Human Molecular Genetics, 2018, Vol. 0, No. 0 1–11

doi: 10.1093/hmg/ddy155
Advance Access Publication Date: 28 April 2018
Invited Review

INVITED REVIEW

The pathogenesis of lysosomal storage disorders: beyond the engorgement of lysosomes to abnormal development and neuroinflammation

Maria Teresa Fiorenza[1,2], Enrico Moro[3] and Robert P. Erickson[4,*]

[1]Division of Neuroscience, Department of Psychology and "Daniel Bovet" Neurobiology Research Center, Sapienza University of Rome, 00185 Rome, Italy, [2]IRCCS Fondazione Santa Lucia, 00179 Rome, Italy, [3]Department of Molecular Medicine, University of Padova, I-35131 Padova, Italy and [4]Department of Pediatrics, University of Arizona, Tucson, AZ 85724-5073, USA

*To whom correspondence should be addressed. Tel: +1 5206262314; Fax: +1 5206267205; Email: erickson@peds.arizona.edu

Abstract

There is growing evidence that the complex clinical manifestations of lysosomal storage diseases (LSDs) are not fully explained by the engorgement of the endosomal-autophagic-lysosomal system. In this review, we explore current knowledge of common pathogenetic mechanisms responsible for the early onset of tissue abnormalities of two LSDs, Mucopolysaccharidosis type II (MPSII) and Niemann-Pick type C (NPC) diseases. In particular, perturbations of the homeostasis of glycosaminoglycans (GAGs) and cholesterol (Chol) in MPSII and NPC diseases, respectively, affect key biological processes, including morphogen signaling. Both GAGs and Chol finely regulate the release, reception and tissue distribution of Shh. Hence, not surprisingly, developmental processes depending on correct Shh signaling have been found altered in both diseases. Besides abnormal signaling, exaggerated activation of microglia and impairment of autophagy and mitophagy occur in both diseases, largely before the appearance of typical pathological signs.

Introduction

For many years the engorgement of lysosomes with undigested macromolecules and the subsequent functional cell impairment have been accepted as primary causes for the pathogenesis of many lysosomal storage diseases (LSDs). However, the lysosomal-dependent cellular engulfment of undigested material has never fully explained the variety of phenotypes displayed by different LSDs. In recent decades, a more nuanced approach has disclosed the important roles of altered autophagy and stimulation of inflammatory responses in the pathology of LSDs.

The interplay between the accumulation/mislocalization of substrates and alteration of signaling pathways is also gaining attention in the context of LSDs (1). Glycosaminoglycan (GAG) fragments accumulating in Mucopolysaccharidoses are typically attached to proteoglycans, which acting as co-receptors, finely regulate the activity of major signaling pathways (2). Gangliosides, sphingolipids and cholesterol (Chol) accumulating in Lipidoses assemble cell surface microdomains, including *caveolae* (3), lipid rafts (4) and glycosphingolipids-enriched microdomains (5), which serve as platforms for signal transduction. Therefore, aberrant signaling cascades contribute to the phenotypes of LSDs affected patients with alterations in autophagy and mitophagy, leading to neuroinflammation and progressive lysosomal substrate storage.

Among signaling pathways modulated by GAGs, glycoconjugated lipids and Chol, Sonic hedgehog (Shh) and Wnt/β-

Received: March 22, 2018. **Revised:** April 24, 2018. **Accepted:** April 24, 2018

© The Author(s) 2018. Published by Oxford University Press. All rights reserved.
For permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

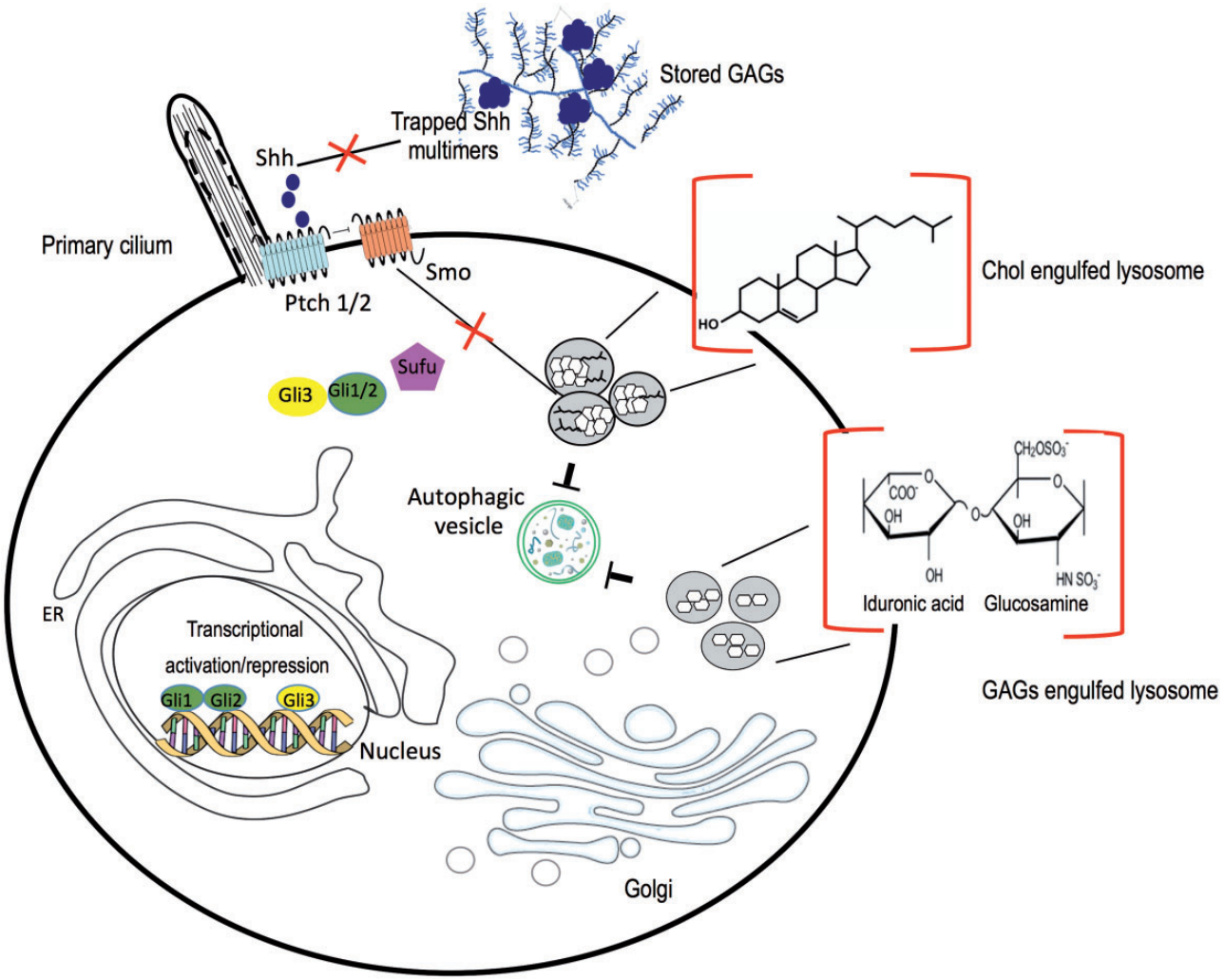

Figure 1. Model for the hypothetical role of lysosomal engorgement in the onset of Sonic Hedgehog (Shh) pathway alterations. When cholesterol (Chol) progressively accumulates inside lysosomes it may not bind and activate Smoothened (Smo). For simplicity, we omitted the potential prevention of Hedgehog ligand cholesterylation, due to lysosomal storage. On the other hand, lysosomal Chol, as well as GAGs engorgement, may perturb the autophagic flux, leading to cell damage. Progressively uncleared GAGs may also interfere extracellularly with Shh ligand, preventing its binding to Patched (Ptch), and therefore blocking Shh pathway transduction (see text for more details).

catenin play a key role in morphogenetic processes (6) and adult tissue homeostasis (7). Both Shh and Wnt morphogens are covalently linked to lipids and associate with lipoprotein particles, which are used as vehicles to ensure their movement within the extracellular space. Extracellular matrix GAGs with distinct modification patterns generate a specific code that shapes the gradient-based activity of both Shh and Wnt signals (8). The current view posits that Shh monomers associate with lipoprotein particles via palmitoyl and Chol moieties forming multimeric complexes that further interact with GAG chains (8), by means of positively charged aminoacid residues of the Cardin-Weintraub (CW) motif (9). This interaction is particularly relevant for proper Shh gradient formation during tissue morphogenesis (8). Hence, perturbations of GAG catabolism or intracellular movement of endocytosed Chol may thereby alter Shh signaling. Progressively uncleared GAGs may interfere extracellularly with Shh ligand, preventing its binding to Patched (Ptch), and therefore blocking Shh pathway transduction at the primary cilium. On the other hand, when Chol is trapped in endo/lysosomes it may not bind and activate both Shh and Smoothened (Smo). Also, lysosomal Chol, as well as GAG engorgement, may perturb the autophagic flux, leading to cell damage (Fig. 1).

