Genetic dissection of a cell-autonomous neurodegenerative disorder: lessons learned from mouse models of Niemann-Pick disease type C

Manuel E. Lopez¹,* and Matthew P. Scott¹

Understanding neurodegenerative disease progression and its treatment requires the systematic characterization and manipulation of relevant cell types and molecular pathways. The neurodegenerative lysosomal storage disorder Niemann-Pick disease type C (NPC) is highly amenable to genetic approaches that allow exploration of the disease biology at the organismal, cellular and molecular level. Although NPC is a rare disease, genetic analysis of the associated neuropathology promises to provide insight into the logic of disease neural circuitry, selective neuron vulnerability and neural-glial interactions. The ability to control the disorder cell-autonomously and in naturally occurring spontaneous animal models that recapitulate many aspects of the human disease allows for an unparalleled dissection of the disease neurobiology in vivo. Here, we review progress in mouse-model-based studies of NPC disease, specifically focusing on the subtype that is caused by a deficiency in NPC1, a sterol-binding late endosomal membrane protein involved in lipid trafficking. We also discuss recent findings and future directions in NPC disease research that are pertinent to understanding the cellular and molecular mechanisms underlying neurodegeneration in general.

Introduction

Worldwide, millions of new cases of neurodegenerative disease are reported every year (Meikle et al., 1999; Van Den Eeden et al., 2003; Logrosino et al., 2008; Mayeux and Stern, 2012). Affected individuals typically experience gradual disease progression and require increasing levels of health service and supportive care, which places long-term strain on the individuals and their families. Although treatments to manage the symptoms of neurodegenerative disease are available, there is an urgent need for mechanism-based treatments. Unfortunately, the underlying mechanisms of neurodegeneration and the pathways that can be manipulated to control disease are not well understood. Genetics offers an excellent tool for research and, in reality, it is the rare inheritable forms of the more common age-related dementia and motor disorders – i.e. Alzheimer’s disease (AD), Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS) – that researchers attempt to engineer animal models of and rely on for biological insight (Wong et al., 2002). However, these age-related diseases are predominantly idiopathic and various genetic plus environmental factors contribute to overall risk. The lack of genetic in vivo models that can truly recapitulate the full complexity of these disorders prevents in-depth studies.

As our understanding of the biology of these diseases progresses, many parallels between different neurodegenerative disorders are becoming apparent. Neurodegenerative lysosomal storage diseases (LSDs) are rare inborn metabolic disorders that are usually caused by a gene defect that leads to deficiency of a particular lysosomal enzyme. Interestingly, gene mutations associated with LSDs, notably those in the glucocerebrosidase gene (GBA), have been identified as genetic risk factors for age-related disorders. GBA mutations cause Gaucher disease, the most common disorder among LSDs, which is linked to PD (Mazzulli et al., 2011). Mutations in ATP13A2, known to cause a form of juvenile Parkinsonism, also cause the LSD neuronal ceroid lipofuscinoses (NCL) (Bras et al., 2012; Dehay et al., 2012), which has a PD-related spontaneous dog model (Wöhlke et al., 2011). The increasing recognition that defects in lysosome-related pathways might underlie age-related dementia and motor neuron disease neuropathology (Nixon et al., 2008) provides incentive to thoroughly investigate the biology of LSDs.

In this Review we highlight recent advances in exploring the pathology of a rare (estimated incidence of ~1 in 120,000-150,000 live births) neurodegenerative LSD, Niemann-Pick disease type C (NPC). NPC causes dementia in children and adults born with an autosomal recessive mutation in either of two genes, NPC1 or NPC2. NPC, which is informally referred to as ‘childhood Alzheimer’s’, shares several mechanistic and biomarker similarities with AD (Reddy et al., 2006; Cologna et al., 2012). These include altered amyloid precursor protein (APP) processing and the presence of neurofibrillary tangles (Kate, 2011). Unlike AD, NPC and other LSDs have naturally occurring mammalian models, which can be genetically manipulated and have proven to be highly useful for testing therapies (Haskins et al., 2006; Farfel-Beer et al., 2011).
There is mounting evidence to suggest that NPC caused by mutations in NPC1 can be modeled in a cell-autonomous fashion. We posit that this unique feature facilitates a detailed exploration of the underlying disease biology, which will ultimately enhance our understanding of general neurodegenerative processes.

