard to the amyloid-β (Aβ) peptide. Aβ is a central molecule in AD pathogenesis according to the "amyloid cascade hypothesis", which postulates that accumulation and aggregation of Aβ trigger a pathological cascade that ultimately produces the complete pathological and clinical symptoms of AD (Hardy and Higgins, 1992; Hardy and Selkoe, 2002). Aβ is generated through the amyloidogenic pathway that involves sequential cleavage of APP by β-secretase (BACE1) followed by γ-secretase complex with presenilin 1 (PS1) or PS2 in the active site. The processing of APP by β-secretase generates a 99-residue membrane bound C-terminal fragment CTFβ (C99) that is further cleaved by γ-secretase to generate 40-residue Aβ40 or 42-residue Aβ42. The amyloidogenic CTFβ and Aβ40/42 are prone to aggregation and are implicated in neurodegeneration. However, Aβ40 is less hydrophobic, less toxic, and less prone to aggregation, compared with Aβ42, and may also be processed further to even less aggregation-prone C-terminally truncated Aβ species (Aβ37/38/39) by γ-secretase (reviewed in Andreasson et al., 2007). Alternatively, APP can be processed through the non-amyloidogenic α-secretase pathway which precludes the formation of Aβ peptides. While extracellular aggregation of Aβ into amyloid plaques was primarily postulated as the cause of neurodegeneration, more recent studies have suggested that the formation of small soluble oligomeric Aβ assemblies (reviewed in Hayden and Teplow, 2013), or the intraneuronal accumulation of amyloidogenic APP fragments – CTFβ and Aβ (reviewed in LaFerla et al., 2007) – is an early toxic event in AD.

Both APP and the proteases involved in its processing: α-, β- and γ- secretases are transmembrane proteins. Lipids have vital roles in the plasma membrane. Indeed, lipid rafts,

cholesterol- and sphingolipid rich membrane microdomains, have been implicated in the pathogenesis of AD. It has been shown that lipid rafts serve as a site of Aβ production and that all three key proteins involved in Aβ formation (APP, BACE1 and PS1) are localized in lipid rafts (Ehehalt et al., 2003; Kosicek et al., 2010; Lee et al., 1998; Riddell et al., 2001). On the other hand, the endocytosis of APP has been shown to be critical for Aβ production both in cultured cells and in vivo (Cirrito et al., 2008; Koo and Squazzo, 1994). In addition, endosome abnormalities and altered endocytic APP trafficking were reported to be involved in early steps of AD (Cataldo et al., 2000, 2004; Ginsberg et al., 2010). From the cell surface APP is internalized via clathrin-mediated endocytosis and BACE1 cleaves wild-type APP during transit in the endosomes (Koo and Squazzo, 1994), the site of optimal activity of BACE1. The inhibition or activation of endocytic pathway decreases or increases the production of Aβ peptide, respectively (Carey et al., 2005; Grbovic et al., 2003). It has been shown that increased cholesterol concentration in the plasma membrane results in reduced membrane fluidity and increased endocytosis, which both may increase β-secretase-mediated APP processing (Weber et al., 2006). It is, however, also possible that cholesterol may fuel APP processing by directly stimulating the proteolytic activity of β-secretase (Kalvodova et al., 2005). Using an enzymatic assay that quantifies both free cholesterol and cholesteryl esters, Xiong et al. (2008) demonstrated that AD brain extracts contain more cholesterol than control brains and that this may contribute to high β- and γ-secretase activities and Aβ production in AD. More recently, however, a study by Marquer et al. (2011) showed that plasma membrane cholesterol content, measured by a fluorescence lifetime imaging microscopy-Förster resonance energy transfer technique, does not increase cellular Aβ production by having a direct impact on BACE1 catalytic activity but rather by altering the accessibility of BACE1 to its substrate, APP. This change in accessibility of APP to BACE1 is mediated by clustering in lipid rafts, followed by rapid endocytosis (Marquer et al., 2011). Altogether, multiple studies suggest that cholesterol may stimulate AD-associated amyloidogenic APP processing, but the details on the exact molecular mechanisms remain uncertain.

Although APOE-ε4 remains the most well-established genetic risk factor for late onset AD (LOAD), more recent genome-wide association studies (GWAS) identified several other loci to be significantly associated with LOAD: CLU, PICALM and BIN1 (Harold et al., 2009; Lambert et al., 2009). While CLU encodes clusterin (also called apolipoprotein J) which is, like ApoE, a lipoprotein expressed in the brain involved in cholesterol metabolism (Nuutinen et al., 2009), PICALM and BIN1 encode proteins involved in clathrin-dependent internalization and endocytic recycling, respectively (Harold et al., 2009; Lambert et al., 2009). Another putative susceptibility gene for AD, SORL1, encodes the neuronal ApoE

receptor and is an important mediator of APP localization and its access to secretases (Andersen et al., 2005). Thus, the two most prominently affected pathways in NPC disease, lipid metabolism and endocytic transport, seem to play essential roles in regulating the molecular events thought to underlie AD.

Alzheimer-like phenotype in Niemann-Pick type C disease

The most vulnerable neurons in NPC disease are Purkinje cells in the cerebellum, accounting for the prominent ataxia seen clinically. Neurodegeneration, however, is progressive and widespread within cortical and subcortical neuronal populations. On the other hand, in AD brains cerebellum seems to be spared, and early neurodegeneration is foremost noticed in the medial temporal lobe and later spreading to other parts of the temporal and parietal cortices, and finally to most association cortices, including the frontal lobe. Although NPC neuropathology at the anatomical level seems to differ considerably from that of AD, NPC disease is, like AD, characterized by progressive neurodegeneration, involvement of cholesterol, hyperphosphorylation of tau and accumulation of Aβ, thus posing the question whether the pathogenesis of these two disorders shares any commonalities in their etiology.

