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Review

# Modeling neuronopathic storage diseases with patient-derived culture systems

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A B S T R A C T

Lysosomes are organelles involved in the degradation and recycling of macromolecules, and play a critical role in sensing metabolic information in the cell. A class of rare metabolic diseases called lysosomal storage disorders (LSD) are characterized by lysosomal dysfunction and the accumulation of macromolecular substrates. The central nervous system appears to be particularly vulnerable to lysosomal dysfunction, since many LSDs are characterized by severe, widespread neurodegeneration with pediatric onset. Furthermore, variants in lysosomal genes are strongly associated with some common neurodegenerative disorders such as Parkinson's disease (PD). To better understand disease pathology and develop novel treatment strategies, it is critical to study the fundamental molecular disease mechanisms in the affected cell types that harbor endogenously expressed mutations. The discovery of methods for reprogramming of patient-derived somatic cells into induced pluripotent stem cells (iPSCs), and their differentiation into distinct neuronal and glial cell types, have provided novel opportunities to study mechanisms of lysosomal dysfunction within the relevant, vulnerable cell types. These models also expand our ability to develop and test novel therapeutic targets. We discuss recently developed methods for iPSC differentiation into distinct neuronal and glial cell types, while addressing the need for meticulous experimental techniques and parameters that are essential to accurately identify inherent cellular pathologies. iPSC models for neuronopathic LSDs and their relationship to sporadic age-related neurodegeneration are also discussed. These models should facilitate the discovery and development of personalized therapies in the future.

## 1. Introduction

Lysosomal storage disorders are a group of rare, inherited diseases that are caused by the dysfunction of lysosomal proteins leading to accumulation of specific substrates by which LSDs are categorized. LSDs can originate from deficiencies in hydrolases, channel or membrane proteins, cofactors, or trafficking components that deliver lysosomal proteins (summarized in Fig. 1). The majority of LSDs demonstrate neurodegeneration as a prominent feature (Wraith, 2002), indicating the sensitivity of neurons toward dysfunctional cellular clearance. Due to recently discovered genetic and biochemical similarities between rare LSDs and common neurodegenerative disorders, such as the link between Gaucher disease (GD) and Parkinson's disease (Pitcairn et al., 2018), there have been focused efforts on using LSD models as simplified systems to study general neurodegenerative mechanisms and the relationship to sporadic neurodegenerative diseases characterized by complex etiology. Below we summarize some of the methods that can be used to differentiate disease-specific iPSCs into appropriate neuronal or oligodendroglia cell types that match the pathology of LSDs, and review recent studies employing these methods to discover novel phenotypes.

## 2. Cellular culture models of neurodegenerative disease

### 2.1. Immortalized and human primary culture models

The discovery of methods to reprogram somatic cells into pluripotent stem cells has opened several opportunities for the study of rare monogenic diseases of the nervous system, as well as common neurodegenerative disorders. Prior to the discovery of methods to generate iPSCs, human culture systems were limited to the use of immortalized cell lines that were obtained from tumor biopsies, or primary cells from patients that had been genetically transformed *in vitro* to induce immortality. Although these models are valuable tools in some respects, they are limited for the study of disease mechanisms by the presence of genetic and epigenetic aberrations that occur as a result of prolonged exposure to culture conditions, unstable karyotypes, and the expression of oncogenes that may complicate phenotype identification. Models of neurodegenerative diseases using immortalized neuronal cell lines are often generated by artificially manipulating a disease-linked gene through transgenic modification, knock-in or knock-out, using recombinant DNA technology. For example, mutations that result in loss-of-function, as often occurring in lysosomal disorders, could be modeled

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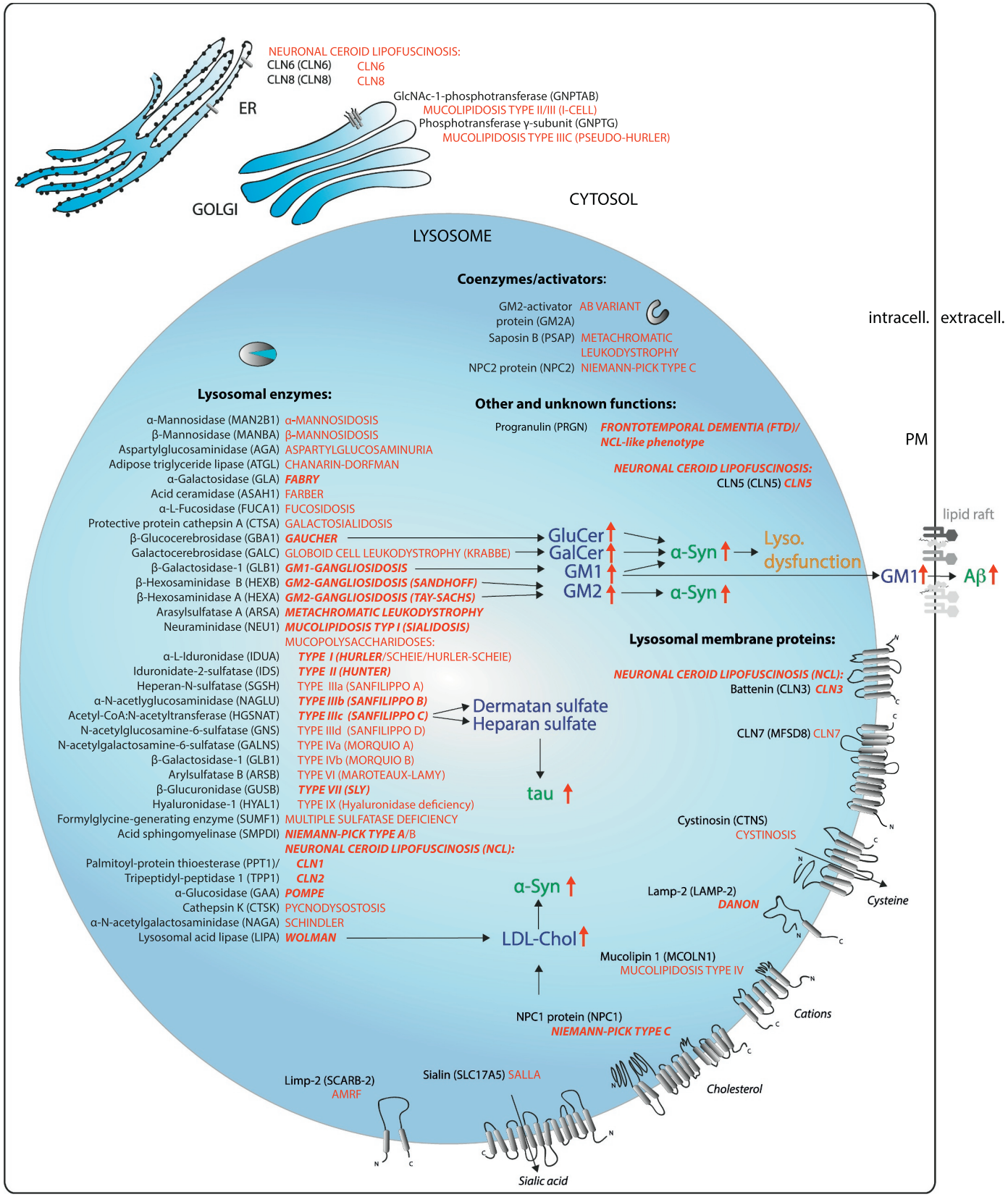


**Fig. 1.** Overview of LSDs, their affected proteins and localization within the cell organelles.
Name of lysosomal storage diseases are depicted in red and the respective dysfunctional proteins in black; in brackets: gene name. Most LSDs are caused by mutations in lysosomal enzymes, but mutations are also found in lysosomal membrane proteins, coenzymes and in proteins, which functions are not well understood to date (*e.g.* PGRN, CLN3, CLN5). Molecules described to be transported *via* the lysosomal membrane by their respective transporter/channel are indicated in italic writing. Accumulating substrates are shown in blue, resulting in aggregation of a-synuclein (a-syn), amyloid-beta (A*β*) and tau (all in green). Respective iPSC models have been established for diseases that appear in bold/italic font. PM, plasma membrane; GluCer, glucosylceramide; GalCer, galactosylceramide; LDL-Chol, LDL-Cholesterol.

by knocking out the gene of interest and studying the downstream cellular pathologies. Putative gain-in-toxic function mutations could be modeled by transgenic overexpression of the disease linked gene, such as a-synuclein (a-syn) accumulation in PD (Lazaro et al., 2017; Polymeropoulos et al., 1997; Spillantini et al., 1997), tau accumulation or amyloid-beta (a-beta) production that occurs in frontotemporal dementia or Alzheimer's disease (Hardy and Higgins, 1992; Mann et al., 1992). While these studies have led to important clues into disease pathophysiology, one limitation is that artifacts may arise through unnatural genetic manipulations. This could be particularly true in protein aggregation or storage diseases, where dramatic overexpression of disease-linked proteins is often required to force artificial protein aggregation. This may result in phenotypes that are not associated with the human disease, by changing the kinetic requirements of protein aggregation into an unnatural time course and dramatically accelerating disease progression. This presents the possibility of aggregate formation in cellular locations where they would not otherwise form, or force protein-protein interactions that would not occur in the disease state. In diseases caused by loss-of-function mutations, which often occurs in LSDs, an unstable protein is produced that gets rapidly degraded by the proteasome *via* endoplasmic reticulum-associated degradation (Asano et al., 2000; Ron and Horowitz, 2005; Tropak et al., 2004; Zhang et al., 2000). This may activate stress pathways involved in the unfolded protein response (UPR) and cause endoplasmic reticulum stress (Sano et al., 2009; Tessitore et al., 2004; Vitner et al., 2010). Culture systems that attempt to model loss-of-function mutations by gene knock-out, while useful to study the function of the gene, will not induce these stress responses and therefore will not be capable of modeling all aspects of disease pathogenesis.