The enrichment of Chol in lipid rafts, as a means to set the necessary microenvironment for signal transduction, is being re-framed in light of the discovery of a high-affinity Chol-binding motif in the intracellular juxtamembrane domain of receptor proteins (10). The precise arrangement of this motif, which is called 'cholesterol recognition aminoacid consensus (CRAC)' and its mirror motif, CARC, in the inner and outer leaflet of the plasma membrane, respectively, suggests a direct role of Chol in the activation processes.

We here overview current findings supporting a novel concept, whereby alterations of different key biological processes, including morphogenetic pathways (see Table 1), autophagic flux and mitophagy, may account for the onset of early LSD-related tissue manifestations. To this purpose, we examine two distinct LSD diseases: Mucopolysaccharidosis type II (MPSII; Hunter syndrome/disease) and Niemann-Pick C 1 and 2 (NPC1, 2), which despite affecting the GAG catabolism and intracellular

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

Table 1. LSDs with documented Hedgehog signaling impairment

Lysosomal storage disease	Molecular defect	Phenotype	Reference
Niemann-Pick C1	Shh dysregulation(reduced Ptch and Smo mRNA and protein levels)	Shortened primary cilium length in human patient fibroblasts	Canterini *et al.* (11)
Niemann-Pick C1	Reduced Ptch1 protein levels	Shortened primary cilium length in human patient fibroblasts	Formichi *et al.* (12)
Mucopolysaccharidosis Type II	Reduced Shh transduction (decreased Shh protein and Gli, Ptch1 mRNA levels)	Reduced cardiac trabeculation in IDS KO mice and Ids deficient fish	Costa *et al.* (13)
Mucopolysaccharidosis Type I	Aberrant Indian hedgehog protein distribution in the growth plate	Structural abnormalities in the growth plate of MPSI mice	Kingma *et al.* (14)
Mucopolysaccharidosis Type VII	Dysregulated Ihh and Ptch1 mRNA levels	Impaired endochondral ossification in MPSVII dogs vertebrae	Peck *et al.* (15)

trafficking of endocytosed Chol, respectively, appear to share common related pathogenic mechanisms.

Mucopolysaccharidosis type II (Hunter syndrome)

MPSII was originally described by its physical feature of 'gargoylism' (16). Since the discovery of GAGs in the urine of affected patients, the result of lysis of cells sloughed from the kidney, ureters and bladder (17), and the identification of the defective enzyme, diagnosis became more precise, enabling the discrimination between the autosomal recessive Hurler's disease (MPS I) due to the lack of alpha-L-iduronidase and the sex-linked Hunter disease, characterized by the lack of iduronate-2-sulfatase (IDS). The latter shares delayed motor development, hepatosplenomegaly, heart disease and short stature with the former, but MPSII patients also display a high frequency of diarrhea (18). In addition, MPSII patients exhibit joint problems with contractures, pseudopapilloedema or papilloedema without visual loss, myocardial enlargement and pulmonary dysfunction (19). The early separation of the disorder into severe and mild forms (18) was later explained by the occurrence of residual enzymatic activity and by different types of mutations (20). Nonetheless, 14% of affected patients are not classifiable (18) and there are families in which, due to environmental variables or modifying genes, both severe and mild cases occur (21,22). Enzyme replacement therapy (ERT) first attempted with fresh frozen plasma (23) represents the current gold standard therapeutic approach, despite being unable to treat the neurological symptoms due to the failure of the recombinant enzyme to cross the blood brain barrier (BBB) (24).

While also prominent in MPSI (25), heart problems are very common in MPSII, ranging from 75% of mildly affected cases (18) to 91% of all cases (26). Mitral and aortic valve disease are the most frequent findings, but myocardial thickening, conduction disturbances, including arrhythmias, coronary arterial narrowing and myocardial infarction have been described (27). And sudden death can also occur.

Impaired cell signaling and cardiac defects in Hunter syndrome

The first example of ultrastructural defects of the ECM related to developmental cardio-vascular abnormalities is provided by the great similarity of Loeys-Dietz syndrome with Marfan syndrome (28). While the connective tissue fibrillin 1, defective in Marfan syndrome, was initially thought to cause the cardiac pathology by decreasing the structural strength of the connective tissue, it now seems that alterations of the TGFβ signaling are more clearly responsible for the disease, in agreement with the pathogenic role of TGFβ mutations in Loeys-Dietz syndrome (29).

Similarly, GAGs that are structurally abnormal in MPSs alter different intracellular signaling pathways, including Shh (30). In particular, impaired Shh signaling whose activity is tightly related to sulfated iduronic acid (2) has been recently shown associated with impaired heart development (13). Indeed, IDS knockout mice exhibit impaired Shh signaling and ECG abnormalities, particularly a prolonged PR interval, before the onset of a large buildup of GAGs in lysosomes (13).

A close link between impaired extracellular matrix GAGs composition and heart development is also illustrated by a new storage disease found in Yakuts, a Siberian population. Mutations in VPS33A, a protein involved in endocytic and autophagic pathways, result in increased heparan sulfate levels (31). This severe disorder shows classic signs of MPSs and most patients exhibit congenital heart disease, with coarse facial features, barrel-shaped chest, and mental retardation with delayed myelination. Early obstructive lung disease occurs as well, and most patients die before 2 years of age due to cardiorespiratory disease. Although it has not been yet demonstrated in any vertebrate animal model for the disease, the severe heart disease leading to early death is likely to be due to heparan sulfate-mediated changes in cell signaling.

Perhaps, similar alterations of cell signaling caused by matrix-induced alterations of cell signaling contribute to other organ pathologies in Hunter syndrome. For instance, the severe diarrhea may not just be secondary to compression of the gut by splenomegaly. Shh and Indian hedgehog are essential for smooth muscle formation, while Shh is essential for gut innervation (32). Mesenchymal Wnt signaling in the developing gut seems to decrease as extracellular matrix formation increases (33). While only increased membranous cytoplasmic bodies have been observed in an ultrastructural study of the rectal wall in MPS II (34), decreases or alterations in the enteric nervous system or smooth muscle might be responsible for the diarrhea so frequently seen.

The role of autophagy in Hunter syndrome CNS and heart-related manifestations

There are several interesting studies implicating alterations of autophagy in the onset of neurodegeneration of MPS patients

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

(35). These studies have mostly been performed in MPSs I, IIIA, IIIB and VII. In these MPSs, accumulation of GAGs is strikingly accompanied by accumulation of gangliosides in the brain (36) as occurs in NPC1, 2 (see below), while increased Chol, which is implicated in the failure of phagosome-lysosome fusion, is also detected in neuronal cell membranes of affected patients (37). Among major consequences of impaired phagosome-lysosome fusion is mitophagic impairment (digestion of dysfunctional mitochondria), which may lead to apoptosis of affected cells. The accumulation of damaged mitochondria has been shown in MPS with subsequent elevation of damaging reactive oxygen species (ROS) (38). The mitochondrial dysfunction in multiple LSDs and its consequences for neurodegeneration have been well reviewed by Saffari *et al.* (39). Altered mitochondrial function also seems important in NPC1, 2 (see below). However, it is the balance of apoptosis [which is not a major process to occur in NPC1 (see below)] with non-apoptotic death, which is important since the latter stimulates the immune system while the former does not. The pattern recognition receptors (PRR) of the innate immune system recognize the extracellular GAGs by the addition of damage associated molecular partners (reviewed in 40), which are known to be involved in microglial activation and neuroinflammation (41). This microglial activation is responsible of the proinflammatory/neurodestructive pathway involving the so called A1 astrocytes (42), which has been found in multiple lysosomal storage disorders (43).

Autophagic processes are very important in early cardiac morphogenesis, as *Atg5*-deficient mice and *atg5* zebrafish morphants exhibit abnormal heart structures, impaired heart looping and heart valve development (44). Moreover, defects in autophagy have been shown in the aging heart and enhancing autophagic processes delays cardiac aging. Many cardiac pathologies resulting in heart failure (ventricular dilation, cardiac hypertrophy and fibrosis) involve re-modelling, which is dependent on autophagy (45). The mitophagic pathway seems to have a homeostatic role in the heart (46). Based on the emphasized role of autophagy in cardiac health, a number of autophagy-targeted drugs are being considered in heart diseases. Although not yet studied in MPSs, altered autophagy may well contribute to the heart disease as well as to the neurodegeneration in MPSs.

Thus, while the precise role of mitophagic versus general autophagic defects remains unclear (see further discussion below for NPC1), the contribution of one or both to MPS pathology is clear. The role of activated microglia, with their recruitment of macrophages and other inflammatory cells (47) in neurodegeneration seems paramount. In this light, it is relevant that a drug targeted at inflammation, pentosan polysulfate, has shown promising results in the MPS VI rat model (48). The addition of drugs targeted to inflammatory pathways, especially if they penetrate the BBB, to enzyme therapy would appear hopeful.

