Niemann-Pick Type C Disease: At the Nexus of Neurodegenerative and Neurodevelopmental Disorders

**Abstract**

Rare genetic diseases can provide valuable insights into more common disorders by linking specific genes and pathways to shared disease phenotypes. The rare Niemann-Pick Type C disease (NPC) is a neurological disorder that has often been compared to Alzheimer’s Disease (AD) because both diseases are characterized by cognitive impairment in the presence of tau pathology and altered Amyloid Precursor Protein (APP) processing and Aβ metabolism. Here we review the molecular pathology of NPC and critically examine the similarities between NPC, AD and other neurological disorders. Besides the phenotypic overlap between AD and NPC, there is substantial evidence that cholesterol metabolism is altered in both diseases. Specifically, the epsilon 4 allele of the brain cholesterol transport protein Apolipoprotein E (ApoE4) is the strongest risk factor for late onset AD (LOAD) whereas NPC disease is caused by point mutations in the cholesterol transport proteins NPC1 and NPC2. In contrast to AD, NPC encompasses a broad neurovisceral disease phenotype having a diversity of penetrance, age of onset, and both central and peripheral manifestations. In addition to features that are in common with AD, NPC frequently exhibits close phenotypic overlap with neurodevelopmental disorders such as schizophrenia. Understanding the mechanistic links shared by NPC, AD, and neurodevelopmental disorders should enable a more holistic approach to therapeutic strategies to diseases which superficially appear very different.

**Introduction**

Neurodevelopmental and neurodegenerative diseases are generally thought to differ fundamentally in cause, course of disease and disease phenotype. Neurodevelopmental diseases arise from perturbed development of the nervous system while neurodegenerative diseases emerge from chronic degenerative changes in the brain resulting from stress, injury, altered metabolism or other maladaptive processes. As will be discussed in detail below, the Niemann-Pick type disease C (NPC) clinical phenotype includes psychosis and dementia [1,2], features of the common neurodevelopmental disease schizophrenia and the most common neurodegenerative disease Alzheimer’s Disease (AD) [3,4], respectively. NPC results from mutations in either of two cholesterol transport proteins, NPC1, or NPC2, highlighting the fundamental role played by cholesterol homeostasis in brain function and disease [5,6]. These neurodevelopmental and neurodegenerative processes may engage components of lipid metabolism in reciprocal ways frustrating the therapeutic targeting of many pathway components however, comparing the NPC phenotype to AD brings to light symptoms not normally associated with AD, such as psychosis [7-9]. This suggests that certain AD features or population subtypes may have characteristics of neurodevelopmental diseases such as alterations in developmental signaling pathways [10-14].

**Niemann-Pick Type C Disease Clinical Phenotype (Table 1)**

Niemann-Pick disease is a rare autosomal recessive disease with an incidence of 1:120,000 live births. Mutations in NPC1 account for 95% of disease and number >140 clinical variants. NPC2 mutations are less common with >40 known variants. Infantile, juvenile and adult onset forms are recognized. Early infantile forms result in death by 6 years of age. late infancy by 12 years of age and juvenile forms typically cause death in the third decade of life. Age at diagnosis from a cohort of 200 NPC patients showed that 45% were aged between 2 and 10 years [15]. Cerebral MRI reveals distinct patterns of brain atrophy associated with each form [16]. Infantile and juvenile forms of NPC present with anatomical abnormalities in the liver, spleen (hepatosplenomegaly) and cerebellar Purkinje cell atrophy.

Behaviorally, there is motor developmental delay, language delay, mental regression, vertical supranuclear gaze palsy and cataplexy [17]. Mutations in NPC1 or NPC2 cause cholesterol transport dysfunction that results in sequestration and accumulation in the late endosome and lysosome. Adult forms of the disease have also been identified in subjects in their 60’s suggesting variable penetrance [18,19]. In adult onset NPC, patients have cortical, cerebellar and midbrain, and liver abnormalities, but hepatosplenomegaly is less pronounced than in the younger forms. Adult onset NPC has increased neurofibrillary tangles, neurodegeneration and CSF Aβ. In some cases, diffuse Aβ plaques were found in the brain of the adult NPC patients that carried two copies of the ApoE4 allele [20].