Aβ metabolism in Niemann-Pick type C disease

In the quest for the mechanism that would clarify the link between cholesterol and amyloid metabolism, a growing number of studies employed different NPC models, all showing alterations of APP metabolism involving the accumulation of Aβ and CTFβ. Although amyloid plaques were not reported in NPC brains, probably due to the lack of the aging process (i.e., the time necessary for plaque formation as in AD), the study by Yamazaki et al. (2001) was the first to reveal a remarkable Aβ accumulation in NPC model cells and in the brain of NPC mice using Western blotting of cell and tissue extracts. The mouse data are particularly interesting, since mice, in contrast to humans, do not naturally accumulate Aβ in the brain during aging. Interestingly, Yamazaki et al. demonstrated the intracellular accumulation of Aβ, especially Aβ42, in cholesterol-laden late endosomes of NPC1mutant CHO cells and cells treated with U18666A — a class 2 amphiphile drug that prevents the translocation of cholesterol from lysosomes to the ER, giving rise to an NPC-like phenotype. Accumulated Aβ in late endosomes appeared to be in an aggregated form and strongly influenced by cholesterol levels. Although in this work, Yamazaki and colleagues postulated that free cholesterol directly interacts with the aggregated Aβ thus causing its accumulation, the following work by different authors, albeit with some discrepancies, showed that the disruption of normal cholesterol trafficking in NPC can alter APP processing and cause greater Aβ production (Burns et al., 2003; Jin et al., 2004; Malnar et al., 2010; Mattsson et

al., 2011; Runz et al., 2002). Two studies reported an increase in γ-secretase activity in extracts from neuroblastoma cells (Runz et al., 2002) and mice brains upon NPC1 dysfunction (Burns et al., 2003). In accordance with these findings, our analysis of APP metabolites in the CSF from 38 NPC patients as compared to 14 matched controls revealed increased levels of Aβ38, Aβ40, and Aβ42 and unaltered levels of β-cleaved soluble APP, irrespective of disease duration (1.5–24 years) or severity, which is consistent with increased γ-secretase-dependent Aβ release in a manner not affected by disease stage (Mattsson et al., 2011). In contrast, the analysis of U18666A-treated primary neurons demonstrated that the accumulation of CTFβ, Aβ40 and Aβ42 was most probably due to enhanced β-secretase activity (Jin et al., 2004). Additionally, a recent study by Kodam et al. (2010) identified increased β-secretase activity along with increased levels of APP, BACE1, and also all four components of the γ-secretase complex in NPC mice cerebellar and hippocampal tissue extracts as compared to controls. Our study of CHO cells in which NPC1 gene had been deleted, CHO-NPC1−/− cells (Millard et al., 2000), revealed an increased level of the β-secretase-generated APP metabolites: sAPPβ and CTFβ, and an intracellular Aβ accumulation in CHO-NPC1−/− versus CHO-wt cells indicating an enhanced β-secretase cleavage of APP upon NPC1 dysfunction (Malnar et al., 2010; Mattsson et al., 2012a). The finding that the overexpression of CTFβ, a direct γ- secretase substrate, does not lead to increased intracellular Aβ levels, as determined by ELISA of cell extracts, in NPC1−/− vs. CHOwt cells suggests that the effect on intracellular Aβ in NPC1−/− cells is not due to increased cleavage by γ-secretase (Malnar et al., 2010). Furthermore, we demonstrated that increased CTFβ and intracellular Aβ levels in CHO-NPC1−/− cells are dependent on cholesterol accumulation because cholesterol depletion, achieved by incubation of cells for 48 h in lipoprotein-deficient medium, corrects aberrant Alzheimer-like APP processing in NPC1−/− cells to that as in wt cells (Malnar et al., 2010). Thus, although NPC1 dysfunction seems to favor β-secretase cleavage of APP in CHO-NPC1−/− cells, these results indicate that this effect is not due to NPC1 loss, but rather to cholesterol accumulation.

In sum, pharmacologically or genetically induced NPC phenotypes show consistent accumulation of Aβ peptides and altered patterns of APP degradation products, but the specific results differ between models and studies. This is confirmed by our most recent study, where we performed a detailed characterization of APP metabolic products in the cell media from pharmacologically (U18666A) and genetically (NPC1−/−) induced NPC cell models, CSF from NPC cats and human NPC patients (Mattsson et al., 2012a). We presume that the discrepancies between models may be a consequence of complex interactions between APP metabolism and NPC-induced pathways in which the effect of either NPC1

loss-of-function (as in NPC1−/− cells or NPC1-mice) or NPC1 dysfunction (as in some NPC patients, NPC cats and U-treated cells) may have distinct effects on APP metabolism.

Mis-trafficking of AD-related proteins upon NPC1-dysfunction

It is plausible that the initial defect in lipid trafficking upon NPC1 dysfunction, results in endocytic retention of transmembrane proteins that transiently associate with the late endosomes in normal cells, on their way to other cellular destinations. Consequently, this defect in protein sorting may result in aberrant processing.

While in vitro data implicate late endosomes as the site for Aβ and PS1 accumulation in NPC cells (Runz et al., 2002; Yamazaki et al., 2001), a study by Burns and colleagues using confocal microscopy of brain tissue from NPC mice found accumulation of PS1 in early endocytic compartments (Burns et al., 2003). Moreover, the intraneuronal accumulation of amyloidogenic APP fragments — CTFβ and Aβ, was reported in post mortem human NPC brains with accumulations again occurring in early endosomes, endocytic compartment upstream of massive cholesterol accumulation upon NPC1 dysfunction (Jin et al., 2004). Thus, the analysis of NPC mice or NPC human brains does not support a direct or close interaction between Aβ deposits and accumulated cholesterol. These results also suggest that a defect in the endocytic pathway, caused by the accumulated cholesterol, makes a more direct contribution to CTFβ/Aβ accumulation and/or their aggregation.

