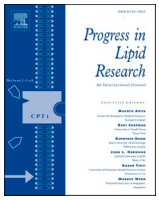
Progress in Lipid Research 90 (2023) 101224

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres



Review

Sphingolipids and impaired hypoxic stress responses in Huntington disease

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ARTICLE INFO

Keywords:
Sphingolipids
Huntington disease
hypoxia
conditioning
mitochondria

ABSTRACT

Huntington disease (HD) is a debilitating, currently incurable disease. Protein aggregation and metabolic deficits are pathological hallmarks but their link to neurodegeneration and symptoms remains debated.

Here, we summarize alterations in the levels of different sphingolipids in an attempt to characterize sphingolipid patterns specific to HD, an additional molecular hallmark of the disease. Based on the crucial role of sphingolipids in maintaining cellular homeostasis, the dynamic regulation of sphingolipids upon insults and their involvement in cellular stress responses, we hypothesize that maladaptations or blunted adaptations, especially following cellular stress due to reduced oxygen supply (hypoxia) contribute to the development of pathology in HD. We review how sphingolipids shape cellular energy metabolism and control proteostasis and suggest how these functions may fail in HD and in combination with additional insults. Finally, we evaluate the potential of improving cellular resilience in HD by conditioning approaches (improving the efficiency of cellular stress responses) and the role of sphingolipids therein.

Sphingolipid metabolism is crucial for cellular homeostasis and for adaptations following cellular stress, including hypoxia. Inadequate cellular management of hypoxic stress likely contributes to HD progression, and sphingolipids are potential mediators. Targeting sphingolipids and the hypoxic stress response are novel treatment strategies for HD.

1. Introduction

Deficits in lipid and energy metabolism in the brain are common and early phenomena of neurodegenerative diseases. Metabolic alterations both on the systemic and the tissue/cellular level also characterize Huntington disease (HD) and are among its earliest symptoms [1]. HD is an autosomal dominant neurodegenerative disease characterized by progressive motor (in particular involuntary movements – "chorea"), cognitive and psychiatric symptoms. Besides the neurological symptoms, HD is associated with metabolic impairments in various other organs and tissues, including skeletal muscle, heart, gastrointestinal tract, liver and adipose tissue [2]. Metabolic dysfunctions are considered central features of HD progression since early reports on clinical cohorts of whole-body metabolic alterations [3] as well as of impaired cerebral glucose metabolism, nowadays readily detectable by positron emission tomography (PET) and for example reflecting synaptic deficits [4]. Impaired lipid metabolism, including sphingolipid metabolism, has emerged as an early feature of HD and other neurodegenerative diseases that may be central in disease pathogenesis and is a potential target for novel pharmacological treatments [8–10]. Also the role of cholesterol in HD has received great public attention recently with the launch of a gene therapy clinical trial (ClinicalTrials.gov Identifier: NCT05541627) aiming to improve cholesterol catabolism in the brain by increasing cholesterol 24-hydroxylase expression. In addition, deficits in the lipid-rich (particularly rich in the sphingolipid sphingomyelin) insulation layer around many neuronal axons, the oligodendrocyte-derived myelin-sheath, is heavily investigated in the context of HD. Several recent reports demonstrated oligodendrocyte and myelination deficits in

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https://doi.org/10.1016/j.plipres.2023.101224
Received 21 November 2022; Received in revised form 20 February 2023; Accepted 5 March 2023
Available online 8 March 2023

rodent models of HD [11–16] and in human HD brain [17] (older results summarized by Wilson and colleagues [18]).

HD is caused by an abnormally expanded trinucleotide CAG repeat in the huntingtin gene (*Htt*), coding for the ubiquitous huntingtin protein (Htt), and leading to the expression of polyglutamine (poly-Q) stretches in Htt [19,20]. The *Htt* gene consists of 67 exons and the Htt protein can be subjected to multiple types of proteolytic cleavages and other post-translational modifications, which determine Htt location, function and activity as discussed in detail elsewhere [21].

If the expansion in Htt contains more than 39 CAG repeats, this almost always triggers disease processes leading to HD-associated motor signs [22], which usually manifest during midlife [24]. Diagnosis of HD is based on motor symptoms and genetic testing [24]. The physiological function of Htt is still not fully elucidated but it is involved in microtubule-mediated transport and vesicle function [25] as well as in many other cellular processes, such as cell division, ciliogenesis and the regulation of transcription (for a detailed review see [21]). Htt further is a regulator of selected macroautophagy [26] and consistent with this role, impaired autophagy is also an important feature of HD [27]. Still, further physiological functions of Htt keep being discovered and just recently, its involvement in mediating neurotrophic signals from peripheral sites in the cell to the nucleus has been demonstrated [28].

HD symptoms are a result primarily of the degeneration of striatal medium spiny neurons (that are exquisitely vulnerable to mutated Htt (mHtt)) and of neuronal populations in the cerebral cortex [29]. The toxicity of mHtt is associated with its proneness to form aggregations and its altered interaction with other biomolecules, leading to impaired transcription, immune and mitochondrial functions [30] and reduced neurotrophic support by brain-derived neurotrophic factor (BDNF) [31]. The intercellular localization, transport, aggregation propensity and molecular interactions of wild-type Htt are regulated by a wide array of posttranslational modifications, and this regulation is different for mHtt (for review see [21]). Location and toxicity of mHtt further depend on the cellular lipid environment and its interaction with lipids, including sphingolipids [32]. Sphingolipid metabolism further regulates the management of cellular stress, which is impaired in HD.

Despite increasing knowledge on both the biochemical underpinnings of mHtt aggregation and the energy- and lipid-metabolic deficits associated with HD, their individual or inter-dependent contributions to neurodegeneration and HD symptomatology remain poorly understood. The aim of this review is first to assess the state of knowledge on lipids and sphingolipid metabolism dysregulation in HD. Secondly, based on recent literature, we evaluate how compromised sphingolipid metabolism may be connected to deficits in cellular stress adaptations (with a special emphasis on hypoxic stress), impacting on mHtt aggregation and mitochondrial functions, which then may synergistically induce a pathogenic cascade and drive disease progression. We highlight sphingolipid involvement in metabolic stress responses as a possible crucial factor in the adverse systemic and cellular alterations in HD, reflect on potentials of pharmacological and life-style interventions related to metabolic deficits and endogenous adaptation capacities and discuss clinical implications.

2. Lipid metabolism in aging, Huntington disease, and other neurodegenerative diseases

Lipid metabolism is an important regulator of aging, and is often impaired in age-related diseases [33]. Sphingolipids may be of special importance for aging processes and their role in senescence has previously been reviewed [34]. While it is assumed that certain sphingolipids (e.g. ceramide) promote senescence and others (e.g. sphingosine-1-phosphate (S1P) and sphingomyelin) antagonize it [34,35], specifics of the regulation of mammalian aging by sphingolipids are still largely unexplored. However, an interesting hypothesis, how sphinglipids regulate aging is related to the reciprocal regulation of sphingolipids and lysosomal activity [36]. Lysosomal activity is closely related to aging and clearance of misfolded proteins, such as mHtt in HD. The observation in rats that enzyme activities to produce ceramide and consequently ceramide levels increase during aging [37,38] are in line with a pro-aging role of ceramide, while high levels of sphingomyelin in centenarians suggest a protective role of these sphingolipids [35]. In the human hippocampus potential sex-specific differences in sphingolipid-levels have been reported with increasing ceramide possibly being more important for brain aging in males and decreasing S1P levels in females [39].

We know from invertebrate models that reduced expression of genes involved in the synthesis of ceramide and overexpression of fatty acid amide hydrolase, lysosomal lipase or diacylglycerol lipase extend lifespan in those species [33]. Conversely, excessive lipid levels are associated with impaired degradation of cellular waste products, as reviewed by Vanier [40] for the neurodegenerative lysosomal storage disorder Nieman-Pick disease type C. In a mouse model of this disease cholesterol-mediated impairment of lysosomal transport into axons, leading to axonal dystrophy, was recently demonstrated [40,41]. Increasing impairment of lipid metabolism during aging (e.g. related to sphingolipids [37–39]) is also a contributor to the progression of other aging-related neurological diseases. Alterations in lipid metabolism potentially modify pathogenesis and disease progression in several neurodegenerative diseases [9,10,43]. Major deficits in HD brain lipid metabolism notably include cholesterol [44,46–48] and sphingolipid metabolism [8,9]. mHtt for example interacts with sterol regulatory element-binding proteins, possibly contributing to lipid metabolism deficits and inflammatory processes [44]. Reports on the consequences of this interaction, however, are conflicting and have been prominently discussed [48]. While mHtt in a striatal neuron model of HD has been shown to increase sterol levels [49], Valenza and colleagues [50] reported a robust downregulation of cholesterol biosynthesis and transport, resulting in reduced cholesterol in cerebrospinal fluid in multiple mouse models of HD.

Deterioration of lipid metabolism homeostasis is intimately connected to compromised energy metabolism, mitochondrial dysfunction and impaired Ca^{2+} signaling and has been shown to be a direct consequence of mHtt in a Drosophila model of HD [51,52], linking the main pathological hallmarks of HD.

In the following sections, we will argue that the dysregulation of sphingolipid metabolism is a crucial factor in HD pathogenesis and progression.

3. Sphingolipid metabolism and neurodegeneration

Sphingolipid metabolism is tightly regulated and highly responsive to cellular stress, as discussed in this section. Impairments interfere with cellular homeostasis and can lead to neurodegeneration [9]. The possibility that dysregulated components of sphingolipid metabolism are involved in the pathogenesis and disease progression of Alzheimer and Parkinson disease are subject of intense discussion [9,10]. Since the main risk factor for these most common neurodegenerative diseases is age, the increasingly acknowledged involvement of sphingolipids in the aging process [33] supports a role also in the development of these age-related neurological diseases. Although less prominently investigated, strong evidence shows impaired sphingolipid metabolism in the early development of the hereditary neurodegenerative disease HD. Such deficits are among the earliest detectable cellular abnormalities in animal models of HD and precede symptom onset, thus suggesting a fundamental role of sphingolipids in disease progression. Before summarizing the knowledge on specific sphingolipid-related deficits in HD, a brief overview of relevant elements of the sphingolipid metabolism is provided, followed by a short chapter on general aspects of sphingolipid involvement in neurodegeneration.

3.1. Sphingolipid synthesis and metabolism

Lipids with a long-chain sphingoid base as structural backbone are termed sphingolipids [53]; ceramides, sphingomyelins, and glycosphingolipids, such as gangliosides, cerebrosides, and sulfatides are prominent representatives of this category that overall comprises more than 300 different lipids. The name is derived from the sphingosine base, which refers to the common 18 carbon long, non-polar, amino-alcohol component of sphingolipids [54]. If a fatty acid group is added to sphingosine, ceramide, hub of sphingolipid metabolism, results and addition of a further choline residue yields sphingomyelin (see Fig. 1) in vertebrates [55]. Gangliosides are more complex sphingolipids with sugar-residues. Furthermore, the length of the carbon chain, saturation level, stereochemical features and number of hydroxyl groups are variable in sphingosines, contributing to their variability and complexity in terms of structure and bioactive function.

While previously considered mainly structural lipids, many sphingolipid-derived molecules are now known to be bioactive (bioactive lipids are involved in the response to specific stimuli and thus contribute to the regulation of cellular homeostasis [56]) and include ceramide, ceramide-1-phosphate (C1P), sphingosine, and S1P; as well as less well studied types such as sphingosylphosphorylcholine, psychosine, lactosylceramide, and cerebroside [56,57].

The synthesis of ceramide can be *de novo* (most common pathway) or, alternatively, through hydrolysis of sphingomyelin or recycling of gangliosides or S1P via salvage pathways [59]. These pathways can be differentially activated due to the different intracellular localization of their key enzymes. *De novo* synthesis occurs in the endoplasmic reticulum, where palmitoyl-coenzyme A (palmitoyl-CoA) – which in the brain can be substituted by steraoyl-CoA [60] – and serine (or alanine or glycine with stearate or myristate [56]) condensation is catalyzed by serine palmitoyl transferase (importantly, a substrate shift of serine palmitoyl transferase to alanine or glycine as a result of various missense mutations of the enzyme is associated with neuropathy, such as in Hereditary sensory and autonomic neuropathy type 1 [61]). This yields 3-ketodihydrosphingosine that can be transformed to dihydrosphingosine (= sphinganine), reducing the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), and dihydroceramide by ceramide synthases. The dependence of this process on NADPH suggests that the oxidative status of the cell is a determinant of sphingolipid metabolism [62] and is an indication that *de novo* synthesis is modulated by cellular stress. Cellular stress, notably heat stress, oxidative stress or pharmacologically induced stress indeed upregulate *de novo* synthesis, as do high levels of serine or palmitate [63]. Conversely, sphingomyelin hydrolysis occurs primarily in the plasma membrane and the salvage pathway is partly located in the lysosomal/late endosomal compartment [63].