NPC is characterized by extensive visceral and neurological signs including dementia in the presence of extensive neurodegeneration with tau pathology, increased Aβ levels and increased secretase activity reminiscent of AD; the disease has been referred to as a “childhood Alzheimer’s disease” [21]. However, distinct from AD, cerebellar Purkinje cells are heavily impacted in NPC leading to ataxia [22], a pattern that is recapitulated in animal models [23]. Heterozygous carriers of NPC mutations may exhibit visceral manifestations of dysregulated cholesterol metabolism, such as obesity [24]. Parkinsonism has been reported in NPC1 heterozygotes further expanding the spectrum of disease phenotypes associated with...
NPC mutations [25]. Genetic screening studies reveal that a late onset phenotype might be present with a much higher incidence, between 1:19,000-1:36,000 [26], and may be overrepresented and under diagnosed among adults with neurological and psychiatric symptoms [27]. These findings suggest that there is a wealth of opportunity to explore modulators of disease severity.

Molecular Pathology of NPC (Figure 1)

Both NPC1 and NPC2 work in concert as cholesterol binding proteins that regulate transport of LDL-derived cholesterol to the endosome and from the late endosome to various intracellular targets including the endoplasmic reticulum, lysosome, Golgi and mitochondria [28,29]. Expression of both proteins is regulated by cholesterol levels via SREBP pathways.

NPC1 is an intrinsic membrane protein having an N-Terminal Domain (NTD), three luminal domains and 13 transmembrane helices several of which comprise a sterol sensing domain [30]. NPC1 mutations alter not only cholesterol binding and transport functions but also expression levels, processing and localization in a mutation-related manner [31,32]. NPC1 protein has significant homology to the Patched1 (Ptc1) morphogen receptor that is part of the Hedgehog (Shh) pathway [33] and, along with Ptc1, significant homology to the resistance-nodulation-division (RND) family of permeases (All forms) Mutations in NPC1 and NPC2 cholesterol transport proteins resulting in cholesterol sequestration and accumulation in the late endosome and lysosome.

Table 1: Phenotypic similarities between NPC Disease, AD & Schizophrenia.

<table>
<thead>
<tr>
<th>Neurological Disorder age of onset</th>
<th>Anatomical Pathology</th>
<th>Neuropathology</th>
<th>Behavioural Pathology</th>
<th>Lipid Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-C Infantile and Juvenile</td>
<td>Liver, spleen (hepatosplenomegaly), cerebellar Purkinje cells</td>
<td>Cerebral and cerebellar atrophy</td>
<td>Motor developmental delay, language delay, mental regression, cataplexy</td>
<td>(All forms)</td>
</tr>
<tr>
<td>Adult</td>
<td>Cortex, cerebellum and midbrain, in liver hepatosplenomegaly is less pronounced than the younger forms</td>
<td>Neurofibrillar tangles, neurodegeneration, CSF Aβ, diffuse Aβ plaques found in some ApoE4/E4 carriers</td>
<td>Dementia, psychiatric cerebellar ataxia, vertical supranuclear gaze palsy (VSGP), movement disorders, dysarthria, dysphagia, and cataplexy</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>Hippocampus, entorhinal cortex, neocortex</td>
<td>Neurofibrillar tangles, Aβ plaques, neurodegeneration</td>
<td>Cognitive impairment, dementia, psychosis, agitation, apathy, depression, and sleep disturbances</td>
<td>ApoE4 carriers have a high risk of AD and AD + psychosis. 40-60% of AD patients carry at least one ApoE4 allele</td>
</tr>
<tr>
<td>AD + Psychosis Adult</td>
<td>Hypofrontality</td>
<td>Lewy Bodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizophrenia Adult</td>
<td>Prefrontal cortex, hippocampus, pyramidal cells, neuro-oligodendrocytes, interneurons, cerebellum</td>
<td>Decreased white matter and brain volume, enlarged ventricles, lower spine density, fewer oligodendrocytes, reduced GABA transmission</td>
<td>Psychosis, delusions, hallucinations, thought disorder, cognitive deficits and apathy</td>
<td>Elevated free fatty acids; metabolic syndrome, obesity, type II diabetes and cardiovascular disease prevalent in drug-naïve patients</td>
</tr>
</tbody>
</table>