We hypothesized that increased formation of APP-CTFs and Aβ in NPC disease is due to cholesterol-mediated altered endocytic trafficking of APP and/or BACE1. First, using internalization-defective APP mutants, we showed that APP endocytosis is a prerequisite for enhanced Aβ secretion in CHO-NPC1−/− cells, measured using ELISA (Malnar et al., 2012). Moreover, using immunocytochemistry and confocal microscopy, we observed that NPC1−/− cells show cholesterol-dependent sequestration and colocalization of APP and BACE1 within enlarged transferrin-receptor positive endosomes. The increased interaction between the substrate APP and the enzyme BACE1 in endosomes of NPC1−/− cells can lead to increased β-secretase-mediated processing of APP. We demonstrated that increased endocytic localization of APP in NPC1−/− cells is likely due to both its increased internalization and its decreased recycling to the cell surface (Malnar et al., 2012). Our results demonstrate that the accumulation of cholesterol due to NPC1 dysfunction contributes to increased production and/or accumulation of Aβ by modulating endocytic trafficking of APP and BACE1. Moreover, our cholesterol-loading experiments (using U18666A- and MβC cholesterol- treatment) indicate that increased cholesterol levels can alter endocytic trafficking of APP and BACE1 even in a non-NPC1-deficient environment (Malnar et al., 2012).

Tau metabolism in Niemann-Pick type C disease

Neurofibrillary tangles (NFTs) consist of paired helical filaments (PHFs) formed by hyperphosphorylated microtubular protein tau (PHF-tau) and are considered to be one of the hallmarks in AD, although they are present also in many other neurodegenerative diseases. In later onset chronic cases (juvenile/adult) of NPC disease, neurodegeneration with the formation of NFTs, in addition to lipid storage, is the usual pathological feature, although distribution and numbers of NFT expressing neurons vary considerably between patients (Suzuki et al., 1995). The NFTs in NPC patient brains contain PHF-tau, which is structurally and immunologically similar to that in AD tangles (Auer et al., 1995; Love et al., 1995) suggesting that similar mechanisms may play a role in the formation of NFTs in these disorders. There is evidence that tangle-bearing cells in both diseases show higher levels of free (i.e. filipin-positive) cholesterol than adjacent tangle-free nerve cells (Distl et al., 2001). Interestingly, although Purkinje cells are the most prominently affected neurons, classic NFTs have not been noted in the cerebellum of NPC patients (Auer et al., 1995; Bu et al., 2002a; Suzuki et al., 1995). This is in concordance with the findings that neurons of the cerebellum in individuals with AD also do not form NFTs (Braak et al., 1989). Additionally, a notable difference between the NPC1-deficient mouse and human NPC is the complete absence of NFTs in the mouse brains (German et al., 2001). However, hyperphosphorylation of tau and other cytoskeletal proteins like MAP2 has been detected immunocytochemically in the cerebellum of NPC patients and brains of NPC mice (Bu et al., 2002a, 2002b). These data support the idea that a similar pathological process takes place in NPC cerebellum as in the cortex and hippocampus of AD, although NFTs are not an end result of the cascade in Purkinje neurons.

We showed that CSF levels of total-tau (T-tau), a neuro axonal injury marker, are elevated in NPC patients (Mattsson et al., 2011, 2012b), like in AD. CSF T-tau levels were also increased in patients with lysosomal diseases in another survey of pediatric neurological diseases (Shahim et al., 2013). There was no association of CSF T-tau levels with disease severity, but patients with longer disease duration (N6 years) had lower CSF T-tau levels (Mattsson et al., 2011), which may indicate a less intense neurodegenerative process in this subgroup. We also examined the association of CSF T-tau levels with miglustat treatment. Miglustat inhibits glucosylceramide synthase, reduces the brain load of GM2 and GM3 gangliosides in NPC and seems to stabilize the neurological disease in a majority of NPC patients (Patterson et al., 2012). In our study, miglustat-treated patients had lower CSF T-tau than untreated patients, which suggests that treatment might have reduced axonal degeneration (Mattsson et al., 2011). Similarly, in AD trials, CSF T-tau was reduced in antibody responders in the AN1792 trial with active immunization against Aβ (Gilman et al.,

2005), and reductions were also seen for CSF T-tau (a trend) and P-tau (significant) in the bapineuzumab trial with passive immunization (Blennow et al., 2012), interpreted as possible reductions of neuroaxonal degeneration. In NPC patients followed longitudinally with repeated CSF samplings, T-tau decreased over 6 to 15 months in patients starting treatment after the first CSF sampling, but not in patients who were already on treatment at the time of the first CSF sampling (Mattsson et al., 2012b). This suggests that the start of miglustat treatment may have lowered the rate of axonal degeneration, and that this effect is saturated over time. However, there were important differences in demographics between patients starting treatment and patients on continuous treatment, which may have confounded the results.

These observations are consistent with high rates of axonal degeneration in NPC, and indicate that CSF biomarkers may be used to monitor the neurodegenerative process. In contrast to increased CSF P-tau levels found in AD, CSF P-tau levels were unchanged in patients with NPC (Mattsson et al., 2011). This is actually not surprising, since several neurodegenerative conditions, including frontotemporal dementia, have NFTs despite normal CSF P-tau levels. Increased CSF P-tau appears to be a rather AD-specific finding.