Niemann-Pick C diseases

Niemann-Pick type C (NPC) disease is an autosomal recessive, neurodegenerative lysosomal storage disorder with variable clinical phenotypes (49). The most common presentation of NPC disease is a child of either sex developing coordination problems, dysarthria, and hepatosplenomegaly during early school-age years. Phenotypic manifestations are accompanied by abnormal intracellular accumulation of Chol and glycosphingolipids in a variety of tissues, including the liver and spleen, and progressive cerebellar degeneration (for a general review, see 50). The neurological progression of the disorder is relentless and characterized by an increasing severity of ataxia, dysarthria and dementia until death occurs, usually during the second decade of life. Seizures are a common manifestation (49). More severe infantile forms, where liver involvement is severe (50,51), and late-onset forms including Niemann-Pick D disease (NPD, see below) also occur. The gene underlying 95% of the cases of this disorder is *NPC1*, which codes for a multi-pass transmembrane protein, containing a sterol-sensing domain. It shows homology to proteins with sterol-binding domains including Ptch, HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, and SCAP (SREBP cleavage-activating protein) (52,53). Ptch is a transmembrane receptor, which interacts with Shh, a signaling molecule with covalently attached Chol, while HMG-CoA reductase and SCAP interact with Chol. Functional analysis of NPC1 suggests that it is involved in late endosomal lipid sorting and trafficking (54). While NPC disease is most commonly associated with mutations in the NPC1 gene (55), it can also be caused by mutations in a second gene, NPC2, which encodes a smaller, soluble lysosomal protein, which presents Chol to NPC1 (56,57). The much greater rarity of NPC2 (5% of NPC cases) may be due to decreased fertility of male carriers since NPC2 is a very abundant protein in seminal fluid. NPD is a milder mutant version of the *NPC1* gene that apparently seemed different, with a large number of cases in an inbred population in Nova Scotia, until Chol storage was also identified in the disorder and mutations in *NPC1* were found.

Although there has been some controversy as to whether Chol storage is causative for the pathology (versus sphingosine or gangliosides), it now seems clear that ganglioside accumulation is secondary, as in MPSs (58). The success of the Chol sequestering hydroxypropyl-beta-cyclodextrins (HPβCD) in treating NPC1 also confirms the primary role of Chol storage. HPβCD has been previously used to modulate Chol trafficking across cell membrane (59). Over a decade ago, an efficacious effect of HPβCD on slowing neurodegeneration in a mouse model of NPC1 was shown (60). HPβCDs are substituted rings of seven glucoses and do not cross the BBB. Since it seems counterintuitive to treat a CNS disorder with a drug that does not cross the BBB, and because the effects are small when the drug is only started at the time of weaning, this drug appeared not particularly promising. Only when therapy was started at postnatal day 7, with higher doses, results were more dramatic. At this age the sealing properties of the BBB are not fully mature (61) and the drug is more slowly eliminated (6 h instead of 3 h; 62). This Chol-interacting compound is now in Phase I/II trials in humans, both using intravenous and intrathecal delivery. Intrathecal delivery has recently been found to significantly delay the pathological progress of NPC1 disease (63,64).

Altered development in Niemann-Pick C diseases

Neurological symptoms, including ataxia, cognitive loss, seizures and dementia represent the most severe clinical manifestations of the NPC disease. The cerebellum is particularly affected, with Purkinje cells (PC) appearing more vulnerable than other cell types (65).

Compared to other tissues/organs, the metabolism of Chol inside the brain is rather unique. Since Chol does not cross the BBB, which completely forms after birth (61), brain Chol comes exclusively from *de novo* synthesis. During embryonic and early postnatal neurogenesis Chol synthesis occurs in both neurons

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

and glial cells, while subsequently Chol produced by astrocytes is delivered to neurons via the LDL pathway and made available to the various cell compartments by NPC1/2-mediated Chol efflux from lysosomes (66,67). This shift is expected to unmask the neuronal vulnerability to the deficiency of either proteins and to affect developmental processes. The morphogenesis of the cerebellar cortex is paradigmatic of such a condition, since it encompasses the 3 weeks after birth and is marked by intense neuronal/glial cell proliferation and migration, neurite outgrowth, synaptogenesis and myelin formation, which likely maximize the need for Chol (68).

Our recent studies in mouse models have shown that Npc1 deficiency affects later steps of cerebellar morphogenesis, in spite of normal cerebellum development at earlier stages (69). We found various subtle developmental defects, including the premature exit from cell cycle of granule neuron (GN) precursors (69) and abnormal differentiation of Bergman glia (BG) (70).

Dysregulated development of both GN and BG is likely to affect the integrity of PC, contributing to their subsequent neurodegeneration. In fact, the reduced proliferation of GNs affects the constant ratio of 175 GN for each PC (71) typical of the normal mouse cerebellum, whereas the defective scaffolding activity of BG does not properly support PCs (72).

Defects of cerebellar morphogenesis of Npc1 mice are linked to altered Shh signaling (11,12,69). This finding is consistent with the tight relationship between Chol metabolism and Shh signaling as indicated by the following evidence. First, active Shh protein is modified by covalent addition of Chol during its intracellular processing (73). Shh Chol modification is relevant for generating temporal and spatial gradients of this protein (74), which likely mediate pleiotropic signaling activities within the developing cerebellum. Starting from mouse embryonal day 17.5 (E17.5), Shh is continuously secreted by PC and diffuses upward to the external granule layer (EGL) and downward to the so-called prospective white matter (PWM, a secondary germinal zone of the postnatal cerebellum), driving the generation of excitatory (GNs) and inhibitory interneurons, respectively. Fate mapping studies have shown that neural stem cell-like 'astroglial cells' in the PWM generate these late-born interneurons, called basket and stellate cells that provide GABAergic input to the PCs. In response to Shh, a niche of progenitors residing in the PWM, also generates astrocytes (75).

Second, genes encoding *Ptc1* and *Npc1* share sequence homology, mostly confined to their 12 transmembrane segments, including the sterol-sensing domain, a motif implicated in lipid intracellular trafficking (52). Accordingly, it was reported that Ptc1 contributes to Chol efflux from the cell (76). Third, a selected group of compounds inhibits both Shh signaling, regulated by Ptc1, and late endosomal lipid sorting, regulated by NPC1, suggesting that Ptch1 regulates Smo activity through a common late endosomal sorting pathway also used by NPC1 protein (77). Fourth, the morphology/function of primary cilium, a crucial center of Shh signaling transduction, depends on lipid composition (78). Of note, two studies have recently reported that the primary cilium length is significantly reduced in NPC patient fibroblasts and brain sections of Npc1-deficient mice (11,12).

A recent study further emphasizes the relevance of Chol for Shh signaling, identifying Smo as the second Chol-modified protein and showing that the Chol modification of Smo is essential for Shh signal transduction and proper embryonic development (79). The homozygous mutation of the aspartate aminoacid residues, to which the Chol moiety is bound, is embryonic lethal due to severe cardiac defects.

The phenotype of a mouse model in which a mutation in the NAD(P)-dependent steroid dehydrogenase-like (*Nsdhl*) gene affects the Chol synthesis pathway further strengthens the essential role that Chol has in Shh signaling (80). Similar to Npc1-deficiency the *Nsdhl* mutation markedly affects the proliferation of GN precursors (80).

The requirement of Npc1 function for normal cell movements at the beginning of gastrulation in zebrafish embryos suggests that this protein might play a relevant role at very early stages of embryo development. The mouse Npc1 RNA is able to rescue these zebrafish developmental defects (81), indicating an evolutionarily conserved function.

The role of autophagy and mitophagy in the neurodegeneration of Niemann-Pick C diseases

There have been multiple studies implicating altered autophagy in NPC1 but with conflicting results and interpretations. Early studies suggested enhanced autophagosome activity in the brain of the NPC1 mouse model with elevated cathepsin D and LC3-II activity (82). This was confirmed in brain and liver by Pacheco and Lieberman (83), who found the enhanced autophagy to be associated with increased levels of Beclin-1. Goldstein's group knocked down NPC1 function in neurons derived from human embryonic stem cells, finding a strong activation of autophagy and accumulation of fragmented mitochondria (84). The autophagic inhibitor, 3-methyladenine and, also, cyclodextrin reversed the defect (84). The elevated autophagosome activity was not due to increased formation of autophagosomes, but rather to a lack of fusion with lysosomes (85), which seems to be a general defect in LSDs. These authors emphasized the disagreement in their results and interpretation of altered autophagy with the results of Pacheco *et al.* (83) and Ordonez *et al.* (84). In particular, they could not reproduce the finding of elevated levels of Beclin-1. Further studies implicated the lack of SNARE (SNAp [Soluble NSF Attachment Protein] REceptor) protein recruitment to the endo-lysosome as the cause of lack of fusion. Importantly, these *in vivo* studies confirmed that there was decreased autophagosome activity due to the lack of fusion with lysosomes in cerebellum and liver (86). These authors pointed out that an excess of cyclodextrin could be harmful by preventing the key role of normal autophagy in cellular homeostasis (86). The Lieberman group came to agreement with these conclusions when they studied the effects of the tau knockout with Npc1 deficiency (87) and demonstrated impaired proteolysis (88). Autophagic flux involves microtubules which interact with tau and the lack of tau exacerbated the Npc1 phenotype. While studying the defect in autophagosome flux in induced pluriportent stem cells from NPC1 patients, Maetzel *et al.* (89) found that carbamazine, a known stimulants of autophagy, decreased Chol storage, especially when combined with HPβCD.