**Clinical, genetic and biochemical features of NPC**

In the early 1900s, a pediatrician named Albert Niemann reported the occurrence in an infant of a newly identified metabolic storage disorder that resembled Gaucher disease (Niemann, 1914). Physician Ludwig Pick later distinguished this unknown disease from other recognized metabolic storage disorders (Pick, 1926). Individuals with Niemann-Pick disease, as the cases were commonly referred to, showed signs of sphingomyelin storage in cells and foamy lipid deposits in body tissues, with varying degrees of organomegaly and neurological symptoms. Based on variability in clinical presentation, Niemann-Pick disease was subdivided into types A, B and C (Pentchev, 2004). In the 1960s, Niemann-Pick types A and B were identified as variants of acid sphingomyelinase deficiencies: both forms are caused by mutations in the gene encoding the lysosomal enzyme sphingomyelin phosphodiesterase 1 (SMPD1). Intriguingly, a mutation in SMPD1 has recently been described as a previously unknown risk factor for PD (Gan-Or et al., 2013). This finding further suggests that defects in lysosome-related pathways might underlie the pathology of more common disorders.

The genetic cause of Niemann-Pick type C was not discovered until the late 1990s (Loftus et al., 1997). Prior to this discovery, several spontaneous mouse models of NPC had been identified. These models played key roles in advancing our understanding of the disease. One such model was identified as a laboratory colony of BALB/c mice, whose progeny developed progressive ataxia while juvenile (3-8 weeks old), and demonstrated weight decline and early death as young adults (Morris et al., 1982). The cells of these mice exhibited a distinct cholesterol-storage disorder, and a subsequent in vitro survey of cell lines of human metabolic storage disorders revealed that NPC patient fibroblasts display a similar cholesterol-storage phenotype (Pentchev et al., 1986). The *NPC1* gene was subsequently identified by using an integrated human-mouse positional candidate approach (Loftus et al., 1997). It is now known that mutations that lead to partial deficiency or complete loss of function of NPC1, a 13-transmembrane endosomal cholesterol-binding protein, account for 95% of NPC cases. Defects in NPC2, a secreted cholesterol-binding protein that is believed to interact with NPC1, account for the remaining 5% of NPC cases (Naureckiene et al., 2000; Sleat et al., 2004; Cheruku et al., 2006; Deffieu and Pfeffer, 2011).

The function of NPC1 remains unclear. However, biochemical studies have shown that the protein binds sterols, particularly oysterols and cholesterol (Ohgami et al., 2004; Infante et al., 2008; Kwon et al., 2009). On this basis, NPC1 is hypothesized to be required for cholesterol egress from the lysosome. In line with this hypothesis, cells that are deficient in NPC1 accumulate a wide array of lipids, including cholesterol (Fig. 1A,B) and sphingomyelin within the endocytic system (Lloyd-Evans et al., 2008). Loss of NPC1 function also renders a cell unable to sense or effectively utilize exogenously derived cholesterol (Reddy et al., 2006; Kulinski and Vance, 2007). Thus, loss of NPC1 causes lipidosis and defects in cholesterol homeostasis.

NPC1 might act as a transporter to exchange lipid molecules between cellular compartments, to facilitate the integration of lipids into membranes, or to promote the sorting of lipids through budding and fusion of membranes. Indeed, in the absence of NPC1, endosomal organelle transport is notably perturbed (Ko et al., 2001) and multilamellar vesicle bodies abound (Fig. 1C) (Blom et al., 2003; Liao et al., 2007; Tang et al., 2009). A structural and functional

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**Fig. 1. Comparison of cell-autonomous and non-cell-autonomous rescue of the lipid-storage defect in NPC disease.** (A,B) The severe intracellular accumulation of unesterified cholesterol caused by the loss of NPC1 function is demonstrated here by comparing filipin-stained (blue) (A) wild-type K1 CHO cells and (B) Npc1 mutant M12 CHO cells. Abundant multivesicular structures and multilamellar bodies (MLBs) accompany the storage disorder. (C) A transmission electron microscope image of a single elaborate MLB (pseudo color) is shown. (D,E) Although loss of either NPC1 or NPC2 function elicits a nearly identical cellular phenotype in NPC, (D) NPC1, a non-secreted late endosomal/lysosomal (LE/L) membrane protein, acts cell-autonomously, whereas (E) NPC2, a secreted cholesterol-binding protein, acts non-cell-autonomously. Thereby, production of NPC1 in a cell (D; cell 1) cannot correct the storage defect, depicted here by an MLB, in a neighboring NPC1-deficient cell (D; cell 2). By contrast, an NPC2-producing cell (E; cell 1) can rescue the storage defect in an NPC2-deficient cell, indicated by the absence of an MLB (E; cell 2).
comparison with related proteins, such as the membrane cholesterol transporter NPC1L1 (Smith and Levitan, 2007; Krishnan et al., 2012), or even distant relatives with conserved domains, such as the sterol-sensing domain of the Hedgehog morphogen receptor Patched (Hausmann et al., 2009), could ultimately lead to a better understanding of the molecular mechanism of action of NPC1.

