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Niemann–Pick type C disease proteins: orphan transporters or membrane rheostats?

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Abstract

Niemann–Pick type C (NPC) disease is a panethnic lysosomal lipidosis, which results in severe cerebellar impairment and death, and is proposed to be a consequence of defective metabolite transport. Numerous models of this disorder have defined the phenotypic impact of misfunction of the NPC proteins, however, their mechanism of action and definition of substrate(s) remain vague and disputed. The proteins may be lipid chaperones, nonspecific transporters, orphan transporters or membrane-sensing regulators ('rheostats') of other transport reactions. These issues pertain to the nature or even existence of a toxic metabolite as causative to this disorder and thus ultimately to treatment of the disease. This review will present the issues that underpin NPC disease and current or future avenues of treatment.

Keywords

cholesterol; gangliosides; lysosome; Niemann–Pick; Niemann–Pick type C1; Niemann–Pick type C2; rheostat; resistance-nodulation-division-transporter

Our understanding of the factors that determine vesicular transport of proteins is both clear and detailed [1]. By marked contrast, lipid movement between organelles, a critical component of eukaryotic membrane homeostasis, has very few known molecular players. Niemann–Pick type C (NPC) disease is a monogenic, autosomal recessive, fatal, neurodegenerative disorder caused by independent defects in two genes, *NPC1* and *NPC2* [2–4]. As the disease progresses, lipid accumulation in the lysosomes of a variety of cells leads to neurological, gastrointestinal, liver and respiratory problems, consistent with a role of *NPC1* and *NPC2* in lipid transport. Studies on NPC disease have exponentially increased our understanding of intracellular lipid transport that reaches beyond this devastating rare disorder to other forms of neuronal dysfunction such as Alzheimer's or Huntingtons disease [5,6]. However, surprisingly little is known about the precise mechanisms of action or substrates of the NPC pathways. In electrical circuits, a rheostat allows for and adjusts to variation in resistance; we hypothesize that NPC1

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is a membrane rheostat that senses and adjusts the fluidity of membranes in which it resides by increasing or decreasing the flux of appropriate lipids. In this review, we will describe the basis for this theory and the manner in which our knowledge of this process may guide future treatments of this devastating disorder.

Human Niemann–Pick type C disease: a transport disorder?

The accepted biochemical hallmark of NPC disease is the lysosomal accumulation of low-density lipoprotein-derived, unesterified cholesterol in fibroblasts. Consequently, the reactions responsible for sterol biosynthesis, uptake and esterification are misregulated. Although the defect in egress of cholesterol from the endosomal/lysosomal system is the predominant mutant phenotype, NPC disease is also typified by the subcellular accumulation of sphingomyelin, sphingosine and complex glycosphingolipids [7]. In balance, the weight of current research into NPC disease has led some to propose a mode of action of NPC1 and NPC2 proteins as cholesterol pumps and chaperones, respectively. When the NPC proteins are defective, cholesterol accumulates, often to the point of crystallization in these organelles; while other lipids with physical affinity for sterols could also accumulate.

It is equally plausible that cholesterol is not cargo. The low affinity of the NPC1 rheostat for cholesterol suggests it acts in a catalytic or allosteric fashion to activate transport of other lipids, which restore membranes to their optimal fluidity. Accordingly, cholesterol itself could be a significant bystander of defective transport of another hydrophobic compound, likely one transported during endocytosis (markers of endocytosis, such as sucrose, exhibit retarded transport to extralysosomal sites in *NPC1*-defective cells [8]). Interestingly, fibroblasts from a ‘variant’ subgroup of NPC individuals with profound neurodysfunction exhibit severe sphingolipid transport aberrancies but only modest cholesterol mislocalization [9]. The converse situation has yet to be described.

NPC genes: structure informs function?