In addition to experimentally induced genetic aberrations, the metabolic state and division rates of the cell line to be used for modeling must be carefully considered in the study of storage or protein aggregation diseases. In immortalized cell lines, it can be difficult to detect mature, insoluble protein aggregates or accumulation of other storage materials due to the dilution effects that can occur through rapid cell division. Dividing cell culture models generated from lines such as Hela or HEK293 cells that undergo rapid division may not have the opportunity to accumulate macromolecules as they would in human disease. Phenotypes of neurodegenerative diseases are often dependent on protein structural changes from physiological forms into misfolded, aggregated oligomers or fibrils. These structural changes can be dictated by many factors, however elevated protein concentration is one of the most critical elements (Jarrett and Lansbury, 1993). Due to rapid degradation rates or cell division, protein levels may not surpass the concentration thresholds required for aggregation and subsequent down-stream toxicity. Neuronal cells have dramatically different baseline metabolic rates compared to immortalized cell lines, and this characteristic may alter the levels of protein or other toxic material stored within the cell, rendering neurons vulnerable to lysosomal dysfunction.

LSDs are often studied in patient-derived primary fibroblasts. These models have the advantage of harboring the natural disease causing mutations in a genetic background that is permissive for disease, and can be utilized to study essential disease phenotypes that may be common to all cell types. Fibroblast models of LSDs that are caused by destabilizing mutations in proteins resulting in loss-of-function can provide a useful system to explore therapies aimed at improving protein stability and function of these enzymes, such as molecular chaperones (Valenzano et al., 2011). As with cell lines, one limitation of fibroblast models is related to metabolic differences as compared to neuronal cell types that are effected in disease. Storage phenotypes can be observed in fibroblast models of some LSDs, however it is possible that cell division mitigates or changes these phenotypes that would otherwise be prominent in neurons. For example, previous studies of GD fibroblasts have indicated that the glucocerebrosidase substrate, glucosylceramide, does not accumulate to a significant degree in patient fibroblasts (Sasagasako et al., 1994). This is significantly different compared to more recently developed iPSC-derived models of the effected cell types in GD, including neuronal or macrophage models, where lipid storage is prominent (Aflaki et al., 2014; Mazzulli et al., 2016a; Schöndorf et al., 2014; Zunke et al., 2018). Furthermore, there have been differences in pathogenic phenotypes observed in fibroblast or neuronal models, such as the observation of UPR-related stress in GD fibroblasts but not neurons (Farfel-Becker et al., 2009; Wei et al., 2008). This underscores the importance of utilizing a model system of the appropriate disease-related cell type to study the downstream pathologies of storage diseases.

Differentiation of immortalized cell lines can be utilized to partially circumvent complications that may arise from cell division. Early studies on the development of clonal cell lines with neuronal properties were developed by the addition of growth factors, such as nerve growth factor (NGF), that is capable of inducing neurites (Dichter et al., 1977; Greene and Tischler, 1976). NGF treatment of rat pheochromocytoma cell lines (PC12) could cease cell division and induce several features that are similar to neuronal cells including the synthesis, storage, and regulated release of neurotransmitters, along with electrical activity (Dichter et al., 1977). Similarly, the differentiation of human neuroblastoma cell lines, such as SH-SY5Y, can be achieved by treatment with retinoic acid, which induces the expression of tyrosine kinase receptors for brain derived growth factor (BDNF) and other trophic factors to bind (Kaplan et al., 1993). These studies have contributed toward our understanding of neuronal differentiation and development, as well as having provided more accurate culture models that recapitulate certain features of neurons.

Developmental studies in animal models have greatly accelerated our understanding of the factors required to induce the differentiation of cells, and have helped to refine *in vitro* human neuronal culture models. These have led to the discovery of bone morphogenic protein (BMP) inhibitors, such as noggin (Lamb et al., 1993), as critical in the direct induction of neural development. Similarly, other key studies found that sonic hedgehog (SHH) and fibroblast growth factor (FGF) act as patterning factors that could drive the development of midbrain dopamine and serotonin neurons (Andersson et al., 2006; Ye et al., 1998). Seminal work using mouse embryonic stem cells showed that isolation and application of key developmental factors to cultures could induce specific types of neural cells, including the development of dopamine (Kim et al., 2002) or motor neurons (Wichterle et al., 2002), providing a foundation for translating these studies into human cells. Soon after the establishment of human embryonic stem cell (hESC) lines (Thomson et al., 1998), these methods were further refined and applied to induce the differentiation of human neuronal subtypes including dopaminergic (Perrier et al., 2004), GABA and glutamatergic (Reubinoff et al., 2001), as well as motor neurons (Li et al., 2005). Subsequent to the discovery of methods to induce pluripotency from human somatic cells (Takahashi et al., 2007), it was found that similar differentiation methods used for hESCs could also be applied to induced pluripotent stem cells (iPSCs) (Dimos et al., 2008; Ebert et al., 2009; Wernig et al., 2008). This opened up novel opportunities to generate patient-specific models of disease that employ specific cell types matching those affected in the corresponding diseases.

### 2.2. iPSC differentiation protocols to generate neuronal cell types and oligodendrocytes

Significant advances in the efficiency of neuralization from iPSCs have been made in the past ten years that have contributed to robust and highly reproducible differentiation protocols. Based on previous development studies *in vivo* (Camus et al., 2006; Valenzuela et al., 1995), it was found that combined inhibition of SMAD signaling with simultaneous BMP inhibition utilizing isolated noggin, and inhibition of the Lefty / Activin / TGFβ pathways with SB431542, could result in near-complete differentiation of iPSCs into neural lineages (Chambers

**Table 1**
Differentiation methods of pluripotent cells and their potential utility in modeling lysosomal and neurodegenerative diseases.

| Cell type | Method | Relevant disease | Citation |
|---|---|---|---|
| Basal Forebrain Cholinergic | Trk B (RA)-Neurosphere ➔ SHH/FGF8 + BMP9 | AD, NP-C, GD, FTD | (Bissonnette et al., 2011) |
| Hippocampal (DG and CA3) | Wnt/BMP/TGFβ/SHH inhib ➔ Wnt3a/BDNF/astrocytes | AD, NCL, Epilepsy, SCHZ | (Sarkar et al., 2018; Yu et al., 2014b) |
| Cortical Excitatory (Glu) | Ngn2 overexpression ➔ puro selection | AD, PD, NCL, Tay Sachs, FTD | (Zhang et al., 2013) |
| Midbrain DAergic | dual SMAD inhib ➔ SHH/FGF8/Wnt ➔ BAGTC + D | PD, NCL, GD | (Kriks et al., 2011) |
| DAergic (non-region specific) | EB / spherical neural mass | PD, NCL, GD | (Cho et al., 2008) |
| Motoneurons | Dual SMAD inhib ➔ Trk B (RA) ➔ BASF | ALS | (Chambers et al., 2009; Li et al., 2005) |
| Purkinje cells | Dual SMAD inhib ➔ FGF8/Wnt1, mouse granule cells | NCL, NP-C, a-Man, SCA | (Sundberg et al., 2018) |
| Purkinje cells | Dual SMAD inhib ➔ SHH inhib ➔ FGF8/Wnt1/Neph3 purif. | NCL, NP-C, a-Man, SCA | (Muguruma et al., 2010) |
| Forebrain GABAergic Interneurons | Dual SMAD ➔ Wnt (tankyrase) ➔ SHH | SCHZ, autism | (Maroof et al., 2013) |
| Hypothalamic Neurons | Dual SMAD ➔ Wnt (tankyrase) ➔ delayed SHH | | (Maroof et al., 2013) |
| Striatal Medium Spiny GABAergic Neurons | Dual SMAD inhib / SHH (purm) of EBs | Htt, GM1 | (Stanslowsky et al., 2016) |
| Oligodendroglia | Dual SMAD inhib ➔ SHH / Trk B (RA) ➔ T3 ➔ SHH, IGF-1, NT-3, cAMP | Krabbe, GM1, MLD, Wolman, NPC | (Najm et al., 2011) |

Abbreviations:
*Diseases*: AD, Alzheimer's disease; PD, Parkinson's disease; ALS, amyotrophic lateral sclerosis; NP-C, Niemann-Pick type C; NCL, Neuronal ceroid lipofuscinosis; GD, Gaucher disease; Htt, Huntington's disease; a-Man, alpha-mannosidosis; SCHZ, schizophrenia; GM1, GM1 gangliosidosis; SCA, spinocerebellar ataxia; MLD, metachromatic leukodystrophy; FTD, frontotemporal dementia;
*Differentiation factors*: Trk B, tyrosine kinase B; RA, retinoic acid; EB, embryoid bodies; SHH, sonic hedgehog; purm, purmorphamine; puro, puromycin; T3, Thyroid hormone T3; BAGTC + D: BDNF, Ascorbic acid, GDNF, TGFb, cAMP, DAPT; BASF: BDNF, Ascorbic acid, SHH, FGF8a; inhib, inhibition; purify, purification by cell sorting.
*Cell types*: DG, dentate gyrus; Glu, glutamatergic; DA, dopaminergic.

et al., 2009). Importantly, the neural stem cells generated by dual SMAD inhibition demonstrated patterning capabilities when stimulated with SHH and FGF8 into dopaminergic neurons, or even into motor neurons with the addition of retinoic acid. Subsequently, more precise methods to develop specific midbrain type dopaminergic neurons that co-express markers FOXA2 and LMX1a were discovered. This was achieved by developing floor-plate precursors through early exposure to SHH (Fasano et al., 2010) followed by activation of Wnt signaling (Cooper et al., 2010; Kriks et al., 2011), which was known to be important for midbrain DA development *in vivo* (Joksimovic et al., 2009). These advances have not only been critical in the field of regenerative medicine for cell replacement therapies where dopamine neurons degenerate, like in PD and GD, but have also provided ways to develop scalable levels of nearly homogenous populations of midbrain dopamine neurons from patients to study in the laboratory. These methods have dramatically facilitated the study of disease phenotypes by expanding the utility of models for use in both single cell-type analysis as well as other biochemical studies that require significant amounts of patient-derived material.