Sphingolipids are abundant membrane lipids and besides cholesterol and glycerophospholipids make up a main part of biological membranes. They are regulators of membrane structure and organization and are involved in the formation of specialized membrane domains, including “lipid rafts” (signaling platforms in membranes composed of

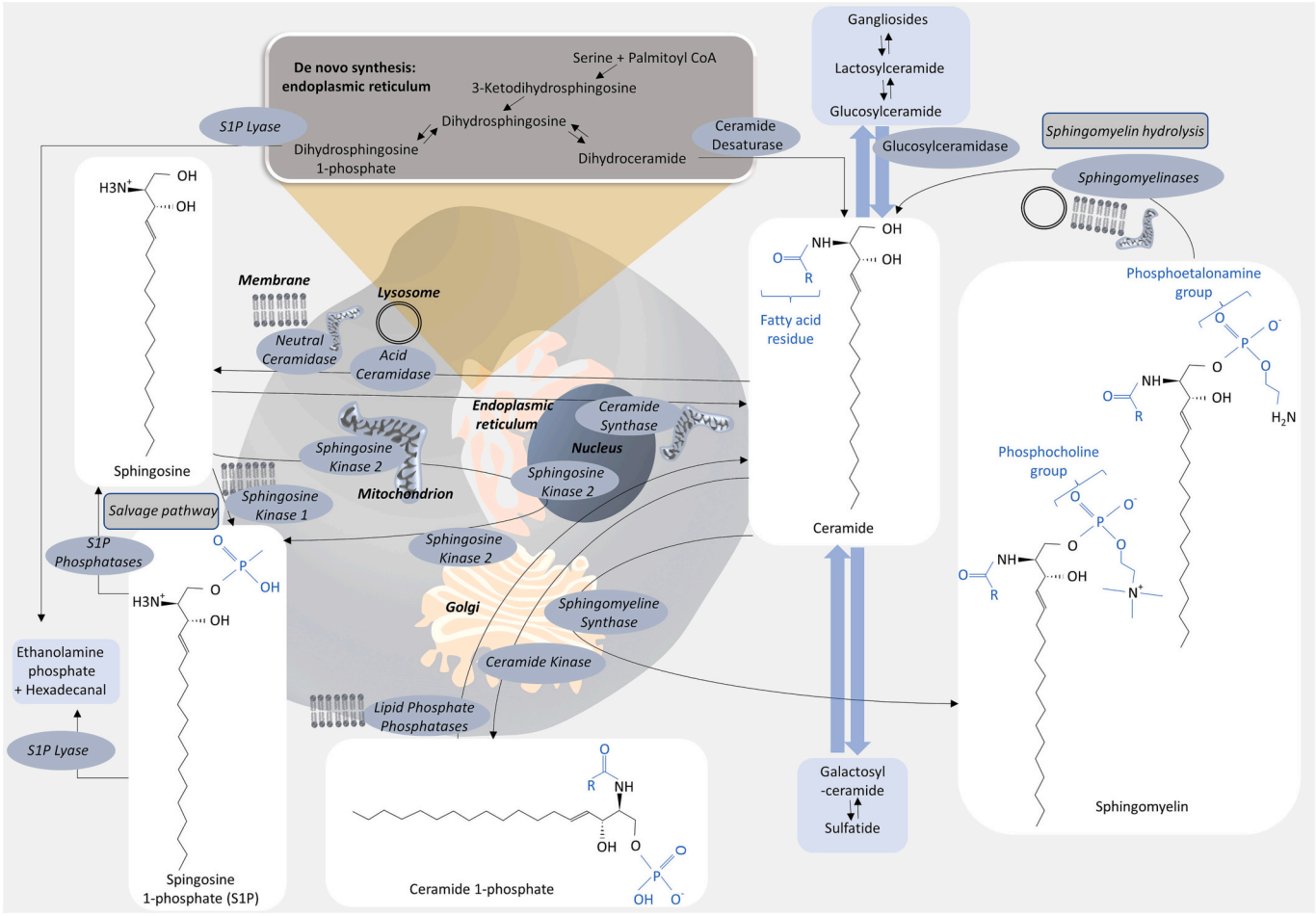

Fig. 1. Overview on sphingolipid metabolism. *De novo* sphingolipid synthesis or transformation of different other sphingolipids results in formation of ceramide, a starting point of the generation of many sphingolipid types. Turquoise ellipses contain the names of some of the most important involved enzymes. According to their function and location of substrates, these enzymes are associated with different cellular compartments, such as the plasma membrane, endoplasmic reticulum, Golgi apparatus, nucleus, lysosomes or mitochondria.

lipids and enriched in protein receptors, which they form together with cholesterol)[64], and in the dynamic integration of associated proteins in cell membranes. Further, they also are well-established key components of various other intracellular signaling modalities [65] and exosome-mediated cell-to-cell communication [66] and are emerging as integral players in crucial brain functions, such as cognition [67].

Sphingolipid synthesis and modification are highly regulated and maintenance of balanced levels is crucial for cellular function and survival. Sphingolipids are enriched in the outer leaflets of plasma membranes but also fulfill a variety of functions in other regions of the cell [53]. While they can rapidly diffuse in membranes (lateral diffusion), the subcellular transport regulating sphingolipid distribution requires vesicle-based transfer, soluble lipid transfer proteins, transfer across juxtaposed membranes, such as contact sites between endoplasmic reticulum and mitochondria, or other means of transport [53].

Some major elements of sphingolipid metabolism and their localization and functions are discussed below.

3.1.1. Ceramide

Ceramides are synthesized in the endoplasmic reticulum and are the central hub of sphingosine synthesis and catabolism (Fig. 1). Their sphingoid long-chain base is linked to an acyl chain via an amide bond, with the length of the acyl chain determining biological effects, such as shaping membrane curvature [68]. Due to the high hydrophobicity of ceramides, ceramide transfer proteins are required for shuttling and regulating intracellular distribution, in particular to the Golgi, where ceramide conversion to sphingomyelin occurs [69]. Ceramide levels change dynamically in response to various stressors. Increased ceramide levels are often associated with reduced cell survival, higher cell differentiation, proliferation, and autophagy (summary in supplementary table 2 of [56]). As modulators of cell death and inhibitors of proliferation [68,70], ceramides are also associated with dysregulated neuronal death control in conditions of sphingolipid metabolism deficits [10]. On the other hand, ceramides are important regulators of fatty acid translocation across cellular membranes via CD36 and facilitators of free fatty acid incorporation into acyl-Coenzymes A or triglycerides and storage in lipid droplets [71].

Ceramides further crucially regulate senescence of mammalian cells [72] and are key players in autophagy-regulation by modulating nutrient transport, endoplasmic reticulum stress, and mitochondria-specific autophagy (mitophagy) [56]. In addition, ceramides are involved in cytoskeleton rearrangement and cancer migration/invasion, and insulin resistance has also been linked to high ceramide levels [56]. Ceramides further activate protein phosphatases (types PP1 and PP2A), thereby regulating the cellular phosphoproteome and overall cell function [56].

3.1.2. Glycosphingolipids, sphingomyelin and C1P

Ceramides can be converted into glycosphingolipids (reviewed in [73]), such as gangliosides (oligosaccharide and up to 7 *N*-acetylgalactosamine residues, for a detailed review on ganglioside metabolism see [74]) and cerebrosides (glucose or galactose residues added). Among important biosynthetic enzymes for glycosphingolipids are sialyltransferases, galactosyltransferases and glucosyltransferases, some of which may be dysregulated in HD (see below).

After biosynthesis of ceramide (involved enzymes are located on the cytosolic leaflet of endoplasmic reticulum membranes), glucosylceramide can be produced by glucosyltransferase, an enzyme in the cytosolic membrane leaflet of the Golgi apparatus [75]. Additional transfer of a galactose moiety on glucosylceramide to yield lactosylceramide occurs on the luminal leaflet of Golgi membranes [76], therefore requiring glucosylceramide to translocate through the Golgi membrane. Lactosylceramide is the precursor of most glycolipids, including gangliosides (except GM4). In the lumen of the Golgi apparatus, glycosyltransferases can add sugar residues, such as sialic acid moieties, to lactosylceramide, generating a variety of gangliosides [74].

Gangliosides are the most abundant lipid class in neurons [67], make up around 10% of the total lipid mass in the brain and are particularly dense in neuronal synapses [70,77]. They are essential for maintenance of myelinated axons [78], with their regulating roles in membrane dynamics, signaling, and myelin-axon interactions being indispensable for brain function [79]. A detailed overview on the ganglioside biosynthetic pathway and discussion of its role in brain physiology has been recently provided [80].

The most abundant sphingolipids in mammalian cells are sphingomyelins [67]. They can be hydrolyzed to ceramides by sphingomyelinases (mostly in the luminal part of the Golgi), enzymes that can be classified in acid, neutral, and alkaline sphingomyelinases. As this classification suggests, these enzymes differ by maximal capacities according to the pH, which depends on their intracellular localization and the cellular environment [54,67].

3.1.3. From ceramide to sphingosine to S1P

Ceramides can be furthermore transformed into sphingosine by neutral and acid ceramidases via acyl chain group removal [81]. Ceramide synthase can convert sphingosine back to ceramide but sphingosine can also be phosphorylated, yielding S1P, a key messenger molecule [82], regulator of cell growth [82] and inhibitor of apoptosis [83]. The cytosolic sphingosine kinase 1 is associated with neuronal survival, and via its presynaptic localization, influences cognitive processes. Depletion of sphingosine kinase 1 results in perturbed endocytic recycling [85] and thus impairs autophagic efficiency. The functions of sphingosine kinase 2 depend on its subcellular localization. It is involved in adaptations to stress, for example due to low oxygen availability, by regulating hypoxia-inducible factor (HIF)-linked signaling [86] and histone acetylation via S1P mediated inhibition of histone deacetylases [87] in the nucleus. In the endoplasmic reticulum, sphingosine kinase 2 contributes to apoptosis induction during cellular stress [88] and mitochondrial sphingosine kinase 2 regulates the assembly of complex IV of the mitochondrial electron transport system via binding to the mitochondrial protein prohibitin 2 [89]. In addition, membrane-bound sphingosine kinase 2 may be involved in neoplastic transformations [90].

S1P signaling is conveyed through intra- and extracellular pathways [91,92] via at least five G protein-coupled receptors, $S1PR_1$ - $S1PR_5$ [93,94]. These receptors have received increasing attention as drug targets [56]. Perturbed control of S1P balances and signaling via its generating or degrading enzymes is associated with impaired overall brain homeostasis and blood-brain barrier dysfunction [8,95]. Dysregulation of sphingolipid metabolism is thus emerging as an early marker of neurodegenerative diseases, including Alzheimer disease [9,10,62,96], Parkinson disease [10,97] and HD [8,10].

S1P breakdown is catalyzed by S1P lyase, 2 types of S1P-specific phosphatases, and 3 types of lipid phosphate phosphatases [98]. Dephosphorylation of S1P replenishes sphingosine levels, while degradation of S1P by S1P lyase yields ethanolamine phosphate and the apoptosis-inducing molecule hexadecanal [99], highlighting the risk of high S1P lyase levels/activity. The S1P lyase catalyzed permanent breakdown of S1P is the only known irreversible breakdown of sphingolipid metabolites.

3.2. Deficits in sphingolipid metabolism in neurodegenerative diseases

Neurodegenerative diseases, including HD are characterized by deficits in neuronal signaling and neurotrophic support, ultimately leading to the demise of vulnerable neuronal populations [100]. These are processes that are importantly regulated by sphingolipids as previously summarized [101–103].

Sphingolipids are highly enriched in the central nervous system and are required for brain development and maintenance, in part by modulating cell death and differentiation processes, cell cycle and proliferation, cell migration and cytoskeleton organization, autophagy and

senescence [54]. By regulating membrane fluidity, receptor composition (e.g., in lipid rafts) and neurotransmitter homeostasis, sphingolipids are fundamental for signaling within the brain [67], which allows cognition, emotion and the coordination of movements, all of which can deteriorate in neurodegenerative diseases. Accordingly, dysregulation of sphingolipid synthesis and signaling pathways is increasingly acknowledged to compromise neuronal plasticity [67] and to contribute to the pathology of various neurodegenerative diseases [104], such as Parkinson disease [97,105], Alzheimer disease [105] and HD [8]. The general role of sphingolipids in neurodegenerative processes has recently been reviewed in more detail [9], therefore only some relevant examples of sphingolipid-related alterations in neurodegeneration will be discussed in the next section before an in-depth analysis of sphingolipid metabolism in HD will be presented in the subsequent section.

Based on the central role of ceramides in sphingolipid metabolism, it is not surprising that its levels are of special importance for brain health, and alterations affect levels of other sphingolipid classes. Of great relevance for neurodegeneration is the involvement of ceramide in the brain and its associated dysregulation of sphingolipid balances in neuroinflammation, a common hallmark of many neurological diseases. For more information on this topic, we refer the reader to previous reviews [68,106].

Ceramide levels are regulated by synthesis (ceramide synthases) and its conversion into other sphingolipid types. Of the six ceramide synthase isoforms, which generate ceramides with different acyl chain lengths [107], ceramide synthase 1 is the most important isoform in the brain [108]. Its brain-region-specific expression pattern, with especially high densities in hippocampus and neocortex [109], has been suggested to play a role in characteristic brain-region specific vulnerabilities in neurodegenerative diseases [62]. In mice with mutations leading to reduced activity of ceramide synthase 1, lipofuscin formation and neurodegeneration was reported [110]. Lipofuscin aggregates are lipid, protein and metal-containing accumulations, which become more frequent at advanced age and are associated with senescence-related alterations of cell physiology. In the brain, they have been linked to protein aggregation and neurodegenerative processes [111]. Among the ceramide-converting enzymes, acid ceramidase (catalyzing ceramide to sphingosine conversion) activity may play a vital role in neurodegeneration since it has been shown to be increased in Alzheimer disease and co-localized with neurofibrillary tangles, a pathological hallmark of Alzheimer disease [112]).

Sphingosine kinase mediated phosphorylation of sphingosine yields S1P, one of the most important bioactive sphingolipids in the brain, which is involved in fundamental cerebral functions such as neurogenesis and neurite outgrowth, survival of different brain cell types and control of the blood-brain barrier [8,95]. As opposed to high ceramide levels, S1P promotes growth and survival and its levels are positively associated with autophagy, cell migration/invasion and cytoskeleton rearrangement, as summarized by Hannun and Obeid [56].

Ceramide can be further converted into complex glycosphingolipids, either via galactosyl-ceramide into sulfatide, or via glucosyl-ceramide into gangliosides. Gangliosides have early been linked to Alzheimer disease pathogenesis [113]. Especially the monosialo-tetrahexosyl-ganglioside GM1 has been demonstrated to exert various beneficial effects in diseases of the nervous system, including HD, with the best clinical evidence of benefits coming from stroke [114]. Its efficiency in treating brain injury associated with ischemia/hypoxia is further substantiated by its benefits in ischemic neuronal and spinal cord injuries, or subarachnoid hemorrhage [114]. While the mechanistic basis for these benefits is not yet sufficiently understood, GM1 is thought to be neuroprotective primarily by regulating apoptosis and the release of neurotrophins [114]. Much previous research was restricted to the direct benefits of GM1 in neurons but important effects on other brain cell types have been recently highlighted, such as its capacity to reduce inflammatory responses of microglia [115].

Sulfatide is predominantly synthesized by oligodendrocytes [62], a brain cell type best known for myelinating axons and thus supporting neuronal function. Since in cortical white matter sulfatide concentrations decrease during adulthood more than those of gangliosides [116], their role in age-related neurological diseases may be substantial. Levels of sulfatide are indeed massively depleted in gray and white brain matter of people with mild cognitive impairment, a prodromal state of Alzheimer disease [117]. This has been linked to the role of the Alzheimer disease risk gene Apolipoprotein E, encoding a protein involved in sulfatide transport [117,118]. The reduction of sulfatide notably was associated with a more than 3-fold increase of ceramide levels in white matter of these subjects [117]. In line with these findings, increased ceramide levels also characterize Alzheimer disease patient brains and are even higher in individuals suffering from additional other neuropathologies [119]. The changes in ceramidase level may result from increased ceramide synthase expression [120]. But also increased levels of amyloid beta (a protein that accumulates in Alzheimer disease brain and forms amyloid plaque pathology) may perturb sphingolipid homeostasis by stimulating sphingomyelin hydrolysis, leading to higher ceramide generation [96].