What is clear about the NPC1 protein is that it is not secreted or transmitted across membranes. Thus, the loss of NPC1 function cannot be corrected by the provision of NPC1 by a neighboring cell (Fig. 1D). In contrast, the exogenous provision of NPC2 can correct the NPC lipid storage defect in cells that lack NPC2 (Fig. 1E) (Naureckiene et al., 2000). Although the cell-autonomous function of NPC1 limits the application of non-autonomous therapeutics, such as enzyme replacement therapy (Vanier, 2010), the ability to control the disorder cell-autonomously by providing NPC1 function to specific cell or tissue types enables the identification of cells that are important for neurodegenerative disease progression and, potentially, for recovery. Other useful treatments, such as substrate reduction therapies (Nietupski et al., 2012), might then be appropriately targeted to these crucial cells.

**Cell-autonomous neuronal toxicity in NPC: evidence from mouse models**

A detailed understanding of what promotes cell-autonomous and non-autonomous neuron toxicity is crucial to understanding neurodegenerative disease pathogenesis. Most studies of neurodegeneration in NPC have focused on one population of highly affected cells, the Purkinje neurons of the cerebellum. However, the disease is systemic, affecting visceral endocrine tissue such as the liver as well as glial cells and neurons throughout the CNS. In disorders involving non-cell-autonomous neuronal toxicity, glial defects as well as invading toxic factors generated elsewhere in the body might cause or exacerbate CNS disease progression. These harmful cells or toxins can be targets for treatment (Ilieva et al., 2009). In disorders in which glial and immune cells contribute less to disease pathogenesis, targeting these cells might decrease the effectiveness of a treatment, because glia can have a high endocytic or phagocytic
capacity for drug compounds. In different disease cases, both cell-autonomous and non-autonomous mechanisms of neurodegeneration are suspected to be present. However, autonomous and non-autonomous neurodegenerative disease mechanisms have proven difficult to tease apart and, prior to studies of NPC, a disease predominantly characterized by cell-autonomous neuronal toxicity had not been modeled. The NPC1 disease animal models, combined with cell- or tissue-specific rescue experiments, might be useful for comparing and contrasting neurodegenerative disease pathways in order to discover universal or distinct mechanisms. Thereby, NPC serves as the prototype model for cell-autonomous toxicity in a neurodegenerative disease.

Multiple complementary studies support the occurrence of cell-autonomous neurodegeneration in NPC1-deficient mice. An early NPC1 rescue study using a prion-promoter-driven Npc1 transgene (Loftus et al., 2002) suggested that visceral tissue disease does not affect the progression of the disease in the brain. Transgene prion-promoter-directed expression, which reduced brain pathology, was widespread and not limited to neurons in the CNS. However, in mice that showed continued visceral disease progression, no neurodegeneration could be observed. This led to the conclusion that the visceral disease pathology does not impact CNS degeneration. A later study using a highly targetable Tet-inducible Npc1 transgene further demonstrated that correction of visceral tissue pathology alone does not alter CNS disease progression (Lopez et al., 2011). Thus, CNS neurodegeneration in the NPC mouse model occurs independently of disease progression elsewhere in the body. This seems to be equally true for humans: in clinical studies, bone marrow and liver transplantation in NPC1-deficient patients altered bone marrow and liver tissue pathology but did not affect neurological signs and symptoms (Patterson, 1993; Hsu et al., 1999).

Studies focusing on the death of Purkinje neurons in NPC mice pinpointed a neuron-intrinsic cell-autonomous phenotype. In an elegant chimeric mouse experiment (Fig. 2A) that created a system in which the cerebellum contained a mixed population of normal (Npc1+/-) and NPC1-deficient (Npc1-/-) cells, Npc1-/- cerebellar Purkinje neurons died, whereas NPC1-producing Npc1+/- Purkinje neurons persisted (Ko et al., 2005). This experimental system made it possible to test whether wild-type glia, astrocytes and particularly microglia can rescue neurons, or whether neurons die regardless of the presence or absence of NPC1 function in adjacent glia. The results of this investigation showed that Npc1+/- glia are not able to rescue Npc1-/- neurons, and, conversely, Npc1+/- neurons are not damaged via proximity to Npc1-/- glia. Importantly, both Npc1-/- and Npc1+/- microglia accumulated selectively near dead or dying neurons. No gross difference in morphology or behavior was noted for reactive astrocytes and microglia with or without NPC1, suggesting that NPC1 normal and deficient glia act similarly when exposed to the same environment. It was concluded that neuron-intrinsic mechanisms are involved in neuron death in NPC disease. In turn, this cell-autonomous neuron injury exerts non-cell-autonomous effects that attract inflammatory glia, which then target dead or dying neurons.