Table 2: NPC Modulation of Pathology in AD and AD Mouse Models.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Human Studies</th>
<th>Mouse Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kagedal et al., 2010 [109]</td>
<td>NPC1 protein and mRNA expression is upregulated in neurons of the hippocampus and frontal cortex of AD patients. No difference was found in cerebellum</td>
<td></td>
</tr>
<tr>
<td>Fu et al., 2012 [154]</td>
<td>Significant associations were found in NP-C patients between ApoE polymorphism and phenotypic severity. Patients with an ApoE4 allele have greater disease severity than NPC3 and those with ApoE2 are less severely effected than those with ApoE3</td>
<td></td>
</tr>
<tr>
<td>Yao et al., 2012 [151]</td>
<td>HP-β-CD improved learning and memory deficits in Aβ over expressing mice, reduced Aβ plaque deposition, and reduced tau immunoreactive dystrophic neurites were reported</td>
<td></td>
</tr>
<tr>
<td>Yang et al., 2017 [152]</td>
<td>Amelioration of intra - lysosomal accumulations of Aβ-immunoreactive material and lipids in TgCRNDB mouse by cyclodextrin.</td>
<td></td>
</tr>
<tr>
<td>Maulik et al., 2012 [153]</td>
<td>Mutant human APP exacerbates pathology in a mouse model of NPC and is reversed by cyclodextrin</td>
<td></td>
</tr>
<tr>
<td>Borbon et al., 2011 [116]</td>
<td>NPC1 heterozygous knockout mice crossed to PSAPP mice accumulate Aβ plaques more rapidly and plaques are larger than PSAPP alone</td>
<td></td>
</tr>
<tr>
<td>Nunes et al., 2011 [112]</td>
<td>Loss of APP in NPC +/− mice (APP +/− : NPC+/−) worsens NPC pathology; specifically motor coordination, cholesterol regulation, tau homeostasis</td>
<td></td>
</tr>
<tr>
<td>Pacheco et al., 2009 [111]</td>
<td>NPC1 +/− mice with tau depletion (crossed with MAPT+/−) had exacerbated NPC1 pathology by exhibiting an enhanced systematic phenotype and younger mortality rate</td>
<td></td>
</tr>
</tbody>
</table>
for neuropsychiatric compounds including haloperidol, ketamine, the endoplasmic reticulum (ER) TMEM97/σ2 is itself a robust target chaperone protein for NPC1 limiting its generation and export from

been employed. It has been suggested that TMEM97/σ2 may act as a

influence NPC1 levels

in vivo

chaperone.

levels would have responded if brain penetrant ASOs had

influenced by numerous interacting proteins suggesting possible

of mammalian target of rapamycin  (mTOR) [43,44]. The expression and protein stability of NPC proteins are regulated by cathepsin B and L [42], and it interacts with neural precursor cell expressed developmentally downregulated gene 4-like (Nedd4L), a regulator of epithelial sodium channels (ENaC) and of mammalian target of rapamycin (mTOR) [43,44].

Mutations in NPC1 and NPC2 proteins impair cholesterol transport functions leading to endosomal accumulation of cholesterol [45], impairing lysosomal activity and autophagy, and impacting multiple signaling pathways leading to neuronal cell death [40]. A basic relationship between genotype and phenotype for NPC1 and NPC2 mutations have been reported [31,35].