In Parkinson disease, high levels of ceramides and sphingomyelin were observed in patient brains [121]. Mutated glucocerebrocidase (GBA) is the most common genetic risk factor in sporadic Parkinson disease [122]. GBA catalyzes the breakdown of glucosylceramide into glucose and ceramide; therefore, altered GBA activities are associated with changes in sphingolipid homeostasis. In non-GBA mutation carriers, alterations in sphingolipid concentrations were also reported, and high ceramide levels were found especially in Parkinson disease patients with cognitive impairments [123].

Whether the neurodegeneration-related changes in sphingolipid metabolism are driving the respective diseases or whether they are compensating mechanisms, at least initially attenuating pathology, remains to be clarified for the individual diseases [124]. In the next sections, we aim to dissect sphingolipid-related brain alterations and their roles in the disease progression, especially for HD, and will argue that impaired sphingolipid metabolism in HD may impair cellular stress adaptations. In this sense, failed compensatory mechanisms turn into drivers of disease progression.

3.3. *Sphingolipid metabolism in Huntington disease*

A wide array of perturbations in glycosphingolipid metabolism has been reported in both cellular and animal models of HD and in post-mortem HD tissues. A summary of such studies on alterations in sphingolipid metabolism-related enzymes is provided in Table 1 and a summary on studies on sphingolipid levels themselves in Table 2.

3.3.1. *De novo synthesis*

Changes in the *de novo* synthesis of sphingolipids have been reported to be specific for different disease stages. Serine palmitoyltransferase activity requires the transcription of serine palmitoyltransferase long chains 1 and 2. Serine palmitoyltransferase long chain 1 mRNA levels were found to be increased in presymptomatic R6/2 striatum and cortex and for chain 2 also in cortex [128]. In symptomatic R6/2 mice reduced serine palmitoyltransferase long chain 1 mRNA was recorded [128]. Accordingly, dihydrosphingosine levels were decreased as well [128].

These results not only show a dysregulation of sphingosine synthesis associated with HD progression but may also be indicative of stress-induced sphingolipid upregulation at early disease stages that might represent compensatory or (mal-)adaptive changes, which are lost at later disease-stages. This dynamic also could indicate fluctuating sphingolipid levels throughout (early) disease stages, highlighting the importance of assessing functional changes at a better temporal resolution. Various selective PET tracers that could be useful to track alterations of different sphingolipid metabolism components for this purpose are emerging, e.g. for ceramide [141] or S1P receptor 2 [142].

Pharmacological inhibition of serine palmitoyltransferase with

Table 1
Alterations of sphingolipid-related enzymes in Huntington disease (HD) and HD models.

Model	Tissue/cells	Sphingolipid metabolism associated enzyme	Effect (vs. control)	References
STHdh Q111 cells	Striatal cells	S1P lyase 1 (protein and mRNA)	↑	[125–127]
		Sphingosine kinase 1 (protein and mRNA)	↓	
		Sphingosine kinase 2 (protein and mRNA)	↑	
		Beta-1,3-Galactosyltransferase 4 (mRNA)	↓	
		UDP-Glucose Ceramide Glucosyltransferase (mRNA)	↓	
		Glucosylceramidase Beta 1 (mRNA)	↑	
HD human fibroblasts	Human fibroblasts	Beta-1,3-Galactosyltransferase 4 (mRNA)	↓	[126]
Early symptomatic R6/2 mice (6 weeks old)	Striatum, cortex	Serine palmitoyl-transferase long chain 1 (mRNA)	↑	[128,129]
		Serine palmitoyltransferase long chain 2 (mRNA)	↑ (only in cortex)	
		Ceramide synthase 1 (mRNA)	↓ (only in cortex)	
		S1P lyase 1 (protein)	↑	
	Small intestine	S1P lyase 1 (protein)	↑	[130]
		Serine palmitoyl-transferase long chain 1 (mRNA)	↓	
		Serine palmitoyl-transferase long chain 2 (mRNA)	↓	
Symptomatic R6/2 mice	Striatum, cortex	Serine palmitoyltransferase long chain 1 (mRNA)	↓	[128,129]
		UDP-Glucose Ceramide Glucosyltransferase (mRNA)	↓ (only in striatum)	
		Ceramide synthase 1 (mRNA)	↓	
		Ceramide synthase 2 (mRNA)	↓ (only in striatum)	
	Small intestine, large intestine	S1P lyase 1 (protein)	↑ (only in small intestine)	[130]
		Sphingosine kinase 1 (protein)	↓ (only in small intestine)	
Symptomatic R6/1 mice	Cerebellum	Beta-1,4-N-Acetyl-Galactosaminyltransferase 1 (mRNA)	↓	[131]
		UDP-Glucose Ceramide Glucosyltransferase (mRNA)	↓	
	Striatum	ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 1 (mRNA)	↑	[132]
		ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 3 (mRNA)	↓	
		Beta-1,4-N-Acetyl-Galactosaminyltransferase 1 (mRNA)	↓	
Symptomatic YAC128 mice	Striatum, cortex	UDP-Glucose Ceramide Glucosyltransferase (mRNA)	↓ (only in striatum)	[126,127]
		ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 5 (mRNA)	↓ (only in striatum)	
		Beta-1,4-N-Acetyl-Galactosaminyltransferase 1 (mRNA)	↓ (only in striatum)	
		Beta-1,3-Galactosyltransferase 4 (mRNA)	↓ (only in cortex)	
		ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 1 (mRNA)	↓ (only in cortex)	
		ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 3 (mRNA)	↓ (only in striatum)	
		S1P lyase 1 (protein)	↑ (only in striatum)	
		Sphingosine kinase 1 (protein)	↓ (only in striatum)	
HD post-mortem, N=3	Caudate	ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 5 (mRNA)	↓	[132]
		ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 3 (mRNA)	↓	
		Beta-1,4-N-Acetyl-Galactosaminyltransferase 1 (mRNA)	↓	
		ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 2 (mRNA)	↓	
HD post-mortem, N=3	Cerebellum	Beta-1,4-N-Acetyl-Galactosaminyltransferase 1 (mRNA)	↓	[131]
		ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 1 (mRNA)	↓	
		ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 5 (mRNA)	↓	
HD postmortem, N=38	Cortex	S1P lyase 1 (protein)	↑	[125]
post-mortem cases HD (40-45 CAG repeats, N=13)	Caudate, putamen	Ceramide synthase 1 (protein)	↓	[133]

Notes: striatum corresponds to caudate nucleus and putamen together, in human anatomy these regions are usually considered differentially, while in rodents the striatum is a more uniform structure. S1P; sphingosine-1-phosphate

myriocine has further been shown to increase the vulnerability of the striatal control STHdh Q7 cells to apoptosis to a similar vulnerability as the HD model cell line STHdh Q111, striatum-derived cells expressing Htt with 111Qs [128]. This could suggest that perturbation of the sphingolipid *de novo* synthesis pathway deprives cells of their intrinsic ability to respond adequately to and survive cellular stress.

3.3.2. *Ceramides and S1P-generating and degrading enzymes*

Lower expression of the ceramide-synthesizing enzyme ceramide synthase 1 (generates long-chain ceramides) was reported in cortex of presymptomatic R6/2 mice and a similar trend was observed in the striatum [128]. Levels were even lower in symptomatic mice, in which also the striatum was affected [128]. Reduced expression of ceramide synthase 1 was also found in human HD striatum and was associated with earlier mortality and longer CAG repeats [133]. Expression of ceramide synthase 2 was not altered in HD brain, however changes in posttranslational modification patterns might still indicate a role in altered composition of sphingolipid types [133].

Conversely, the S1P degrading enzyme S1P lyase was found to be upregulated both in STHdh Q111 cells and in cortex of HD patients [125]. The resulting decreased levels of S1P together with concomitant increased levels of ceramide, have been repeatedly described in HD models [127,134], and this combination is considered neurotoxic [68].

Experiments showing that inhibition of S1P lyase in mHtt exon1-expressing neurons and in STHdh Q111 cells was neuroprotective [127] support detrimental outcomes of too high levels of S1P lyase – or too low S1P levels – for neurons. Interestingly, although the inhibition of S1P lyase was not clearly detectable *in vivo*, treatment with the inhibitor 2-acetyl-5-tetrahydroxybutyl imidazole (THI) ameliorated both motor performance and neuropathology and preserved normal levels of glucosylceramide in R6/2 HD mice [129]. On the other hand, the stimulation of sphingosine kinase 1 to support S1P synthesis slowed down progressive motor deficits, reduced mHtt aggregates in the brain [143] and improved perturbed intestinal homeostasis associated with the R6/2

Table 2
Alterations of sphingolipid levels in Huntington disease (HD) and HD models

Model	Tissue/cells	Sphingolipid species	Effect (vs. control)	References
STHdh Q111 cells	–	Ceramide (d18:1, C24:1, C24:0 and C16:0)	↑	[134]
		Sphingomyelin (C24:1)	↑	
		S1P	↓	
STHdh Q111 cells, HD patient fibroblasts	–	Plasma membrane and total GM1	↓	[126]
YAC128 primary cell cultures	Striatum	GM1, GT1b	↓	[126]
	Cortex	GM1, GD1a	↓	
	Neurons	GM1	↓	
	Astrocytes	GM1	↔	
Sheep (73 CAG in full-length human cDNA transgene), presymptomatic	Plasma	Hydroxylated (OH) and non-hydroxylated sphingomyelins ((OH) C14:1; (OH) C16:1; (OH) C22:1; (OH) C22:2; (OH) C24:1; C16:0; C16:1; C18:0; C18:1; C24:0; C24:1; C26:0; C26:1)	↓	[135]
Early-symptomatic zQ175 and YAC128	Striatum, cortex	Glucosylceramide	↑ (only in cortex)	[129]
Early symptomatic YAC128 and R6/2 mice	Corpus callosum	GD1a	GD1a in R6/2 ↓	[136]
Early-symptomatic YAC128 and R6/2 mice	Corpus callosum	GD1a, and GT1b	↓	[136]
Early symptomatic and symptomatic R6/2 mice (CAG repeat length 320–350)	Striatum	S1P (d18:1, d20:1 and total)	↓	[125]
Symptomatic YAC128 and R6/2 mice	Corpus callosum	GM1, GD1a, and GT1b	↓	[136]
Symptomatic R6/2 mice	Cortex	GM1	↓	[136]
		GD1a and GT1b	↑	
	Striatum	GM1 and GD1a	↓	
Symptomatic R6/2 mice (CAG repeat length 160 ± 5)	Striatum, cortex	S1P	↓	[127,129]
		Sphingosine	↑ (only in striatum)	
		Ceramide (C20:0, C22:0, C24:1)	↑ (only in cortex)	
		Ceramide C24:0	↑	
		Hexosylceramide (C18:0, C24:1)	↑	
		Hexosylceramide C18:1	↓	
		Glucosylceramide	↑	
Symptomatic R6/2 mice	Striatum, cortex	Dihydrosphingosine	↓	[128]
		DihydroCeramide (C18:0)	↓	
		DihydroS1P	↓	
Symptomatic R6/2 mice on BCF1-background (CAG repeat length 120-150)	Total brain	GD1a	↑	[137]
	Serum	GM2-NeuGc	↓	
Symptomatic R6/1	Cerebellum	GM1, GD1a, GD1b, GT1b, GQ1b	↓	[131]
Symptomatic R6/1 mice	Forebrain	GM1	↓	[132]
		Cerebrosides, sulfatides	↓	
Rats injected with kainic acid in the striatum	Striatum	Gangliosides (N-acetylneuraminic acid)	↓	[138]
post-mortem cases HD (40-45 CAG repeats, N=13)	Caudate	Ceramide C16:0, sphingomyelin (most species), dihexosylceramide C16:0	↑	[133]
		Very long ceramide (C22:1; C24:1; C24:2), Sphingomyelin (C24:0; C24:1; C25:0 and C26:0), Dihexosylceramide (C24:0) and Galactosylceramide (C22:0; C24:0 and C24:1)	↓	
	Putamen	Ceramide (C16:0) and Sphingomyelin (C14:0; C15:0; C16:0; C16:1; C17:0; C18:1; C22:1 and C26:2)	↑	
	Cerebellum	Dihexosylceramide (C16:0, C22:0, C24:1)	↑	
Post-mortem cases HD, N=3	Caudate	Gangliosides (N-acetylneuraminic acid)	↓	[138]
Post-mortem cases HD, N=4	Putamen	Gangliosides (N-acetylneuraminic acid)	↓	
Post-mortem cases HD (Vonsattel grade 3, N=3)	Caudate	Total gangliosides and GD3	↓	[132]
Post-mortem cases HD, N=13	Caudate	Glucosylceramide	↑	[139]
Post-mortem Vonsattel grade III cases HD, N=4	Myelin layer, sub-ventricular zone	Accumulation of sphingomyelins (d34:0; d36:4; d38:4; d38:3; d35:0; d36:0) and ceramide-1-phosphate	↑	[140]
		Sulfatides (36:1; 40:1; 40:1(2OH); 42:2; 42:1; 42:2 (2OH))	↓	
		Triglycerides (39:3; 56:13; 58:14)	↓	
Post-mortem Vonsattel grade III cases HD, N=3	Cerebellum	GM1	↑	[131]

model [130]. Another strategy to tap into S1P-mediated benefits is targeting S1P receptors. Chronic low-dose intraperitoneal administration of a selective agonist of the S1P receptor 5 (A-971432, 0,1mg/kg) in R6/2 mice prolonged the animals' lifespan and was associated with improved blood-brain barrier integrity, reduced mHtt aggregation, and reduced motor symptoms [144]. These effects were most substantial when A-971432 administration was started in the pre-symptomatic disease stage. The FDA-approved drug FTY720 (against multiple sclerosis) upon phosphorylation by sphingosine kinase 2 acts as unselective agonist at four S1P receptors ($S1PR_1$, $S1PR_3$, $S1PR_4$, and $S1PR_5$) but leads to the selective downregulation of S1PR1, making it a functional antagonist of S1PR1 [145,146]. We have recently reviewed its beneficial effects in the brain with potential benefits for HD, including improved synaptic transmission, brain connectivity and BDNF signaling in both

cortex and striatum, protection from mHtt pathology and brain atrophy via restoring GM1 levels [8].