In addition to midbrain-type dopamine neurons, the development of other types of neurons has been achieved through distinct differentiation methods of iPSCs (Table 1). Basal forebrain cholinergic neurons, critical for modeling diseases characterized by dementia, have been generated by treating neural stem cells that are pre-patterned with SHH / FGF8 and BMP9 (Bissonnette et al., 2011). This protocol was derived from the fact that these factors were shown to be important for the development of cholinergic neurons *in vivo* (Lopez-Coviella et al., 2000). A simplified method to generate excitatory cortical neurons from hESCs or iPSCs is based on overexpression of a single transcription factor, either neurogenin-2 (ngn2) or NeuroD1. Fusing ngn2 to a puromycin resistant gene allowed for the selection of pure populations of neurons, which results in a homogenous population of neurons and greatly facilitates biochemical studies or other assays that cannot be done on a single-cell level. One of the limitations of iPSC-derived neurons is the apparent lack of functional synapses that are formed *in vitro*. However, it was found that co-culturing ngn2-induced neurons with mouse glial cells could faithfully induce neurons with pre- and post-synaptic features, providing a more physiologically relevant model of human neurons (Zhang et al., 2013).

The generation of inhibitory forebrain GABAergic interneurons from iPSCs has been accomplished through dual SMAD inhibition followed by tankyrase inhibition to block Wnt signaling (Maroof et al., 2013; Nicholas et al., 2013), which can be useful for the study of psychiatric disorders including schizophrenia and autism. Distinct types of ventral progenitors could be further resolved simply by changing the timing of SHH addition to the media, allowing for the generation of neural progenitors with characteristics of ganglion eminence, preoptic, or hypothalamic type cells (Maroof et al., 2013). Medium spiny GABAergic neurons have also been developed through similar protocols that involve dual SMAD inhibition / Wnt antagonist treatment of embryoid bodies, followed by ventral patterning with purmorphamine, an agonist for the SHH receptor Smoothened (Stanslowsky et al., 2016). These cultures exhibited several features of medium spiny neurons including the expression of glutamate decarboxylase (GAD67) in 80% of neurons and DARPP32 expression in ~40% of neurons (Stanslowsky et al., 2016). Methods to develop medium spiny neurons will be useful for the study of several neurodegenerative diseases that involve the basal ganglia, including Huntington's disease and adult onset GM1 gangliosidosis.

Relevant to the development of iPSC-neuronal models for epilepsy,

depression, schizophrenia, or Alzheimer's disease, protocols have been developed to produce dentate gyrus (DG) hippocampal cultures. This occurs by first producing telencephalic precursors through blocking Wnt, BMP, and TGFβ pathways, along with SHH antagonists (Watanabe et al., 2005). These precursors can be further differentiated into mature DG neurons by treating with Wnt3a and BDNF and co-culturing with human astrocytes (Yu et al., 2014b), factors that were previously shown to be required for DG development in animals (Erickson et al., 2010; Lee et al., 2000). More recently, iPSC-derived neurons were shown to be capable of forming functional connections that model the mossy fiber pathway of the hippocampus (Sarkar et al., 2018). This was achieved by differentiating iPSCs into CA3 type neurons through a protocol similar to that of DG neurons, except that lower levels of Wnt3a were used to mimic the Wnt3a gradient observed *in vivo* (Sarkar et al., 2018). CA3 cultures exhibited functional capabilities including electrophysiological properties consistent with evoked and spontaneous action potentials, and the ability to form connections with DG cultures when plated in microfluidic devices (Sarkar et al., 2018). This model system was employed to uncover novel disease-linked phenotypes of schizophrenia-derived cultures related to changes in neuronal activity and connectivity (Sarkar et al., 2018). This shows how patient-derived neuronal cell models, which mimic the neural connectivity features observed in the brain, can reveal new phenotypes in neurological diseases. The development of these protocols from various groups has greatly accelerated our ability to produce specific neuronal subtypes that are relevant to individual diseases, and should produce more accurate models to study downstream phenotypes in the future.

In the majority of neurodegenerative diseases, selective regions of the nervous system appear to be more vulnerable or degenerate at accelerated rates. One neuronal cell type that appears to be particularly susceptible to lysosomal dysfunction and storage are Purkinje cells of the cerebellum. For example, ataxia observed in many LSDs including Niemann Pick type C, alpha-mannosidosis and Batten's disease, are thought to primarily occur from degeneration of the cerebellum, although other brain regions also degenerate in these diseases. Robust methods for the efficient development of Purkinje cells have been more challenging compared to other cell types. However, some success has been achieved through the use of methods that recapitulate the environment of the developing midbrain / hindbrain junction that express patterning factors FGF8 and Wnt1, that are critical for cerebellar development (Muguruma et al., 2010; Simeone, 2000). In this system, inhibition of SHH was used to promote dorsal specification and prevent the patterning of dopaminergic or 5HT type neurons. Purification was done by cell sorting for Neph3 (Mizuhara et al., 2010; Muguruma et al., 2010), a plasma membrane protein selective for Purkinje cells (Mizuhara et al., 2010). Other protocols have been developed that utilize the dual SMAD inhibition strategy to generate neural precursors, followed by Wnt1 activation and FGF8b treatment (Sundberg et al., 2018). Subsequent cell sorting using the thyroid hormone receptor was found to achieve 60–90% of neurons expressing Purkinje cell phenotypes when co-cultured with mouse granule cells (Sundberg et al., 2018).

Critical for the study of neurodegenerative storage disorders that involve myelin dysfunction, including Krabbe disease, GM1 gangliosidosis and metachromatic leukodystrophy, are differentiation protocols for the development of oligodendrocytes from disease-specific iPSCs. Early studies demonstrated that oligodendrocyte progenitor cells (OPCs) could be efficiently generated from mouse epiblast stem cells. As with many of the protocols used for generating neurons from iPSCs, neuroectoderm was first generated by dual SMAD inhibition followed by patterning with SHH and retinoic acid to mimic developmental cues of the ventral ventricular zone of the neural tube (Najm et al., 2011; Orentas and Miller, 1996). OPCs generated by this method expressed specific markers important for oligodendrocyte development including Olig2 and Nkx2.2, and could be expanded for several passages in the presence of SHH, PDGF, and FGF. Differentiation of OPCs into functional oligodendrocytes with myelination capabilities occurred through the addition of thyroid hormone T3, which was previously shown to be critical for oligodendrocyte differentiation (Barres et al., 1994), along with a cocktail of growth factors including SHH, IGF-1, NT-3, and cAMP (Najm et al., 2011). Subsequent studies demonstrated that these differentiation protocols could also be used for the development of human OPCs and oligodendrocytes derived from iPSCs or ESCs, and have aided in the discovery of compounds capable of stimulating remyelination (Najm et al., 2015). Oligodendrocytes derived from human iPSCs were found to be capable of restoring the myelination of a mouse model with genetic hypomyelination that occurs from a deletion in myelin basic protein (Wang et al., 2013). The majority of oligodendrocyte differentiation protocols are longer compared to neuralization protocols, involving up to 150 days in culture. More recent studies have shown that modifications can accelerate these protocols to 75 days (Douvaras et al., 2014). These studies demonstrate the potential of iPSC-derived oligodendrocytes in regenerative medicine of demyelinating disorders, however further studies are required that address disease-specific phenotypes in order to discover novel therapeutic pathways.

In summary, the development of different human cell culture models used to study and phenocopy neurodegenerative diseases over the past several decades has dramatically evolved from simple, immortalized and primary cell lines, into human derived iPSC-neurons that harbor naturally occurring, disease causing mutations. The establishment of iPSCs from human somatic cells and their differentiation potential into various cell types of the CNS have opened novel opportunities that provide more accurate models of neurodegenerative disorders including neuronopathic LSDs. We provide a summary of established protocols for differentiation of iPSCs into various neuronal and glial cell types and their potential applications to different diseases (Table 1). In the future, employing these strategies and model systems will aid in our understanding of disease etiology and contribute to the discovery of novel therapeutic strategies.

## 3. Identifying inherent cellular pathologies in patient-specific iPSC models

A major challenge in identifying clear phenotypes from iPSC-based disease models comes from the variability that can be observed between lines from distinct individuals, between clones of lines from the same individual, and between the same clones of different cell passages. This is particularly important when measuring dynamic processes that are related to the metabolic state of the cell such as autophagic flux, lysosomal enzyme activity, and the turnover of macromolecular substrates. The multiple levels of variability that are possible in these systems underscore the importance of careful study design with appropriate number of lines, technical replicates, and normalization methods. These parameters often depend on the robustness and reproducibility of the assay that is utilized to identify phenotypes, which can only be determined after baseline variabilities are established in preliminary studies. The recent progress in genome editing using zinc finger nucleases or CRISPR/Cas9 systems have greatly accelerated our ability to produce more accurate disease models from iPSCs, where even subtle phenotypes can be uncovered. The generation of isogenic control lines accounts for variations in genetic backgrounds between patients with the same disease-causing mutation. Depending on the severity of the mutation on protein function, and if heterozygotes carriers are to be analyzed, it is essential to include isogenic corrected lines in the study design.