An alternative mechanism of inhibition of autophagosome-lysosome fusion was proposed to be due to sphingosine accumulation (90). Evidence was provided for a decreased level of VEGF a protein well known in lymphatic and blood vasculature formation, with subsequent depression of sphingosine kinase activity. A mechanism for this surprising decrease of VEGF was not given but cross-breeding with a VEGF over-expressing transgenic line led to a modest increase in survivial rates of the Npc1 null mice (90). Necroptosis, a form of cell death involving RIP1 and RIP3 kinases has also been implicated in the death of NPC1

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

neurons and its inhibition with a RIP1 antagonist extended survival in Npc1 model mice (91).

A primary initiator of autophagic irregularities in NPC1 may be the accumulation of altered mitochondria. The mitochondrial role in autophagy is well recognized as they supply the membranes for the formation of the autophagosomes during starvation (92). As studied in neurons, there is increased mitochondrial membrane Chol in NPC1 mutant cells and brains (93). The route of this Chol to mitochondrial membranes obviously does not require the missing NPC1 protein but rather the NPC2 (94) and the MNL64 proteins (95). When over-expressed, MLN64 leads to increased mitochondrial membrane Chol with subsequent impairment of function and smaller and rounded mitochondria (96). The route for Chol movement to the mitochondrial outer membrane involves the Chol binding proteins StARD1 (the major regulator of Chol biosynthesis) and StarD3 (97), while the former, along with the translocator protein, TSPO, is involved in the transport of Chol from the outer to the inner mitochondrial membrane (98). Importantly, StarD1 expression is increased in Npc1 deficient mice (99) providing one possible mechanism for the increased mitochondrial Chol (reviewed in 100). Mitochondria with increased membrane Chol show multiple metabolic abnormalities (93,96), including decreased membrane potential, decreased ATP synthase activity, fragmentation and increased ROS. Quite opposite results, with increased mitochondrial activity, were found by Wos *et al.* (101) in cultured primary fibroblasts from patients. ROS have often been implicated in NPC1 pathology (102) and glutathione, which reduces ROS, extends life expectancy in the Npc1 model mice (99). Since caveolin 1 has been shown to have a role in mediating autophagy and preventing ROS activity (at least in endothelial cells; 103), the elevation of caveolin 1 seen in NPC1 (104) may be a secondary cellular response to ROS-dependent stress. Defective mitochondria, with increased levels of oxidative stress markers and decreased survival (shortened chronological life span) were also found in the yeast model of NPC1 (105). These defects seemed to be secondary to elevated sphingolipid levels.

Alterations in in lysosomal calcium levels due to sphingosine accumulation have been previously shown by Lloyd-Evans *et al.* (106). A defect in calcium release was found to be a more general defect in LSDs, including NPC1, but lysosomal calcium levels were found to be normal (107). A third group found low lysosomal calcium levels which could be raised, and Chol storage corrected, by stimulation of the adenosine receptor A_{2A} (108). Thus, although there is a disagreement about whether lysosomal calcium is decreased, there is agreement that calcium release is abnormal, whether or not it is due to initially low levels of calcium.

There are many other proteins involved in mitophagy which could be altered in NPC1. These include the outer mito membrane receptor Atg32, its partner Atg11, and CK2 kinase need to phosphorylate it (reviewed in 109). The PINK1-PRKN/PRK2 pathway, well studied in Parkinson's disease, senses mito depolarization, which leads to outer mito membrane ubiquitination and mitophagy (109).

In addition to 'classical' mitophagy of defective mitochondria in the autophagosome, there is some evidence for an alternative route for removing them involving mitochondria-derived vesicles. These incorporate oxidized mitochondrial fragments and are delivered directly to the lysosome (110). Alterations of this pathway in LSDs has not been explored yet to our knowledge. A further pathway of interest involves the small GTPase Rab7, which influences endosome motility and is also implicated in mitochondrial-lysosome contact (111). Its overexpression corrected the abnormalities of Chol storage in cultured NPC1 cells (112).

It is clear that defective mitophagy allows these abnormal mitochondria to persist when they should be removed (86) and the defect may be due to increased Chol in membranes as outlined above for MPSs pathogenesis. However, mitochondrial alterations may themselves cause altered autophagy. Mitochondria associate with a special portion of the endoplasmic reticulum named mitochondria-associated ER membranes (MAM). This location is the major site of phospholipid, Chol ester, and fatty acid metabolism (113). Experimental evidence has suggested that MAMs-related abnormalities, especially as indicated by the differences between ApoE4 and ApoE3 effects are the primary problem in both sporadic late-onset Alzheimers disease (SLAD) and genetic Alzheimers disease (presenilin and amyloid precursor protein mutations) (113). NPC1 has been called 'juvenile Alzheimers'. The similarity between NPC and Alzheimer is based on the presence of neurofibrallary tangles, influence of APOE-4 genotype on severity, and the association of variants in the human *NPC1* gene with the onset of sporadic, late onset Alzheimers (114,115). Thus, it might be anticipated that similar alterations in lipid and phospholipid synthesis, affecting phagosome-lysosome fusion and leading to the same pro-inflammatory events described above in MPSs, may be present in NPC1.

Altered mitochondrial respiration may also be important for impaired autophagy. As studied in T cells, markedly reduced mitochondrial respiration is associated with decreased autophagosome-lysosome fusion with subsequently increased inflammatory responses (116). Thus, altered mitochondria have multiple roles in autophagy.

Another possibly anomalous relationship of NPC1 to autophagy concerns the target of rapamycin, TOR. The TOR kinase is the major inhibitory signal of autophagy in the presence of abundant nutrients. NPC1 is part of a signaling complex that activates TOR in the presence of Chol (117). While free Chol is abundant in NPC1 lysosomes, in normal cells, NPC1 binds to a TOR regulating protein, SLC389, to inhibit TOR. Thus, autophagy would not be expected to be decreased. Again, *in vivo*, autophagy is increased but abnormal. These studies have been performed in cultured cells under oxidative stress i.e. at 20% oxygen instead of the physiological 5% level. Also, it is possible that the very excessive levels of free Chol present in NPC1 cells would overcome the absent/decreased TOR signaling due to the down-regulation of autophagy. Finally, it must be remembered that autophagy is an ancient process and involves redundancy with over 100 proteins involved. Combined loss of function of different autophagic-related proteins is likely to be needed to evaluate the relation between autophagic defects and NPC disease.

A somewhat alternative view of the role of mitochondrial dysfunction in LSDs has been presented by Saffari *et al.* (39). In their view, the MAM-related, altered Ca^{++} flux plays a major role in mediating the subsequent neurodegeneration. They also emphasize the release of mitochondrial contents, which can stimulate apoptosis. However, as mentioned above (MPSII section), apoptosis does not stimulate the immune response, while autophagy does. In addition, it has been previously shown that the major process of cell death in a Npc1 mouse model does not involve apoptosis since over-expression of Bcl2, an inhibitor of apoptosis, did not change the rate of PC death or the progress of the disease (118).

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

The subsequent stimulus to inflammation, primarily mediated by microglia (50) follows the pathway described above for MPSII with the additional wrinkle that over expression of VEGF, as mentioned above, seems to have a protective effect (119). The neuroinflammatory pathway has also been specifically studied in the olfactory bulb of NPC1 model mice. Seo *et al.* (120) explored the mechanism of activation of the neuropathic microglial response in the olfactory system, finding that the cysteine endopeptidase, Ctss, is involved in the activation of its target, Cx3cl1 (also known as neurotactin), resulting in p38 mitogen-activated protein kinase signaling (120). The nasal delivery of antibody to Cx3cl1 was shown to decrease the number of damaged OB neurons and slightly enhance olfaction (120).

Given the abundant evidence for neuroinflammation, it is also important to note that drugs primarily directed against inflammation, such as vitamin E (121), curcumin (122), and non-steroidal anti-inflammatory drugs (123), have only had modest effects in alleviating neurodegeneration. In contrast, the group studying neuroinflammation in the olfactory bulb found that a 4 weeks treatment with cyclosporine A markedly reduced neuroinflammation in the olfactory epithelium (124).