The lack of neuron toxicity due to diseased astrocytes was definitively demonstrated using a conditional Cre-mediated Npc1 knockout mouse model of NPC (Fig. 2B). Animals that lacked NPC1 in astrocytes did not exhibit any gross signs of disease progression or neurological decay, despite evidence of an astrocytic cholesterol-accumulation phenotype (Yu et al., 2011). In a complementary experiment, providing functional NPC1 to astrocytes alone in an otherwise NPC1-deficient mouse did not significantly alter disease progression, despite a slight delay in astrocyte reactivity (Fig. 2C) (Lopez et al., 2011). Taken together, these studies demonstrate that astrocytes are weakly, if at all, involved in triggering or exacerbating the initial disease pathology.

Using the conditional knockout mouse model of NPC1 disease described above, it was also shown that loss of NPC1, specifically from neurons, is sufficient to cause neurodegeneration and to recapitulate neurological signs of the disease (Fig. 2B) (Elrick et al., 2010; Yu et al., 2011). Conversely, using the Tet-inducible Npc1 mouse model, neuron-specific NPC1 in an otherwise NPC1-deficient animal was shown to be sufficient to prevent and halt local neurodegeneration, altering neurological signs in an animal with continued systemic disease progression (Fig. 2C) (Lopez et al., 2011; Lopez et al., 2012b). These complementary results, which were obtained independently using distinct experimental methods and different mouse backgrounds, provide compelling evidence for predominant neuron-autonomous mechanisms of toxicity in NPC disease.

Dissecting NPC disease neural circuitry in mice
As outlined above, NPC disease progression can be carefully controlled with targeted neuronal NPC1 knockout and rescue. The ability to control neuron survival in a cell-autonomous, temporal and spatial manner in the NPC mouse model offers a way to address questions of disease neural circuitry and behavior. At what stage can a neurological condition be halted or reversed? Which brain regions are involved and should be targeted therapeutically? Can surviving functional neurons in a neuronal network compensate for those that are lost? It seems possible that only partial correction of neural networks would be sufficient to elicit profound beneficial effects. Indeed, chimeric Npc1 mice in which at least 30% of Purkinje neurons are wild type do not develop severe ataxia (Ko et al., 2005). The aspects of neural circuitry that can be corrected and maintained in these mice to halt the progression of neurological signs remain unclear.

Current attempts to decipher neural circuits rely heavily on inactivating or activating defined cell types in a normal or diseased brain (Luo et al., 2008; Tye and Deisseroth, 2012). These studies rely on the existence of a causal relationship between acute neuronal activity and behavior. Neurons, however, are much more than transmitters of electrical impulses elicited by the opening and closing of ion channels; they also serve as metabolic and endocrine centers. For example, Purkinje neurons produce neurosteroids and secrete morphogens capable of tissue remodeling and modulating glial behavior (Dahmane and Ruiz i Altaba, 1999; Sakamoto et al., 2001; Bouslama-Oueghlani et al., 2012). Thus, controlling the neuronal defect and subsequent cell-autonomous survival or death of discrete neurons in a diseased animal offers a powerful tool to investigate the developmental and behavioral effects of restoring or maintaining normal aspects of neuronal network function in a disease setting (Fig. 3).

There are several challenges to be overcome in order to be able to interpret results of neural circuit rescue. First, discrete neurons must be effectively targeted. Viral-mediated delivery and transduction, a commonly used method in optogenetic
investigations of neural circuits, can limit expression to the injection site or spread rescue through neuronal connections, depending on the design and capacity of the virus (Tye and Deisseroth, 2012). Adenovirus delivery of recombinant NPC1 in the mouse brain has already been attempted (Paul et al., 2005). To target discrete neuronal populations, it might be more practical to deliver a neuronally driven transcription factor, such as tetracycline-sensitive transactivator (tTA), which can be applied in the existing Tet-inducible Npc1 transgenic mouse, or Cre in the conditional Npc1 knockout mouse model of NPC. Second, it is necessary to develop behavioral assays that can detect altered phenotypes. For example, Purkinje-neuron-specific rescued mice exhibit significant but temporary improvements in various disease progression parameters, such as weight and survival, but continue to have motor deficits (Lopez et al., 2011). One explanation could be the persistence of dystonic features and hind limb paralysis, which have been shown to progress independently of cerebellar involvement (Fig. 3) (Lopez et al., 2011). As a result, Purkinje-neuron-rescued mice are expected to perform poorly on rotor rod or similarly automated tests of motor skills. Other types of behavioral recordings would then be required to more accurately identify differences. The continued degeneration of other brain regions and timing of rescue would also need to be taken into account (Lopez et al., 2011; Lopez et al., 2012b).