Mechanistic explanations for the benefits of improving S1P synthesis and signaling include the importance of S1P in immune functions [106,147]. With regard to HD sphingosine kinase 1-mediated stimulation of autophagic clearance of mHtt, as shown in primary striatal neurons, may be a primary protective feature, with S1P lyase upregulation having the opposite effect [148]. In addition, sphingosine kinase 2 and increased S1P levels have been demonstrated to inhibit histone deacetylases [87]. However, in contrast to sphingosine kinase 1, sphingosine kinase 2 can induce DNA double strand breaks and its inhibition attenuated neurodegeneration in neuronal models of HD [127,149].

In summary, expression patterns of S1P regulating enzymes have been shown to be altered in patient brain and HD models, with sphingosine kinase 1 being consistently down- and S1P lyase 1 being consistently up-regulated, yielding reduced levels of S1P [125,127,134]. This constellation of S1P-related enzymes that especially characterizes the striatum but also cortical regions in HD has been suggested to be a crucial factor for the vulnerability of the striatum to mHtt toxicity [8]. The evidence that modulation of sphingolipid metabolism-related enzymes has the potential to improve cellular metabolism with benefits for HD pathology has been previously discussed in more detail [8].

3.3.3. *Shifts in ceramide and sphingomyelin*

Consistent with enzymatic alterations, changes in sphingolipid compositions have been observed by mass spectrometry studies in R6/2 cortex and striatum, in which levels of dihydrosphingosine, dihydrosphingosine-1-phosphate and dihydroceramide [C18:0] were decreased [128]. Similar alterations have been reported in postmortem brains of HD patients [133]. While the cortex was less affected, a shift from very-long-chain (C22-C26) ceramides, sphingomyelins and lactosylceramides towards long (C13-C21) was recorded in the striatum and cerebellum [133]. Using a network-based metabolomics approach, Pirhaji and colleagues [134] also found perturbations in sphingolipid metabolism in STHdh Q111 cells, including significantly upregulated ceramide (C24:1, C24:0 and C16:0) and sphingomyelin (C24:1) levels. Abnormalities in sphingolipid metabolism were also reported by metabolic profiling of transgenic, presymptomatic HD model sheep (73 CAG repeat expansion in a full-length human cDNA transgene) [135].

Overall, reduced levels of not only S1P but also of very long types of sphingolipids are common in human postmortem HD brain and HD models. Concurrently, shorter ceramide and sphingomyelin species tend to be upregulated.

3.3.4. *Gangliosides*

The importance of gangliosides in the brain – and the relevance in the context of HD – is highlighted by observations that mice unable to synthesize gangliosides develop neurodegeneration and various neurodegeneration-associated symptoms [150], including movement [151] and cognitive deficits [152]. Higatsberger and colleagues [138] described reduced levels of gangliosides in HD patients in 1981. An impaired ganglioside synthesis and associated reduced ganglioside levels were subsequently confirmed for HD postmortem brain, HD patient-derived fibroblasts and in the brain of different animal models of the disease [126,132,136]. In these studies, particularly GM1 levels were reduced in HD patient forebrain [132], patient fibroblasts [126] and R6/2 mouse cortex and corpus callosum [136]. Importantly, decreased levels of GM1 were found in neurons, while they remained stable in astrocytes [126]. This reduction rendered the affected neurons more vulnerable to cell death. In the cerebellum, Denny and colleagues [131] found substantially reduced levels of many gangliosides in R6/1 mice, while in the human HD cerebellum, most ganglioside levels were similar to controls, except for GM1, which was even more abundant in this brain region in HD patients. This finding highlights brain region-specific differences in sphingolipid-metabolism alterations in HD and cautions about the validity of sphingolipid changes in mouse models for human HD. The upregulation of GM1 may indicate a neuroprotective mechanism that is successful in human HD cerebellum but not in more vulnerable brain regions in HD. GM1 has been demonstrated to be a particularly potent neuroprotective agent, e.g. by intraventricular administration of GM1 in different mouse models of HD [154,155]. In R6/2 mice such treatment reduced striatal neurodegeneration and white matter atrophy, prevented body weight loss and motor dysfunction and increased survival [154]. It further attenuated abnormal microglial and astroglial activation in these mice. Close to complete abolishment of motor symptoms was observed in Q140 mice, and this effect was accompanied by a reduction in mHtt aggregation, similar like in R6/2 mice [154,155]. Various cognitive, anxiety-like and depression-like symptoms were also ameliorated after GM1 treatment, especially in Q140 and YAC128 mice [154]. Alpaugh and colleagues [154] also studied the levels of various neuroactive amino acids (glutamate, GABA, glycine, serine) in YAC128 mice and observed a trend towards the restoration of these molecules to wild-type levels after GM1 administration.

Exogenous additions of sphingomyelin and GM1 further modulated the aggregation and toxicity of Htt in total brain lipid extracts. Increased sphingomyelin and GM1 levels reduced Htt propensity to insert into lipid monolayers [32]. Total brain lipid extract vesicles were increasingly vulnerable to permeabilization with growing sphingomyelin concentrations while GM1 did not modulate permeabilization [32]. Exogenously added sphingomyelin and GM1 in the presence of Htt-Exon1 induced different structural alterations of membranes and both conditions facilitated Htt aggregation [32]. Increased sphingomyelin contents were associated with less stable and reduced numbers of Htt oligomers at plateau-like structures forming specifically in this condition as compared to rougher membrane patches resulting from Htt-exposure alone [32]. Increasing levels of GM1 slowed down Htt-induced membrane alterations and no plateau-like structures were observed like in the sphingomyelin conditions. Still, high GM1 levels did not significantly preserve membrane integrity in this study [32], suggesting that its interaction with receptors or its ability to modulate posttranslational modifications of Htt [126] are important for the described neuroprotective effects. GM1 treatment has been shown to modify levels of Htt phosphorylation at Ser13 and Ser16 [155], posttranslational modifications that affect both aggregation propensity and toxicity of mHtt [156–158] and may thus be especially beneficial in HD.

The reported potential neuroprotective effects of GM1 include preservation of myelin integrity, maintenance of Ca^{2+} homeostasis [159], modulation of autophagy [160], “neurotrophin-like” benefits on neuronal plasticity and repair [161], membrane integrity [79,159] and anti-inflammatory effects, notably mediated by microglia [115]. Recently it has been shown that GM1 promotes glycolysis by stimulating the expression of various genes linked to glucose metabolism in primary astrocytes [77]. This study indicated that the resulting release of lactate enhanced mitochondrial activity and survival of co-cultured primary neurons [77].

It is important to highlight that despite these promising results on increasing GM1 levels in models of HD the efficiency of – particularly systemic – administration of GM1 in human patients remains unclear. The amphiphilic properties of GM1 limit its capacity to penetrate the blood-brain barrier and, whether demonstrated central benefits (summarized in [114]) are due to GM1 itself of associated metabolites or other indirect effects has not been clarified in humans. Clinical trials of GM1 administration required high doses to elicit modest beneficial effects for Parkinson disease patients [162–165] and intraventricular administration for Alzheimer disease patients [166]. GM1 treatment in stroke commonly was associated with improved neurological outcomes but never with improved survival, as reviewed by Magistretti and colleagues [114]. Despite these limitations, GM1 remains an interesting pharmacological target for HD and the exploration of alternative routes of administration or use of modified molecules that are more stable and

more readily cross the blood-brain barrier (e.g., the hydrophilic GM1-oligosaccharide [167]) may render GM1 administration an even more promising treatment strategy for HD.

Another point that requires unambiguous clarification are potential negative effects of gangliosides on neurons. Several gangliosides, including GM1, have been demonstrated to inhibit Ca^{2+} uptake via the sarco/endoplasmic reticulum Ca^{2+}-ATPase [168–170]. This effect may contribute to neurodegenerative processes observed in lysosomal storage disorders that are characterized by a pathological accumulation of gangliosides [171]. Conversely, the unability to synthesize complex gangliosides is also associated with neuronal deficits, as has been described e.g. for a mutation leading to the premature termination of GM3 synthase (required for the biosynthesis of most complex gangliosides) resulting in an infantile-onset epilepsy syndrome [172].

Distinct tissues, brain regions and brain cell types have differential glycosphingolipid profiles [70], with neurons being particularly enriched. The metabolism of gangliosides has been consistently shown to be perturbed in HD human post-mortem brain and experimental models of the disease but how these changes may contribute to disease progression remains to be elucidated. Differential enzymatic equipment of specific cells to generate and degrade gangliosides may determine selective vulnerabilities in neurodegenerative diseases and specifically of the striatum in HD, where an inhibition of ganglioside synthesis also occurs physiologically [126]. Taken together, sphingolipid metabolism defects are increasingly recognized as central - and drugable - factors in HD pathogenesis. Despite great potential, particularly in modulating S1P-related metabolism and ganglioside levels, the field is still in its infancy, and better understanding of the alterations of sphingolipid metabolism and consequences on cells and brain is necessary for the development of efficient and safe clinical strategies. We suggest that sphingolipid-related alterations in HD interfere with cellular stress responses and with adequate adaptive processes that usually allow cells to deal with potentially harmful stressors. The following sections are subject to how sphingolipid metabolism modulates cellular stress responses and how that may lead to impaired adaptive capacities in HD and drive disease progression.

4. Proteotoxicity and mitochondrial dysfunction impair the hypoxic stress responses in HD

Classical molecular hallmarks associated with neurodegeneration in HD are aggregation of mHtt and various mitochondrial dysfunctions. How these phenomena mechanistically contribute to neurodegeneration and disease progression remains elusive. However, both are stressors and harmful to vulnerable cells that fail to adapt adequately, for which sphingolipids are essential. This section will discuss these characteristic stressors in HD pathogenesis and explore the importance of the lipid environment - specifically sphingolipid metabolism (see following chapter) - to deal with them. We aim to discuss these aspects with a particular emphasis on the role of hypoxic stress and cellular adaptations to overcome reduced oxygen availability, since sphingolipids are important modulators of corresponding stress responses.

To maintain cellular homeostasis, the cellular capacity to respond and adapt to stressors is crucial [173]. Among the most important factors regulating cellular adaptations to hypoxic stress are the transcription factors HIFs and nuclear factor (erythroid-derived 2)-like 2 (NRF2) [173]. Among key parameters of cellular health are the preservation of sufficient oxygen and nutrient supply and associated cellular energy levels, pH and ion-balance regulation, oxidative stress and inflammation, control of transcription and translation, proteostasis and cellular waste disposal, as well as cell death pathways. Perturbation of any of these factors can be detrimental for cells, particularly for vulnerable neurons that primarily rely on oxygen-dependent mitochondrial respiration for energy generation. It is becoming increasingly evident that the lipid environment plays a key role in maintaining cellular homeostasis, and in the context of neurodegeneration, efficient energy metabolism and mechanisms to prevent protein aggregation toxicity. In HD, several mechanisms to regulate these balances have been reported to be impaired and a brief overview on this is provided in the subsequent chapters.

4.1. Lipids and protein aggregation pathology in HD brain

Despite the causal role of CAG-repeat extension in the *Htt* gene for HD, the associated misfolding, aggregation and the consecutive sequestration of mHtt into inclusion bodies, the contribution of mHtt aggregation pathology to neuronal cell death and HD symptoms is still not clear.

On the one hand, reduced aggregation load has been shown to be beneficial in HD models [174], supporting the view of toxic gain of function by mHtt aggregation. On the other hand, the inclusion-load does not evidently correlate with cell death in HD models of striatal neurons [175]. Accordingly, it has been argued that the aggregation of mHtt and segregation into inclusion bodies may reduce mHtt toxicity [175–177]. In line with these observations, also soluble N-terminal mHtt fragments can be detrimental to cells, e.g, by impairing transcription after translocation into the nucleus (reviewed in [178]) and via stalling ribosomes [179]. Finally, the depletion of soluble and functional Htt could lead to toxicity due to the loss of physiological functions of Htt (see introduction).

The interaction of mHtt with lipids and membranes is likely involved in HD pathogenesis in several ways, including through modulation of aggregation pathology [180,181] and a hazardous disruption of membrane structures [182,183]. Lipids are major components of proteinaceous inclusions in different neurodegenerative diseases, including mHtt-containing inclusions in HD. [184,185]. In addition, mHtt via interaction with lipids can damage membranes, including nuclear membranes [182,186], the endoplasmic reticulum [187] or mitochondrial membranes [188] and thus interfere with cellular signaling, gene expression, proteostasis and energy metabolism. According to a recent study [189], lipids with anionic headgroups, POPS (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho- L-serine) and POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol)) appear to be particularly suited to influence Htt aggregation, as compared to lipids with zwitterionic headgroups, POPC (1-palmitoyl-2-oleoyl- sn -glycero-3-phosphocholine) and POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine) [189]. The authors of this study concluded that increased membrane charge densities result in specific Htt-lipid interactions that facilitate fibrilization [189].

4.2. Energy metabolism, mitochondrial dysfunctions and hypoxic stress in HD brain

Although energy metabolism is clearly impaired in HD, the placement of specific energy-metabolic deficits in the sequence of events leading to neurodegeneration has been proven to be complicated. It appears that pathogenesis and disease progression in HD are characterized by metabolic shifts, with changes between more glycolytic and oxidative energy metabolism and changes of substrate supply/availability for these processes [190].

It is likely that a high oxygen demand, e.g., for the clearance of aggregates, is a crucial factor in HD progression [191]. In particular, combined with early deficiencies in glycolytic ATP-production [192,193], this may result in reduced ATP levels and an energetic crisis. Impaired homeostasis of ATP and phosphocreatine was observed during disease progression in R6/2 mice, where creatine and phosphocreatine levels increased starting from presymptomatic stages but ATP levels dropped starting from about the onset of motor symptoms [194]. Importantly, it may be primarily a lack of adaptive changes to energetic stress, such as of the ratios of inorganic phosphate to phosphocreatine (Pi:PCr), in response to stimulation, which was observed in patient brains and could be reversed with the anaplerotic agent triheptanoin

[195].