Conclusions

Since the discovery of the pathological hallmarks, clinical manifestations of MPSII and NPC1, 2 have been related to the accumulation of GAGs and unesterified Chol, respectively. More recently this perspective has been enlarged thanks to a number of studies showing that authophagic flux and signaling pathways are altered in both diseases. These anomalies largely precede the onset of symptoms and are likely to also affect developmental trajectories that are essential for proper organ morphogenesis. The occurrence of developmental defects in affected patients should be further considered to secure a more exhaustive comprehension of the diseases. The finding that the genetic defect of either MPSII or NPC1, 2 leads to an impairment of the Shh signaling is particularly significant since it provides the basis for a 'convergent hypothesis' to partly explain the pathogenesis of these diseases. In addition, it might be also relevant for finding novel therapeutic avenues.

Acknowledgements

We are grateful to Jessica Dragotto for help with figure preparation. We apologize to all the authors whose work could not be cited due to limitations of space.

Conflict of Interest statement. None declared.

Funding

This work was supported by Telethon Foundation, Italy (grant no. GGP13183) and Ateneo La Sapienza (grant no C26V127RC3) to M.T.F, and by the Italian Ministry of Health (Ricerca Finalizzata GR-2008–1139743) and PRID2017 (University of Padova) to E.M.

References

1. Ballabio, A. and Gieselmann, V. (2009) Lysosomal disorders: from storage to cellular damage. *Biochim. Biophys. Acta*, **1793**, 684–696.
2. Witt, R.M., Hecht, M.I., Pazyra-Murphy, M.F., Cohen, S.M., Noti, C., van Kuppevelt, T.H., Fuller, M., Chan, J.A., Hopwood, J.J. and Seeberger, P.H. (2013) Heparan sulfate proteoglycans containing a glypican 5 core and 2–O–sulfoiduronic acid function as Sonic hedgehog co-receptors to promote proliferation. *J. Biol. Chem.*, **288**, 26275–26288.
3. Anderson, R.G. (1998) The *caveolae* membrane system. *Annu. Rev. Biochem.*, **67**, 199–225.
4. Simons, K. and Toomre, D. (2000) Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell. Biol.*, **1**, 31–39.
5. Hakomori, S., Yamamura, S. and Handa, A.K. (1998) Signal transduction through glyco(sphingo)lipids. Introduction and recent studies on glyco(sphingo)lipid-enriched microdomains. *Ann. N.Y. Acad. Sci.*, **845**, 1–10.
6. Panáková, D., Sprong, H., Marois, E., Thiele, C. and Eaton, S. (2005) Lipoprotein particles are required for Hedgehog and Wingless signalling. *Nature*, **435**, 58–65.
7. Petrova, R. and Joyner, A.L. (2014) Roles for Hedgehog signaling in adult organ homeostasis and repair. *Development*, **141**, 3445–3457.
8. Whalen, D.M., Malinauskas, T., Gilbert, R.J. and Siebold, C. (2013) Structural insights into proteoglycan–shaped Hedgehog signaling. *Proc. Natl. Acad. Sci.*, **110**, 16420–16435.
9. Chang, S.-C., Mulloy, B., Magee, A.I. and Couchman, J.R. (2011) Two distinct sites in Sonic hedgehog combine for heparan sulfate interactions and cell signaling functions. *J. Biol. Chem.*, **286**, 44391–44402.
10. Di Scala, C., Baier, C.J., Evans, L.S., Williamson, P.T.F., Fantini, J. and Barrantes, F.J. (2017) Relevance of CARC and CRAC cholesterol-recognition motifs in the nicotinic acetylcholine receptor and other membrane-bound receptors. *Curr. Top. Membr.*, **80**, 3–23.
11. Canterini, S., Dragotto, J., Dardis, A., Zampieri, S., De Stefano, M.E., Mangia, M.F., Erickson, R.P. and Fiorenza, M.T. (2017) Shortened primary cilium length and dysregulated Sonic hedgehog signaling in Niemann-Pick C1 Disease. *Hum. Mol. Genet.*, **26**, 2277–2289.
12. Formichi, P., Battisti, C., De Santi, M.M., Guazzo, R., Tripodi, S.A., Radi, E., Rossi, B., Tarquini, E. and Federico, A. (2018) Primary cilium alterations and expression changes of Patched1 proteins in niemann-pick type C disease. *J. Cell Physiol.*, **233**, 663–672.
13. Costa, R., Urbani, A., Salvalaio, M., Bellesso, S., Cieri, D., Zancan, I., Filocamo, M., Bonaldo, P., Szabò, I., Tomanin, R. and Moro, E. (2017) Perturbations in cell signaling elicit early cardiac defects in mucoppolysaccharidosis type II. *Hum. Mol. Genet.*, **26**, 1643–1655.
14. Kingma, S.D.K., Wagemans, T., IJlst, L., Bronckers, A.L.J.J., van Kuppevelt, T.H., Everts, V., Wijburg, F.A. and van Vlies, N. (2016) Altered interaction and distribution of glycosaminoglycans and growth factors in mucopolysaccharidosis type I bone disease. *Bone*, **88**, 92–100.
15. Peck, S.H., O'Donnell, P.J.M., Shore, E.M., Pacifici, M., Haskins, M.E., Malhotra, N.R. and Smith, L.J. (2015) Failed vertebral bone formation in mucopolysaccharidosis VII dogs is associated with impaired chondrocyte hypertrophic differentiation. *Mol. Genet. Metabolism*, **114**, S91–SS9.
16. Nja, A. (1946) A sex-linked type of gargoylism. *Acta Paed. Scand.*, **33**, 267–286.
17. Erickson, R.P., Sandman, R., Epstein, C.J. and Robertson, W. v B. (1975) Lack of relationship between serum and urine levels of glycosaminoglycans and lysosomal enzymes. *Biochem. Med.*, **12**, 331–339.