Apolipoprotein E-ε4 – a common risk factor for Alzheimer's and Niemann-Pick type C disease

Apolipoprotein (Apo) E is the major cholesterol transport molecule in the brain. There are three major isoforms of this carrier expressed in humans: ApoE2, ApoE3, and ApoE4. These variants differ from each other only by a Cys to Arg amino acid substitution at positions 112 or 158 and are encoded by three alleles called ε2, ε3 and ε4. In humans, the ε3 allele is the most common and is considered the “neutral” APOE genotype. APOE-ε4 is associated with increased risk of AD (Corder et al., 1993), while APOE-ε2 may be protective against AD (Talbot et al., 1994). The mechanism by which ApoE4 increases the risk for AD is not entirely understood. ApoE was found in amyloid plaques and neurofibrillary tangles in AD brain. ApoE isoforms differentially modulate Aβ production and clearance and ApoE4 appears to influence the development of neurofibrillary tangles (reviewed in Liu et al., 2013).

Although AD characteristic amyloid plaques are not observed in NPC, Saito et al. (2002) reported the presence of diffuse plaques in 3/9 NPC patients (two sisters and an unrelated man). Of particular note, the APOE genotype for these three patients was ε4/ε4. Diffuse plaques in brain tissue of these three APOE-ε4 homozygous NPC patients were accompanied by an earlier onset of NFT formation and increased tangle load. This finding suggests that ApoE4 may be the cause of aberrant Aβ deposition as well as accelerated tauopathy in NPC. The same authors observed α-synucleinopathy and Lewy bodies in the

majority of NPC1 cases (Saito et al., 2004). Lewy bodies are a pathological hallmark of Parkinson disease and dementia with Lewy bodies, and the major protein constituent is phosphorylated α-synuclein. Lewy bodies are also seen in a subset of patients with AD (Hamilton, 2000; Lippa et al., 1998). Interestingly, in NPC, Lewy body pathology correlated with tauopathy, the accumulation of Aβ and the APOE-ε4 allele (Saito et al., 2004).

Consistent with the finding that the APOE-ε4 allele was associated with more severe neuropathological features, a correlation between APOE genotype and neurological onset of NPC was recently reported (Fu et al., 2012). NPC1 subjects with an APOE-ε4 allele had earlier neurological disease onset than those without a ε4 allele. In contrast, APOE-ε2 carriers had later onset than non-carriers. Thus, like in AD, the APOE-ε2 allele seems to be protective while APOE-ε4 allele contributes to an increased risk of the disease. A limitation of these APOE studies in NPC disease is the small number of cases. Unfortunately, this is a limitation that is inherent to the study of rare genetic diseases. However, acknowledging this weakness, these studies support that ApoE may play a role in modulating the occurrence of Aβ pathology in NPC.

NPC1 changes in Alzheimer's disease

In addition to numerous studies showing an Alzheimer-like phenotype in NPC disease, NPC1 changes have recently been demonstrated in several AD mouse models and AD patients (Ginsberg et al., 2010; Kågedal et al., 2010; Yao et al., 2012), indicating a bidirectional link between these two neurodegenerative disorders. Although the molecular network(s) which connect AD and NPC are yet to be determined, a recently discovered role for APP in controlling cholesterol metabolism (Pierrot et al., 2013) implicates again that the interplay between APP and cholesterol metabolism may be important for neurodegeneration, at least in AD and NPC disease.

NPC1 genetic variations in Alzheimer's disease

In recent GWAS, genetic markers of the NPC1 region were not found associated with late-onset AD (LOAD) (Harold et al., 2009; Lambert et al., 2009, 2013). Previously, a significant association of SNPs in NPC2 gene with LOAD was found in one population but not in several others (Wollmer et al., 2007). In a study conducted in the Polish population, which used centenarians as additional controls, an association of genetic variation in NPC1 with LOAD and/or aging was detected (Erickson et al., 2008). In this preliminary study, there were gradients of two-nonsynonymous (rs18050810 in exon 6 and rs1788799 in exon 12) SNP's allele frequencies in NPC1 from centenarians through normal controls to LOAD, indicating a role for NPC1 in AD and/or aging. Indeed, altered mRNA/protein levels of NPC1 were

detected in the brain extracts from AD patients and AD mouse models (described in more detail below). Additionally, increased tau phosphorylation and neurodegeneration were reported in aged heterozygous NPC1 mice (104–106 weeks of age) (Yu et al., 2005), indicating that human heterozygous NPC1 mutations or NPC1 genetic variants that cause malfunction of NPC1, in the aged population, may have some influence on the risk of neurodegenerative disorders.

Although NPC1 genetic variations were not found in AD GWAS studies, this does not disapprove its role in heritability of AD. Genome-wide significant SNPs in complex traits generally explain only a proportion of the heritability of that disorder (Yang et al., 2010). A substantial proportion of SNPs that do not achieve genome-wide significance may constitute residual heritability, meaning that the associated genetic signal(s) hidden below the threshold of genome-wide significance may comprise multiple contributing factors within the same pathway involved in the pathogenesis of the disease. Statistical (pathway-based) approaches have recently been developed to identify sets of functionally related genes containing genetic variants that collectively show evidence for association (Wang et al., 2007). These analyses may highlight non- GWAS significant SNPs that could explain some disease heritability which current GWAS do not have the power to detect. Jones et al. (2010) used the ALIGATOR algorithm to examine SNPs in two previous ADGWAS (Harold et al., 2009; Lambert et al., 2009) for enrichment in related gene categories. The two main themes that emerged were cholesterol metabolism and immune response, implicating that genetic variation in these two categories could lead to LOAD susceptibility. Among cholesterol- and lipid-related genes, NPC1 SNPs (rs1808579 and rs12970899) were shown to contribute to the association signals in both GWAS. While this does not mean that NPC1 gene with significant SNPs is a true susceptibility gene for AD, it rather implicates that this category is likely relevant to the disease etiology since it contains an excess of nominally associated SNPs. It is, thus, likely that a number of SNPs, in a set of genes within a common biological pathway, may be associated with disease risk and that affected individuals need not have the same combination of risk alleles. Indeed, the interactive effect between polymorphisms of two genes (epistasis), namely ABCA1 (encoding an ATP binding cassette transporter that removes cholesterol from cells) and NPC1, was recently demonstrated to contribute to AD susceptibility (Rodríguez-Rodríguez et al., 2010). The subjects carrying both the ABCA1 TT genotype (rs2422493) and the NPC1 GG genotype (rs18050810), NPC1 AA genotype (rs4800488), NPC1 AA genotype (rs2236707) or NPC1 GG genotype (rs2510344) had a higher risk of developing AD than subjects without these risk genotypes, suggesting ABCA1 NPC1 gene interaction. Although there are no functional studies of these ABCA1/NPC1 polymorphisms, the most likely mechanism of the association of these genetic variants with