In HD iPSCs mitochondrial fragmentation, an apparent shift to glycolysis and pyruvate dehydrogenase inactivation were reported [196]. The latter suggests a shunt of pyruvate away from mitochondria and thus downregulation of oxidative phosphorylation, which was confirmed by reduced mitochondrial respiration and ATP-levels, and which may partially explain glycolytic abnormalities in HD. The main consumers of oxygen and the main producers of ATP in neurons are mitochondria, which therefore are integral for brain energy metabolism. Several functions of mitochondria in HD patient brains are compromised and deficits in their morphology and physiology have been described early [45,197], particularly in the caudate nucleus [198]. Mitochondria are further important components in the regulation of cellular homeostasis by disposing of specific molecular programs to protect from proteotoxicity [199].

Treatment of mice with the mitochondrial electron transport system (complex II) inhibitor 3-nitropropionic acid (3-NP) results in a pathology typical for HD; the degeneration of striatal medium spiny neurons [200]. This discovery sparked interest into mitochondrial dysfunctions as potential underlying mechanisms in HD pathogenesis. Indeed, in human postmortem HD striatum, the activity of the mitochondrial electron transport system components complex II, III and IV are markedly reduced [197,198,201,202]. Dysregulation of components of the mitochondrial respiratory system has been reported on the RNA, protein, and enzyme activity level from peripheral patient tissues [203], in human post-mortem brain and mouse models of HD, such as the Q175 model [204]. Recently, Gardiner and colleagues reported respirational deficits in HD patient fibroblasts that correlated with the age of disease onset [205].

In 3-NP HD rat models, mitochondrial dysfunction has been reported to be associated with an altered lipid composition of striatal mitochondria that could be rescued with the mitochondrial modulators alpha-lipoic acid and acetyl-l-carnitine [206]. Furthermore, preventing lipid peroxidation using the antioxidant nordihydroguaiaretic acid restored mitochondrial functions in male R6/2 mice [207]. These findings show the importance of the lipid environment and the danger of oxidative damage of lipids in HD.

Besides deficits in the electron transport system and associated oxidative stress, mitochondrial protein import is impaired in HD, contributing to proteotoxic stress. Most mitochondrial proteins are expressed by nuclear genes and thus have to be imported into mitochondria (for review see [208]) and mitochondrial transfer of proteins is essential for cellular stress response signaling via the mitochondrial unfolded protein response and thereby for cellular proteostasis [209]. Full-length and exon1 of wild-type Htt and mHtt have been reported to translocate into the mitochondrial inter-membrane space, bind the mitochondrial protein complex translocase of the inner mitochondrial membrane 23 (TIM23) and thereby inhibit mitochondrial import [210,211]. Furthermore, a dysregulation of mitochondrial protein expression in undifferentiated HD embryonic stem cells before observable mHtt pathologies suggests mitochondrial dysfunctions to be a very early event in HD pathogenesis [212]. Similarly, early reports indicate reduced membrane potential and impaired mitochondrial Ca^{2+}-handling in HD patient lymphoblast mitochondria and mHtt overexpressing mice, an effect that could be reproduced by exposure of normal mitochondria with glutathione S-transferase fusion proteins containing up to 62 Q residues [213]. Lipid components of mitochondrial membranes further regulate mHtt aggregation. In particular, cardiolipin binding of mHtt has been suggested to modify its aggregation kinetics, thus possibly representing an important pathological process in disease progression. Using mitochondrial membrane mimics with different concentrations of cardiolipins, Adegbuyiro and colleagues [214] showed that while molar ratios of <5% of cardiolipin slightly increased fibrillization propensity of mHtt, higher cardiolipin concentrations reduced it. Furthermore, the membrane composition affected the morphology of resulting mHtt aggregates, suggesting that mitochondrial membranes are involved in the formation of specific aggregate types. Specifically, inner mitochondrial membrane mimics (characterized by high cardiolipin content) not only inhibited aggregation of Htt but underwent a morphological transformation upon Htt exposure that was associated with perforation of the membranes.

In addition, mHtt interferes with the normally highly dynamic nature of mitochondria. Soluble N-terminal mHtt fragments were shown to interact with mitochondria in Hdh(CAG)150 knock-in mouse brain, an effect that was exacerbated with increasing age, impaired intracellular trafficking of mitochondria, leading to mitochondrial mis-location within the cell and to reduced ATP availability [215]. Increased expression levels of mitochondrial-fission-related proteins (Drp1 and Fis1) and reduced levels of mitochondrial-fusion-related proteins (Mfn1, Mfn2, Opa1) are also characteristic for HD patient striatum and frontal cortex [216]. This suggests that mitochondrial shape changes, which are important for the adaptation to environmental conditions [217], are impaired in HD.

mHtt has further been reported to repress transcription of the transcriptional coactivator PGC-1α, a master regulator of mitochondrial biogenesis, in striatum of HD mouse models and patients [218]. Mitochondrial biogenesis is crucial to increase mitochondrial biomass in order to address for example elevated demand for mitochondria in altered environmental or cellular conditions. Reduced PGC-1α has been shown to impair mitochondrial biogenesis, respiration, and adaptive energy metabolism in mice; for example, PGC-1α-deficient hepatocytes lost their capacity to respond to hormone-regulated feeding controls [219]. In contrast, autophagic clearance of mitochondria (mitophagy) is essential to remove dysfunctional mitochondria from cells and thus to protect from harmful products of damaged mitochondria that include ROS and other damage-associated molecular patterns. Maintaining the balance between mitochondrial biogenesis and mitophagy is crucial in cellular stress responses. Impaired neuronal autophagy in HD may be due to impaired cargo recognition of autophagosomes, resulting in reduced cellular turnover and higher levels of lipid droplets and damaged mitochondria [220].

Despite the described mitochondrial deficits, changes in mitochondrial respiration during HD pathogenesis in preclinical models and patient brains have not been consistently observed, and temporal patterns, besides the extent of causal involvement in disease progression, are not well understood [221,222]. For example, Guidetti and colleagues [223] report no changes in electron transport system activity in the striatum of HD patients and model mice. Preclinical models often fail to reproduce overt functional mitochondrial deficits as well [221,222], or additional metabolic stress is required to elicit overt respirational deficits [224]. This may indicate that mitochondrial dysfunction is an outcome or epiphenomenon on HD pathology rather than a causal factor inducing neurodegeneration. Also, mitochondrial dysfunction in HD patients is relatively mild, which may be one reason for failures of clinical trials to improve early HD mitochondrial function, as, for example recently reported in the mitochondrial enhancer SBT-020 [225]. Therefore, despite many reports on mitochondrial dysfunction associated with mHtt aggregation in human HD brain and HD models, causal consequences of Htt on mitochondrial respiration, Ca^{2+}-handling and other mitochondrial functions are still debated [222,226].

The energy-metabolism changes in HD are partially reminiscent of hypoxic insults [203], based on the switch to glycolysis and mitochondrial responses, as recently reviewed [191]. It is likely that the energy demand of brain cells has to reach a distinct threshold before it results in mitochondrial deficits in HD [227] or additional oxygen and substrate limitations or metabolic stress in vivo cause bioenergetics crises more easily in HD [222,224]. Hypoxic stress has various consequences on brain energy and lipid metabolism that may be relevant for HD pathology [228], including an impaired oxygen-dependent fatty acid and sterol metabolism [229], with especially cholesterol synthesis requiring high levels of oxygen, as outlined in a recent review [230].

4.3. Impaired gene expression and waste disposal in HD brain affect cellular stress responses

mHtt has been shown to disrupt 3D-chromatin structure and, thereby transcriptional regulation [231]. Recently, the induction of a transcriptional proteotoxic stress response as a consequence of mHtt aggregation has been characterized in detail in HEK293T cells transfected with GFP tagged mHtt with 134Q, leading to the authors' conclusion that protein aggregation in HD likely results in maladaptative cellular stress responses [232].

In HD knock-in mice expressing Htt with different numbers of CAG-repeats, it was observed that striatal transcriptional deficits are progressive and depend on the length of the CAG repeats [233]. Consequently, cells, particularly vulnerable medium spiny neurons [234], in the striatum of HD patients [235–237] or HD model mice [233,234,236,238,239] have been reported to downregulate expression of their cellular identity genes and exhibit epigenetic abnormalities. Gene expression studies further identified pathways related to metabolism, inflammation and cellular stress responses to be upregulated in HD striatum [235] and the striatum of HD model mice [233,234,238]. These patterns of upregulated genes have recently been attributed primarily to astroglial and microglial cells [234]. The authors of that study suggest that the transcriptional changes may reflect an adaptation of glial metabolism as a response to mHtt in order to improve resilience of the HD cellular environment.

Evidence implicating physiological Htt in the regulation of selected macroautophagy (aggregophagy, lipophagy and mitophagy) in response to cellular stress [26] supports the notion of impaired cellular stress responses in HD. Htt was shown to directly interact with the autophagy cargo receptor p62 to facilitate cargo recognition and to increase autophagy levels by binding the autophagy-initiating kinase ULK1, promoting ULK1 activation by dissociating the mTOR-ULK1 complex [26].

In line with this observation, strengthening the cellular stress response, for example by allosteric activation of heatshock protein 70 (Hsp70) with the compound YM-1 improved mHtt clearance and reduced accumulation of mHtt in the nucleus [240].

5. Sphingolipid metabolism at the crossroads between hypoxic stress adaptation and HD

Since cellular stress responses especially to hypoxia are impaired in HD and HD models with profound consequences on metabolic and cellular homeostasis, the question arises, which molecular changes are responsible and are potential targets for therapeutic strategies to modulate cellular resilience. In this chapter we argue that early changes in spingolipid metabolism during HD progression are central for disrupted or maladaptive cellular responses to stress in HD and associated with aggregation pathology and neurodegeneration.

5.1. Sphingolipids regulate lipid transport and proteostasis

Sphingolipids indirectly influence Htt aggregation in various ways, including due to modulating lipid transport and availability, as well as energy metabolism that is crucial for correct protein folding and disposal of misfolded and aggregated proteins. It also influences Htt aggregation by regulating its posttranscriptional modifications. The N-terminal 17 amino acids of Htt (Nt17) can form an amphipathic alpha-helix, they are major interactors with membranes, responsible for retention of the protein in the endoplasmic reticulum and function as a nuclear export signal [241]. Aggregation of mHtt is likely amplified by its proteolytic cleavage that yields aggregation-prone N-terminal mHtt fragments [242], which translocate to the nucleus, where they interfere with DNA transcription and induce cell-death [178]. Phosphorylation of the Nt17 residues, Thr3, Ser13, and/or Ser16, reduces mHtt fibrillization and phenotypes in HD models. Using phosphomimetic mutations, it has further been demonstrated that these posttranscriptional modifications modulate Htt's interaction with membranes and its aggregation, depending on membrane compositions [243]. Gangliosides (specifically GM1) increase Htt phosphorylation at Ser13 and Ser16 [155], which may be one mechanism by which increasing ganglioside levels is beneficial in HD models.

In addition, the regulation of lipid transport via membrane contact sites by components of the sphingolipid metabolism may affect cholesterol availability. Specifically, the downregulation of sphingosine kinases in HeLA cells was associated with expected low S1P and elevated ceramide and sphingosine levels (alterations similar to sphingolipid level changes in HD models, see Table 2), which resulted in reduced membrane contact sites and cholesterol accumulation. In this model, however, a compensatory mechanism involving enhanced recruitment of the cholesterol transfer protein Aster-B to the plasma membrane preserved cellular cholesterol availability [244]. It is noteworthy that the interaction of poly-Q with organelle membranes has been shown to lead to their disruption, possibly contributing to neuronal death in HD [187]. Thus, the impairment of membrane contact sites and therefore of intracellular lipid transport in HD may be induced and aggravated by parallel mechanisms, including protein aggregation pathology and dysregulation of sphingolipid levels. Cholesterol content, in addition, influences Htt interaction with membranes as well as Htt aggregation. Gao and colleagues [245] used synthetic Htt peptides and a full-length Htt-exon1 recombinant protein to study interactions with model membranes in dependence of exogenously added cholesterol. Higher cholesterol content of membranes reduced the interaction of Htt and prevented Htt-induced membrane permeabilization [245].

Taken together, sphingolipid metabolism is essential in maintaining favorable lipid environments and regulation of cellular proteostasis, including the determination of mHtt toxicity.

5.2. Sphingolipids and mitochondrial functions in HD brain

While mitochondria are able to synthesize several lipids autonomously (phosphatidylglycerol, cardiolipin and in part phosphatidylethanolamine, phosphatidic acid and CDP-diacylglycerol), they have to import other lipids, including sphingolipids [246]. Although sphingolipids make up only a small portion of the mitochondrial liposome [247], they are crucial for cellular metabolism, including mitochondrial functions [248]. Many sphingolipids and their associated enzymes appear to be located in mitochondria and functionally interact with them. Ceramides and sphingomyelins were found to be components of mitochondrial membranes and with advancing age their concentrations in mitochondrial membranes increased in mice [247]. A differential distribution within mitochondrial membranes has been described for ceramides, with a 3-fold higher concentration in outer than in inner mitochondrial membranes [249]. Several ceramide-producing enzymes, such as ceramide synthases [250,251], neutral sphingomyelinases [252–254] and neutral ceramidases [255] have also been suggested to be located within mitochondria.

The best understood effects of sphingolipids on mitochondria are those of ceramides. While very long-chain ceramide species are considered benign in most cases [256], long-chain ceramides can have detrimental effects on mitochondria. This has been demonstrated for ceramide synthase 6-generated C16:0 ceramide that, via binding mitochondrial fission factor, triggered mitochondrial fission in liver, specifically in high-fat diet-fed mice [257]. Knockout of ceramide synthase 6 protected from hepatic mitochondrial fragmentation, systemic insulin resistance, non-alcoholic liver steatosis and obesity in those mice [257]. High levels of ceramides disrupt the electron transport system, causing an increase of ROS [248] and may promote mitochondrial cell-death pathways, particularly if associated with mitochondrial membranes [258]. Ceramides also importantly control cellular metabolism by enhancing fatty acid uptake and storage and inhibition of glucose and amino acid uptake, thereby favoring fatty acid utilization for energy metabolism [71]. Concomitantly, ceramide reduces mitochondrial

membrane potential and inhibits oxygen consumption via oxidative phosphorylation [63]. The resulting lower efficiency of mitochondria to utilize fatty acids leads to reduced ATP production, despite an increased break-down of fatty acids [71]. Thus HD-associated elevated ceramide levels may partially explain impaired energy metabolism and increased resting energy expenditure [259], despite characteristically increased calorie-intake and paradoxical concomitant weight loss in HD [260].