### *3.1. Quality control analyses to ensure culture integrity and comparability of iPSC-neurons*

In iPSC models of LSDs and other neurodegenerative disorders characterized by protein accumulation, assessing the biochemical

nature of the storage material, as well as the functionality of cellular clearance pathways, can provide important insights into pathogenic mechanisms. However, prior to any attempts at identifying cellular phenotypes between patient and control lines, the culture quality and integrity should be firmly established for each culture batch. This will help to ensure that any differences observed in cellular function or morphology occurs as a result of the disease-causing mutation, as opposed to differences in culture populations or differentiation efficiencies. For example, differences in neuronal development, neurite extension, and synapse number can unintentionally occur by simple technical variations. This might include inadvertent changes in culture density that result from differences in the way that neurons attach to a substrate, or subtle differences in media volume that alter oxygen exposure to cells. Although the precise reasons for developmental variability of iPSCs *in vitro* are unknown, it could partly be due to the fact that once developed, neurons rely on autocrine growth factors such as BDNF that can amplify neurite outgrowth and maintain viability *in vitro* (Cheng et al., 2011). Simple measures can be made to test culture integrity and neuralization efficiencies, such as quantification of neuron-specific markers β-iii-tubulin, synaptophysin, or synapsin, and comparing their levels to a ubiquitously expressed protein such as GAPDH or alpha-tubulin by western blot. If unequal neuralization occurs during initial differentiation stages, or if the culture population changes during the incubation, this will be evident in the ratios of neuronal / non-neuronal markers. It will then be difficult to compare control and patient lines using biochemical assays such as western blotting, or any other assay that requires a cell homogenate. It is important to note that even using isogenic matched pairs does not guarantee equal neuralization, as the efficiency of differentiation protocols utilized are often sensitive to technical variations noted above. For example, it was previously shown that at the initial stages of iPSC differentiation, cells seeded at high density and treated with dual SMAD inhibitors could efficiently generate neural precursors capable of patterning, while those seeded at lower density could lead to a mix of neural precursors and other cell types with neural crest fates including melanocytes (Chambers et al., 2009). Therefore, if two distinct iPSC lines are to be compared in an experiment but are seeded at different densities, it is possible to inadvertently achieve different types of cell populations. Some studies have eliminated these concerns by showing that fluorescence assisted cell sorting could be employed to generate pure populations of neurons (Schöndorf et al., 2014). Furthermore, there are simple differentiation methods for some neuron types, such as the cortical-Ngn2 method (Zhang et al., 2013), that involves puromycin selection to assure that only neuronal cells are present. These methods provide an example of ways to overcome inherent variability of iPSC systems, and future studies should be focused on developing iPSC-differentiation methods for additional neuronal subtypes that are simple and reproducible. However, most of the current iPSC differentiation protocols are complex and require exposure to distinct types of media that contain defined and undefined factors. Cells are exposed to different culture vessels that are coated with various substrates such as Matrigel, which can show lot variations, or defined factors such as poly-D-lysine, polyornithine, or laminin. These protocols provide multiple opportunities to introduce technical variations that could ultimately culminate into dramatic differences at the cellular level. As an experimentalist, it is therefore critical to carry out these culture procedures in a meticulous manner, using consistent reagents and materials, to help eliminate batch variations that could interfere with identifying cellular pathologies in patient cells.

### 3.2. Analysis methods for the study of iPSC models of LSDs and neurodegenerative disorders

Multiple methods can be employed to study the functionality of cellular clearance pathways, which may be related to the development of cellular pathology in neurodegenerative disorders and LSDs. One powerful method to determine functionality of the lysosomal system is a proteolytic pulse-chase assay, since it can be done in living neuronal cultures and therefore eliminate potential artifacts that could be obtained in measuring hydrolase activities from lysates. For example, activity measurements in lysates *in vitro* can be informative, however they typically involve the use of buffers and cofactors that artificially optimize enzyme activity, which may be different from the environment of an intact cell. Cell-permeable, artificial substrates that fluoresce upon cleavage and accumulate within lysosomes can be used to measure the activity of specific hydrolases, such as 5-PentafluorobenzoylaminoFluorescein Di-β-D-Glucopyranoside (PFB-FD-Glu) to measure glucocerebrosidase activity (Steet et al., 2006). Measuring the response to lysosomal inhibitors such as bafilomycin A1, and quantifying the change observed, can provide a more robust and reproducible phenotype compared to measuring total enzyme activities, as we have previously shown in phenotyping Parkinson's disease midbrain neurons (Mazzulli et al., 2016a). Using this method also enables one to obtain activities that occur outside of lysosomes resulting from problems in protein trafficking or enzymes leaking outside of lysosomes, such as what has been shown to occur in different models of neurodegenerative disease (Abeliovich and Gitler, 2016). An additional factor to consider in quantifying lysosomal activity from iPSC neurons is the normalization method. There are methods available that allow for the selective quantification of lysosomes in living cultures, and some of these reagents are insensitive to changes in lysosomal pH such as dextran blue. Normalizing activity to lysosomal mass with dextran blue as opposed to total cell number can uncover distinct phenotypes that may arise from differences in lysosomal biogenesis, size, or efficiency. Together with complimentary imaging assays such as confocal immunofluorescence or electron microscopy to directly visualize lysosomes and related organelles, these methods can be powerful tools to study changes in the lysosomal system.

A soundly documented feature of LSDs and neurodegenerative disorders is the accumulation of storage material. Depending on the enzyme or pathway that is deficient in a particular disease, storage material can be detected and quantified using reagents selective for certain accumulating metabolites. For example, oligosaccharide accumulations that include glycogen, or glyco-containing proteins or lipids, can be measured with periodic acid-schiff staining (Fairbanks et al., 1971). Specific oligosaccharides can be detected *in situ* or as protein-bound moieties with the use of lectins such as concanavalin A, which detects internal or terminal a-D-mannose or glucose residues, or wheat germ agglutinin that detects *N*-acetyl-D-glucosamine and sialic acid. Lipophilic accumulations that occur as a result of deficiencies in lipid-metabolizing enzymes can be accurately quantified through mass spectrometry, which has the advantage of uncovering distinct sphingolipid subspecies that differ by fatty acid chain lengths (Sullards et al., 2011). Other lipophilic dyes exist that are capable of detecting neutral lipid accumulations in fixed cultures such as Sudan Black B or BODIPY probes. Cholesterol accumulation that occurs in Niemann-Pick diseases can be detected by cytochemical staining techniques using filipin, a highly fluorescent compound with specific cholesterol-binding properties. Some LSDs are characterized by deficiencies in trafficking components, such as I-Cell disease and CLN8 Batten's disease (di Ronza et al., 2018; Hickman and Neufeld, 1972), or putative lysosomal ion channels such as CLN3 (Kyttala et al., 2006), and are characterized by non-specific storage materials. Pathogenic material in these diseases develops as a result of multiple enzyme deficiency, and often does not result in the accumulation of one particular substrate. In this case, storage material can be detected by ultrastructural examination of the *endo*-lysosomal system using electron microscopy, which often demonstrates the accumulation of electron lucent or electron dense vesicles. Autofluorescent pigment is a frequent characteristic of these diseases and can be detected in standard GFP-fluorescent channels in microplate readers or by direct epifluorescence. Such fluorescent material can occur from lipofuscin or ceroid accumulation, which is a highly insoluble polymer comprised of oxidized lipids and proteins (Seehafer and Pearce, 2006).

The detection of pathological proteins in storage diseases can be challenging, since it is necessary to detect not only total protein abundance, but also conformational changes that occur during the transition into insoluble aggregates. This renders simple methods to measure protein quantity, such as ELISA or western blot of lysates, inadequate at detecting pathological proteins. This is mainly because physiological conformations can increase if more neurite extensions or synapses are present in the culture dish. Even small changes in neuralization are important to consider, especially when studying changes in the levels of disease-linked proteins, such as synaptic a-syn or microtubule-associated protein tau that is normally found in axons. For example, physiological levels of a-syn have been shown to change in response to synaptic activity (Fortin et al., 2005). Therefore methodologies that are specific for probing conformation, or biochemical changes that are associated with conformational changes, should be employed for accurate detection of protein pathology. Disease-linked proteins become highly insoluble when converting from their physiological conformations into inclusion bodies or amyloid fibrils (Jarrett and Lansbury, 1993). These alterations can be indirectly detected by sequentially extracting lysates in detergents of increasing solubilizing strengths, followed by immunodetection. Other useful techniques comprise pathogenic-specific antibodies that can detect disease-linked post translational modifications, including phosphorylation or oxidization (Fujiwara et al., 2002; Giasson et al., 2000). Detail on changes in molecular size can be obtained by size exclusion chromatography, which measures space occupancy in solution and molecular radius, and is useful for the detection of oligomeric intermediates (Conway et al., 2001). Limited proteinase K digests can be used to assess protein stability, which often increases during pathological conformational changes. These methods have been utilized to uncover distinct strains of prion proteins (Bessen et al., 1995), as well as identifying oligomeric intermediates that are formed during a-syn aggregation (Cremades et al., 2012). Our recent studies have employed proteinase K assays to uncover differences between the physiological and pathogenic a-syn species in iPSC-neurons of Gaucher disease (Zunke et al., 2018).

## 4. Modeling neuronopathic lysosomal storage diseases with iPSCs

There are > 50 distinct types of LSDs. Due to their extremely rare incidence, iPSC models for many of these diseases have yet to be established. Some LSDs await a comprehensive neuropathological characterization that clearly defines the degeneration of specific cell-types, the nature of storage material and protein pathologies, and the correlation of pathology with clinical phenotypes. The dearth in our knowledge of many LSDs at the neuropathological level precludes the ability to develop and characterize accurate iPSC models. Below we summarize recent data from some of the more common neuronopathic LSDs that have been sufficiently characterized at the neuropathological and clinical levels, and have employed iPSCs to study phenotypes in neural precursors, distinct neuronal subtypes, or glial cells. We summarize neuropathological phenotypes, neuronal differentiation protocols that were utilized, as well as therapeutic strategies for some diseases. An overview of this information can be found in Table 2.

### 4.1. Gaucher disease

GD is the most frequent and therefore most studied LSD. Patients present with visceral as well as neurological symptoms, which are caused by a mutation within the gene of β-glucocerebrosidase (*GBA1*) leading to aggregation of the glycolipid glucosylceramide. Traditionally, GD is classified as non-neuronopathic (type 1), acute neuronopathic (type 2) and chronic neuronopathic (type 3). Interestingly, age of onset, symptoms, and severity can vary significantly between and within the different types of disease. Traditional classifications of GD are distinguished by the involvement of the nervous system (types 2 and 3), whereas type 1 is mainly characterized by visceral symptoms including hepatosplenomegaly, interstitial lung disease, anemia, thrombocytopenia and bone abnormalities (Goker-Alpan et al., 2003). However, it is now recognized that patients with type I GD have an increased incidence of developing PD. Type 2 (acute neuronopathic) is the most severe form of the disease and is characterized by severe neurological symptoms and neurodegeneration within the first six months of life. The acute neuronopathic type 3 presents with milder forms of the disease and later onset than type 2. In total, there have been 300 disease-associated mutations found in *GBA1*. The most frequent are p.N370S, p.L444P, c.84GG and IVS2 + 1 (O'Regan et al., 2017).