Another concern for neuron-specific studies in NPC mouse models is the potential effect of non-cell-autonomous factors on neuronal function. For example, diseased glia or other cell types could fail to support proper neuron function and, ultimately, their survival. Despite the evidence for cell-autonomous toxicity described above, a role for non-cell-autonomous toxicity has not been ruled out. In fact, non-cell-autonomous toxicity has been widely suggested to contribute substantially to disease pathogenesis of other LSDs. Recently, using a conditional Cre-mediated deletion of the sulfatase-modifying factor 1 gene (Sumf1), which causes the LSD mucosulfatidosis, researchers were able to demonstrate that SUMF1-deficient astrocytes failed to support function and survival of neurons in a wild-type mouse (Di Malta et al., 2012). However, the authors did acknowledge evidence demonstrating an earlier and more severe cell-autonomous degenerative phenotype when Sumf1 deletion was limited to neurons. This indicates that non-cell-autonomous and autonomous processes might combine in varying degrees and at various times to contribute to a neurodegenerative disorder. For NPC, the initial disease severity seems to be driven mainly by cell-autonomous factors. Whether neuron rescue alone can completely correct the disorder is uncertain because the criteria for a fully rescued or recovered state in mice is yet undetermined.

Neuronal rescue could delay early progression of the disease, but both neuronal and glial correction might ultimately be required for complete rescue. Studies using an Npc1 transgene whose expression is driven by a neuron-specific enolase promoter allowed animal survival for more than a year, representing a reported >fivefold improvement in survival age (Borbon et al., 2012; Erickson, 2013). However, neurological defects were still observed. The possibility exists that not all critical neurons were targeted. Many ‘pan-neuronal’ drivers do not show the robust ubiquitous expression desired and often have variegated and partly silenced expression patterns (Lopez et al., 2011). Different studies might produce different results owing to the different expression patterns of the promoter and enhancer control elements used to drive cell-type-limited transcription. An alternative explanation is that non-cell-autonomous mechanisms exert greater influence with age. A recent study, using Cre-mediated...
**Exploring selective neuron vulnerability**

The ultimate pathological event in all neurodegenerative disorders is neuronal cell loss. However, not all neurons are equally vulnerable to each disease. Despite the broad and widespread presence of potential disease-causing factors, only a subset of neuron classes or distinct brain regions degenerate, and degeneration occurs at varying rates (Ilieva et al., 2009; Saxena and Caroni, 2011). This selective neuronal vulnerability is a major phenomenon of neurodegenerative disorders but is not well understood. In mice with NPC disease, the Purkinje neurons of the cerebellum or thalamic neurons seem to be particularly vulnerable to loss of NPC genes, but the reasons behind this are unknown (Sarna et al., 2003; Lopez et al., 2011). In light of the involvement of NPC proteins in intracellular trafficking and lipid accumulation, one might expect motor neurons that are sensitive to transport defects (LaMonte et al., 2002), or hippocampal neurons with potentially greater lipid accumulation defects (Lopez et al., 2011), to be more susceptible. It has been proposed that selective neuron vulnerability results in part because of heterogenous injury to supporting glial cells (Ilieva et al., 2009; Saxena and Caroni, 2011). Although data to support this argument can be found in disease models that exhibit non-autonomous toxicity, e.g. SOD1-linked ALS (Nagai et al., 2007) and spinocerebellar ataxia 7 (Custer et al., 2006), the idea does not seem to hold true for all disorders or across different experimental scenarios.

The NPC mouse model provides an ideal neurodegenerative environment for studying neuron-intrinsic factors that mediate selective neuron vulnerability. In NPC1-deficient mice, although the lysosomal defect causes the accumulation of cytoplasmic and lysosomal inclusions, vesicle transport defects (Reid et al., 2003), mitochondrial alterations (Yu et al., 2005), accumulation of oxidative byproducts (Porter et al., 2010), and even altered immune and inflammatory signaling (Sagiv et al., 2006; Liao et al., 2010) in most cells, neurodegeneration follows a particular pattern. For example, the rate and sequence of Purkinje neuron degeneration can be predicted in the NPC mouse model, and particular Purkinje neuron populations always remain resistant to cell death (Sarna et al., 2003; Ko et al., 2005). This patterned neuron loss is not easily explained by defects in glia cells or the accumulation of stressors in particular areas but does correlate with differences in gene expression across Purkinje neurons (Sarna et al., 2003). We propose that, in a cell-autonomous neurodegenerative situation, comparing gene expression changes between susceptible and non-susceptible neuronal subsets could facilitate the identification of modifiers of neuron vulnerability. It would be interesting to determine whether neurons inherently fated to die can be reprogrammed to be as resilient as their non-vulnerable counterparts. If so, this would open up avenues of treatment for mitigating disease progression, even without treating the underlying cause.