In summary, cellular-stress-related changes in ceramide levels exert pronounced effects on cellular energy metabolism, substrate utilization and survival, and affect oxygen utilization and dependence of cells. While these changes might likely be harnessed for protective adaptations in healthy cells against hypoxic stress, it is possible that the combination with additional stressors, such as proteotoxic stress in cells expressing mHtt, leads to metabolic maladaptations that promote disease progression. While presently mainly the detrimental effects of long ceramides on mitochondrial functions have been investigated, research on shifting "sphingometabolism" towards higher levels of more favorable sphingolipids (e.g., very long ceramides and especially S1P and gangliosides) may aid the development of novel strategies to improve cellular stress responses and therefore brain resilience in HD.

5.3. *Sphingolipids in the cellular stress response with a focus on hypoxic stress*

As outlined above, hypoxic stress is a dangerous condition for neurons and is likely involved in HD pathogenesis. Hypoxia induces profound adaptations in cells, including of mitochondria [261] and also substantially alters lipid metabolism. Acute hypoxia for example increases circulating triglycerides [262] and oxidative stress related both to hypoxia and reperfusion [261] increases lipid peroxidation, mediated partly by increased 12/15-Lipoxygenase, which is associated with reduced mitochondrial integrity [263]. Among the mediators of cellular stress responses, sphingolipids are of particular importance [57,264–266] and alterations in enzyme activities and levels of different species contribute to cellular adaptations to overcome challenging conditions, including hypoxia. In response to many cellular stressors, sphingolipid compositions in cells change dynamically, rendering them interesting biomarkers for many diseases [9]. Sphingolipid homeostasis is a balance of sphingosine *de novo* biosynthesis, recycling and degradation [62] and is crucial for cellular homeostasis in the brain [9]. Deficits in sphingolipid metabolism cause sphingolipidoses leading to lysosomal storage disorders [56]. Changes in sphingolipid levels in the brain profoundly affect cell physiology and have been suggested to be involved in the pathogenesis of various neurological diseases [68,70], besides HD, including ischemic injuries in the central nervous system [67] and Alzheimer disease [62]. Alterations of sphingolipid metabolism during aging [68,70] suggest a prominent role specifically in age-related diseases. The homeostasis of sphingolipids and interconversion rates of sphingolipid subtypes has direct consequences on membrane dynamics and trafficking [267] and affects both aging and neurodegenerative processes [62]. The regulation of sphingosine subtypes by a specific stress stimulus often modulates the activity of more than one sphingolipid metabolism-associated enzyme [56]; overall about 40 enzymes and fluxes of substrates/metabolites are involved in the regulation of sphingolipid levels [54,56].

Stressors, to which the sphingolipid system has been shown to be sensitive, include hypoxic/ischemic and oxidative stress, nutritional stress and free fatty acid excess, heat, pathogens and various cytokines and inflammatory mediators [106,256,268]. Resultant sphingolipid level changes modulate nutrient uptake, metabolic and epigenetic remodeling and exert reciprocal effects on the immune system. The modulating effects of the DNA damage response on ceramides is one of the earliest recognized inducers of sphingosines' bioactive functions [56]. Sphingomyelinases have been shown to be receptive to many receptor-mediated and extracellular stress cues, with neutral sphingomyelinase upregulation having been linked robustly to the extracellular signal regulated kinase (ERK) cascade and pro-inflammatory responses, while acid sphingomyelinases seem to be particularly responsive to the stress-activated protein kinase/c-jun kinase (SAPK/JNK) cascade [269,270]. Similarly, the salvage pathway (catabolism of complex sphingolipids via sphingosine to ceramide) is activated by cellular stress, including due to TNF, radiation, and oxidative stress [63].

Neutral sphingomyelinase 2 is prominently regulated by DNA damage responses [271], by epigenetic mechanisms [272] and by serine phosphorylation via ROS and tumor necrosis factor (TNF) signaling [273]. Alkaline ceramidase 2 is also induced by DNA damage responses and the resultant increased sphingosine levels were associated with increased oxidative stress, autophagy and apoptosis [274]. Acid sphingomyelinase secretion is stimulated by various cytokines, such as TNF and interleukin-1 [275] but the function of secreted acid sphingomyelinase is not well understood yet. Cellular stress increases ceramide levels in a dose-dependent manner [269] by sphingomyelinase upregulation (leading to sphingomyelin hydrolysis) [106] and increased *de novo* synthesis or ceramide production via the salvage pathway [276]. In complex interdependence with other second messenger systems, ceramide then functions as intercellular second messenger, acts as stress signal and mediates responses such as growth arrest and survival [265], ultimately regulating whether a stressed cell undergoes programmed cell death or not [268].

Cerebral ischemia/reperfusion injury is robustly associated with increased ceramide biosynthesis, leading to elevated ceramide levels and deficits in mitochondrial respiration [250]. These effects required c-Jun N-terminal kinase 3 (*JNK3*), a regulator of neuronal apoptosis [250]. The role of ceramide synthases in mitochondrial Ca^{2+} homeostasis and mitochondrial pathways involved in cell death regulation [250,251,277] is additional evidence for the important involvement of ceramide level regulation in apoptotic and necrotic cell death modulation via mitochondrial pathways, as occurs in stroke and ischemia/reperfusion injuries in the brain [278–280]. In contrast, administration of exogenous GM1 protected lipid rafts and rat brain myelin structure from hypoxia/ischemia-induced damage [281]. The role of ceramide metabolism in severity and progression of ischemic stroke has recently been reviewed [278]. These authors highlight the technical limitations of investigation of the role of ceramides in stroke, including the complexity of the system, owed to the interconnected and cell-type specific networks and interactions of different components of sphingolipid metabolism and the technically challenging analysis.

The ratio of ceramide and S1P levels functions as a crucial cellular rheostat, with higher ceramide favoring cell-death pathways while higher S1P levels promote survival and growth [83]. Like ceramide, S1P is an important signaling molecule of stress responses [191], but in contrast to elevated ceramide, increased S1P levels have been linked to protective outcomes related to vessel formation, inflammatory processes, and neuronal survival. The balance of ceramide and S1P regulates cell-death pathways, with the pro-apoptotic effects of ceramide being opposed by anti-apoptotic actions of S1P [282]. Ceramide and S1P levels further control tissue perfusion, which is essential to counteract hypoxic threats. In endothelial cells of blood vessels, ceramide is involved in the regulation of vasoconstriction and vasodilation. Rising lipid levels in vessels lead to ectopic ceramide formation in endothelial cells. This ceramide, via breaking the interaction of protein phosphatase 2A (PP2A) with inhibitor 2 of PP2A (I2PP2A), induces increased dephosphorylation of endothelial nitric oxid synthase (eNOS), which reduces NO production and leads to endothelial cell dysfunction [283,284]. In addition, ceramide increased ROS in endothelial cells (possibly via inducing NADPH oxidase), which resulted in lower NO-levels too [256]. This process is implicated in hypertension and atherosclerosis [256] and potentially plays a role in hypoxic insults in neurodegenerative diseases as well.

S1P-producing Sphingosine kinases 1 and 2 are highly sensitive to hypoxic stress and are involved in the coordination of related adaptive responses. Sphingosine kinase 1 expression and activity have been

demonstrated to be stimulated by reduced oxygen availability in endothelial and smooth muscle cells via promoters that contain putative hypoxia-inducible factor-responsive-elements [285,286]. Sphingosine kinase 1 was further required for migration effects of endothelial cells in response to hypoxia [285]. Conversely, Sphingosine kinase 1 and S1P are important modulators of HIF-1, an effect depending on Akt/GSK3beta signaling, [287–289] and HIF-target genes, such as vascular endothelial growth factor (VEGF) [290]. Conversely, both HIF-1 and HIF-2 regulate sphingosine kinase 1 [285,291], HIF-2 possibly by direct binding to the sphingosine kinase 1 promoter, as shown in cobalt chloride ($CoCl_2$) – mediated chemically mimicked hypoxia in glioma-derived U87 cells [291]. Sphingosine kinase 1 activation further seems to depend on ROS, the levels of which are increased as a consequence of both hypoxia and reperfusion [261], as ROS scavenging prevented its upregulation in cancer cells [292]. The protective effects of sphingosine kinase 1 stimulation in ischemic/hypoxic insults have been extensively demonstrated in cardiomyocytes [293–296] but lack investigation in HD models.

Also, sphingosine kinase 2 has been shown to be activated by hypoxia both in lung cancer cells [297] and in brain [298,299]. Extracellular signal regulated kinase 1/2 (ERK1/2) usually phosphorylates and thereby activates sphingosine kinase 1 and 2 [300,301]. Since ERK1/2 pathways are impaired in HD [302], this may further deregulate sphingosine signaling and contribute to a shift towards a higher ceramide: S1P ratio.

Another requirement of efficient adaptations to hypoxia is a flexible switch to alternative energy metabolism substrates. S1P-generating enzymes may also be involved in related metabolic switches. Such a function could be assumed from recent findings suggesting that sphingosine kinase 1 is required for adaptations to low serine levels in human colon carcinoma cells [303]. In response to serine starvation, sphingosine kinase 1 was down-regulated, which resulted in increased sphingosine levels, led to reduced mitochondrial respiration and increased ROS production, although it also improved cell survival [303]. Whether similar adaptations to decreased substrate availability are relevant for HD pathogenesis remains to be elucidated.

Sphingosine kinase 1 can be activated by a broad array of molecules, including cytokines, hormones, growth-factors, and many G-protein coupled receptor ligands. Such activation often results in the induction of ERK, which in turn phosphorylates serine 225 of sphingosine kinase 1, effectuating its translocation to the plasma membrane and S1P generation by the phosphorylation of sphingosine [304]. These pathways or the stimulation of S1P receptors include suitable targets to modulate ceramide: S1P balances with beneficial outcomes in HD [8,104,127,130,143,144]. Whether rescuing S1P levels is capable of restoring cellular stress responses and thereby renders cells more resilient to hypoxic insults in HD, which in turn may modify disease progression, remains to be established.

Taken together, sphingolipids are crucial players in the maintenance of cellular energy metabolism and proteostasis. The dysregulation of sphingolipid levels contributes to a reduced efficiency of cells to deal with cellular stress, particularly stress caused by impaired oxygen or nutrient supply to vulnerable brain regions, such as the striatum. Consequently, alterations in sphingolipid levels are likely involved in the development of neuroinflammation and neurodegeneration and therefore in the disease progression of HD (see fig. 2).

6. Hormesis and conditioning in HD

Hormesis describes a biphasic response of a stressor, with high levels of this stressor being detrimental, while levels that can be dealt with by the cellular environment and the entire organism through adequate adaptations, even render cells and organism more resilient to subsequent injuries of similar quality, a process termed “conditioning” [173].

The possibility that hormetic processes are at work during HD disease progression or that the adaptive capacities are impaired in HD, thereby attenuating beneficial molecular and systemic responses, has been previously suggested [191,305,306]. Zuchner and Brundin [305] argued that acquired higher resilience to excitotoxicity in several HD mouse models may be due to reduced cellular vulnerability due to alterations of unknown features of N-terminal Htt. Extracting commonalities between the resilience-inducing models, Zuchner and Brundin [305] identified the expression of *exon 1* human Htt with poly-Q stretches in the pathological range, which however does not lead to

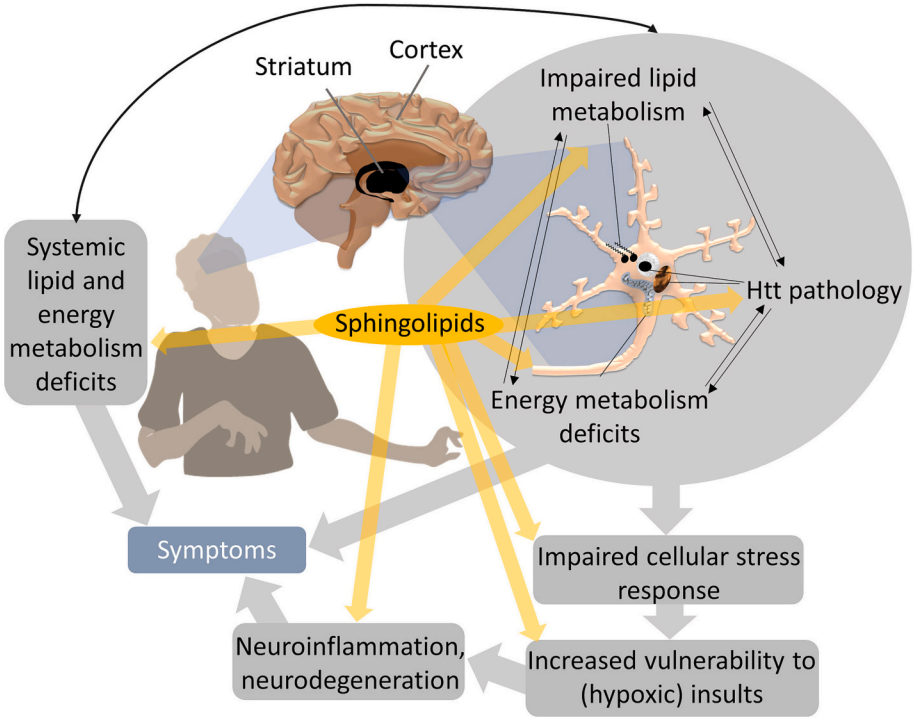

Fig. 2. The central role of sphingolipids in the impaired cellular stress response and neuro-degeneration in HD. Htt – Huntingtin protein.

neuronal protection in all models. Especially the expression of long Htt forms was not associated with protection from excitotoxicity.

The increasing resilience to quinolinic acid in the murine HD models R6/1 and R6/2 had been described by the same group around Patrick Brundin [307,308]. This and other groups later demonstrated similar effects in other HD model mice and increased resistance also to other neurotoxic compounds, as reviewed by Zuchner and Brundin [305], and also to global cerebral ischemia [309]. Hansson et al. [308] further demonstrated that mHtt conferred age- and CAG-repeat-dependent protection of striatal cells in R6/1 and R6/2 mouse models of HD and they proposed that mild metabolic stress due to mHtt increased cellular resistance to excitotoxicity in a hormetic-like manner.

Zuchner and Brundin [305] consider the possibility that toxic poly-Q stretches generally induce resilience by adaptative mechanisms but deem it unlikely based on the fact that other, similar HD models do not exhibit the same neuroprotective features and models without toxic Htt-fragments can also develop comparable neuronal resilience. They reason that rather modifications of Htt, such as conformational changes, aggregation or Htt location within the cells, determine the development of resilience. Another possibility, however, could be the efficiency of cellular stress responses in the respective animal models that likely are associated with characteristic sphingolipid metabolism patterns. We hypothesize that low cellular stress response capacities are associated with faster disease progression.