Mutations in a single complementation group on human chromosome 18 confer 95% of NPC cases, which led to the identification of the *NPC1* gene by positional cloning in 1997 [10]. *NPC1* encodes a 1278 amino acid, 13 transmembrane domain protein localizing to late endosomes and lysosomes (Figure 1A). The sequence of *NPC1* reveals a close relationship with several other human proteins that are influenced by sterols, including a component of dietary sterol absorption, Niemann–Pick C1-like 1 (NPC1L1; 39% overall identity), sterol regulatory element binding protein (SREBP) cleavage activating protein (SCAP; 26% identity, residues 615–797), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, 7-dehydrocholesterol reductase, translocation in renal carcinoma chromosome 8/ring finger protein 139 and, inexplicably, the morphogen receptor Patched (23% identity over several domains). These proteins all retain putative ‘sterol sensing domains’ (SSDs; for alignments see [11,12]), which mechanistically appear to act as membrane-sensitive protein interaction motifs. Although a partner protein is yet to be identified for NPC1 (or its orthologs and paralogs), patients with mutations in the SSD of *NPC1* typically have a severe disease phenotype [13]. Similarly, a functionally important region of *NPC1* based on clustering of mutant alleles is a cysteine-rich luminal loop (residues 857–1015 [14]). Patients with severe and variant disease phenotypes have mutations in this ‘RING’ domain, which has been shown to interact with phospholipids [13,15]. Thus, genotype–phenotype correlations loosely link the SSD with accumulation of cholesterol (the hallmark of NPC disease), while regions of the cysteine-rich luminal loop may be more relevant to sphingolipid transport. Perhaps these biochemical parameters are separable?

The role of NPC2 in lipid transport is also poorly understood. This protein (Figure 1B) is responsible for 5% of NPC cases and was formerly known as HE-1, a soluble 151 amino acid

lysosomal protein that is highly concentrated in mammalian epididymal fluid and bovine milk [16]. Similarly to NPC1, NPC2 contains a predicted lipid interaction motif, the MD-2-related lipid-recognition (ML) domain (residues 24–145). The connection between mutations in the ML domain and disease is unclear, although patients with a truncated NPC2 protein present with severe neonatal onset of respiratory or hepatic manifestations and/or neurological disease with death prior to 4 years of age. The NPC2 protein, for which a 1.7-Å resolution crystal structure has been solved [17], binds cholesterol and other sterols at a high affinity [18,19]. Thus, its proposed activity as a cholesterol transfer protein *in vivo* appears more than plausible, perhaps acting as a lipid chaperone to prevent cholesterol crystals from forming. Nevertheless, the mechanism by which NPC2 influences lipid transport is obscure and recent studies indicate it acts as a dimer to bind glycosphingolipids [20]. Could NPC2 transfer cholesterol (or other lipids) to NPC1? The epistatic interactions of NPC1 and NPC2 in mice place them in a common linear lipid transport pathway [21], however, there is no evidence for a physical interaction and, at the cellular level, overexpression of one protein is not sufficient to compensate for a lesion in the other.

NPC genes: relics of an ancient transport reaction?

NPC1 was the first mammalian member of the resistance-nodulation-division (RND) family of prokaryotic permeases to which proteins such as NPC1L1 and Patched have now been included based on their sequence and structure. These observations provided the only hypotheses on the mechanism of action of NPC1 and its relatives [22]. Members of the bacterial RND protein family use a proton motive force to transport lipophilic substrates against concentration gradients. Since NPC1 has not been shown to directly transport sterols, one proposed function has been as a permease for fatty acids [23]. This hypothesis remains contentious, with different groups disputing fatty acid accumulation as a characteristic of this syndrome [24,25]. Amino acids conserved between the RND family members do not correspond to NPC disease alleles, suggesting that a permease function may not be the ancestral activity of NPC1 [15,26]. It is possible that the eukaryotic RND proteins use proton motive force to transport hydrophobes across the membranes of their resident organelles. Indeed, dogma on the substrates of NPC1L1 action, and thus by inference those of NPC1, is rapidly accruing, primarily because the former protein is a likely target of the cholesterol absorption inhibitor ezetimibe [27]. The proteins probably act similarly in that 66% of missense mutations and 23% of missense polymorphisms in NPC1 occur at amino acids shared with NPC1L1 [15].

The *NPC1* and *NPC2* genes are constitutively and ubiquitously expressed in animal tissues and conserved from yeast to humans. Despite the striking molecular dissimilarity of *NPC1* and *NPC2* (Figure 1), the disease phenotypes in patients with *NPC1* and *NPC2* mutations are virtually identical at biochemical, cellular and clinical levels [28]. Moreover, the low incidence of these panethnic disorders (1:150,000), and the virtual absence of any founder effect, has meant that linkage of structure and function is error prone. Thus, much of our insight on protein function stems from analyses of orthologs of NPC1 (Table 1) and NPC2 in model systems. This approach is validated by complementation studies of the yeast orthologs to NPC1 and NPC2 in mammalian cells [12,29], and further by reports that 43% of missense mutations and 15% of missense polymorphisms in *NPC1* in human NPC patients are at phylogenically conserved residues [15]. Together, these data suggest an evolutionarily conserved function is disturbed in NPC patients.