Interestingly, recent clinical, molecular and genetic studies emphasize a link between GD and PD, as *GBA1* mutations are currently considered as one of the main risk factor for developing PD (Aflaki et al., 2017). The mechanisms involved in *GBA1*-linked PD are thought to involve lysosomal dysfunction that leads to a-syn aggregation, which can be potentiated by interaction of the GCase substrate, glucosylceramide, with physiological conformers of a-syn (Mazzulli et al., 2011; Zunke et al., 2018). Mitochondrial dysfunction is also another prominent feature of GD and *GBA1*-PD, which may occur through combined loss-of-function and ER-stress related mechanisms, and are covered in depth within another article of this issue (Baden et al., 2019). The majority of studies using GD iPSC neurons have involved the generation of midbrain dopamine neurons due to the pathological link with PD, however phenotypes have been observed in neural precursors and generalized neuronal cultures. Lysosomal dysfunction has been observed in the majority of models, with some studies demonstrating deficiencies in neural differentiation that can be rescued by Wnt activation (Awad et al., 2017). Protocols that utilize neural precursor cells derived from floating embryoid bodies, which are subsequently differentiated into mature neurons with SHH and FGF8a, demonstrated GCase deficiency and substrate accumulation, recapitulating key features of neuronopathic GD (Sun et al., 2015). This phenotype is similar to that observed in GD neurons that were generated by a distinct differentiation protocol using the floor-plate based strategy (Kriks et al., 2011), which showed GCase deficiency, elevations in glycosphingolipids, and a-syn accumulation (Mazzulli et al., 2016a; Schöndorf et al., 2014). This indicates that GD iPSC-neuronal models provide robust and reproducible models for the study of pathogenesis that can be independent of the differentiation protocol or other technical variations. Moreover, these cell models can be used to test new therapeutic strategies. For instance, small compounds that activate GCase have been tested in iPSC-neurons from GD, *GBA1*-PD, and PD patients with *SNCA* mutations, and have demonstrated reduction of glycosphingolipids and a-syn pathology (Mazzulli et al., 2016). Additionally, inhibition of glycosphingolipid synthase could reduce a-syn pathology in GD and *SNCA* mutant iPSC-neurons (Zunke et al., 2018), as well as in mouse models of GD (Sardi et al., 2017), demonstrating the importance of glycosphingolipids in the development of GD pathology. Taken together, this indicates the potential of iPSC-derived neuronal cell models for the discovery and validation of novel therapeutic approaches in GD and PD, and are an important stage in the pipeline to translate these strategies into the clinic.

### 4.2. GM1 gangliosidosis

GM1 gangliosidosis (GM1) is caused by mutations in the gene that encodes lysosomal β-galactosidase (*GLB1*), and similar to other LSDs, is characterized by severe neurodegeneration. Insufficient enzyme activity leads to abnormal accumulation of GM1 gangliosides particularly in the central nervous system, resulting in progressive neuropathology, along with hepatosplenomegaly, skeletal abnormalities and seizures (Brunetti-Pierri and Scaglia, 2008). The cerebral cortex is prominently involved in the infantile forms of GM1 (type 1 and 2), while late-stage onset (type 3) is associated with other regions of the brain including the basal ganglia (Suzuki, 1991). Clinically, type 3 patients exhibit dystonia with speech and facial abnormalities, and nearly half of these patients also exhibit akinetic-rigid parkinsonism (Roze et al., 2005). Pathological studies of type 3 GM1 have indicated that storage occurs mainly in

**Table 2**
Summary of iPSC models of neuronopathic LSDs.

| Disease | Protein | Function | Affected Region / Cell type | Modeled Cell type | Phenotype | Therapeutic Strategies | Citation |
|---|---|---|---|---|---|---|---|
| **Gaucher** | Glucocerebrosidase (GBA1) | Degradation of Glucosylceramide and Glucosylsphingosine | Midbrain DA neurons, Hippocampus, Cortex | Midbrain DA neurons | Substrate and a-syn accumulation, reduced sodium and potassium currents | | (Sun et al., 2015) |
| | | | | | Substrate and a-syn accumulation neurodegeneration | | (Mazzulli et al., 2011; Mazzulli et al., 2016a; Schöndorf et al., 2014) |
| | | | | | | GCase activation | (Aflaki et al., 2016; Mazzulli et al., 2016) |
| | | | | | | Substrate reduction | (Zunke et al., 2018) |
| | | | | Neuronal cells | Deficient neural differentiation | Wnt activation | (Awad et al., 2017) |
| | | | | Neuronal cells | Enzyme depletion, autophagic block, decreased TFEB | Recombinant GCase | (Awad et al., 2015) |
| **GM1-Gangliosidoses** | β-Galactosidase-1 (GLB1) | Degradation of GM1 Gangliosides | Cortex, Basal Gangila | Neural Precursors, Neuronal cells | Reduced β-Galactosidase activity, substrate accumulation, increased lysosomes | | (Son et al., 2015) |
| **GM2-Gangliosidoses** | | | | | | | |
| Sandhoff | Hexosaminidase A/B | Degradation of GM2 Gangliosides | Thalamus, Substantia Nigra, Cerebellum, Cortex | Mouse Neural Precursor cells | Impaired neural differentiation, substrate accumulation | Overexpression of HexB | (Ogawa et al., 2017) |
| | | | | Cerebral Organoids | Substrate accumulation, enlarged organoid size, impaired neural development | Hex B isogenic correction | (Allende et al., 2018) |
| Tay Sachs | Hexosaminidase A/S | Degradation of GM2 Gangliosides | | Neural Stem Cells | Substrate accumulation, cholesterol accumulation | Recombinant HexA, cyclodextrin | (Vu et al., 2018) |
| **Neuronal Ceroid Lipofuscinosis** | | | | | | | |
| CLN1 | Palmitoyl-protein thioesterase 1 (PPT1) | Removal of thioester-linked palmitoyl groups from proteins | Widespread neurodegeneration, Retinal degeneration | Neural Stem Cells | Lipid accumulation, lysosomal enlargement | Cyclodextrin, tocopherols | (Sima et al., 2018) |
| CLN2 | Tripeptidy-peptidase 1 (TPP1) | Removal of N-terminal tripeptides from proteins | | Neural Precursors, Neuronal cells | Curvilinear and lipid accumulation | TPP1 overexpression | (Lojewski et al., 2014) |
| CLN3 | Battenin/CLN3 (CLN3) | Transport of endosomes and lysosomes (Uusi-Rauva et al., 2012) | | Neural Precursors, Neuronal cells | Mitochondrial, Golgi, ER abnormalities | AAV expression of CLN3 | (Lojewski et al., 2014) |
| CLN5 | CLN5 (CLN5) | unknown | Widespread neurodegeneration, Cerebellum | Neuronal cells | Lipid accumulation, altered sphingolipid transport | | (Uusi-Rauva et al., 2017) |
| **Frontotemporal Dementia (FTD)** | Progranulin (PGRN) | Modulates Proteolytic function, Cathepsin D activator (granulin E) | Widespread neurodegeneration, mainly Frontotemporal lobe | Neuronal cells | Staurosporin sensitivity | | (Almeida et al., 2012) |
| | | | | Excitatory cortical cells | Insoluble TDP43, lipofuscin, fingerprint-like accumulations | | (Valdez et al., 2017) |

(*continued on next page*)

**Table 2** (*continued*)

| Disease | Protein | Function | Affected Region / Cell type | Modeled Cell type | Phenotype | Therapeutic Strategies | Citation |
|---|---|---|---|---|---|---|---|
| **Mucopoly-saccharidoses** | | | | | | | |
| Type 1 (Hurler) | α-L-Iduronidase (IDUA) | Breakdown of glycosaminoglycans (GAGs) | Widespread neurodegeneration | Hematopoietic cells | Substrate accumulation | | (Tolar et al., 2011) |
| Type 2 (Hunter) | Iduronidate-2-sulfatase (IDS) | | | Neuronal cells | Substrate accumulation, skewed X-inactivation | | (Reboun et al., 2016) |
| | | | | Neuronal cells, astrocytes, oligodendrocytes | Substrate accumulation, abnormal structure of lysosomes | Enzyme replacement | (Rybova et al., 2018) |
| Type 3 B (Sanfilippo) | α-N-acetlyglucosaminidase (NAGLU) | | | Neural Stem Cells | Storage vesicles, disorganization of Golgi, changes in gene profile | | (Lemonnier et al., 2011) |
| Type 3C (Sanfilippo) | AcetylCoA:*N*-acetyltransferase (HGSNAT) | | | Neuronal cells | Substrate accumulation, lysosomal alterations, defects in neuronal activity | | (Canals et al., 2015) |
| Type 7 (Sly) | β-Glucuronidase (GUSB) | | | Neural Stem Cells | | Genetic correction and transplantation in Sly mouse: reversal of phenotype | (Griffin et al., 2015) |
| **Wolman Disease** | Lysosomal acid lipase (LIPA) | Cholesterol ester and triglyceride metabolism | Brainstem, spinal cord, and cortex | Neural Precursors | Substrate accumulation | Enzyme replacement therapy, compound study | (Aguisanda et al., 2017) |
| **Niemann-Pick type C (NPC)** | NPC1 protein (NPC1) | Cholesterol transport | Widespread neurodegeneration, mainly cerebellum | Neural Precursors | Cholesterol storage | | (Trilck et al., 2013) |
| | | | Purkinjie, Oligodendrocyte | Neuronal cells | Reduction of VEGF / sphingosine kinase (SphK) activity | VEGF treatment | (Lee et al., 2014) |
| | | | | Neuronal cells | Cholesterol storage, autophagic dysfunction | | (Maetzel et al., 2014) |
| | | | | Neural Precursors | Cholesterol storage | Small compounds | (Yu et al., 2014a) |
| | | | | Neuronal cells | Dysfunction in Calcium and WNT signaling | | (Efthymiou et al., 2015) |
| | | | | Neuronal cells | Substrate accumulation, autophagic dysfunction | | (Soga et al., 2015) |
| **Niemann-Pick type A** | Acid sphingomyelinase (SMPDI) | Breakdown of sphingomyelin | Widespread neurodegeneration | Neural Stem Cells | Substrate accumulation, enlarged lysosomes | Substrate reduction, enzyme replacement | (Long et al., 2016) |
| **Metachromatic Leukodystrophy** | Arylsulfatase A (ARSA) | Degradation of cerebroside sulfate | Widespread demyelination of cells in CNS and PNS | Neural Precursors, Neuronal cells, astrocytes, oligodendrocytes | Impaired neuronal and glial differentiation capacity | Lentiviral transduction with ARSA | (Frati et al., 2018) |

neuronal cells of the brain, with relative specificity to medium spiny neurons of the caudate nucleus and putamen (Goldman et al., 1981). While the mechanisms responsible for the specific degeneration and storage within striatal neurons are unknown, it has been suggested to occur from differences in the metabolic demands or utilization of GM1 ganglioside within the affected cell types (Yoshida et al., 1994). Other studies also indicated the involvement of dysfunctional oligodendrocytes in neurodegeneration (Folkerth et al., 2000).