NPC Protein Interactions and Cellular Pathways (Figure 2)

The expression and protein stability of NPC proteins are influenced by numerous interacting proteins suggesting possible strategies for therapeutic intervention through the manipulation of NPC protein levels.

NPC1

Levels of NPC1 are regulated by TMEM97/α2 receptor [46]. Knockdown of TMEM97/α2 increases NPC1 protein levels in cell culture but anti-sense oligomers (ASOs) to TMEM97/α2 failed to influence NPC1 levels in vivo in rat liver. It is unclear whether brain NPC1 levels would have responded if brain penetrant ASOs had been employed. It has been suggested that TMEM97/α2 may act as a chaperone protein for NPC1 limiting its generation and export from the endoplasmic reticulum (ER) TMEM97/α2 is itself a robust target for neuropsychiatric compounds including haloperidol, ketamine, methamphetamine and phencyclidine (all agonists).

TMEM97/α2 is involved in one of several complexes with the low-density lipoprotein receptor (LDLR) upstream of NPC1 that may be differentially regulated among different tissues. TMEM97/α2 forms a complex with progesterone receptor membrane component 1 (PGRMC1) and LDLR to promote internalization of LDL [47]. LDLR also forms clathrin-dependent internalization complexes with propionate convertase subtilisin/kexin-9 (PCSK-9) and the adaptor protein autosomal hypercholesterolemia (ARH)/ receptor associated protein (LDLRAP). LDLRAP protein levels are high in liver but low in brain and may provide an alternative to TMEM97/α2 for LDLR internalization in liver.

Levels of LDLR are promoted in NPC1 defective cells by the metastatic suppressor N-myc downstream regulated gene-1 (NDRG1) which is required for caspase activation by tumor protein p53 (TP53) [48]. NDRG1 effects balance degradation of the receptor by the inducible degrader of the LDLR (IDOL) [49]. TP53 protein is reduced in Niemann Pick’s disease as a result of abnormal p38MAPK activation and subsequent Mdm2 activation resulting in TP53 degradation [50]. In contrast in AD, there is evidence that TP53 activity is increased and that there are direct interactions with tau and Aβ while others have reported conformational alterations of TP53 associated with AD [51-53].

Transcriptional profiling of NPC1 knockout mice links NPC1 to levels of tau, apolipoprotein C1 (ApoC1), sortlin 1, nexins 12, 13, 17, and ATP-binding cassette sub-family A (ABCA) members 2, 5, and 8B which are all related to active targets for intervention in AD. ABCA2, for example, is reported to regulate amyloid precursor protein (APP) expression via sphingolipid metabolism [54,55]. APP protein increases in cerebellum and hippocampus of NPC1 knockout mice [56]. Reduction of NPC1 levels by proteasomal degradation [57], is a consequence of Akt activation.

Figure 2: Regulators of NPC1 and NPC2 protein levels.

NPC1 levels appear to be reciprocally regulated by TMEM97/α2 receptor. TMEM97 forms a complex with PGRMC1 and LDLR. LDLR levels are enhanced by NDRG1, and down regulated by IDOL (left). NPC1 directly interacts with SLC38A9 to mediate cholesterol activation of mTORC1 which in turn regulates SREBP activity. Activation of SREBP by SCAP yields an amino-terminal fragment which translocates to the nucleus to activate transcription of NPC1 and NPC2 genes (right).

NPC2 levels are stabilized by Nogo B receptor (left) while NPC1L1 protein promotes its degradation. NPC1 deficiency promotes NPC2 levels and vice versa.
**NPC2**

Mutations in NPC2 yield “compensatory” increases in NPC1 protein [31]. NPC2 is stabilized by the Nogo- B receptor which has an independent role in lipogenesis by enhancing nuclear transport of liver X receptor alpha (LXRA) while NPC1-Like 1 protein (NPC1L1) down-regulates the expression and secretion of NPC2 [58-60].