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

18. Young, I.D., Harper, P.S., Archer, I.M. and Newcombe, R.G. (1982) A clinical and genetic study of Hunter's syndrome 1 heterogeneity. *J. Med. Genet.*, **19**, 401–407.
19. Martin, R., Beck, M., Eng, C., Giugliani, R., Harmatz, P., Munoz, V. and Muenzer, J. (2008) Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome). *Pediatrics*, **121**, e377–e386.
20. Lissens, W., Seneca, S. and Liebaers, I. (1997) Molecular analysis in 23 Hunter disease families. *J. Inherit. Metab. Dis.*, **20**, 453–456.
21. Thurmon, T.F., DeFraites, E.B. and Anderson, E.E. (1974) Clinical heterogeneity in mucopolyssachiridosis II: evidence for epistasis. *Birth Defects Orig. Artic Ser.*, **10**, 125–127.
22. Yatziv, S., Erickson, R.P. and Epstein, C.J. (1977) Mild and severe forms of Hunter syndrome within the same sibship. *Clin. Genet.*, **11**, 319–326.
23. Erickson, R.P., Sandman, R., Robertson, W.B. and Epstein, C.J. (1972) Inefficacy of fresh frozen plasma therapy of mucopolysaccharidosis II. *Pediatrics*, **50**, 693–701.
24. Wraith, J.E., Scarpa, M., Beck, M., Bodamer, O.A., De Meirleir, L., Guffon, N., Meldgaard Lund, A., Malm, G., Van der Ploeg, A.T. and Zeman, J. (2008) Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy. *Eur. J. Pediatr.*, **167**, 267–277.
25. Krovetz, L.J., Lorincz, A.E. and Schiebler, G.L. (1965) Cardiovascular manifestations of Hurler syndrome: hemodynamic and angiocardiographic observations in 15 patients. *Circulation*, **31**, 132–141.
26. Young, I.D. and Harper, P.S. (1982) Mild form of Hunter's syndrome: clinical delineation based on 31 cases. *Arch. Dis. Child.*, **57**, 828–836.
27. Gross, D.M., Williams, J.C., Caprioli, C., Dominguez, B. and Howell, R.R. (1988) Echocardiographic abnormalities in the mucopolysaccharide storage diseases. *Am. J. Cardiol.*, **61**, 170–176.
28. Loeys, B.L., Chen, J., Neptune, E.R., Judge, D.P., Podowski, M., Holm, T., Meyers, J., Leitch, C.C., Katsanis, N., Sharifi, N. *et al.* (2005) A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat. Genet.*, **37**, 275–281.
29. Mizuguchi, T., Collod-Beroud, G., Akiyama, T., Abifadel, M., Harada, N., Morisaki, T., Allard, D., Varret, M., Claustres, M., Morisaki, H. *et al.* (2004) Heterozygous *TGFBR2* mutations in Marfan syndrome. *Nat. Genet.*, **36**, 855–860.
30. Linhardt, R.J. and Toida, T. (2004) Role of glycosaminoglycans in cellular communication. *Add. Chem. Res.*, **37**, 431–438.
31. Kondo, H., Maksimova, N., Otomo, T., Kato, H., Imai, A., Asano, Y., Kobayashy, K., Noijma, A., Hamada, Y. and Irahara, K. (2017) Mutation in VPS33A affects metabolism of glycosaminoglycans: a new type of mucopolysaccharidosis with severe systemic symptoms. *Hum. Molec. Genet.*, **26**, 173–183.
32. Romalho-Santos, M., Melton, D.A. and McMahon, A.P. (2000) Hedgehog signals regulate multiple aspects of gastrointestinal development. *Development*, **1227**, 2763–2772.
33. Ormestad, M., Astorga, J., Landgren, H., Wang, T., Johansson, B.R., Miura, N. and Carlsson, P. (2006) *Foxf1* and *Foxf2* control murine gut development by limiting mesenchymal Wnt signaling and promoting extracellular matrix production. *Development*, **133**, 833–843.
34. Elsner, B. (1970) Ultrastructure of the rectal wall in Hunter's syndrome. *Gastroenterol*, **56**, 856–862.
35. Ward, C., Martinez–Lopez, N., Otten, E.G., Carroll, B., Maetzel, D., Singh, R., Sarkar, S. and Korolchuk, I. (2016) Autophagy, lipophagy and lysosomal lipid storage disorders. *Biochim. Biophys. Acta*, **1861**, 269–284.
36. McGlynn, R., Dobrenis, K. and Walkley, S.U. (2004) Differential subcellular localization of cholesterol, gangliosides, and glycosaminoglycans in murine models of mucopolysaccharide storage disorders. *J. Comp. Neurol.*, **480**, 415–426.
37. Sobo, K., Le Blanc, I., Luyet, P.P., Fivaz, M., Ferguson, C., Parton, R.G., Gruenberg, J. and van der Goot, F.G. (2007) Late endosomal cholesterol accumulation leads to impaired intra–endosomal trafficking. *PLos One*, **2**, e851.
38. Rego, A.C. and Oliveira, C.R. (2003) Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: implications for the pathogenesis of neurodegenerative diseases. *Neurochem. Res.*, **28**, 1563–1574.
39. Saffari, A., Kolker, S., Hoffman, G.F. and Ebrahimi–Fakhari, D. (2017) Linking mitochondrial dysfunction to neurodegeneration in lysosomal storage diseases. *J. Inherit. Metab. Dis.*, **40**, 631–640.
40. Deretic, V., Saitoh, T. and Akira, S. (2013) Autophagy in infection, inflammation and immunity. *Nat. Rev. Immunol.*, **13**, 722–737.
41. Ohmi, K., Greenberg, D.S., Rajavel, K.S., Ryazantsev, S., Li, H.H. and Neufeld, E.F. (2003) Activated microglia in cortex of mouse models of mucopolysaccharidoses I and IIIB. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 1902–1907.
42. Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.-S., Peterson, T.C. *et al.* (2017) Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*, **541**, 481–487.
43. Pshezhetsky, A.V. (2016) Lysosomal storage of heparin sulfate causes mitochondrial defects, altered autophagy, and neuronal death in the mouse model of mucopolysaccharridosis III type C. *Autophagy*, **12**, 1059–1050.
44. Lee, E., Koo, Y., Ng, A., Wei, Y., Luby-Phelps, K., Juraszek, A., Xavier, R.J., Cleaver, O., Levine, B. and Amatruda, J.F. (2014) Autophagy is essential for cardiac morphogenesis during vertebrate development. *Autophagy*, **10**, 572–587.
45. Nishida, K. and Otsu, K. (2016) Autophagy during cardiac re-modelling. *J. Mol. Cell. Cardiol.*, **95**, 11–18.
46. Dorn, G.W. II (2016) Parkin-dependent mitophagy in the heart. *J. Mol. Cell. Cardiol.*, **95**, 42–49.
47. Archer, L.D., Langford-Smith, K.J., Bigger, B.W. and Fildes, J.E. (2014) Mucopolysaccharide diseases: a complex interplay between neuroinflammation, microglial activation and adaptive immunity. *J. Inherit. Metab. Dis.*, **37**, 1–12.
48. Schuchman, E.H., Ge, Y., Lai, A., Borisov, Y., Faillace, M., Eliyahu, E., He, X., Iatridis, J., Vlassara, H., Striker, G. and Simonaro, C.M. (2013) Pentosan polysulfate: a novel therapy for the mucopolysaccharidoses. *PLos One*, **8**, e54459.
49. Vanier, M.T. (2010) Niemann–Pick disease type C. *Orphanet J. Rare Dis.*, **5**, 16.
50. Kelly, D.A., Portmann, B., Mowat, A.P., Sherlock, S. and Lake, B.D. (1993) Niemann-Pick disease type C: diagnosis and outcome in children, with particular reference to liver disease. *J. Pediatr.*, **123**, 242–247.
51. Erickson, R.P., Bhattacharyya, A., Hunter, R.J., Heidenreich, R.A. and Cherrington, N.J. (2005) Liver disease with altered bile acid trans- port in Niemann–Pick C mice on a high-fat, 1% cholesterol diet. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **289**, G300–G307.