The appearance of intranuclear Htt aggregates in the cortex and striatum of R6/1 and R6/2 mice that correlates with the onset of protection from quinolinic acid [308] is an indication for a role of Htt-aggregation localization in hormetic resilience development.

Intriguingly, 3-NP, which at higher doses results in HD-like phenotypes, promoted health and extended survival if administered at sub-harmful doses to R6/2 mice with 400 CAG repeats [310]. The outcomes were less consistent for R6/2 mice with 250 CAG repeats, where female mice benefited substantially, while in male mice survival was even reduced [310]. The conditioning effects of 3-NP at lower doses and their detrimental neurodegenerative consequences at higher doses are reminiscent of hormetic effects, i.e. the characteristic biphasic response with beneficial outcomes at lower doses (that cells or the organisms can tolerate and adapt to) and detrimental outcomes at higher doses. Various compounds that showed benefits in rodent models of HD have been suggested to exhibit hormetic-like biphasic responses [306]. This suggests that HD progression is amenable to conditioning-like interventions. In line with this assumption, the induction of a cellular heat shock stress response in the R6/2 mouse HD model by 15 doses of Withaferin A over a month in adult mice ameliorated HD-related symptoms and increased life-span [311].

In HD cybrids (cytoplasmic hybrid systems) cells derived from HD patients with 42-44 CAG repeats in *Htt* slightly decreased mitochondrial membrane potential and slightly increased reactive oxygen levels were observed at baseline [312]. These cells also were characterized by different cell death mechanisms as compared to control cybrids in response to 3-NP or staurosporine, involving higher cytochrome c release and thus suggesting higher susceptibility to reduced membrane integrity [312]. These differences indicate chronically elevated cellular stress levels that may interfere with beneficial conditioning effects, rendering otherwise mild challenges (e.g., oxidative stress or ischemic/hypoxic episodes) hazardous for these cells. In support of this hypothesis, striatal neurons of young adult Hdh150 knock-in mice displayed no impairments in mitochondrial Ca^{2+} buffer capacity, but in energy-demanding conditions (NMDA receptor activation) they were more vulnerable to excessive Ca^{2+} [227].

Furthermore, impaired mitochondrial respiration *in vivo* in a HD mouse model was absent unless induction of additional metabolic stress [224]. In YAC128 mice, increased mitochondrial Ca^{2+} uptake capacities were observed, possibly a consequence of fatty acids sequestered by mHtt, which was associated with mitochondria [313]. While this might indicate increased resilience against Ca^{2+} mediated cellular damage, it could also be indicative for a reduced sensitivity of the mitochondria in response to variation in Ca^{2+} level changes, which in turn may result in reduced adaptations to associated cellular stress. Subtle and age-dependent reductions in Ca^{2+} sensitivity to induce mitochondrial permeability transition in mice expressing mHtt with more than 92 Q have indeed been reported by Brustovetsky and colleagues [314]. Although results from different HD models on mitochondrial Ca^{2+} handling vary [226], a dysregulation in HD is likely and, therefore, probably impairs homeostatic processes that are required for successful adaptation to cellular stress and protective hormesis responses.

We hypothesize that increased basal metabolic and proteotoxic stress associated with HD progression and mHtt pathology elicits continuous cellular stress responses in HD. This chronic activation of adaptive mechanisms and the associated changes in sphingolipid metabolism potentially contribute to a delay of symptomatic manifestations in early disease stages and some HD models. This may mask mitochondrial dysfunction but also renders the chronically stressed environment vulnerable to additional stressors. Among the most common cellular stressors is hypoxia and related oxidative stress that can result from reduced oxygen inspiration, for example, due to environmental conditions, such as at high altitude or due to pathological systemic (e.g., sleep apnea, respiratory or cardiovascular diseases) or tissue (e.g., inflammation, stroke, cancer) hypoxia. In addition, motor-cognitive challenges can result in "functional" hypoxia in specific brain regions [315,316] that in HD might be sufficient to exceed specific cell populations' adaptive capacities, which in turn rely on sphingolipid metabolism. Therefore, protocols to improve the adaptive responses to hypoxic stress in controlled settings may be suitable to re-calibrate sphingolipid homeostasis and improve cellular resilience to insults related to compromised oxygen supply. Although direct evidence for this hypothesis is currently lacking, a few reports suggest benefits of targeting pathways related to hypoxia adaptations, namely HIF and NRF2 pathways. Activation of HIF-1 signaling using betulinic acid hydroxamate (which deactivates prolyl hydroxylase 2 that otherwise would hydroxylate HIF-1α to label it for degradation) for example was shown in striatal HD cells ($STHdh^{Q111/Q111}$) to induce expression of the HIF-1 regulated *Vegf* and *Bnip3* genes (the gene products of which are involved in angiogenesis and mitophagy/apoptosis, respectively) and to reduce cell toxicity [317]. In a mouse 3-NP model of HD, daily i.p. injections of betulinic acid hydroxamate (30 mg/kg) improved motor symptoms, attenuated neuronal loss, [318] mitigated reactive astrogliosis and microglial activation and improved antioxidant defenses and inflammation markers in the brain [317]. The pharmacological induction of an anti-oxidative stress response by inducing the hypoxia-regulated transcription factor NRF2, a key transcription factor in antioxidative cellular responses, has been reported to be beneficial in primary astrocytes derived from zQ175 knockin or wild-type mice [319]. In conditions of additional oxidative stress, elevating NRF2 partially rescued mitochondrial membrane potential and ATP-levels in both genotypes [319], while astrocytic mitochondria were not substantially impaired in the HD condition alone.

In summary, sphingolipid metabolism and associated cellular stress response capacities in HD are impaired and results from numerous HD models suggest that HD progression is amenable to conditioning effects and modulation of hypoxia adaptations. It is thus conceivable that specific conditioning approaches improve sphingolipid metabolism or – conversely – that restoring sphingolipid homeostasis ameliorates cellular stress responses, allowing more efficient adaptations, and both could lead to a beneficial modification of HD disease progression.

7. Conclusion

HD is caused by mutation of *Htt*. Despite this clear causality, hitherto poorly understood factors modulate disease onset and progression, development and severity of symptoms. Herein, we reasoned that dysregulation of the sphingolipid metabolism is a crucial factor in HD

progression and closely associated with cellular stress tolerance in the brain, especially to low oxygen availabilities, regulation of proteostasis and neurodegenerative processes. Among the main consequences of impaired sphingolipid metabolism are various mitochondrial dysfunctions, such as impaired mitochondrial dynamics, quality control and ATP-production capacity, as well as protein aggregation pathology. We summarized the pronounced alterations of sphingolipid-levels in HD brain and in tissues and cells of HD models that reveal a characteristic sphingolipid pattern change in HD, including increased levels of long ceramides and reduced levels of S1P and various gangliosides, notably GM1. Detrimental effects of long ceramides on cellular resilience by compromising mitochondrial functions and cellular oxygen consumption are relatively well acknowledged. The potential to rescue cellular resilience by enhancing levels of more favorable sphingolipids (e.g., very long ceramides and especially S1P and gangliosides) to improve HD remains a future challenge.

Although much research in HD is still neurocentric, the important roles of other brain cell types in HD progression is increasingly recognized. This is also relevant for changes in sphingolipid metabolism in HD that have been recently shown to be specific for different cell types. Unsurprisingly, glial cell types such as astrocytes and microglia, are also crucial players in hypoxic stress responses, also due to the metabolic support they can provide to neurons and their involvement in clearance of HD-associated waste products. Therefore, the characterization of sphingolipid levels and specific effects of sphingolipid-imbalances in specific cell-types and cell populations in HD will help to better understand the "sphingolipid factor" in HD and possibly enable highly specific treatment approaches targeting only the mainly affected cells to rescue favorable sphingolipid patterns.

In conclusion, lipids are emerging as major players in HD, shaping pathologies and modulating symptom onset and severity. Sphingolipids play a key role in cellular resilience to stress and their alterations in HD are probably linked to the compromised beneficial adaptations to stress in HD. This affects responses to endogenous cellular stress, arising for example from proteotoxicity but also from impaired mitochondrial functions. In addition, reduced oxygen and/or nutrient supply following exogenous insults, such as immune reactions, trauma or reduced blood flow (e.g. due to cardiovascular events) pose greater risk factors than usual in absence of efficient cellular adaptations. Pharmacological targeting of sphingolipids (ideally cell type/population specific), "conditioning" or modulating the hypoxic stress responses of the brain represent potential interventions to optimize the sphingolipid balance and improve the cellular stress response.

Funding information

No funding was received for this work.

Author contributions

Conceptualization – JB and NR; Project administration, Supervision – JB, VM and GPM; Visualization and Writing of the original draft – JB; Writing review & editing – JB, GP, NM, ADP, VM

Submission declaration and verification

The article has not been published previously and is not under consideration for publication elsewhere. All authors have read the final version of the article and approved its publication.

Declaration of Competing Interest

The authors declare no conflicts of interest.

References

[1] Hult S, Schultz K, Soylu R, Petersén A. Hypothalamic and neuroendocrine changes in Huntington's disease. Curr Drug Targets 2010;11(10):1237–49.
[2] Chuang CL, Demontis F. Systemic manifestation and contribution of peripheral tissues to Huntington's disease pathogenesis. Ageing Res Rev 2021;69:101358.
[3] Sanberg PR, Fibiger HC, Mark RF. Body weight and dietary factors in Huntington's disease patients compared with matched controls. Med J Aust 1981;1(8):407–9.
[4] Delva A, Michiels L, Koole M, Van Laere K, Vandenberghe W. Synaptic damage and its clinical correlates in people with early Huntington disease: a PET study. Neurology 2022;98(1):e83–94.
[8] Di Pardo A, Maglione V. The S1P axis: new exciting route for treating Huntington's Disease. Trends Pharmacol Sci 2018;39(5):468–80.
[9] Alaamery M, Albesher N, Aljawini N, Alsuwailm M, Massadeh S, Wheeler MA, et al. Role of sphingolipid metabolism in neurodegeneration. J Neurochem 2021;158(1):25–35.
[10] Czubowicz K, Jęśko H, Wencel P, Lukiw WJ, Strosznajder RP. The role of ceramide and sphingosine-1-phosphate in Alzheimer's disease and other neurodegenerative disorders. Mol Neurobiol 2019;56(8):5436–55.
[11] Jin J, Peng Q, Hou Z, Jiang M, Wang X, Langseth AJ, et al. Early white matter abnormalities, progressive brain pathology and motor deficits in a novel knock-in mouse model of Huntington's disease. Hum Mol Genet 2015;24(9):2508–27.
[12] Teo RT, Hong X, Yu-Taeger L, Huang Y, Tan LJ, Xie Y, et al. Structural and molecular myelination deficits occur prior to neuronal loss in the YAC128 and BACHD models of Huntington disease. Hum Mol Genet 2016;25(13):2621–32.
[13] Huang B, Wei W, Wang G, Gaertig MA, Feng Y, Wang W, et al. Mutant huntingtin downregulates myelin regulatory factor-mediated myelin gene expression and affects mature oligodendrocytes. Neuron 2015;85(6):1212–26.
[14] Ferrari Bardile C, Sidik H, Quek R, Yusof N, Garcia-Miralles M, Pouladi MA. Abnormal spinal cord myelination due to oligodendrocyte dysfunction in a model of Huntington's disease. J Huntington's Dis 2021;10(3):377–84.
[15] Benraiss A, Mariani JN, Osipovitch M, Cornwell A, Windrem MS, Villanueva CB, et al. Cell-intrinsic glial pathology is conserved across human and murine models of Huntington's disease. Cell Rep 2021;36(1):109308.
[16] Ferrari Bardile C, Garcia-Miralles M, Caron NS, Rayan NA, Langley SR, Harmston N, et al. Intrinsic mutant HTT-mediated defects in oligodendroglia cause myelination deficits and behavioral abnormalities in Huntington disease. Proc Natl Acad Sci U S A 2019;116(19):9622–7.
[17] Gabery S, Kwa JE, Cheong RY, Baldo B, Ferrari Bardile C, Tan B, et al. Early white matter pathology in the fornix of the limbic system in Huntington disease. Acta Neuropathol 2021;142(5):791–806.
[18] Wilson H, Dervenoulas G, Politis M. Structural magnetic resonance imaging in Huntington's disease. Int Rev Neurobiol 2018;142:335–80.
[19] MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993;72(6):971–83.
[20] DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel J, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science (New York, NY) 1997;277(5334):1990–3.
[21] Saudou F, Humbert S. The biology of Huntingtin. Neuron 2016;89(5):910–26.
[22] Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR, et al. Huntington disease. Nat Rev Dis Primers 2015;1(1):1–21.
[24] Ross CA, Aylward EH, Wild EJ, Langbehn DR, Long JD, Warner JH, et al. Huntington disease: natural history, biomarkers and prospects for therapeutics. Nat Rev Neurol 2014;10(4):204–16.
[25] Gutekunst CA, Levey AI, Heilman CJ, Whaley WL, Yi H, Nash NR, et al. Identification and localization of huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. Proc Natl Acad Sci U S A 1995;92(19):8710–4.
[26] Rui YN, Xu Z, Patel B, Chen Z, Chen D, Tito A, et al. Huntingtin functions as a scaffold for selective macroautophagy. Nat Cell Biol 2015;17(3):262–75.
[27] Martin DD, Ladha S, Ehrnhoefer DE, Hayden MR. Autophagy in Huntington disease and huntingtin in autophagy. Trends Neurosci 2015;38(1):26–35.
[28] Scaramuzzino C, Cuoc EC, Pla P, Humbert S, Saudou F. Calcineurin and huntingtin form a calcium-sensing machinery that directs neurotrophic signals to the nucleus. Sci Adv 2022;8(1):eabj8812.
[29] Han I, You Y, Kordower JH, Brady ST, Morfini GA. Differential vulnerability of neurons in Huntington's disease: the role of cell type-specific features. J Neurochem 2010;113(5):1073–91.
[30] Tabrizi SJ, Flower MD, Ross CA, Wild EJ. Huntington disease: new insights into molecular pathogenesis and therapeutic opportunities. Nat Rev Neurol 2020;16(10):529–46.
[31] Gauthier LR, Charrin BC, Borrell-Pagès M, Dompierre JP, Rangone H, Cordelières FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 2004;118(1):127–38.
[32] Chaibva M, Gao X, Jain P, Campbell WAT, Frey SL, Legleiter J. Sphingomyelin and GM1 Influence Huntingtin Binding to, Disruption of, and Aggregation on Lipid Membranes, ACS. Omega 2018;3(1):273–85.
[33] Johnson AA, Stolzing A. The role of lipid metabolism in aging, lifespan regulation, and age-related disease. Aging Cell 2019;18(6):e13048.
[34] Trayssac M, Hannun YA, Obeid LM. Role of sphingolipids in senescence: implication in aging and age-related diseases. J Clin Invest 2018;128(7):2702–12.