**Relationship to Cholesterol Sensing by mTORC1**

NPC1 forms a complex with the lysosomal transmembrane protein SLC38A9 which mediates cholesterol activation of mTOR complex 1 (mTORC1) [61]. mTORC1 regulates sterol regulatory element binding proteins (SREBP)1 and SREBP2 activity [62,63]. mTOR is a link between AD, in which mTOR is chronically activated with detrimental impact on autophagy and tau phosphorylation [64-66], and schizophrenia which is characterized by hypofunction of the mTOR pathway [67].

**NPC Proteins as pharmacological targets**

NPC protein levels are sensitive to treatment with amphiphilic psychotropic and antidepressant drugs [68,69]. The cationic amphiphile U18666A binds to NPC1 [70], inhibits cholesterol binding and recapitulates features of NPC disease phenotype [71]. The antidepressant amitriptyline induces the accumulation of cytoplasmic cholesterol levels and increases expression of NPC2 mRNA [69].

Amitriptyline treatment increases the secretion of NPC2, causes neurogenesis and improves cognition in 3XTg Alzheimer’s mice [72]. It also causes functional improvement in a Huntington’s disease mouse model via increased neurotrophin signaling [73]. Amitriptyline has also shown benefit in the context of another neurodegenerative disease, progressive supranuclear palsy (PSP) [74, 75].

**Current treatment strategies**

Efforts to standardize disease diagnosis and treatment strategies have been reported [76]. Diagnosis of NPC disease typically involves histopathological analysis using filipin, a fluorescent macrolide antibiotic that binds to cholesterol. The compound has also been used extensively for chemical screening of compounds for the treatment of NPC [77-80]. A positive filipin test would prompt genetic testing to be the result of NPC1 or NPC2 mutations. Specific blood oxysterol profiles are associated with NPC disease [81]. The Phase 1-2 clinical trial for Intracerebroventricular (ICV) β-cyclodextrin employed plasma hydroxycolesterol [82], cerebrospinal fluid (CSF) fatty acid binding protein (FABP) and calbindin, a marker for Purkinje cell degeneration as biomarkers. In addition, various magnetic resonance modalities such as MRI have been employed as imaging biomarkers [83,84].

Robust treatment approaches include ICV injections of β-cyclodextrin (BCD) which acts essentially as a cholesterol chaperone. BCD treatment is the result of novel research collaboration between academic, government and industry researchers and family members called Support of Accelerated Research-NPC (SOAR-NPC) [85].

Emerging therapies include: adenoiviral-expressed wild-type NPC1 to provide functional NPC1 protein [86], stimulation of exosomal secretion of cholesterol to ameliorate abnormal accumulation of cholesterol [87], and knock-down of genes associated with ESCRT III (especially knock-down of VPS4B [88]) to increase exosome secretion [89].

NPC is associated not only with accumulation of cholesterol, but also with sphingosine, sphingomyelin and glycosphingolipids (GSL’s) resulting in altered endolysosomal calcium homeostasis as a result of inhibiting the mucolipin TRP channel 1 (TRPML1) [90-93]. The glycosylceramide synthase inhibitor Miglustat is approved for the treatment of NPC and reportedly stabilizes or improves neurological manifestations [76,94-96]. In the feline NPC model, the adverse neurological phenotype was delayed with miglustat treatment without having a significant impact on cholesterol accumulation or visceral endpoints suggesting that neurological manifestations and cholesterol accumulation, as well as central and peripheral manifestations are separable phenomena [97].

Treatment with fingolimod (FTY720), a sphingosine analog and sphingosine- 1-phosphate receptor agonist, increases NPC1 and NPC2 expression, and reduces both cholesterol and sphingolipids in NPC mutant cells [98]. Fingolimod, becomes a potent histone deacetylase (HDAC) inhibitor once phosphorylated. It is being evaluated in clinical trials for NPC disease and has been tested in the context of AD models [99]. Dysregulation of sphingolipid metabolism is observed in AD where it correlates with CSF Aβ levels and contributes to impairment of autophagy [100-103].