Many model organisms, including murine, feline, nematode, insect and yeast, have been used to analyze *NPC1* and *NPC2* (Table 1). In addition, multiple full genome sequences, ranging from plants (*Arabidopsis thaliana*) to sea urchins (*Strongylocentrotus purpuratus*), suggest the conservation of NPC pathways. The diversity of mutant phenotypes in metazoan model

systems [30–32] reiterates the complexity of this disease, however, some common concepts emerge. All multicellular organisms retain two representatives of the *NPC1* gene, at least one of which is involved in dietary sterol absorption [33,34]. All mammalian models exhibit an accumulation of cholesterol and phospholipids in the liver and an accumulation of gangliosides, glycolipids and, to a lesser extent, cholesterol in the brain. Sterol accumulation, however, is clearly not universal, although sterol-derived products (e.g., neurosteroids and ecdysones) are frequently deficient when both genes are defective.

Unlike the other models, yeast cells have single orthologs to NPC1 and NPC2, encoded by the Niemann–Pick C-related gene 1 (*NCR1*) and *NPC2* genes, respectively, that are functionally analogous to their mammalian counterparts. Expression of the yeast genes corrects the lipid trafficking defect of NPC1^{−/−} CHO cells or human NPC2^{−/−} patient fibroblasts, respectively [12,29]. However, sterol imbalance was not associated with loss of these pathways in yeast [12,35,36]. By contrast, the phenotypes of a dominant mutation in Ncr1p led us to propose that the primordial function of these proteins is to recycle sphingolipids and that defects in this process in multicellular eukaryotes secondarily result in cholesterol accumulation [12]. Under certain conditions, sterols may be substrates for Ncr1p or Npc2p, but these lipids rarely pass through the vacuolar compartment where the proteins reside. Alternatively, a model for the lack of a striking null mutant phenotype is that the proteins function in a redundant pathway for membrane lipid recycling.

The substrates of NPC1, although likely to be conserved throughout evolution and an obvious target for intervention in this disease, remain elusive. A photoactivable form of cholesterol has been crosslinked to NPC1, however, the affinity is several orders of magnitude lower than for NPC2 [37,38]. This is much more in keeping with a catalytic role of cholesterol binding (in NPC1) as opposed to bulk transfer or solubilization by NPC2. Could NPC1 act as a rheostat, initiating the transport of lipids by other proteins to optimize membrane fluidity? This would be consistent with the function of a related protein, SCAP, which in response to cholesterol binding regulates the transfer of a transcription factor (SREBP) to an activation compartment. NPC disease complementation groups other than NPC1 and NPC2 may also exist. Interestingly, several NPC patients and as many as 11 out of 37 tested cell lines from NPC patients have been described where the coding sequence of neither NPC1 or NPC2 was variant [15].

Therapies based on the deprivation of hypothetical substrates or end-product replenishment

The variety and complexity of lipids trapped in the lysosomes of cells from different tissues in this disease are too complex to determine the offending metabolites. Despite this, and because there is no cure for NP-C, therapy modalities have focused on decreasing accumulation of putative toxic metabolites (Table 2 & Figure 2). In several model systems, pharmacological or genetic reduction of serum cholesterol or inhibition of brain cholesterol biosynthesis had no detectable impact on disease progression. Cholesterol-lowering agents reduced hepatic and serum cholesterol levels in human NPC patients but did not lead to any delay in the onset of neurological symptoms [39,40]. Liver or bone marrow transplantations were of no benefit, providing only a brief period of stabilization with eventual recurrence of lipid storage and progression of neurological symptoms [41]. These observations all point to cholesterol not being an offending metabolite in NPC disease.

Unesterified cholesterol levels are not dramatically increased in the brains of NPC models, although loss of myelin might obscure the true cholesterol status of the diseased brain [42]. Thus, recent therapies have focused on modulating other lipids that accumulate in this disease (Figure 3). Gangliosides and glycosphingolipids accumulate in NPC cells and neuropathological findings in NPC are similar to primary gangliosidoses. Feline and murine

NPC models were treated with N-butyldeoxynojirimycin (NB-DNJ), an inhibitor of glucosylceramide synthase, which is an enzyme in the glycosphingolipid pathway [43]. In both animal models, treatment with NB-DNJ reduced ganglioside accumulation, delayed onset of neurological dysfunction and pathology, and increased average lifespan (Figure 3). In one NPC patient treated aggressively with NB-DNJ, lipid storage and trafficking in lymphocytes was improved [44]. The efficacy of miglustat (a derivative of NB-DNJ and an approved treatment for Gaucher's disease) is now being tested in NPC patients. Preliminary results are promising; patients treated with miglustat have shown improvements in lipid levels [44], horizontal saccadic eye movements, mental status, motor function (swallowing) and slower progression of disease compared with palliative care [Patterson M, Pers. Comm.].