AD is their loss of function. The authors have postulated that these genetic variations in ABCA1 and NPC1 genes could act in concert and that the underexpression of NPC1 together with the underexpression of ABCA1 could result in increased cholesterol accumulation and increased AD risk. Indeed, increased cholesterol levels in AD brains were recently reported (Lazar et al., 2013; Panchal et al., 2010) and epidemiological studies have confirmed midlife high serum cholesterol levels as a risk factor for AD (Pappolla et al., 2003). The functional links between ABCA1 and NPC1 were previously demonstrated by the finding that both ABCA1-deficient cells and NPC1-deficient cells show an excessive storage of free cholesterol in late endosomes/lysosomes and that in NPC1-deficient cells, ABCA1 expression is decreased and the ABCA1-dependent efflux of cholesterol is impaired (Choi et al., 2003). However, the analysis of the expression of ABCA1 and NPC1 at the brain level in subjects with different genotypes is needed to gain mechanistic insights into ABCA1 and NPC1 epistasis and the risk of AD. It also remains to be determined whether genetic interaction of NPC1 with other gene(s) within the cholesterol metabolic pathway, especially those previously identified by genetic/GWAS studies, such as CH25H, ABCA7 and ACAT (www.alzforum.org), or genes functionally involved in transfer of cholesterol/lipids across the plasma membrane, such as LDLR, LRP, SR-B1, NPC1L1, ABCG1/5/8, or encoding cholesterol hydroxylase, such as CYP46A1 and CYP27A1 (Dietschy and Turley, 2004) contributes to AD risk. Overall, these studies indicate that although NPC1 genetic variations per se are not sufficient to cause AD susceptibility, they most likely in combination with other SNPs of genes within the lipid-related pathway contribute to the etiology of LOAD. It would thus be important to identify which SNP combinations, including the NPC1 genetic variations, lead to LOAD susceptibility.

Altered *NPC1* expression in Alzheimer's disease

Variations in NPC1 expression in AD were detected both on mRNA and protein levels. Microarray analysis, which specifically assessed the expression of the endosomal–lysosomal genes and neurotrophin Trk receptors of microdissected hippocampal CA1 neurons harvested from subjects who died with a clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI) or AD, revealed significant downregulation of NPC1 expression as an early event occurring in AD (MCI and AD b NCI) and no differential regulation of NPC2 gene (Ginsberg et al., 2010). The NPC1 downregulation in MCI and AD CA1 neurons is consistent with the previously described role of NPC1 dysfunction on enhanced amyloidogenic APP processing and the pathogenesis of AD (Burns et al., 2003; Jin et al., 2004; Malnar et al., 2010; Mattsson et al., 2011; Yamazaki et al., 2001), but it is so far unknown if it translates to the accumulation of unesterified cholesterol, which would be important to address in future studies. Among other endosomal–lysosomal genes, Ginsberg

et al. (2010) found rab5 and rab7 to be significantly upregulated in CA1 neurons and these alterations were considered early changes, i.e. observed already in MCI cases. In addition, endosomal–lysosomal markers displaying significant differential regulation included an upregulation of cathepsin D (Cstd), early endosome antigen 1 (Eea1), extracellular signal-regulated kinase 1 (Erk1), lysosomal-associated membrane protein 1 (Lamp1) and dynein cytoplasmic light chain 1 (Dnclc1), and a downregulation of calpain inhibitor calpastatin (Cast) and the vacuolar proton pump homologue 1 (Vpp1). In parallel, a marked downregulation of the brain-derived neurotrophic factor (BDNF) receptor TrkB was found within CA1 MCI neurons and, thus, was considered an early gene expression alteration. Further in vitro analysis demonstrated a functional interrelationship between increased endocytic drive and decreased neurotrophin receptor expression. While wild-type and constitutively active rab5 downregulated TrkB at the transcriptional level, no feedback onto Rab expression was observed upon TrkB knockdown, suggesting a mechanistic link in which the expression of rab5 (and potentially other differentially regulated Rab GTPases) is an upstream regulator of TrkB levels. What drives a downregulation of NPC1 in CA1 neurons in MCI and AD and whether upregulation of rab5/7 can transcriptionally regulate NPC1 are still not known. We propose a model in which enhanced endocytosis due to increased rab5/7 transcription impairs endocytic flux and causes accumulation of late endosomes/lysosomes due to their inefficient fusion. Potentially, the downregulation of NPC1 and the upregulation of CstD can act as a response to the late endosome/lysosome dysfunction, leading to accumulation of internalized molecules and/or lysosomal substrates. Indeed, lysosomal dysfunction and the accumulation of aggregation prone proteins have been considered as a common mechanism of neurodegeneration in a number of neurodegenerative diseases including AD. We recently found increased CSF levels of six endosomal and lysosomal proteins (EEA1, LAMP-1, LAMP-2, LC3, Rab3, and Rab7) in AD patients compared to controls, and most of these (with the exception of EEA1) appeared to be specifically increased in AD, rather than general markers of neurodegeneration (Armstrong et al., 2014).