Cellular treatment with sphingolipids causes a “molecular trap” for cholesterol Sphingolipid treatment results in SREBP cleavage by SREBP cleavage-activating protein (SCAP) [48], and subsequent upregulation of LDL receptors which is the source of the elevated cholesterol. LDL receptors as well as TIMEM97/ε2, a protein that interacts with, and might regulate NPC1 levels are targets of SREBP [104]. In turn the lipogenic activity of SREBP1 is regulated by mTORC1 and promotes cell growth via Akt signaling [62].

**NPC and AD (Table 2)**

The observation of AD-like neurofibrillary tangles and diffuse amyloid deposits in NPC have prompted numerous studies to search for molecular links between these two diseases. For example, while there is little evidence for a direct genetic relationship between NPC and AD, there is reported epistasis between NPC1 and ATP-binding cassette type A1 (ABCA1) and AD risk [105]. ABCA1 is a critical lipidating gene for ApoE. ApoE4 is the strongest risk factor for sporadic AD and has been found to be poorly lipidated compared to the other common human ApoE isoforms, ApoE2 and ApoE3 [106]. Increasing ApoE4 lipidation has been suggested as a therapeutic strategy for AD. It has been reported that NPC disease patients have dysregulated ABCA1 expression and reduced ABCA1 activity [107].

The NPC1 inhibitor U18666A, not only inhibits NPC1 cholesterol binding and recapitulate features of NPC disease phenotype [71], treatment with the drug alters APP metabolism resulting in endosomal - lysosomal processing of APP [108]. Knockout of the NPC1 gene has similar effects in vivo all suggesting that NPC proteins can influence the amyloidolytic processing of APP. Levels of NPC1 are increased in Alzheimer’s disease and in APP/PS1 transgenic animals [56,109], a surprising finding if the AD phenotype in NPC disease is thought to be the result of NPC1 or NPC2 loss of function. It is possible that such elevations in NPC protein levels reflect a homeostatic response...
to perturbed cholesterol metabolism in AD.

Several additional studies have employed animal models of NPC disease (reviewed by [110]) crossed with models of AD. Deletion of either Tau or APP exacerbates the NPC phenotype [111,112].

The deletion of tau is thought to impair the cytoplasmic transport required for autophagy, while the exacerbation of phenotype caused by the loss of APP suggests that APP may play a compensatory role for the loss of cholesterol transport proteins. Aβ is reported to have a role in regulating lipid homeostasis and furthermore lipid-associated Aβ is increased in NPC suggesting a potential lipid “chaperone” role for the peptide. In contrast to APP deletion [113,114], APP overexpression in NPC-deficient background yields increased Aβ generation and the production of shortened γ-terminal fragments (γ-CTFs) suggesting correspondingly longer and potentially more toxic forms of Aβ [114-116].

APP, APP fragments and APP processing enzymes interact robustly with SREBP2, which in turn regulates NPC expression. Aβ and β-cleaved APP inhibit SREBP2, while α-soluble APP stimulates it [117,118]. The nuclear translocation of SREBP2 N-terminal fragments, which is required for SREBP transcriptional activation is impaired in AD and tau transgenic animals, but not in APP transgenic animals suggesting that AD-related tau dys-homeostasis can alter SREBP2 signaling [119]. Similarly, dysregulation of SREBP2 caused by high cholesterol content can cause an increase in the expression of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) under the same conditions expected to result in an increase in NPC expression [120].

**NPC and other neurodegenerative diseases**

NPC is a lysosomal storage disease, a category of neurodegenerative disorders that includes the sphingolipidoses. This class of disorders is characterized by aberrations in sphingolipid metabolism and includes Niemann-Pick disease types A and B caused by defects in sphingomyelinase (SMPD1), Gaucher’s disease caused by galactosidase (GBA1) deficiency, Fabry’s disease associated with α-galactosidase-A (GLA) deficiency, Krabbe disease associated with galactosidase (GALC) deficiency, Tay-Sachs caused by β-hexosaminidase-A mutations and metachromatic leukodystrophy (MLD) resulting from defects in arylsulfatase A (ARSA) [121]. Although cholesterol is the principle material impacted by NPC mutations, they also influence sphingolipid metabolism and therefore are included in this class of disorders all of which have devastating neurological consequences.