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

52. Carstea, E.D., Morris, J.A., Coleman, K.G., Loftus, S.K., Zhang, D., Cummings, C., Gu, J., Rosenfeld, M.A., Pavan, W.J. and Krizman, D.B. (1997) Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. *Science*, **277**, 228–231.
53. Loftus, S.K., Morris, J.A., Carstea, E.D., Gu, J.Z., Cummings, C., Brown, A., Ellison, J., Ohno, K., Rosenfeld, M.A. and Tagle, D.A. (1997) Murine model of Niemann–Pick C disease: mutation in a cholesterol homeostasis gene. *Science*, **277**, 232–235.
54. Garver, W.S., Heidenreich, R.A., Erickson, R.P., Thomas, M.A. and Wilson, J.M. (2000) Localization of the murine Niemann–Pick C1 protein to two distinct intracellular compartments. *J. Lipid Res*, **41**, 673–687.
55. Park, W.D., O'Brien, J.F., Lundquist, P.A., Kraft, D.L., Vockley, C.W., Karnes, P.S., Patterson, M.C. and Snow, K. (2003) Identification of 58 novel mutations in Niemann–Pick disease type C: correlation with bio-chemical phenotype and importance of PTC1–like domains in NPC1. *Hum. Mutat.*, **22**, 313–325.
56. Naureckiene, S., Sleat, D.E., Lackland, H., Fensom, A., Vanier, M.T., Wattiaux, R., Jadot, M. and Lobel, P. (2000) Identification of HE1 as the second gene of Niemann-Pick C disease. *Science*, **290**, 2298–2301.
57. Infante, R.E., Wang, M.L., Radhakrishnan, A., Kwon, H.J., Brown, M.S. and Goldstein, J.L. (2008) NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid bilayers, a step in cholesterol egress from lysosomes. *Proc. Natl. Acad. Sci.*, **105**, 15287–15292.
58. Erickson, R.P. (2013) Current controversies in Niemann-Pick C1 disease: steroids or gangliosides; neurons or neurons and glia. *J. Appl. Genet.*, **54**, 215–224.
59. Christian, A.D., Haynes, M.P., Phillips, M.C. and Rothblat, G.H. (1997) Use of cyclodextrins for manipulating cellular cholesterol content. *J. Lipid Res.*, **38**, 2264–2272.
60. Camargo, F., Erickson, R.P., Garver, W.S., Hossain, G.S., Carbone, P.N., Heidenreich, R.A. and Blanchard, J. (2001) Cyclodextrins in the treatment of a mouse model of Niemann-Pick C disease. *Life Sci.*, **70**, 131–142.
61. Saunders, N.R., Liddelow, S.A. and Dziegielewska, K.M. (2012) Barrier mechanisms in the developing brain. *Front. Pharmacol.*, **3**, 46.
62. Liu, B., Ramirez, M., Miller, A.N., Repa, J.J., Turley, S.D. and Dietschy, J.M. (2010) Cyclodextrin overcomes the transport defect in nearly every organ of NPC1 mice leading to excretion of sequestered cholesterol as bile acid. *J. Lipid Res.*, **51**, 933–944.
63. Ory, D.S., Ottinger, E.A., Farhat, N.Y., King, K.A., Jiang, X., Weissfeld, L., Berry-Kravis, E., Davidson, C.D., Bianconi, S., Keener, L.A. *et al.* (2017) Intrathecal 2-hydroxypropyl-beta--cyclodextrin decreases neurological disease progression in Niemann-Pick Disease, type C1: a non-randomized, open--label, phase 1/2 trial. *Lancet*, **390**, 1758–1768.
64. Erickson, R.P. and Fiorenza, M.T. (2017) A hopeful therapy for Niemann-Pick C diseases. *Lancet*, **390**, 1720–1721.
65. Higashi, Y., Murayama, S., Pentchev, P.G. and Suzuki, K. (1993) Cerebellar degeneration in the Niemann-Pick type C mouse. *Acta Neuropathol.*, **85**, 175–184.
66. Deffieu, M.S. and Pfeffer, S.R. (2011) Niemann–Pick type C function requires luminal domain residues that mediate cholesterol-dependent NPC2 binding. *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 18932–18936.
67. Jurevics, H. and Morell, P. (1995) Cholesterol for synthesis of myelin is made locally, not imported into brain. *J. Neurochem.*, **64**, 895–901.
68. Mauch, D.H., Nägler, K., Schumacher, S., Göritz, C., Müller, E.C., Otto, A. and Pfrieger, F.W. (2001) CNS synaptogenesis promoted by glia-derived cholesterol. *Science*, **294**, 1354–1357.
69. Nusca, S., Canterini, S., Palladino, G., Bruno, F., Mangia, F., Erickson, R.P. and Fiorenza, M.T. (2014) A marked paucity of granule cells in the developing cerebellum of the Npc1(-/-) mouse is corrected by a single injection of hydroxypropyl-β-cyclodextrin. *Neurobiol. Dis.*, **70**, 117–126.
70. Caporali, P., Bruno, F., Palladino, G., Dragotto, J., Petrosini, L., Mangia, F., Erickson, R.P., Canterini, S. and Fiorenza, M.T. (2016) Developmental delay in motor skill acquisition in Niemann-Pick C1 mice reveals abnormal cerebellar morphogenesis. *Acta Neuropathol. Commun.*, **4**, 94.
71. Wetts, R. and Herrup, K. (1983) Direct correlation between Purkinje and granule cell number in the cerebella of lurcher chimeras and wild-type mice. *Brain Res.*, **10**, 41–47.
72. Yamada, K. and Watanabe, M. (2002) Cytodifferentiation of Bergmann glia and its relationship with Purkinje cells. *Anat. Sci. Int.*, **77**, 94–108.
73. Porter, J.A., Young, K.E. and Beachy, P.A. (1996) Cholesterol modification of hedgehog signaling proteins in animal development. *Science*, **274**, 255–259.
74. Dehart, D.B., Lanoue, L., Tint, G.S. and Sulik, K.K. (1997) Pathogenesis of malformations in a rodent model for Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.*, **68**, 328–337.
75. Fleming, J.T., He, W., Hao, C., Ketova, T., Pan, F.C., Wright, C.C., Litingtung, Y. and Chiang, C. (2013) The Purkinje neuron acts as a central regulator of spatially and functionally distinct cerebellar precursors. *Dev. Cell*, **27**, 278–292.
76. Bidet, M., Joubert, O., Lacombe, B., Ciantar, M., Nehmé, R., Mollat, P., Brétillon, L., Faure, H., Bittman, R., Ruat, M. and Mus-Veteau, I. (2011) The hedgehog receptor patched is involved in cholesterol transport. *PLos One*, **6**, e23834.
77. Incardona, J.P. and Roelink, H. (2000) The role of cholesterol in Shh signaling and teratogen-induced holoprosencephaly. *Cell Mol. Life Sci.*, **57**, 1709–1719.
78. Wang, W., Wu, T. and Kirschner, M.W. (2014) The master cell cycle regulator APC-Cdc20 regulates ciliary length and disassembly of the primary cilium. *Elife*, **3**, e03083.
79. Xiao, X., Tang, J.-J., Peng, C., Wang, Y., Fu, L., Qiu, Z.-P., Xiong, Y., Yang, L.-F., Cui, H.-W., He, X.-L. *et al.* (2017) Cholesterol modification of smoothened is required for hedgehog signaling. *Mol. Cell*, **66**, 154–162.
80. Cunningham, D., DeBarber, A.E., Bir, N., Binkley, L., Merkens, L.S., Steiner, R.D. and Herman, G.E. (2015) Analysis of hedgehog signalling in cerebellar granule cell precursors in a conditional *Nsdh1* allele demonstrates an essential role for cholesterol in postnatal CNS development. *Hum. Mol. Genet.*, **24**, 2808–2825.
81. Schwend, T., Loucks, E.J., Snyder, D. and Ahlgren, S.C. (2011) Requirement of Npc1 and availability of cholesterol for early embryonic cell movements in zebrafish. *J. Lipid Res.*, **52**, 1328–1344.
82. Liao, G., Yao, Y., Liu, J., Yu, Z., Cheung, S., Xie, A., Liang, X. and Bi, X. (2007) Cholesterol accumulation is associated with lysosomal dysfunction and autophagic stress in $Npc1^{-/-}$ mouse brain. *Am. J. Pathol.*, **171**, 962–975.
83. Pacheco, C.D., Kunkel, R. and Lieberman, A.P. (2007) Autophagy in Niemann-Pick C disease is dependent upon