In contrast to NPC1 gene downregulation observed in MCI/AD CA1 neurons in post mortem brains by Ginsberg et al. (2010), two additional reports described its increased mRNA and protein levels in both AD human brains and AD transgenic mouse models (Kågedal et al., 2010; Yao et al., 2012). Kågedal et al. (2010) analyzed mRNA/protein levels of NPC1 in different brain regions of AD patients and APP/PS1-tg mice (APPsw/Ps1ΔE9) and revealed, in both models, a significantly increased NPC1 mRNA/protein expression in the hippocampus and frontal cortex, the two most affected brain areas in AD. Interestingly, in AD human/murine cerebellum, a brain region that is relatively spared in AD and is the most affected in NPC disease, no change in NPC1 expression was detected. NPC1 was

predominantly expressed in CA1 pyramidal neurons in both control and AD cases, suggesting that pathological process characteristic for AD, including altered processing of APP and the accumulation of Aβ in CA1 hippocampal neurons, may be associated with altered NPC1 expression in these cells. However, in vitro analysis using SK-N-SH neuroblastoma cell line did not confirm this association, i.e. no changes in NPC1 mRNA/protein levels were detected upon APP overexpression or after exposure to Aβ, indicating that neither APP nor Aβ induces neuronal NPC1 expression in vitro. Interestingly, Kågedal et al. (2010) found an inverse association between NPC1 and cholesterol levels in AD patients vs. controls. In the hippocampus, a brain region in which NPC1 expression was upregulated, cholesterol levels were found significantly decreased in AD patients compared to controls, while in the cerebellum, in which no obvious alterations of NPC1 were observed, a significant increase of cholesterol levels was detected. Although further mechanistic studies are needed to describe NPC1 alteration in AD, these findings suggest that interplay between NPC1 and cholesterol homeostasis might occur in AD. Indeed, a recent study by Yao et al. (2012) demonstrated a significant upregulation of NPC1 mRNA/protein levels upon cholesterol depletion in AD-tg mouse model (Tg19959: APPsw/APP-V717F). In the brains of AD Tg19959 mice, cholesterol depletion by chronic 2-hydroxypropyl-β-cyclodextrin (HP-β-CD; a cholesterol-sequestering agent that can bypass NPC1) administration was shown to significantly increase mRNA and protein levels of ABCA1 and NPC1. They also observed a trend toward increased mRNA/protein levels of NPC1 in untreated AD Tg19959 mice compared with wt mice. These findings are consistent with an upregulation of NPC1 expression in AD patients and APPsw/PS1 ΔE9 tg-mice previously reported by Kågedal et al. (2010). They further support that NPC1 and ABCA1 expression is coordinated and that NPC1 most likely acts in concert with ABCA1 toward cholesterol excretion. Since both NPC1 and ABCA1 transcription is regulated by liver X nuclear receptor (LXR), it would be important to identify LXR activators which are increased in AD and may be responsible for coordinated NPC1 and ABCA1 upregulation. Indeed, there is evidence that the concentration of oxysterol LXR activator 27-hydroxycholesterol is increased in AD (Heverin et al., 2004).

Although further studies are warranted to define the precise pathways that lead to altered NPC1 expression in AD and to describe the functional consequences of such changes, we speculate that the two most likely scenarios explaining the apparently contradictory NPC1 alterations in AD may involve a response due to: 1) dysfunction of the late endosomal/lysosomal pathway and a “traffic jam” in the endosomal/lysosomal system (in which increased NPC1 expression occurs as a result of NPC1 dysfunction due to alterations in the late endosomal/lysosomal pathway) and/or 2) alterations in cholesterol homeostasis that were recently described in AD brains. The links between NPC1 function and cholesterol

homeostasis were previously demonstrated in NPC1 overexpressing CHO cells (Millard et al., 2000) and in aged NPC1 heterozygous mouse (Yu et al., 2005) in which both NPC1 overexpression and NPC1 deficiency (NPC1+/−) caused increased total cholesterol levels. Since the data on altered NPC1 expression in AD is so far limited and to some extent contradictory (downregulation of NPC1 detected by Ginsberg et al. (2010) compared to its upregulation observed by Kågedal et al. (2010) and Yao et al. (2012)), additional studies including both early-onset and late-onset AD patients and MCI cases, as well as different AD mouse models are needed to precisely determine the role of cholesterol transporter NPC1 in AD pathogenesis. Further, it would be important to focus the autopsy studies on specific cell populations in affected and unaffected brain regions, define if the captured cells are positive or negative for NFT pathology and how close they are to plaques, and study, not only NPC1 and APP expression but also ABCA1, LXR, as well as cholesterol and lipid metabolites. In this manner, clues could be obtained on whether neuronal NPC1 expression changes early or late in AD-affected neurons and in what direction.

Molecular interactions between NPC1 and APP

Since NPC1 dysfunction has been shown to cause an Alzheimer-like phenotype including the accumulation of the two APP metabolites, CTFβ and Aβ, and since in AD brains (both in humans and in mouse models) alterations of NPC1 expression have been reported, it is tempting to speculate that NPC1 and APP might function within the same pathway(s). Indeed, several recent studies on mouse models have supported a bidirectional link between NPC1 and APP in which either NPC1-loss or APP-loss/overexpression exacerbates AD or NPC pathology, respectively. An AD-tg mouse (APPsw/PS1-M146L) was crossed with NPC1-heterozygote (NPC1+/−) to assess whether a partial reduction of NPC1 protein would influence the rate of progression or Aβ42 accumulation and plaque deposition in an AD mouse model (Borbon and Erickson, 2011). There was an earlier onset of the two features in these triple transgenic mice (APPsw/PS1-M146L/NPC1+/−), i.e. the mice showed earlier Aβ42 accumulation as well as more severe plaque load (already at 8-months of age). Although there were no data on whether the partial loss of NPC1 contributes to earlier defects in learning and memory of these mice, these findings demonstrate that NPC1 dysfunction may enhance the progression of AD, at least at the Aβ level, providing further evidence for the link between NPC1 and Aβ metabolism. It is tempting to speculate that genetic variants of NPC1 analyzed in genetic/GWAS studies of LOAD cause decreased NPC1 function and, thus, contribute to the pathogenesis of LOAD. In addition, a more challenging but very important task would be to investigate whether NPC1mutation carriers show cognitive impairment and increased risk of AD. Further, more cell-based studies are needed to address if NPC1 and APP share regulatory pathways in neurons. Future research

should examine specific changes in APP expression and degradation profiles in NPC1 gene-edited stem cell-derived neurons on the one hand, and NPC1- and cholesterol-related changes in APP gene edited neurons on the other.