Due to the essential role NPC proteins play in cholesterol metabolism, it is not surprising that impairment of their function results in phenotypic overlap with numerous neurological diseases such as fronto-temporal dementia (FTD), Parkinson’s disease, multiple sclerosis (MS) and other inflammatory disorders. The presence of frontal lobe atrophy suggests similarities with FTD [36]. A potential link with FTD is further supported by the observations that there is aberrant cytoplasmic localization of the FTD-related protein TAR DNA-binding protein 43 (TDP-43) in NPC models, and that the expression of TDP-43 regulated genes such as transcription factor AP-2 alpha (TFAP2A), ciliary neurotrophic factor receptor (CNTFR), MAP kinase-activating death domain protein (MADD), myocyte- specific enhancer factor 2D (MEF2D), transducin-like enhancer protein 1 (TLE1) and TRAF2 and NCK- interacting protein kinase (TNIK) is altered [122].

Parkinsonism is associated with NPC heterozygosity [25], and NPC cases share features of synucleinopathy associated with PD including Lewy bodies and Lewy neurites as detected by immunoreactivity for phosphorylated synuclein [20]. Ceramide metabolism appears to be perturbed in both NPC and PD [90,91,98]. Mutations in GBA1 cause Gaucher’s disease when homozygous, or a predisposition to PD when heterozygous [123,124]. The accumulation of GSLs caused by GBA1 mutations can be mitigated with the pharmacological chaperone alegostat-tartrate (isofagomine) or with inhibitors of glycosylceramide synthase such as inhibitor GZ667161, which has been tested in models of synucleinopathy [125,126], and miglustat, which has been employed in NPC models and human NPC subjects as discussed above. Impaired mitophagy has been implicated in PD pathogenesis. NPC2 as well as the PD related genes parkin and Pten-Induced kinase (PINK1) are regulators of mitochondrial autophagy suggesting a mechanistic link between NPC and PD [40,127].

Cholesterol is a major component of myelin, so a relationship between diseases that result from dysregulation of cholesterol homeostasis and demyelinating diseases is expected. A case of adult NPC disease originally diagnosed as multiple sclerosis and a report of severe demyelination in a case of juvenile NPC disease illustrate the connection between aberrant cholesterol metabolism and impaired myelination of neurons. Defects in myelination are common both in human NPC disease and in the knockout mouse model and proteomic studies of the corpus callosum from knockout mice have identified specific factors involved in defective myelination including glycolipid transfer protein (GLTP), ceramide synthase 2 (CerS2), and 2-hydroxyacylsphingosine 1-beta-galactosyltransferase (UGT8). Fingolimod, a therapeutic approved for the treatment of MS is under evaluation for its utility in NPC disease. In the context of inflammation, NPC1 mutations are associated with activation of the innate immune system and chronic inflammation [128-132], however NPC2 knockdown reduced lipopolysaccharide (LPS)-induced expression of pro-inflammatory genes suggesting Toll-like receptors (TLR) signaling activation requires NPC2 [40].

**NPC and other neurodevelopmental disorders**

In contrast to the features that are conventionally associated with AD, NPC features psychosis as a component of disease phenotype, and stereotypy is a feature of both psychosis and NPC. Both NPC disease and schizophrenia are associated with cerebellar impairment [133-135]. Likewise seizure is a feature of NPC disease and AD. In a study on glutamatergic function in NPC1 -/- mice, AMPA receptors did not respond to prolonged application of agonist to cause a reduction in synaptic transmission despite normal AMPA receptor protein levels [136]. Similarly, studies on iPSC-derived NPC1 mutant neurons show upregulation of AMPA receptor expression and protein level, but attenuated function. Collectively, these data suggest that NPC protein plays an important role glutamatergic function.