An alternate perception of the NPC cellular lesion is that it induces cholesterol auxotrophy due to sequestration in a metabolically inaccessible site. Accordingly, Maue, and later Mellon, proposed that the severity of the disease results from neurosteroid deficiency [45,46]. In the latter study, NPC disease in the murine model was delayed by supplementation with allopregnenalone, a neurosteroid and substrate for other neurosteroids (Figure 3). A marked improvement in life expectancy was observed when allopregnenalone was given at post-natal day 7 (~14 days prior to weaning) [46]. Enantiomeric allopregnenalone, unlike natural allopregnenalone, does not activate the γ -aminobutyric acid (GABA) receptor but was effective in improving survival, suggesting that the target is independent of the GABA pathway (and thus the known mode of action of this neurosteroid). By contrast, both isomers were equally effective in binding pregnane X receptor, a target of xenobiotics [47]. When allopregnenalone was administered later there was minimal impact on life expectancy, perhaps due to irreversible prior damage to purkinje cells. Interestingly, combination of a relatively ineffective treatment (the liver X receptor agonist, T0901317) with an effective one (allopregnenalone) was synergistic in terms of survival. A combined and early treatment approach appears the best approach to treating this disease.

An alternate approach: identify & modify the modifiers?

To date, there are approximately 230 private mutations in *NPC1* (only three of which are frequent), and 15 private mutations in *NPC2*, of which 11 were homozygous [13,26,48]. More than 50 single nucleotide polymorphisms (SNPs) in *NPC1* have been identified, of which 68% were nonsynonymous [48]. In general, patients with apparently identical NPC alleles experience a similar disease course. However, disparate phenotypes can occur, with variable age of onset and rate of progression. Indeed, NPC disease can present in neonates, juveniles, adolescents or adults, with varying severity (recently surveyed in the UK [49]). Rarely, non-neuropathic adults with NPC disease have been identified suggesting the existence of modifier alleles, intra- or extragenic to the NPC genes.

Interestingly, the combinations of alleles and/or SNPs can provoke a disease phenotype not seen in a patient homozygous for either allele or SNP alone, further suggestive of intermolecular interactions. For example, an I1061T/P1007A heterozygote presented with earlier onset of symptoms and slower progression than an I1061T homozygote. Similarly, the c.2572 A>G polymorphism has a modifying effect on *NPC1* expression and disease caused by the I858V mutation [50]. Furthermore, two patients with mutations in the SSD, the region in which most mutations result in a severe disease phenotype, presented neurological symptoms as juveniles rather than infants, possibly because of being heterozygous with disease alleles outside the SSD.

These observations predict intermolecular associations within NPC1 or between NPC1 and another protein. Furthermore, it is clear that in certain kindreds with identical NPC1 alleles, and generally similar environments, that disease progression can vary significantly, both in

terms of onset and progression [49]. At the molecular level, these pathways have yet to be identified, although genomic and genetic approaches with model systems are promising strategies. For example, global approaches, such as transcriptional profiling of NPC1 mutant and normal fibroblasts, identified genes involved in very basic processes, such as metal metabolism and vesicular transport, as misregulated in these cells [51]. Interestingly, overexpression of two Rab GTPases, Rab9 and Rab7, critical to vesicular protein transport, has been shown to normalize cholesterol and glycosphingolipid trafficking from NPC endosomes [48,49]. This may arise in part from an aberrant export of mannose 6-phosphate receptors from this compartment [52]. Crucially, the role of Rab proteins in disease progression remains to be determined.

Conclusion

NPC diseases will only be cured by gene replacement, stem cell replenishment or other such correction technologies. The prospects of this occurring for any neurodegenerative disorder are disappointingly distant. However, treatments may be closer to fruition. Indeed, the approaches described here of substrate depletion or product replacement already show promise and are well founded in testable scientific hypotheses. A conclusive assignment of substrate (s) to the NPC1 transporters is lacking, hence our regard here of the NPC1 protein in particular as an orphan transporter. Clearly, as a research community we are well versed in the ramifications of the loss of these genes, but the question remains; what is the primary metabolite that accumulates and is it toxic? This is not a worthless pursuit. Even amelioration of what looks like a secondary substrate (GM2) by inhibition of a glucosylceramide synthase appears to significantly impact the disease phenotype. Variation in modifying pathways (regulatory or bypass) are certain to exist and will present a new approach to therapy; the challenge is to identify them.