While cell-type specific iPSC models of GM1 have yet to be generated and fully characterized, some studies have demonstrated phenotypes in iPSC-derived neuronal precursor cells (NPCs) that were further differentiated into generalized neuronal and glial cells from floating embryoid bodies (Son et al., 2015). iPSC-derived disease cell lines recapitulated GM1-disease phenotype by exhibiting decreased β-galactosidase activity, substrate accumulation and an increased number of lysosomes (Son et al., 2015). Importantly, this study also showed that GM1 phenotypes are significantly associated with the activation of inflammasomes in GM1-NPCs. Future analyses employing models of specific neuronal cell types such as medium spinal neurons, or interactions between neurons and oligodendroglia populations in co-culture studies, should provide critical information into the pathogenesis of GM1. Moreover, it will be of interest to examine the nature of the accumulating material in these cultures beyond GM1 gangliosides, such as aggregates associated with age-related neurodegenerative disorders including a-syn, tau, or a-beta. Some studies have demonstrated that a-syn fibrillization can be inhibited by GM1, while causing an accumulation of oligomeric forms (Martinez et al., 2007). A-beta accumulations have also been noted to occur in ganglioside-rich regions of the presynaptic terminal (Yamamoto et al., 2008) (see also Fig. 1), and can tightly bind to GM1 ganglioside (Yanagisawa et al., 1995). These interactions can induce the seeding of a-beta aggregates with fibrillar morphologies and beta-sheet structures (Matsubara et al., 2018; Yanagisawa, 2007). GM1 ganglioside may play a role in the pathogenesis of Alzheimer's disease through these mechanisms, and some studies have shown that a-beta accumulates in GM1 post-mortem brain (Keilani et al., 2012). While one study has demonstrated little-to-no accumulation of a-syn in a GM1 patient brain (Suzuki et al., 2007), future studies are required to determine if a-beta specifically accumulates or if other aggregation prone proteins such as a-syn or tau aggregate in human post-mortem tissues. This may help to assess whether a firm link exists between this LSD and common neurodegenerative disorders, and may provide insight into the specificity of the relationship between lysosomal dysfunction and age-related proteinopathies.

### 4.3. GM2 gangliosidoses

GM2 gangliosidoses (GM2) occur by a deficiency in the acid-β-hexosaminidase (Hex) enzyme, which is expressed as a functional dimer of alpha and beta subunits. There are three Hex enzymes that can be formed depending on the dimer combination including Hex A (alpha-beta), Hex B (beta–beta), and Hex S (alpha-alpha). Substrate specificity has been described for Hex enzymes, where Hex A can cleave both *N*-acetylglucosamine and *N*-acetylgalactosamine of charged and uncharged glycoconjugates, while Hex B cleaves only uncharged glycoconjugates. Different variants of GM2 gangliosidoses occur that are distinguished by the deficiency of certain Hex subunits. For example, the autosomal recessive Sandhoff disease is caused by mutations in the alpha and beta subunits (Hex A) resulting in loss of enzyme activity of Hex A and B, but with functional Hex S. Neuronal accumulation and neurodegeneration of both charged and uncharged substrates is a characteristic feature of Sandhoff disease. In contrast to GM1 gangliosidosis where pathology is relatively selective for the basal ganglia, the pathology of GM2 gangliosidosis is more widespread and effects several regions of the brain including the thalamus, substantia nigra, and cerebellum (Suzuki, 1991). While the molecular mechanisms of Sandhoff disease is not completely understood, mouse models have indicated that accumulation of a-beta, a-syn, as well as phospho-tau can occur (Keilani et al., 2012; Suzuki et al., 2003). Post-mortem analyses of 2 cases of Sandhoff disease brains demonstrate pathology of a-syn in the cortex, cerebellum, and brainstem regions (Suzuki et al., 2007). As these proteins can be metabolized by the *endo*-lysosomal system, it is possible that generalized dysfunction of lysosomes may render aggregation prone proteins susceptible to pathogenic accumulation. Further, the aggregation rates of a-beta and a-syn can be modulated by gangliosides, and it is possible that this interaction in combination with endo-lysosomal dysfunction could play a major role in pathogenesis of these disorders.

iPSCs from Sandhoff mice have been generated and differentiated in neural precursor cells. These cells exhibited significant GM2 ganglioside storage and impaired neuronal differentiation. Either restoration of *Hexb* gene expression or reduction of glycosphingolipid synthesis by glucosylceramide synthase inhibitors could reduce pathological phenotypes and improve neuron development (Ogawa et al., 2017). These studies indicate a prominent role for GM2 metabolism and lysosomal function in the development from iPSCs into neural stem cells, and may lead to further clues related to the pathogenic mechanisms of neurodevelopmental defects in Sandhoff disease.

Recently, cerebral organoids derived from Sandhoff disease iPSCs have been established and similarly indicate neurodevelopmental phenotypes (Allende et al., 2018). The iPSCs were reprogrammed from an infant that carried a compound heterozygous mutation with an IVS10-2A > G resulting in an improper splice site, while the other allele resulted in a ~16 kb deletion in *HEXB*, which includes the promoter region as well as exon 1–5. Organoids were developed through an established protocol involving the generation of embryoid bodies followed by neural ectoderm induction (Lancaster et al., 2013). Neural ectoderm tissues are cultured within Matrigel droplets and further differentiated into cerebral organoids by culturing in neural basal media with B27 and retinoic acid in suspension, with gentle rotation to enhance nutrient delivery (Lancaster et al., 2013). Importantly, isogenic controls were established by correcting the IVS10-2A > G mutation through CRISPR/Cas9 editing. The disease-organoids, but not the *HEXB*-corrected organoids, displayed GM2 ganglioside accumulation and enlarged organoid size (Allende et al., 2018). Furthermore, Sandhoff organoids exhibited increased cellular proliferation, and a whole-transcriptome analysis revealed an impairment in neuronal development. Hence, this suggests that altered neuronal differentiation might be an early developmental event in Sandhoff disease.

Tay-Sachs disease is a distinct GM2 gangliosidosis that is caused by mutations in the alpha subunit, resulting in a loss-of-function of Hex A and S, while Hex B remains functional. Similar to Sandhoff disease, this leads to the accumulation of GM2 gangliosides, as well as a-syn within neurons of multiple brain regions (Suzuki et al., 2007). An iPSC model of Tay-Sachs disease was generated from fibroblasts of a patient with an infantile form of the disease that carried compound heterozygous mutations in Hex A of 1278insTATC and IVS12 + 1G > C, but iPSCs were not further differentiated or characterized (Liu and Zhao, 2016). Another group established two iPSC models from fibroblasts of two patients, one carrying a homozygous mutation of 1278insTATC, and the other carrying a compound heterozygous mutation of 1278insTATC and Trp392Ter. These iPSCs were further differentiated into neural stem cells (NSCs), demonstrating lysosomal GM2 accumulation along with the build-up of secondary metabolites including cholesterol (Vu et al., 2018). These phenotypes could be rescued by enzyme replacement treatment using recombinant human Hex A, as well as by cyclodextrin treatment, enhancing lysosomal exocytosis, resulting in reduction of gangliosides (Vu et al., 2018). Future studies that involve the differentiation into specific neuronal subtypes that are prominently affected in Tay-Sachs disease, such as cortical type neurons, are required to further evaluate the effect of GM2 accumulation and lysosomal dysfunction on neuronal degeneration.

### 4.4. Neuronal ceroid lipofuscinosis (NCL)

Neuronal Ceroid Lipofuscinoses (NCLs), also known as Batten's disease, are a group of LSDs characterized by severe neurodegeneration. At the cellular level, all forms of NCLs demonstrate a common pathology of autofluorescent lipopigment within lysosomes, termed ceroid, that leads to lysosomal dysfunction and cell death through unknown mechanisms (Carcel-Trullols et al., 2015). While the NCLs share the common pathology of ceroid accumulation, there are 13 subtypes of the disease that are caused by different genetic mutations in distinct CLN genes (for Ceroid Lipofuscinosis, Neuronal, followed by the type). Neurological symptoms include motor and cognitive decline, seizures, and typically vision loss. Infantile NCL can be caused by mutations in the palmitoyl-protein thioesterase (PPT1) gene (CLN1) and late-infantile NCL is due to mutations in the tripeptidyl-peptidase 1 (TPP1) gene (CLN2). Deficiency in PPT1 or TPP1 enzyme function results in lysosomal accumulation of pathological lipofuscin-like material in the patient cells. iPSC models were previously established and utilized for drug development and screening in an effort to test potential therapies that could reduce lysosomal storage (Sima et al., 2018). CLN1 as well as CLN2 iPSC-derived neural stem cells phenocopied disease manifestations and exhibited substrate accumulation, lysosomal enlargement and lipid droplet accumulation. Disease phenotypes could be ameliorated by two lipid-reducing compounds (cyclodextrin/tocopherols) indicating their potential use as treatment strategy in CLN1 and CLN2 (Sima et al., 2018). Another study using CLN2 iPSC-derived neuronal precursors and neurons generated by mitogen withdrawal and cAMP incubation, demonstrated a similar phenotype exhibiting accumulation of predominantly curvilinear storage materials and lipid droplets (Lojewski et al., 2014). Retroviral re-introduction of *TPP1* was able to rescue the persisting cellular phenotypes in CLN2 iPSCs, demonstrating the usefulness of this model in testing potential therapies, including TPP1 activators (Lojewski et al., 2014). The same group analyzed iPSC-derived neural precursor cells from a patient carrying a CLN3 mutation demonstrating predominantly fingerprint like accumulations, with additional abnormalities in mitochondria, dispersion of the Golgi, and ER structure that preceded storage phenotypes (Lojewski et al., 2014). Adenoviral reconstitution of functional *CLN3* in iPSC-derived retinal neurons was efficient in restoring the *CLN3* transcript without causing cellular toxicity (Wiley et al., 2016). This suggests that viral transduction could be used as a potential therapeutic approach in CLN3 disease, since to date there are no other treatment strategies available.