Selective neuron vulnerability has been studied in other models of neurodegenerative disease for which neuron-intrinsic mechanisms of neurodegeneration have been implicated. For example, in the TAR-DNA binding protein 43 (TDP-43) model of
frontotemporal degeneration (FTD) (Neumann et al., 2006), producing the mutant form of the protein specifically in forebrain neurons causes selective neuron degeneration in mice (Igaz et al., 2011). TDP-43 is a DNA- and RNA-binding protein that regulates expression of an array of genes and might interfere with cytoplasmic-nuclear signaling (Polymenidou et al., 2011; Tollervey et al., 2011). Despite the global production of the mutant TDP-43 protein in forebrain neurons, only dentate gyrus and deep cortical layer neurons were shown to acutely degenerate. As a result of this neuron-intrinsic control of neuronal loss, similar approaches used in the study of NPC1 can be implemented using the TDP-43 model of FTD. Mouse models for both diseases allow temporal and neuron-specific control of the disease and show reproducible patterns of selective neurodegeneration. It would be intriguing to compare and contrast the NPC1 and TDP-43 models with regards to respective cortical neuron degenerative pathways. Although the two disorders differ greatly in terms of the cause of disease and vulnerability of forebrain neurons, in some cases these neurons display a similar neuronal pathophysiology, such as the occurrence of ubiquitin inclusions (Neumann et al., 2006; Bifsho et al., 2007; Xu et al., 2011). Thus, potential stress pathways that cause neurodegeneration specifically in the TDP-43 model can be identified more precisely.

Beyond the mouse: additional models of NPC

Research on NPC is not limited to spontaneous or engineered mouse models. The NPC field also benefits from a naturally occurring cat model of the disease that is used to test proposed drug therapies (Loftus et al., 1997; Somers et al., 2003; Maue et al., 2012). Although these mammalian models are important tools for understanding the disease biology and therapeutic testing, the use of mammals is expensive and frequently inadequate for molecular, genetic and biochemical approaches. Owing to the high evolutionary conservation of the NPC1 gene among eukaryotes, other more experimentally tractable model organisms are available (Fig. 5). A range of model organisms studied in various laboratory
settings have added to our current understanding of the function of NPC1 and the mechanisms underpinning NPC.

In addition to serving as NPC models, cats happen to host the parasitic protozoan Toxoplasma gondii (Fig. 5), which invades the nervous system and can alter host behavior. T. gondii possesses TgNCR1, a sterol-sensing-domain-containing protein with sequence similarities to mammalian NPC1. Addition of the human NPC1 late endosomal localization sequence generates a chimeric TgNCR1-hNPC1 that is capable of ameliorating the NPC lipid-storage phenotypes in Npc1 mutant Chinese hamster ovary cells (Lige et al., 2011). Parasites lacking TgNCR1 were shown to have abundant lipid storage bodies and altered membrane lipid composition. Unexpectedly, the loss of TgNCR1 induced an increased replication response, which resulted in increased virulence. The study of how TgNCR1 influences membrane lipid composition and subsequent cellular growth signaling could provide clues about the molecular and cell biology of NPC1 function.

Along with parasites, NPC1 can affect the pathogenicity of viruses. Ebola virus and its family members, which use endocytic pathways to infect a cell, require a portion of the NPC1 protein for successful integration (Carette et al., 2011). In cells lacking NPC1, the virus enters but remains trapped inside vesicles and does not replicate. Studies of Ebola infection have revealed a potential specific inhibitor of NPC1 that might be useful for exploring its molecular functions (Côté et al., 2011). Determining how Ebola and other viruses use NPC1 as a scaffold to traverse their genetic material into the cytoplasm could reveal attributes of the membrane function of NPC1.