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

Beclin-1 and responsive to lipid trafficking defects. *Hum. Mol. Genet.*, **16**, 1495–1503.
84. Ordonez, M.P., Roberts, E.A., Kidwell, C.U., Yuan, S.H., Plaisted, W.C. and Goldstein, L.S. (2012) Disruption and therapeutic rescue of autophagy in a human neuronal model of Niemann Pick type C1. *Hum. Mol. Genet.*, **21**, 2651–2662.
85. Meske, V., Erz, J., Priesnitz, T. and Ohm, T.G. (2014) The autophagic defect in Niemann-Pick C neurons differs from somatic cells and reduces neuronal viability. *Neurobiol. Dis.*, **64**, 88–97.
86. Sarkar, S., Carroll, B., Buganim, Y., Maetzel, D., Ng, A.H.M., Cassady, J.P., Cohen, M.A., Chakraborty, S., Wang, H., Spooner, E. *et al.* (2013) Impaired autophagy in the lipid-storage disorder Niemann-Pick C1 disease. *Cell Rep.*, **5**, 1302–1315.
87. Pacheco, C.D., Elrick, M.J. and Lieberman, A.P. (2009) Tau deletion exacerbates the phenotype of Niemann-Pick type C mice and implicates autophagy in pathogenesis. *Hum Mol Genet.*, **18**, 956–965.
88. Elrick, M.J., Yu, T., Chung, D. and Lieberman, A.P. (2012) Impaired proteolysis underlies autophagic dysfunction in Niemann-Pick type C disease. *Hum. Mol. Genet.*, **21**, 4876–4887.
89. Maetzel, D., Sarkar, S., Wang, H., Abi-Mosleh, L., Xu, P., Cheng, A.W., Gao, Q., Mitalipova, M. and Jaenisch, R. (2014) Genetic and chemical correction of cholesterol accumulation and impaired autophagy in hepatic and neural cells derived from Niemann-Pick Type C patient-specific iPS cells. *Stem Cell Rep.*, **2**, 866–880.
90. Lee, H., Lee, J., Park, M., Hong, Y.R., Marti, H.H., Kim, H., Okada, Y., Otsu, M., Seo, E.-J., Park, J.-H. *et al.* (2014) Pathological roles of the VEGF/SphK pathway in Niemann-Pick type C neurons. *Nat. Commun.*, **5**, 5514.
91. Cougnoux, A., Cluzeau, C., Mitra, S., Li, R., Williams, I., Burkert, K., Xu, X., Wassif, C.A., Zheng, W. and Porter, F.D. (2016) Necroptosis in Niemann-Pick disease, type C1: a potential therapeutic target. *Cell Death Dis.*, **7**, e2147.
92. Hailey, D.W., Rambold, A.S., Satpute-Krishnan, P., Mitra, K., Sougrat, R., Kim, P.K. and Lippincott-Schwartz, J. (2010) Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell*, **141**, 656–667.
93. Yu, W., Gong, J.-S., Ko, M., Garver, W.S., Yanagisawa, K. and Michikawa, M. (2005) Altered cholesterol metabolism in Niemann-Pick type C1 mouse brains affects mitochondrial function. *J. Biol. Chem.*, **280**, 11731–11739.
94. Kennedy, B.E., Charman, M. and Karten, B. (2012) Niemann-Pick Type C2 protein contributes to the transport of endosomal cholesterol to mitochondria without interacting with NPC1. *J. Lipid Res.*, **53**, 2632–2642.
95. Charman, M., Kennedy, B.E., Osborne, N. and Karten, B. (2010) MNL64 mediates egress of cholesterol from endosomes to mitochondria in the absence of functional Niemann-Pick Type C1 protein. *J. Lipid Res.*, **51**, 1023–1034.
96. Balboa, E., Castro, J., Pinochet, M.-J., Cancino, G., Matías, N., José Sáez, P., Martínez, A., Álvarez, A.R., Garcia-Ruiz, C., Fernandez-Checa, J.C. and Zanlungo, S. (2017) MNL64 induces mitochondrial dysfunction associated with increased mitochondrial cholesterol content. *Redox Biol.*, **12**, 274–284.
97. Elustondo, P., Martin, L.A. and Karten, B. (2017) Mitochondrial cholesterol import. *Biochem. Biophys. Acta*, **1862**, 90. 900-101.
98. Rone, M.B., Liu, J., Blonder, J., Ye, X., Veenstra, T.D., Young, J.C. and Papadopoulos, V. (2009) Targeting and insertion of the cholesterol-binding translocator protein into the outer mitochondrial membrane. *Biochemistry*, **48**, 6909–6920.
99. Torres, S., Baulies, A., Fucho, R., Garcia-Ruiz, C. and Fernendez-Checa, J.C. (2016) StARD1 overexpression through ACDase downregulation contributes to hepatic mitochondria cholesterol-mediated GSH depletion and liver injury in Niemann-Pick C disease. *Hepataology*, **64**, 361A.
100. Torres, S., Matías, N., Baulies, A., Nuñez, S., Alarcon-Vila, C., Martinez, L., Nuño, N., Fernandez, A., Caballeria, J., Levade, T. *et al.* (2017) Mitochondrial GSH replenishment as a potential therapeutic approach for Niemann Pick Type C disease. *Redox Biol.*, **11**, 60–72.
101. Woś, M., Szczepanowska, J., Pikuła, S., Tylki-Szymańska, A., Zabłocki, K. and Bandorowicz-Pikuła, J. (2016) Mitochondrial dysfunction in fibroblasts derived from patients with Niemann-Pick type C disease. *Arch. Biochem. Biophys.*, **593**, 50–59.
102. Vázquez, M.C., del Pozo, T., Robledo, F.A., Carrasco, G., Pavez, L., Olivares, F., González, M. and Zanlungo, S. (2011) Alteration of gene expression profile in Niemann-Pick type C mice correlates with tissue damage and oxidative stress. *PLoS One*, **6**, e28777.
103. Shiroto, T., Romero, N., Sugiyama, T., Sartoretto, J.L., Kalwa, H., Yan, Z., Shimokawa, H. and Michel, T. (2014) Caveolin–1 is a critical determinant of autophagy, metabolic switching, and oxidative stress in vascular endothelium. *PLoS One*, **9**, e87871.
104. Garver, W.S., Erickson, R.P., Wilson, J.M., Colton, T.L., Hossain, G.S., Kozloski, M.A. and Heidenreich, R.A. (1997) Altered expression of caveolin1 and increased cholesterol in detergent-soluble membrane fractions from liver in mice with Niemann-Pick disease type C. *BBA Mol. Basis Dis.*, **1361**, 272–280.
105. Vilaça, R., Silva, E., Nadais, A., Teixeira, V., Matmati, N., Gaifem, J., Hannun, Y.A., Sá Miranda, M.C. and Costa, V. (2014) Sphingolipid signalling mediates mitochondrial dysfunctions and reduced chronological lifespan in the yeast model of Niemann-Pick type C1. *Mol. Microbiol.*, **91**, 438–451.
106. Lloyd-Evans, E., Morgan, A.J., He, X., Smith, D.A., Elliot-Smith, E., Sillence, D.J., Churchill, G.D., Schuchman, E.H., Galione, A. and Platt, F.M. (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. *Nat. Med.*, **14**, 1247–1255.
107. Shen, D., Wang, X., Li, X., Zhang, X., Yao, Z., Dibble, S., Dong, X.P., Yu, T., Lieberman, A.P., Showalter, H.D. and Xu, H. (2012) Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release. *Nat. Commun.*, **3**, 731.
108. Visentin, S., De Nuccio, C., Bernardo, A., Pepponi, R., Ferrante, A., Minghetti, L. and Popoli, P. (2013) The stimulation of adenosine A2A receptors ameliorates the pathological phenotype of fibroblasts from Niemann-Pick type C patients. *J. Neurosci.*, **33**, 15388–15393.
109. Gatica, D., Lahiri, V. and Klionsky, D.J. (2018) Cargo recognition and degradation by selective autophagy. *Nat. Cell Biol.*, **20**, 233–242.
110. Soubannier, V., McLelland, G.L., Zunino, R., Braschi, E., Rippstein, P., Fon, E.A. and McBride, H.M. (2012) A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr. Biol.*, **22**, 135–141.
111. Wong, Y.C., Ysselstein, D. and Krainc, D. (2018) Mitochondria-lysosome contacts regulate mitochondrial fission via RAB7 GTP hydrolysis. *Nature*, **554**, 382–386.

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018

112. Choudhury, A., Dominguez, M., Puri, V., Sharma, D.K., Narita, K., Wheatley, C.L., Marks, D.L. and Pagano, R.E. (2002) Rab proteins mediate Golgi transport of caveola-internalized glycosphingolipids and correct lipid trafficking in Niemann-Pick C cells. *J. Clin. Invest.*, **109**, 1541–1550.
113. Area-Gomez, E. and Schon, E.A. (2017) On the Pathogenesis of Alzheimer's disease: the MAM hypothesis. *FASEB J.*, **31**, 864–867.
114. Erickson, R.P., Larson-Thome, K., Weberg, L., Szybinska, A., Mossakowska, M., Stycyznska, M., Barcikowska, M., Zekanowski, C. and Kuznicki, J. (2008) Variation in NPC1, the gene encoding Niemann-Pick C1, a protein involved in intracellular cholesterol transport, is associated with Alzheimer disease and/or aging in the Polish population. *Neurosci. Lett.*, **447**, 153–157.
115. Fiorenza, M.T., Dardis, A., Canterini, S. and Erickson, R.P. (2013) Cholesterol metabolism-associated molecules in late onset Alzheimer's disease. *J. Biol. Regul. Homeost. Agents*, **27**, 23–35.
116. Baixauli, F., Acín-Pérez, R., Villarroya-Beltrí, C., Mazzeo, C., Nuñez-Andrade, N., Gabandé-Rodriguez, E., Ledesma, M.D., Blázquez, A., Martin, M.A., Falcón-Pérez, J.M. *et al.* (2015) Mitochondrial respiration controls lysosomal function during inflammatory T cell responses. *Cell Metab.*, **22**, 485–498.
117. Castellano, B.M., Thelen, A.M., Moldavski, O., Feltes, M., van der Welle, R.E.N., Mydock-McGrane, L., Jiang, X., van Eijkeren, R.J., Davis, O.B., Louie, S.M. *et al.* (2017) Lysosomal cholesterol activates mTORC1 via an SLC38A9-Nimann-Pick C1 signaling complex. *Science*, **355**, 1306–1311.
118. Erickson, R.P. and Bernard, O. (2002) Studies on neuronal death in the mouse model of Niemann-Pick C disease. *J. Neurosci. Res.*, **68**, 738–744.
119. Park, M.H., Lee, J.Y., Jeong, M.S., Jang, H.S., Endo, S., Bae, J.S. and Jin, H.K. (2018) The role of Purkinje cell-derived VEGF in cerebellar astrogliosis in Niemann-Pick type C mice. *BMB Rep.*, **51**, 79–84.
120. Seo, Y., Kim, H.-S., Kang, I., Choi, S.W., Shin, T.-H., Shin, J.-H., Lee, B.-C., Lee, J.Y., Kim, J.-J., Kook, M.G. and Kang, K.-S. (2016) Cathepsin S contributes to microglia–mediated olfactory dysfunction through the regulation of Cx3cl1–Cx3cr1 axis in a Niemann–Pick diseasae type C1 model. *Glia*, **64**, 2291–2305.
121. Bascunan-Castillo, E.C., Erickson, R.P., Howison, C.M., Hunter, R.J., Heidenreich, R.H., Hicks, C., Trouard, T.P. and Gillies, R.J. (2004) Tamoxifen and vitamin E treatments delay symptoms in the mouse model of Niemann-Pick C. *J. Appl. Genet.*, **45**, 461–467.
122. Borbon, I.A., Hillman, Z., Duran, E., Jr., Kiela, P.R., Frautschy, S.A. and Erickson, R.P. (2012) Lack of efficacy of curcumin on neurodegeneration in the mouse model of Niemann-Pick C1. *Pharmacol. Biochem. Behav.*, **101**, 125–131.
123. Smith, D., Wallom, K.-L., Williams, I.M., Jeyakumar, M. and Platt, F.M. (2009) Beneficial effects of anti–inflamatory therapy in a mouse model of Niemann–Pick disease type C1. *Neurobiol. Dis.*, **36**, 242–251.
124. Seo, Y., Kim, H.-S., Shin, Y., Kang, I., Choi, S.W., Yu, K.-R., Seo, K.-W. and Kang, K.-S. (2014) Excessive microglial activation aggravates olfactory dysfunction by impeding the survival of newborn neurons in the olfactory bulb of Niemann-Pick disease type C1 mice. *Biochim. Biophys. Acta*, **1842**, 2193–2203.

Downloaded from https://academic.oup.com/hmg/advance-article-abstract/doi/10.1093/hmg/ddy155/4990256
by Università di Padova - Dipartimento di Scienze dell'Educazione user
on 05 June 2018