### 4.5. Frontotemporal dementia/NCL-like phenotype

Frontotemporal dementia (FTD) comprises a group of neurodegenerative disorders characterized by cognitive and behavioral impairments. Loss of function heterozygous mutations in progranulin (PGRN) are a cause of FTD, while homozygous mutations result in neuronal ceroid lipofuscinosis (NCL) (Smith et al., 2012). The relationship between *PGRN* mutation dosage and the contribution to the development of these two different diseases is not well understood. *PGRN* is expressed in microglia as well as neurons and has been implicated in neurite growth and inflammation (He and Bateman, 2003; Yin et al., 2010). Further, it can be cleaved into individual granulin proteins by cathepsins within lysosomes followed by secretion (Lee et al., 2017). Interestingly, mutations in cathepsin D cause another form of NCL disease that is associated with severe neurodegeneration and a-syn accumulation (Cullen et al., 2009; Siintola et al., 2006). Initial studies using patient specific iPSCs harboring *PGRN* mutations utilized an embryoid body culture protocol followed by differentiation of neural rosettes and neurospheres with BDNF, GDNF, cAMP, and ascorbic acid to generate MAP2 positive neural cultures (Almeida et al., 2012). These cells demonstrated reduced expression and secretion of *PGRN* and increased sensitivity to staurosporine, a broad kinase inhibitor that induces apoptosis (Almeida et al., 2012). Another study used patient-derived iPSCs from an FTD patient harboring a heterozygous PGRN mutation of c.26C > A, p.A9D with isogenic controls and developed a cortical model through differentiation by ngn2 overexpression (Valdez et al., 2017). These neurons presented with an FTD and NCL-like pathology as indicated by a decrease in nuclear TDP-43 (TAR DNA-binding protein 43) and increased insoluble TDP-43, a feature that has been observed previously in FTD brain (Dormann and Haass, 2011; Neumann et al., 2006). Ultrastructural analysis also revealed enlarged electron-dense vesicles, lipofuscin accumulation and granular osmiophilic deposits, features that are similar to NCL. Functional analyses revealed deficits in lysosomal proteolysis and decreased activity of the lysosomal enzyme cathepsin D. Since a specific interaction of *PRGN* with cathepsin D was also documented, this might further explain the overlapping phenotype of FTD and NCL in patients with *PGRN* mutations (Valdez et al., 2017). These studies further implicate the close pathological and biochemical connections between rare LSDs and more common age-related neurodegenerative disorders.

### 4.6. Mucopolysaccaridoses

Accumulation of glycosaminoglycans (GAG) occurs in a class of diseases called mucopolysaccharidoses (MPS), which are caused by a deficiency in hydrolases that are involved in GAG breakdown in lysosomes. Multiple types of MPS exist that can be separated based on the deficiency of 1 out of 10 different enzymes and are involved in the stepwise degradation of GAGs including dermatan sulfate, heparan sulfate, keratan sulfate, or chondroitin sulfate (Muenzer, 2011). Most MPS forms are characterized by progressive dysfunction in multiple organ systems and dysmorphism. Mucopolysaccharidosis type I (MPS I; Hurler / Scheie / Hurler-Scheie syndrome) is caused by a deficiency of α-L-iduronidase, leading to extremely heterogeneous pathology of somatic and nervous system involvement, including hepatosplenomegaly, corneal clouding, skeletal deformities, cardiac and respiratory abnormalities, as well as learning disabilities (Coutinho et al., 2012). Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is transmitted in an X-linked manner and results from mutations and loss of function of iduronate-2-sulfatase (IDS), which is important for the degradation of heparan and dermatan sulfate. The clinical features of MPS II are similar to those of MPS I and exhibit prominent somatic and nervous system pathologies, except that MPSII patients often do not show corneal clouding. One study demonstrated that iPSCs from MPSII patients could be generated from reprogrammed peripheral white blood cells which were further differentiated to neuronal and oligodendroglial cells (Rybova et al., 2018). The neuronal protocol involved the development of neurospheres from embryoid bodies (EBs), followed by patterning with retinoic acid, purmorphamine (SHH agonist) and FGF8, resulting in cells that were positive for MAP2, b-iii-tubulin, synapsin, and tyrosine hydroxylase indicating a dopaminergic phenotype (Stacpoole et al., 2011). Oligodendroglial precursors (OPCs) were generated through EBs-neural precursors, followed by treatment with epidermal growth factor using an established protocol (Stacpoole et al., 2011). Elevated levels of lysosomes were apparent in OPCs through LAMP1 immunostaining and electron microscopy revealing vacuole accumulation. Interestingly, GAG accumulation occurred in differentiated neurons but not in iPSCs or neural precursors (NPCs), possibly due to the fact that neurons were post-mitotic and unable to dilute out storage materials like dividing iPSCs or NPCs. A moderate increase in GAG levels in IDS-deficient iPSC-derived neurons and glia cells was observed. For unclear reasons, attempts to reverse GAG accumulation with recombinant IDS were not able to prevent accumulation of GAGs (Rybova et al., 2018). Nonetheless, MPS II-iPSC models should be further evaluated to test future therapeutic targets.

A unique feature of MPS III (Sanfilippo syndrome) is that it primarily affects the central nervous system with very little somatic involvement. Four different subtypes have been described and designated as type A-D. These subtypes share clinical characteristics of severe early onset CNS

degeneration that typically results in death within the second or third decade of life. All MPS III forms are characterized by heparan sulfate accumulation, but with distinct enzyme deficiencies including Type A (heparan N-sulfatase (*SGSH*)), B (alpha-*N*-acetylglucosaminidase (*NAGLU*)), C (acetyl-CoA:alpha-glucosaminide acetyltransferase (*HGSNAT*)), and D (*N*-acetylglucosamine-6-sulfatase (GNS)). MPS IIIB is characterized by severe mental retardation and dementia, and studies of *NAGLU* deficiency in mice have shown that Alzheimer's-like pathology of phosphorylated tau and a-beta aggregates accumulate in these models (Ohmi et al., 2009). Enzyme replacement has been achieved in the brain of *NAGLU*-deficient mice through the fusion of insulin growth factor II fusion, which facilitates passing through the blood brain barrier. This has demonstrated reductions in GAG, a-beta and phospho-tau protein (Kan et al., 2014), suggesting that accumulation of these disease linked proteins likely occurs through NAGLU deficiency. Interestingly, accumulation of tau and a-beta were not observed in another MPS (type VI) model that is characterized by arylsulfatase B (*ARSB*) deficiency with dermatan sulfate accumulation. Previous studies have shown that the microtubule binding ability of tau is reduced in the presence of GAGs using direct *in vitro* binding assays, and that tau assembly can be stimulated by the addition of dextran sulfate, heparan, and heparan sulfate (Hasegawa et al., 1997). Thus, the specificity of tau aggregation in models that accumulate heparan sulfate ($NAGLU^{-/-}$) *vs.* those that accumulate dextran sulfate ($ARSB^{-/-}$) is unclear. However, it is possible that the differences could be due to relative GAG accumulation levels within individual cell types, correlating with variable tau expression levels. For example, vulnerable cell types with high base-line levels of tau may also be susceptible to lysosomal heparan sulfate accumulation, but not dextran sulfate. In this scenario, tau may have more opportunities to interact with heparan sulfate, possibly within lysosomal compartments, as tau can be processed by the autophagic-lysosomal pathway (Lo and Yuen, 1998) (see also Fig. 1). This interaction may stabilize tau, resulting in higher steady-state levels that are required to surpass a pathogenic threshold and lead to pathological fibril formation.

Initial attempts to reprogram MPS III patient fibroblasts into iPSCs required supplementation of the cultured cells with α-*N*-acetylglucosaminidase, as it was found that reprogramming could not be achieved in the absence of this enzyme (Lemonnier et al., 2011). iPSCs, neural stem cells, and mature GABAergic and glutamatergic neurons demonstrated storage vesicles, heparan sulfate accumulation and disorganization of the Golgi (Lemonnier et al., 2011). Secondary accumulation of GM3 ganglioside was detected in perinuclear regions, which may have occurred from reduced lysosomal activity of GM3 degrading enzymes or through disrupted trafficking events as previously suggested from studies in MPS III mouse models (Vitry et al., 2010). Analysis of gene expression indicated changes in extracellular matrix proteins, cell adhesion, and cellular communication / signaling pathways. Other studies have also shown secondary accumulation of glycosphingolipids and cholesterol in MPS III mouse models (McGlynn et al., 2004), and it is possible that disruption of lipid rafts that are critical signal transduction pathways in neurons pathways could explain the alterations in this gene expression network.