Does AD-related, amyloidogenic APP processing influence NPC? The amyloid cascade hypothesis on neurodegeneration in AD suggests that Aβ accumulation is a pathogenic trigger that leads to tau dysregulation and, eventually, to neuronal dysfunction and death. According to this hypothesis, which states that Aβ accumulation indeed is toxic, it could be reasoned that removing the source of Aβ, namely APP, could help ameliorate neuronal dysfunction in NPC and that the overexpression of APP might have an opposite effect. However, as will become clear below, this appears not to be the case.

Mice lacking both APP and NPC1 protein (APPko/NPC1−/−) as well as mice overexpressing human mutant APP (APPsw/APP-V717F) under the NPC1-null background both showed exacerbated NPC phenotype at multiple levels (Maulik et al., 2012; Nunes et al., 2011). Double APPko/NPC1−/− mice displayed shorter cumulative survival, lower birth weight and poorer motor coordination than NPC1−/− mice (Nunes et al., 2011). The APPsw/APP-V717F/NPC1−/− bigenic mice exhibited decreased life span, early object memory and motor impairments, exacerbated glial and astrocyte pathology, significant demyelination and accelerated neurodegeneration in the cerebellum (Maulik et al., 2012). Interestingly, while APPko mice did not show any cholesterol storage abnormalities, the loss of APP in NPC1−/− mice resulted in further cholesterol accumulation throughout the cerebellar cortex, motor cortex and hippocampus (particularly in the dentate gyrus) (Nunes et al., 2011). There was an increase in the number of vesicles accumulating cholesterol in APPko/NPC1−/− mice than in NPC1−/− mice, indicating that cholesterol homeostasis is further disrupted and upregulated upon the removal of APP. This finding is in line with the recent report on APP function in controlling cholesterol homeostasis and turnover which showed that APP downregulation increased cholesterol biosynthesis and SREBP mRNA levels while APP upregulation showed opposite effects (Pierrot et al., 2013). Furthermore, it was demonstrated that APP controls cholesterol turnover via its direct binding to SREBP1 and that recently identified cholesterol binding sites within APP (Barrett et al., 2012) are necessary for this effect (Pierrot et al., 2013). Since SREBP controls transcription of the NPC1 gene, among a number of other genes involved in cholesterol synthesis and uptake, it is likely that APP and NPC1 interact indirectly within the cholesterol pathway, APP being an upstream effector. The intriguing finding that G700XXXG704 motif within the β-secretase generated APP-CTFβ fragment binds to cholesterol (Barrett et al., 2012) and is responsible for controlling cholesterol turnover (Pierrot et al., 2013) suggests that CTFβ levels/function and cholesterol homeostasis may be tightly linked. It is possible that CTFβ, through its binding to cholesterol,

interacts also with NPC1 (which contains a cholesterol sensing domain) in late endocytic compartments in which both proteins are localized. Intriguingly, the purification of an endogenous human functional γ-secretase, which substrate is CTFβ, revealed NPC1 protein among the known partners of the γ-secretase complex (Winkler et al., 2009). Although the presence of NPC1 during this isolation procedure could be nonspecific due to its multi-spanning membrane structure (13 transmembrane domains) and hydrophobicity, the possibility for direct interactions between APP and/or PS1 and NPC1 should be reinvestigated. It is also interesting to note that PS1−/− mice show several similar pathological features as NPC1−/− mice, including increased cholesterol levels, increased ApoE levels in the mouse brains as well as the accumulation of CTFβ (Tamboli et al., 2008). Since CTFβ has previously been shown to mediate endosome dysfunction (Jiang et al., 2010), which is considered to be an early pathological feature of AD (Cataldo et al., 2000), and is responsible for memory impairment and neurotoxicity characteristic for AD (Neve et al., 1996), it is tempting to speculate that the accumulation of CTFβ, in addition to Aβ, could play a role in AD and NPC pathogenesis. The CTFβ-tg mouse models may help us to elucidate the potential role of CTFβ as a signaling molecule which controls membrane trafficking and cholesterol homeostasis.

However, when APP-tg mice expressing human APPsw and APPV717F FAD mutations were crossed with NPC1−/− mice no differences in cholesterol accumulation and total cholesterol levels were observed compared to NPC1−/− mice. Filipin-labeled cholesterol was evident in almost all neurons of the hippocampus and cerebellum in both mice (Maulik et al., 2012). According to Pierrot et al. (2013), APP overload under the wt conditions would lead to decreased cholesterol synthesis and decreased expression of genes under the SREBP activation. Since NPC1 dysfunction causes a slight upregulation of cholesterol synthesis due to cholesterol entrapment in late endosomes/lysosomes, it is possible that these two opposing effects on cholesterol synthesis by APP overexpression and by NPC1 loss generate a net null effect on cholesterol accumulation/levels in these mice or that a slight change in cholesterol homeostasis by overexpression of APP-FADs in NPC1−/− mice is masked by the gross cholesterol accumulation due to NPC1 deficiency. Importantly, the restoration of cholesterol accumulation in APPsw/APP-V717F/NPC1−/− bigenic mice by subcutaneous administration of HP-β-CD increased longevity and attenuated behavioral/pathological abnormalities in these mice (Maulik et al., 2012), confirming that aberrant cholesterol homeostasis is the key trigger of the behavioral/ pathological alterations in the APP-FADs/NPC1−/− mice. It is interesting to note that the same treatment attenuated AD associated alterations in AD-tg mice (Yao et al., 2012). Since HP-β-CD is primarily used to lower cholesterol accumulation, it is tempting to speculate that AD, like NPC disease,

could be primarily a disorder of cholesterol homeostasis and/or that cholesterol dysregulation might represent an early pathogenic event and a primary trigger of AD.