As mentioned, TMEM97/2 is both a molecular partner of NPC1 and a robust target for antipsychotic medications suggesting a link
between NPC and psychiatric disorders, while other psychotropic drugs upregulate expression of NPC1, NPC2 and other cholesterol transport genes through regulation of SREBP [68]. Large numbers of undiagnosed NPC mutations among psychiatric patients have been reported suggesting that psychosis may be a major manifestation of adult onset NPC disease [27,137,138].

The neurodevelopmental - neurodegenerative disorder overlap: Alzheimer’s disease + psychosis

If cholesterol metabolism is truly central to pathogenesis in both NPC and AD, it suggests that there may be additional phenotypic overlaps which are less commonly observed. Psychosis, for example, is a feature of NPC, and distinguishes an AD subtype. AD plus Psychosis (AD+P) is now recognized to be associated with accelerated cognitive decline, hypofrontality and a significant (as much as 61%) heritability [7,8,139,140]. Psychosis is reported in as many as 50% of individuals with AD and is associated with greater cortical synaptic impairment [9]. In the Tg4510 mouse model (P301L mutant human Tau), a psychosis phenotype (pre-pulse inhibition, PPI) correlates with brain load of hyperphosphorylated tau [141].

Treatment with the anti-psychotic haloperidol reduces tau phosphorylation in the same model by inhibiting AMPK consistent with postmortem human observation of reduced neurofibrillary load in subjects treated with haloperidol [142-144]. A psychotic phenotype was also described in the APPsw/ePSI deltaE9 transgenic model, and rescued by knockdown of a protein linked to schizophrenia, kalirin [145].

ApoE genotype appears to correlate with both the occurrence of psychosis in AD and with the presence of Lewy body pathology, with those carrying two ApoE4 alleles at greatest risk [146,147].

Discussion and Conclusion

The NPC proteins regulate the critical transit of cholesterol through the endocytic pathway and as such appear to be prime targets for interventions into many pathogenic processes whether developmental or degenerative in origin [148]. The expression and stability of the NPC proteins are regulated by a diverse network of proteins, and NPC1 itself is the target of small molecule pharmacology efforts. Nevertheless, it is precisely that tie to diverse and potentially reciprocal processes that complicates targeting NPC-related processes and brings with it risks of off-target effects.

Given the central importance of cholesterol metabolism to AD and the fact that many NPC associated proteins are targets for antipsychotics, it should be no surprise that NPC disease is at the nexus of these diverse processes and highlights the heterogeneity of related diseases such as AD. Finding relationships between AD and neurodevelopmental processes is not unprecedented. Alterations of developmentally programmed gene expression and microchimerism have been evoked in claims that AD is neurodevelopmental in origin [10,14]. Moreover, there are clear links between AD and neurodevelopmental diseases based upon APP expression and metabolism, as in the case of Down’s syndrome, in which increases in APP and Aβ due to a gene dosage effect is observed. Conversely excess activity of α-soluble APP is believed to contribute to brain enlargement in autism [149]. Furthermore, the tau pathology which is so central to the link between NPC and AD is also present in numerous other neurodevelopmental disorders, such as hemimegalencephaly, tuberous sclerosis complex and focal cortical dysplasia [150]. NPC disease exemplifies how cholesterol metabolism lies at the nexus of developmental and degenerative processes linking diverse phenotypes to common mechanisms.

References


NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid binding a variety of sterols. J Biol Chem 281: 36710-36723.


Niemann-Pick disease type C: spectrum of HE1 mutations and genotype/molecular pump activity of Niemann- Pick C1 protein. Science 290: 2295-2300.


ISSN: 2376-922X


Acknowledgement

We thank Dominic M. Walsh for his insightful review of the manuscript.