Executive summary

Human Niemann–Pick type C disease: a transport disorder

- Niemann–Pick type C (NPC) disease is a progressive, neurodegenerative disorder caused by a deposition of cholesterol and other lipids in a variety of tissues, indicating a defect in lipid transport.

NPC genes: relics of an ancient transport reaction

- The NPC genes, *NPC1* and *NPC2*, have been identified and are functionally and structurally conserved from yeast to humans, thus allowing for research using various model systems; *NPC1* predates yeast based on homology to the prokaryotic resistance-nodulation-division family.
- It is still not clear whether *NPC1* and *NPC2* physically interact.
- A ‘smoking gun’ regarding assignment of substrate(s) to these transporters is lacking, hence our proposal here of the *NPC1* and *NPC2* proteins as orphan transporter and chaperone, respectively.

Therapies based on deprivation of hypothetical substrates or replenishment of likely end products

- The diversity of lipid accumulation in this syndrome has impeded the assignment of a toxic metabolite that could be effectively targeted in treatment.
- Therapies are now focused on deprivation of hypothetical substrates or replenishment of likely end products.

An alternate approach: identify and modify the modifiers

- NPC models may be applied to identify potential disease modifiers, which can elucidate the pathway and function of the two NPC disease proteins. With this information, substrates may be identified that will direct novel treatment modalities.

Future perspective

The proteins defective in NPC disease have hallmarks of lipid transporters in that their absence provokes these metabolites to accumulate. However, as yet, no molecule has been detected that binds to NPC1, the candidate permease, with an affinity expected for any other transporter. A key future finding will relate the tertiary structure of NPC1 to its ligand and, in addition, interpret the interaction of this process with the NPC2 molecule and other cellular factors.

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Figure 1. The structure of two proteins defective in Niemann–Pick type C disease
 NPC1 and NPC2 play a major role in the transport of cholesterol and/or other hydrophobic molecules through the endosomal/lysosomal system. While structurally diverse, homozygous lesions in either gene result in the same pathophysiology and cellular defect.
(A) Schematic of the structure of NPC1 [55]. The NPC1 cysteine-rich and sterol-sensing domains comprise the majority of the 230 lesions in NPC patients. **(B)** Crystallized structure of bovine NPC2 (PDB accession 1-NEP, [17]), with residues that are required for binding cholesterol (green with black stripes) and observed to be mutated in NPC patients (yellow) indicated.

NPC2 is a 151 amino acid protein; this structure represents the mature 132 amino acids and was constructed using Cn3D 4.1 [56].

NPC: Niemann–Pick type C.

Figure 2. Progression of Niemann–Pick type C disease in mice and humans

Niemann–Pick type C disease is a complex lipid storage disorder where a variety of lipids, including cholesterol and glycosphingolipids, accumulate in the lysosomes of all cells. The sequelae of this cellular defect and onset of disease symptoms are depicted in a timeline for mice and humans homozygous for the disease alleles. In humans, the timeline is exceptionally variable.

Figure 3. Mechanism of action of potential treatments

(A) Miglustat blocks the production and, consequently, the build-up of gangliosides by inhibiting the function of glycosyltransferase. It is currently approved for Gaucher disease and, in preliminary clinical studies in Niemann–Pick type C patients, demonstrates improvement in motor and swallowing function.

(B) Allopregnenalolone, a steroid hormone derivative of cholesterol, activates GABA receptors, however, recent work indicates that the activation of steroid hormone orphan receptors (such as LXR or PXR) may be important in delaying the onset of symptoms.

GABA: γ -aminobutyric acid; LXR: Liver X receptor; PXR: Pregnane X receptor; UDP: Uridine diphosphate.

Table 1

Paralogs and orthologs of the *NPC1* gene, and their corresponding null mutant phenotypes.