Molecular interactions between NPC1 and tau

The presence of tau-positive NFTs represents a striking similarity between AD and NPC pathologies. However, the contribution of tau to the pathogenesis of these disorders has remained poorly defined. For example in AD, data from several laboratories have provided evidence for the amyloid cascade hypothesis, which states that tau pathology represents a secondary effect due to amyloid-β aggregation (Hardy and Higgins, 1992; Hardy and Selkoe, 2002). In contrast, Small and Duff (2008) have recently proposed a dual pathway hypothesis in which both tau and Aβ pathologies occur in parallel and independently of each other. This is supported by the finding that tau pathology may occur decades before amyloid pathology (Braak and Del Tredici, 2011). Also, even if phosphorylation of tau occurs after the abnormal increase of Aβ peptides in the brain, the existence of tau in general is necessary for Aβ-induced toxicity (Roberson et al., 2007). Therefore, both Aβ peptides and tau protein may play crucial roles in the development of Alzheimer's disease. The interrelationship between Aβ and tau pathologies in NPC disease has been revealed by crossing the APP and NPC1−/− mice (Maulik et al., 2012; Nunes et al., 2011). Strikingly, the loss of APP in NPC1−/− mice led to a substantial increase in tau abnormalities, in both cortex and cerebellum, including further reduction in tau levels and an increase of hyperphosphorylated tau (Nunes et al., 2011). A similar deterioration of tau function as in APPko/NPC1−/− mice, was observed in bigenic APPsw/APP-V717F/NPC1−/− mice (Maulik et al., 2012), supporting that APP may affect tau homeostasis independently of Aβ generation. Thus, it seems that in NPC disease Aβ and tau pathologies are not linked.

There is a limited data which explains a contribution of tau in the pathogenesis of NPC disease. The recent study on the aged NPC1+/− mice (104–106 weeks old) showed that these mice, similar to NPC1−/− mice, also develop hyperphosphorylation of tau (at positions Ser-199, Ser-202, Ser-212 and Thr-214). This is mediated by the activation of the mitogen-activated protein kinase (MAPK) (Yu et al., 2005), and suggests that human heterozygous NPC1 mutations may be a risk factor for tauopathy in the aged population. In our study of patients with NPC, the CSF levels of the neuroaxonal damage marker total-tau were higher as compared to controls, but the levels of a phosphorylated form of the protein (P-tau181), which is typically elevated in AD, were unchanged (Mattsson et al., 2011). While this needs to be extensively investigated, possibly with novel tau assays, neuropathological data suggest that NPC1 dysfunction in both NPC patients and aged NPC carriers may cause tau hyperphosphorylation leading to tau pathology and its loss-of-function. To assess the role of

tau dysfunction in the pathogenesis of NPC disease two different approaches have been employed so far: 1) the inhibition of tau phosphorylation and 2) tau deletion. Cyclin-dependent kinase inhibitors reduced tau phosphorylation and attenuated the phenotype of NPC1−/− mice (Zhang et al., 2004), suggesting a role for tau in disease pathogenesis. However, it is still unknown whether the observed effect is due to increased levels of the functional tau, decreased formation of tau toxic species or due to inhibitor effect on a different substrate. Interestingly, the generated NPC1/tau double knockout mice showed exacerbated phenotype compared to a NPC1−/− mice, resulting in a decreased litter size, worsening of the systemic phenotype and early death (Pacheco et al., 2009). This finding is in contrast to the recently reported effect of tau deletion in AD mice which partially ameliorated the AD phenotype (Roberson et al., 2007), demonstrating that the elimination of functional tau does not invariably lead to exacerbation of a neurodegenerative phenotype. This implies the existence of two distinct subclasses within a large and diverse group of tauopathies in which either tau loss of- function or tau gain-of-function mechanisms modulate pathogenesis of the disease. Nevertheless, these findings indicate that the restoration of normal tau function, rather than tau depletion as in AD, could be considered as a novel approach aiming to ameliorate or delay progression of NPC disease. If this approach proves beneficial against NPC, it could be tested for treating multiple other tauopathies which show to be similarly affected by tau loss-of-function. How tau deletion contributes to the worsening of the NPC phenotype is still unknown. However, in vitro studies of tau-knockdown in NPC1-deficient cells have suggested that impaired autophagy may be involved. It has been postulated that the activation of basal autophagy previously observed in NPC disease could represent a prosurvival response that is diminished by the loss of microtubules as a result of tau depletion. Indeed, acute down-regulation of tau in vitro markedly reduced both autophagic induction and flux in NPC1-deficient cells, but not in control cells (Pacheco et al., 2009). Thus, apart from the known function of tau in stabilization of microtubules, the regulation of axonal transport, and anterograde and retrograde trafficking, these results suggest that upon NPC1 deficiency tau may play a role in regulating the activity of the autophagic pathway in NPC disease.

Conclusion

NPC and AD show intriguing neuropathological similarities, including neurofibrillary tangles and deregulated Aβ metabolism. The strongest common denominator, however, is the link to genes involved in cholesterol metabolism. Lipid imbalance in neuronal membranes may be an important driver in the neurodegenerative process of both conditions and may influence both amyloidogenic and tau-related cascades. Further studies of similarities and differences between AD and NPC are encouraged to increase our understanding of the molecular

pathogenesis of these conditions, as well as our chances of finding disease-modifying therapies.

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