Two commonly used laboratory model organisms, the nematode Caenorhabditis elegans and zebrafish Danio rerio, could be useful for studying developmental defects caused by lack of NPC1 (Fig. 5). C. elegans is an excellent model for whole-organism high-throughput chemical and molecular screening (Kwok et al., 2006). The C. elegans NCR-1 and NCR-2 proteins, which are thought to be involved in sterol signaling, can be functionally substituted by human NPC1 expressed in the worm, suggesting a high degree of functional conservation (Li et al., 2004; Smith and Levitan, 2007). Mammalian NPC1 also rescues the developmental defects detected in zebrafish npc1 morphants (NPC1-deficient zebrafish generated by morpholino injection), also suggesting a high degree of functional homology between mammalian NPC1 and its counterpart in fish (Schwend et al., 2011; Louwette et al., 2013). Zebrafish offer the possibility of being able to image, in real time, cell activity in vivo in a vertebrate (Ahrens et al., 2012). Both model organisms require further characterization of the neurological defects caused by NPC1 protein deficiency.

Despite lacking a nervous system, yeast is a powerful single-cell model organism for genome-wide analysis of biological functions (Fig. 5). Unfortunately, loss of the yeast NPC1 ortholog Ncr1 does not cause a phenotype that can be easily explored by high-throughput screening assays to gain insight into NPC1 cellular pathways (Munkacsi et al., 2011). By contrast, the filamentous fungus Fusarium graminearum does have a discernible NPC storage phenotype upon deletion of the NPC1 ortholog (Breakspear et al., 2011). As in yeast, the NPC1 ortholog in F. graminearum localizes to the vacuolar membrane, which is homologous to the lysosome. In contrast with yeast, mutant F. graminearum strains accumulate ergosterol (a fungal sterol), show sensitivity to ergosterol synthesis inhibitors and have a temperature-dependent reduction of growth. Tools that would enable full exploitation of F. graminearum as a model system are currently lacking, but this could change with technological advances in this area.

One organism that has a nervous system and reigns supreme in the world of genetic screens is Drosophila melanogaster. The fly model benefits from fast reproduction times and the availability of a vast number of genetic resources. Fly models of NPC1 and NPC2 have been generated (Fig. 5), revealing sterol-usage and steroid-synthesis defects (Huang et al., 2005; Fluegel et al., 2006; Phillips et al., 2008). dNPC1a, the fly homolog of the NPC1 protein, has been shown by MARCM (mosaic analysis with a repressible cell marker) clonal analysis to be required in a cell-autonomous fashion to prevent intracellular cholesterol-storage defects (Phillips et al., 2008). Moreover, neuron-specific rescue studies in flies have shown a strong neuronal requirement for dNPC1a as well as selective neuron vulnerability (Phillips et al., 2008).

In addition to model organisms, human cell lines from the differentiation of stem cells and other sources provide unique opportunities to perform cellular studies and test therapeutic compounds for neurodegenerative disease (Ordonez et al., 2012). Although these studies often do not mimic natural organs or tissues, they can be coupled with information gleaned from in vivo models. Of course, the greatest system for in vivo study is provided by affected individuals (Fig. 5). Thorough analysis of signs and symptoms along with well-designed clinical trials might help uncover biomarkers of disease progression and identify new drug targets.

Conclusions and future perspectives

The study of rare genetic disorders, although often neglected by biopharmaceutical companies, can facilitate important discoveries in our understanding and treatment of more common idiopathic diseases. For example, investigation of homozygous familial hypercholesterolemia, a rare disease with a prevalence of 1 in 1 million, led to the discovery of the low-density lipoprotein (LDL) receptor (Goldstein and Brown, 2009). This initial finding facilitated dramatic advances in our understanding of cholesterol homeostasis, receptor-lysosome biology and the mechanism by which statins, drugs used to prevent heart attacks, lower plasma LDL cholesterol. Although NPC is a rare neurodegenerative disease, the naturally occurring and engineered genetic models of the disease show great potential for the elucidation of neurodegenerative disease biology that is translatable to humans. The lessons learned from exploring the disease in mice and other organisms could be invaluable for understanding mechanisms of neurodegeneration that are conserved or differ between organisms and among disparate neurological disorders.

Since the discovery of the NPC1 disease gene, remarkable efforts have been made to generate genetic tools to study the neurodegenerative aspects of the disease. In mice alone, multiple targeted transgenics, chimeras, a conditional knockout, and a regulatable cell-type-specific disease-rescue model have been engineered. Despite the advances made, caution should be exercised in analyzing the data generated in mouse-based studies. Altered patterns of transgene expression within a mouse population and with age can affect results (Lopez et al., 2011). In the development...
of future models, or the use of current NPC transgenic mice, multiple approaches can and should be used to assess correct cell and tissue targeting of a particular genetic modification. Strain differences can also account for divergent phenotypes (Liu et al., 2008). For example, \( \textit{Npc1} \)-null mutations put into the C57BL/6 mouse strain cause a more severe early defect (leading to premature death prior to obvious neurodegeneration) than in FVB/N, Balb/c or mixed genetic backgrounds. In the C57BL/6 mouse strain, visceral tissue defects and inflammatory factors might play a more central role (Parra et al., 2011). A novel C57BL/6 mouse strain harboring a humanized mutation of NPC1 has recently been characterized and reported as more closely mimicking the signs and symptoms of the human disorder, probably because the allele is hypomorphic and disease progression is therefore slower in this strain (Maue et al., 2012). In spite of the caveats associated with mice studies, researchers should not be dissuaded from tackling the disease complexity \textit{in vivo} using these or other model organisms. We anticipate that precise targeted manipulations and the ability to accurately trace the affected cell types will lead to a better understanding of disease pathology.