Organism	Gene	GenBank accession	Amino acid identity to human NPC1 (%)	Ref.
<i>Homo sapiens</i> (human)	<i>NPC1</i>	AAD48006	100	[28]
Neurological, gastrointestinal, liver and respiratory problems. Disease onset and severity variable, primarily infant to prebuscent. Accumulation of cholesterol, sphingomyelin, sphingosine and complex glycosphingolipids. Neurodegeneration: Alzheimer's disease, with neurofibrillary tangles and tau phosphorylation.				
<i>Homo sapiens</i> (human)	<i>NPC1L1</i>	AAF20396	39	[28]
No known variants				
<i>Mus musculus</i> (mouse)	<i>NPC1</i>	AAB63372	86	[28]
Neurological, gastrointestinal, liver and respiratory problems. Decreased brain size, disease onset at 4–5 weeks of age, with death at 70–80 days. Brain: accumulation of GM2 and GM3 gangliosides and several neutral glycolipids. Liver: accumulation of cholesterol and phospholipid (largely sphingomyelin). Neurodegeneration, without neurofibrillary tangles and tau phosphorylation.				
<i>Mus musculus</i> (mouse)	<i>Npc1L1</i>	AAR97887	40	[28]
Immune to diet-induced hyperlipidemia. Decrease of 70% in intestinal cholesterol and sitosterol absorption. Cholesterol and sphingolipids trapped in cytoplasmic vesicular structures. Insensitive to sterol absorption inhibitor ezetimibe.				
<i>Felis catus</i> (cat)	<i>NPC1</i>	AAF72187	91	[28]
Neurological, gastrointestinal and liver problems. Disease onset at 8–12 weeks, with death typical before 11 months. Brain: accumulation of GM2 and GM3 gangliosides. Liver: accumulation of lactosylceramide, glucosylceramide, phospholipids and cholesterol.				
<i>Drosophila melanogaster</i> (fruit fly)	<i>NPC1a</i>	NP_609357	44	[31,32]
Life until first larval stage with inability to molt. Life to adulthood restored with 20-hydroxyecdysone, cholesterol and 7-dehydrocholesterol. Accumulation of sterols in punctuate pattern throughout bodies. No neurodegeneration.				
<i>Drosophila melanogaster</i> (fruit fly)	<i>NPC1b</i>	AAF50873	39	[34]
Similar to hNPC1L1 with expression only in mid-gut.				
<i>Caenorhabditis elegans</i> (nematode)	<i>ncr-1</i>	AAK84521	27	[30]
Subtle constitutive dauer formation due to shortage of sterol-derived hormones. Hypersensitive to cholesterol deprivation and progesterone. Slow late-stage embryogenesis.				

Organism	Gene	GenBank accession	Amino acid identity to human NPC1 (%)	Ref.
<i>Caenorhabditis elegans</i> (nematode)	<i>ncr-2</i>	AAK84521	27	[30]
Subtle constitutive dauer formation due to shortage of sterol-derived hormones.				
<i>Saccharomyces cerevisiae</i> (yeast)	<i>NCR1</i>	Q12200	33	[28]
No impact on viability.				[12,35,36]
Resistance to edelfosine.				[35]
Missense allele in sterol-sensing domain confers accumulation of sphingolipids.				[12]

hNPC1L1: Human Niemann–Pick type C1-like 1; NCR: Niemann–Pick C-related gene; NPC: Niemann–Pick type C.

Table 2
Efficacy of therapeutic modalities for Niemann–Pick type C disease.

Treatment	Outcome	Putative mechanism	Ref.
<i>Diet</i>			
Vitamin E supplementation	Delay in weight loss of female mice; No phenotypic changes (humans and mice)	Antioxidant	[53]
Low-cholesterol diet	Reduced lipid accumulation in murine liver	Substrate depletion	[39]
Coenzyme Q10 supplementation	Anecdotal reports of neurological improvement	Antioxidant?	[Porto A, Sturley SL: Ubiquinone metabolism and Niemann–Pick type C disease. Manuscript in preparation (2007)]
<i>Nondiet</i>			
Cholesterol-lowering drugs	Decrease in human and murine serum and liver cholesterol levels; No changes in onset of neurologic symptoms	Substrate depletion	[39]
Liver/bone marrow transplant	Brief stabilization with subsequent progression of neurologic symptoms in humans	Somatic cell genetic therapy (probably not penetrant to neurons)	[53]
Tamoxifen	Delay in weight loss and improved neurologic function in mice	Reducing oxidative damage	[43]
Miglustat	Feline and murine: Delayed neurologic dysfunction and increased lifespan Humans: Slows progression of disease at 1-year follow-up; Improvement in mental status and swallowing	Substrate depletion	[44,46,54]
Allopregnalone	Mice studies are promising with increased life expectancy when administered at 7 days of life; May involve the pregnane X receptor	Metabolite replacement?	[43,52]