Disease-specific iPSCs have been generated from patients with mucopolysaccharidosis type C (MPS IIIC; Sanfilippo C) (Canals et al., 2015), which is caused by mutations in the *HGSNAT* gene. Malfunction of the enzyme acetyl-CoA:alpha-glucosaminide acetyltransferase, which catalyzes the transfer of an acetyl group from acetyl-CoA to a terminal alpha-linked glucosamine residues of heparan sulfate, leads to lysosomal accumulation of heparan sulfate. Differentiation into neurons was done through the formation of embryoid bodies into spherical neural masses of neural precursor cells, by the sequential addition of N2 and B27 neuronal supplements, resulting in > 80% of tyrosine hydroxylase positive (TH + ) dopaminergic neurons (Cho et al., 2008). Patient-iPSC-derived neurons exhibited diminished HGSNAT activity, GAG accumulation occurring gradually over 9 weeks in culture, and accumulation of electron lucent vacuoles consistent with lysosomal dysfunction (Canals et al., 2015). Storage phenotypes were associated with reductions in spontaneous neuronal activity and connectivity, determined by calcium imaging, which may indicate problems in neural development. Lysosomes have been shown to be important for the development and pruning of synapses (Song et al., 2008), and it is possible that GAG-induced lysosomal dysfunction was the cause for aberrant synaptic connections with disrupted neuronal activity. This notion is consistent with studies of MPS III mice, which demonstrate elevations in post-synaptic markers and altered spine morphology with decreased mini-excitatory post-synaptic potentials during key developmental stages in the cortex (Dwyer et al., 2017). An advantage of the iPSC-neuronal model is that the temporal sequence of events can be examined, by correlating distinct pathological changes that occur as neurons degenerate in culture. Future studies applying disease iPSC models should reveal more detail into the mechanistic relationship between GAG accumulation, lysosomal dysfunction, changes in synaptic physiology, and neuronal degeneration in MPS III.

### 4.7. Wolman disease

Wolman disease is caused by loss of function mutations in the *LIPA* gene encoding for the lysosomal acid lipase (LAL) resulting in the accumulation of cholesteryl esters and triglycerides in lysosomes. LAL cooperates with NPC2 protein to mobilize cholesterol, by liberating the fatty acid moiety from low-density lipoproteins (LDL)-bound cholesteryl esters (Goldstein et al., 1975), allowing free cholesterol to bind soluble NPC2 within the lysosomal lumen. The accumulation of cholesteryl esters that result from LAL deficiency can lead to excessive oxidation of LDL-cholesterol, and may play an important role in cytotoxicity (Fitoussi et al., 1994). This may particularly affect cell types that have high levels of LDL receptors, since this will lead to more LDL uptake and prominent accumulation of LAL substrates. Symptoms seen in individuals who are affected include adrenal calcification, hepatosplenomegaly, enlarged lymph nodes with prominent central nervous system involvement leading to cognitive decline. Neuropathological examination has shown the accumulation of lipid droplets within oligodendrocytes and astrocytes of the cerebrum, brain stem, and spinal cord, however accumulation within neurons was not observed (Byrd and Powers, 1979). These accumulations can exist as both membrane delimited (possibly lysosomes) as well as non-membrane delimited structures.

iPSC lines generated from two Wolman patients were developed and differentiated into neural stem cells (NSCs) (Aguisanda et al., 2017). The disease NSCs exhibited the typical phenotypes of severely deficient LAL activity, neutral lipid accumulation, and lysosomal enlargement. Further, enzyme replacement treatment dramatically reduced the phenotype observed in the disease cells. In addition, cyclodextrin and alpha-tocopherol, which have been shown to reduce cholesterol storage through enhancing lysosomal exocytosis, were found to reduce phenotypes such as lysosomal size (Aguisanda et al., 2017). This study indicates the utility of this model for detecting potential therapies of Wolman disease and should be useful for future studies of disease mechanisms focused on the relationship between the storage of cholesteryl esters and cell death.

### 4.8. Niemann-Pick Type C

Niemann–Pick disease type C (NPC) is caused by recessive mutations in the *NPC1* or *NPC2* (or *HE1)* gene that lead to LDL-derived cholesterol accumulation within lysosomes (Carstea et al., 1997; Naureckiene et al., 2000). NPC genes encode lysosomal proteins that traffic cholesterol out of lysosomes into other parts of the cell including the endoplasmic reticulum (ER), Golgi apparatus, and plasma membrane. Once LDL-bound cholesteryl esters are endocytosed into the lysosome and metabolized by LAL, it has been proposed that soluble NPC2 binds LDL-derived cholesterol and subsequently transfers it to membrane bound NPC1 to be delivered throughout the cell (Infante

et al., 2008; Kwon et al., 2009). Disruption of the process leads to cholesterol accumulation culminating in cell death by multiple cellular dysfunctions including disrupted calcium homeostasis in lysosomes (Lloyd-Evans et al., 2008), cellular trafficking (Choudhury et al., 2002), and impaired autophagy (Benussi et al., 2018). Early onset of disease presents primarily with visceral symptoms, such as hepatosplenomegaly, but is followed by progressive intellectual and neurological impairments including cerebellar ataxia that may occur from Purkinje cell loss (Patterson et al., 2012; Sarna et al., 2003). Late onset of the disease is characterized by significant involvement of the nervous system including ataxia (Sevin et al., 2007), dystonia that correlates with striatal pathology, and acute myoclonus that may originate from cortical dysfunction (Canafoglia et al., 2006). Nearly all adult onset NPC patients present with dementia and impaired saccadic eye movements (Sevin et al., 2007), and can also present with psychiatric problems including psychosis and depression (Patterson et al., 2012). While Purkinje cells of the cerebellum seem to be the most prominently effected neuronal cell type in NPC, deficiencies in myelination and oligodendrocyte dysfunction likely play a prominent role in neurodegeneration. Studies from NPC1$^{-/-}$ mice indicate that de-myelination may occur during early developmental stages and precede Purkinje cell loss (Yu and Lieberman, 2013). Since glia cells synthesize the majority of brain cholesterol, oligodendrocytes may be particularly sensitive to alterations in cholesterol transport.

In addition to cholesterol accumulation, proteinaceous inclusions have been documented in NPC brain. The neuropathological features of NPC overlap with common age-related neurodegenerative disorders, including tau and a-syn accumulation, which are characteristic for Alzheimer's (Love et al., 1995), and Lewy body disease (Saito et al., 2004), respectively. While a-beta inclusions are not a common neuropathological feature of NPC brain, it was previously found that patients harboring both NPC and apolipoprotein E4 mutations in combination exhibited prominent a-beta plaques (Saito et al., 2002). The mechanisms that lead to protein aggregates in NPC are unknown, however it is possible that generalized lysosomal dysfunction may preferentially cause the accumulation of aggregation-prone proteins. Both tau and a-syn are relatively abundant proteins that are maintained at the threshold of solubility under physiological conditions (Ciryam et al., 2013) and can be metabolized through the lysosomal system (Cuervo et al., 2004; Kenessey et al., 1997). A-syn, tau, and a-beta possess physiochemical features within their amino acid sequence that render them susceptible to aggregation under favorable conditions, such as stretches of hydrophobic amino acids that promote beta-sheet formation (Pawar et al., 2005; von Bergen et al., 2000; Waxman et al., 2009). Therefore, subtle changes in local concentrations of each protein may dramatically influence the ability of tau and a-syn to aggregate. The accumulation of other toxic metabolites may also potentiate aggregation such as heparan sulfate interactions with tau (Hasegawa et al., 1997), or cholesterol interactions with a-syn (Bosco et al., 2006).

Early models of NPC1 patient-derived iPSCs were reprogrammed from fibroblast lines and developed into neural precursor cells by dual SMAD inhibition to induce neural rosettes, followed by treatment with FGF-2, EGF, and BDNF (Trilck et al., 2013). Similar levels of cholesterol accumulation were observed in parental fibroblasts, iPSCs, and neural precursors, indicating that the cell type does not dictate the level of cholesterol accumulation in culture (Trilck et al., 2013). Using similar differentiation methods, another study demonstrated cholesterol accumulation and lysosomal enlargement of NPC neuralized cells that could be reversed with cyclodextrins, demonstrating the potential for therapeutic development of these compounds (Yu et al., 2014a). Consistent with this, *in vivo* feline cases of NPC1 have shown that 2-hydroxypropyl-b-cyclodextrin can ameliorate cholesterol storage, reduce Purkinje cell death, and improve lifespan (Yu et al., 2014a). More recent studies on patient derived NPC neurons have provided insight into the pathological mechanisms of NPC loss of function. For example, one study found that sphingosine accumulation in NPC cells was the result of reduced vascular endothelial growth factor (VEGF) and sphingosine kinase activity, an effect that could be rescued by replenishment of VEGF, suggesting the potential for VEGF as a therapeutic candidate for NPC (Lee et al., 2014). A separate study performed electrophysiological characterization of NPC-derived neural cells and found reduced AMPA-induced calcium influx, which may have occurred through accumulation of dysfunctional AMPA receptors (Rabenstein et al. 2017). It is possible that these effects may have occurred through potential disruptions of lipid raft composition where AMPA receptors reside on the plasma membrane, since cholesterol and other glycosphingolipids are known to be important structural components of lipid rafts. Accumulation of dysfunctional AMPA receptor subunits may also occur through reduced recycling or degradation by the *endo*-lysosomal system, consistent with lysosomal dysfunction observed in NPC. Together, these studies indicated that iPSC-neuronal models of NPC provide a suitable system to uncover novel phenotypes, as well as discover and test novel therapeutic targets.

## 5. Conclusion

The advent of iPSC technology has provided novel opportunities for the study of disease etiology of neurological storage disorders characterized by protein, lipid, or oligosaccharide accumulation. A major advantage of modeling disease with iPSCs as compared to other cell models is that the pathogenicity of endogenous, disease causing mutations can be studied in a genetic background that is permissive for disease. A second advantage of these models is that various differentiation strategies can be employed to generate the specific cell-type that is effected in the disease of interest. Homogenous cell types can be obtained in sufficient quantities that are scaled to fit the assay of interest. Although these tools can be powerful, we emphasize the need of strict quality standards that includes the careful monitoring of cell culture integrity and neuralization efficiency, as well as the necessity to include isogenic control lines. Due to the recently emerging mechanistic and genetic relationships between LSDs and common neurodegenerative disorders, iPSC models of rare LSDs may provide insight into the etiology of complex, sporadic neurodegenerative disorders. These models also hold promise as accurate prognostic indicators of experimental therapies during preclinical stages, as compared to mouse models that have been used in the past, since they eliminate species-specific barriers that have previously hindered the development of neurodegenerative therapies.

## Disclosure

JRM is a scientific founder of Lysosomal Therapeutics, INC.

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