As is evident for most instances of inherited neurodegenerative disease, in NPC the causative gene factor, \( \textit{NPC1} \), is ubiquitously expressed, yet subsets of neurons and a few other cell types are exceptionally vulnerable to loss of \( \textit{NPC1} \) function. This root problem must be addressed by determining the intracellular events that lead to selective neuron malfunction, injury and cell death. NPC, caused by loss of \( \textit{NPC1} \) function, continues to serve as a prototype for studying cell-autonomous neurodegeneration. As a result of the cell-autonomous function of \( \textit{NPC1} \) and ability to manipulate specific populations of cells genetically, a detailed roadmap of the progression and rescue of neurodegeneration is gradually being generated. Although the remaining obstacles to fully understanding neurodegenerative disease pathology are challenging, the increasingly powerful genetic, molecular and imaging tools available support the optimistic view that a deep understanding of disease neurobiology and methods to control the disease can be obtained.

Fig. 5. Animal models of NPC. The \( \textit{NPC1} \) protein is highly evolutionarily conserved among eukaryotes. Many model systems of NPC, both spontaneously derived and engineered, are available. We have listed the range of organisms in which NPC-related phenotypes have been reported (see in-text references under ‘Beyond the mouse: additional models of NPC’) and have ranked them in the order of \( \textit{NPC1} \) protein homology. Some of the current and potential uses of these model organisms for research are also listed. The phylogenetic tree was drawn using Phylogeny.fr (Dereeper et al., 2008) and the mammalian Patched protein, PTCH1, a related RND permease superfamily member and sterol-sensing-domain-containing protein (Hausmann et al., 2009), was used as an outgroup. For \textit{C. elegans}, the NCR1 protein sequence was used. We apologize if we have failed to mention any additional organisms that have been or are currently being studied in the context of NPC.

### Models

| Organism | NPC1 phylogenetic tree | Utility of model |
|----------|------------------------|-----------------|
| \( \textit{H. sapiens} \) | Patient brain scans | Genetic rescue studies |
| \( \textit{F. catus} \) | Biomarker studies | Therapeutic testing |
| \( \textit{M. musculus} \) | Therapeutic trials | Biomarker analysis |
| \( \textit{D. rerio} \) | Derived cell lines | CNS imaging |
| \( \textit{D. melanogaster} \) | Epistasis | Epistasis |
| \( \textit{S. cerevisiae} \) | | |
| \( \textit{F. graminearum} \) | | |
| \( \textit{C. elegans} \) | | |
| \( \textit{T. gondii} \) | | |

**Spontaneous**

| Organism | NPC1 phylogenetic tree | Utility of model |
|----------|------------------------|-----------------|
| \( \textit{H. sapiens} \) | Patient brain scans | Genetic rescue studies |
| \( \textit{F. catus} \) | Biomarker studies | Therapeutic testing |
| \( \textit{M. musculus} \) | Therapeutic trials | Biomarker analysis |
| \( \textit{D. rerio} \) | Derived cell lines | CNS imaging |
| \( \textit{D. melanogaster} \) | Epistasis | Epistasis |
| \( \textit{S. cerevisiae} \) | | |
| \( \textit{F. graminearum} \) | | |
| \( \textit{C. elegans} \) | | |
| \( \textit{T. gondii} \) | | |

**Engineered**

| Organism | NPC1 phylogenetic tree | Utility of model |
|----------|------------------------|-----------------|
| \( \textit{H. sapiens} \) | Patient brain scans | Genetic rescue studies |
| \( \textit{F. catus} \) | Biomarker studies | Therapeutic testing |
| \( \textit{M. musculus} \) | Therapeutic trials | Biomarker analysis |
| \( \textit{D. rerio} \) | Derived cell lines | CNS imaging |
| \( \textit{D. melanogaster} \) | Epistasis | Epistasis |
| \( \textit{S. cerevisiae} \) | | |
| \( \textit{F. graminearum} \) | | |
| \( \textit{C. elegans} \) | | |
| \( \textit{T. gondii} \) | | |
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COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

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