[35] Montoliu I, Scherer M, Beguelin F, DaSilva L, Mari D, Salvioli S, et al. Serum profiling of healthy aging identifies phospho- and sphingolipid species as markers of human longevity. Aging (Albany NY) 2014;6(1):9–25.
[36] Tang H, Huang X, Pang S. Regulation of the lysosome by sphingolipids: potential role in aging. J Biol Chem 2022;298(7):102118.
[37] Lightle SA, Oakley JI, Nikolova-Karakashian MN. Activation of sphingolipid turnover and chronic generation of ceramide and sphingosine in liver during aging. Mech Ageing Dev 2000;120(1–3):111–25.
[38] Sacket SJ, Chung HY, Okajima F, Im DS. Increase in sphingolipid catabolic enzyme activity during aging. Acta Pharmacol Sin 2009;30(10):1454–61.
[39] Couttas TA, Kain N, Tran C, Chatterton Z, Kwok JB, Don AS. Age-dependent changes to sphingolipid balance in the human hippocampus are gender-specific and may sensitize to neurodegeneration. J Alzheimers Dis 2018;63(2):503–14.
[40] Vanier MT. Complex lipid trafficking in Niemann-Pick disease type C. J Inherit Metab Dis 2015;38(1):187–99.
[41] Roney JC, Li S, Farfel-Becker T, Huang N, Sun T, Xie Y, et al. Lipid-mediated motor-adaptor sequestration impairs axonal lysosome delivery leading to autophagic stress and dystrophy in Niemann-Pick type C. Dev Cell 2021;56(10):1452–1468.e8.
[43] Rickman OJ, Baple EL, Crosby AH. Lipid metabolic pathways converge in motor neuron degenerative diseases. Brain 2020;143(4):1073–87.
[44] González-Guevara E, Cárdenas G, Pérez-Severiano F, Martínez-Lazcano JC. Dysregulated brain cholesterol metabolism is linked to neuroinflammation in Huntington's disease. Mov Disord 2020;35(7):1113–27.
[45] Goebel HH, Heipertz R, Scholz W, Iqbal K, Tellez-Nagel I. Juvenile Huntington chorea: clinical, ultrastructural, and biochemical studies. Neurology 1978;28(1):23–31.
[46] Block RC, Dorsey ER, Beck CA, Brenna JT, Shoulson I. Altered cholesterol and fatty acid metabolism in Huntington disease. J Clin Lipidol 2010;4(1):17–23.
[47] Karasinska JM, Hayden MR. Cholesterol metabolism in Huntington disease. Nat Rev Neurol 2011;7(10):561–72.
[48] Valenza M, Cattaneo E. Emerging roles for cholesterol in Huntington's disease. Trends Neurosci 2011;34(9):474–86.
[49] Luthi-Carter R, Taylor DM, Pallos J, Lambert E, Amore A, Parker A, et al. SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. Proc Natl Acad Sci U S A 2010;107(17):7927–32.
[50] Valenza M, Leoni V, Karasinska JM, Petricca L, Fan J, Carroll J, et al. Cholesterol defect is marked across multiple rodent models of Huntington's disease and is manifest in astrocytes. J Neurosci 2010;30(32):10844–50.
[51] Singh A, Agrawal N. Deciphering the key mechanisms leading to alteration of lipid metabolism in Drosophila model of Huntington's disease. Biochim Biophys Acta Mol basis Dis 2021;1867(7):166127.
[52] Aditi K, Shakarad MN, Agrawal N. Altered lipid metabolism in Drosophila model of Huntington's disease. Sci Rep 2016;6:31411.
[53] van Meer G, Holthuis JC. Sphingolipid transport in eukaryotic cells. Biochim Biophys Acta 2000;1486(1):145–70.
[54] Hannun YA, Obeid LM. Many ceramides. J Biol Chem 2011;286(32):27855–62.
[55] Panevska A, Skočaj M, Križaj I, Maček P, Sepčić K. Ceramide phosphoethanolamine, an enigmatic cellular membrane sphingolipid. Biochim Biophys Acta Biomembr 2019;1861(7):1284–92.
[56] Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. Nat Rev Mol Cell Biol 2018;19(3):175–91.
[57] Hannun YA, Obeid LM. The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J Biol Chem 2002;277(29):25847–50.
[59] Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. Adv Exp Med Biol 2010;688:1–23.
[60] Sonnino S, Chigorno V. Ganglioside molecular species containing C18- and C20-sphingosine in mammalian nervous tissues and neuronal cell cultures. Biochim Biophys Acta 2000;1469(2):63–77.
[61] Penno A, Reilly MM, Houlden H, Laurá M, Rentsch K, Niederkofler V, et al. Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. J Biol Chem 2010;285(15):11178–87.
[62] van Echten-Deckert G, Walter J. Sphingolipids: critical players in Alzheimer's disease. Prog Lipid Res 2012;51(4):378–93.
[63] Kogot-Levin A, Saada A. Ceramide and the mitochondrial respiratory chain. Biochimie 2014;100:88–94.
[64] Simons K, Gerl MJ. Revitalizing membrane rafts: new tools and insights. Nat Rev Mol Cell Biol 2010;11(10):688–99.
[65] Futerman AH, Hannun YA. The complex life of simple sphingolipids. EMBO Rep 2004;5(8):777–82.
[66] Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science (New York, NY) 2008;319(5867):1244–7.
[67] Kalinichenko LS, Gulbins E, Kornhuber J, Müller CP. Sphingolipid control of cognitive functions in health and disease. Prog Lipid Res 2022;86:101162.
[68] Mencarelli C, Martinez-Martinez P. Ceramide function in the brain: when a slight tilt is enough. Cell Mol Life Sci 2013;70(2):181–203.
[69] Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, Fukasawa M, et al. Molecular machinery for non-vesicular trafficking of ceramide. Nature 2003;426(6968):803–9.
[70] van Echten-Deckert G, Herget T. Sphingolipid metabolism in neural cells. Biochim Biophys Acta 2006;1758(12):1978–94.
[71] Summers SA, Chaurasia B, Holland WL. Metabolic messengers: ceramides. Nat Metab 2019;1(11):1051–8.
[72] Huang X, Withers BR, Dickson RC. Sphingolipids and lifespan regulation. Biochim Biophys Acta 2014;1841(5):657–64.
[73] Huwiler A, Kolter T, Pfeilschifter J, Sandhoff K. Physiology and pathophysiology of sphingolipid metabolism and signaling. Biochim Biophys Acta 2000;1485(2-3):63–99.
[74] Sandhoff R, Sandhoff K. Emerging concepts of ganglioside metabolism. FEBS Lett 2018;592(23):3835–64.
[75] Coste H, Martel MB, Got R. Topology of glucosylceramide synthesis in Golgi membranes from porcine submaxillary glands. Biochim Biophys Acta 1986;858(1):6–12.
[76] Lannert H, Gorgas K, Meissner I, Wieland FT, Jeckel D. Functional organization of the Golgi apparatus in glycosphingolipid biosynthesis. Lactosylceramide and subsequent glycosphingolipids are formed in the lumen of the late Golgi. J Biol Chem 1998;273(5):2939–46.
[77] Finsterwald C, Dias S, Magistretti PJ, Lengacher S. Ganglioside GM1 targets astrocytes to stimulate cerebral energy metabolism. Front Pharmacol 2021;12:653842.
[78] Yang LJ, Zeller CB, Shaper NL, Kiso M, Hasegawa A, Shapiro RE, et al. Gangliosides are neuronal ligands for myelin-associated glycoprotein. Proc Natl Acad Sci U S A 1996;93(2):814–8.
[79] Posse de Chaves E, Sipione S. Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS Lett 2010;584(9):1748–59.
[80] Sipione S, Monyror J, Galleguillos D, Steinberg N, Kadam V. Gangliosides in the brain: physiology, pathophysiology and therapeutic applications. Front Neurosci 2020;14:572965.
[81] Mao C, Obeid LM. Ceramidases: regulators of cellular responses mediated by ceramide, sphingosine, and sphingosine-1-phosphate. Biochim Biophys Acta 2008;1781(9):424–34.
[82] Olivera A, Spiegel S. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature 1993;365(6446):557–60.
[83] Cuvillier O, Pirianov G, Kleuser B, Vanek PG, Coso OA, Gutkind S, et al. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature 1996;381(6585):800–3.
[85] Shen H, Giordano F, Wu Y, Chan J, Zhu C, Milosevic I, et al. Coupling between endocytosis and sphingosine kinase 1 recruitment. Nat Cell Biol 2014;16(7):652–62.
[86] Hait NC, Maiti A, Xu P, Qi Q, Kawaguchi T, Okano M, et al. Regulation of hypoxia-inducible factor functions in the nucleus by sphingosine-1-phosphate. FASEB J 2020;34(3):4293–310.
[87] Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, et al. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. Science (New York, NY) 2009;325(5945):1254–7.
[88] Maceyka M, Sankala H, Hait NC, Le Stunff H, Liu H, Toman R, et al. SphK1 and SphK2, sphingosine kinase isoenzymes with opposing functions in sphingolipid metabolism. J Biol Chem 2005;280(44):37118–29.
[89] Strub GM, Paillard M, Liang J, Gomez L, Allegood JC, Hait NC, et al. Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration. FASEB J 2011;25(2):600–12.
[90] Neubauer HA, Pham DH, Zebol JR, Moretti PA, Peterson AL, Leclercq TM, et al. An oncogenic role for sphingosine kinase 2. Oncotarget 2016;7(40):64886–99.
[91] Strub GM, Maceyka M, Hait NC, Milstien S, Spiegel S. Extracellular and intracellular actions of sphingosine-1-phosphate. Adv Exp Med Biol 2010;688:141–55.
[92] Mendelson K, Evans T, Hla T. Sphingosine 1-phosphate signalling. Development 2014;141(1):5–9.
[93] Martin R, Sospedra M. Sphingosine-1 phosphate and central nervous system. Curr Top Microbiol Immunol 2014;378:149–70.
[94] Blaho VA, Hla T. An update on the biology of sphingosine 1-phosphate receptors. J Lipid Res 2014;55(8):1596–608.
[95] Prager B, Spampinato SF, Ransohoff RM. Sphingosine 1-phosphate signaling at the blood-brain barrier. Trends Mol Med 2015;21(6):354–63.
[96] Haughey NJ, Bandaru VV, Bae M, Mattson MP. Roles for dysfunctional sphingolipid metabolism in Alzheimer's disease neuropathogenesis. Biochim Biophys Acta 2010;1801(8):878–86.
[97] Esfandiary A, Finkelstein DI, Voelcker NH, Rudd D. Clinical sphingolipids pathway in parkinson's disease: from GCase to integrated-biomarker discovery. Cells 2022;11(8).
[98] Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 2012;22(1):50–60.
[99] Chipuk JE, McStay GP, Bharti A, Kuwana T, Clarke CJ, Siskind LJ, et al. Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. Cell 2012;148(5):988–1000.
[100] Milnerwood AJ, Raymond LA. Early synaptic pathophysiology in neurodegeneration: insights from Huntington's disease. Trends Neurosci 2010;33(11):513–23.
[101] Buccoliero R, Futerman AH. The roles of ceramide and complex sphingolipids in neuronal cell function. Pharmacol Res 2003;47(5):409–19.
[102] Fuller M, Futerman AH. The brain lipidome in neurodegenerative lysosomal storage disorders. Biochem Biophys Res Commun 2018;504(3):623–8.
[103] Ledesma MD, Martin MG, Dotti CG. Lipid changes in the aged brain: effect on synaptic function and neuronal survival. Prog Lipid Res 2012;51(1):23–35.
[104] Di Pardo A, Maglione V. Sphingolipid metabolism: a new therapeutic opportunity for brain degenerative disorders. Front Neurosci 2018;12:249.

[105] Czubowicz K, Jęśko H, Wencel P, Lukiw WJ, Strosznajder RP. The role of ceramide and sphingosine-1-phosphate in Alzheimer's disease and other neurodegenerative disorders. Mol Neurobiol 2019;56(8):5436–55.
[106] Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. Nature 2014;510(7503):58–67.
[107] Levy M, Futerman AH. Mammalian ceramide synthases. IUBMB Life 2010;62(5):347–56.
[108] Ben-David O, Futerman AH. The role of the ceramide acyl chain length in neurodegeneration: involvement of ceramide synthases. NeuroMolecular Med 2010;12(4):341–50.
[109] Becker I, Wang-Eckhardt L, Yaghootfam A, Gieselmann V, Eckhardt M. Differential expression of (dihydro)ceramide synthases in mouse brain: oligodendrocyte-specific expression of CerS2/Lass2. Histochem Cell Biol 2008;129(2):233–41.
[110] Zhao L, Spassieva SD, Jucius TJ, Shultz LD, Shick HE, Macklin WB, et al. A deficiency of ceramide biosynthesis causes cerebellar purkinje cell neurodegeneration and lipofuscin accumulation. PLoS Genet 2011;7(5):e1002063.
[111] Moreno-García A, Kun A, Calero O, Medina M, Calero M. An overview of the role of lipofuscin in age-related neurodegeneration. Front Neurosci 2018;12:464.
[112] Huang Y, Tanimukai H, Liu F, Iqbal K, Grundke-Iqbal I, Gong CX. Elevation of the level and activity of acid ceramidase in Alzheimer's disease brain. Eur J Neurosci 2004;20(12):3489–97.
[113] Ariga T, McDonald MP, Yu RK. Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease–a review. J Lipid Res 2008;49(6):1157–75.
[114] Magistretti PJ, Geisler FH, Schneider JS, Li PA, Fiumelli H, Sipione S. Gangliosides: treatment avenues in neurodegenerative disease. Front Neurol 2019;10:859.
[115] Galleguillos D, Wang Q, Steinberg N, Zaidi A, Shrivastava G, Dhami K, et al. Anti-inflammatory role of GM1 and other gangliosides on microglia. J Neuroinflammation 2022;19(1):9.
[116] Svennerholm L, Boström K, Jungbjer B, Olsson L. Membrane lipids of adult human brain: lipid composition of frontal and temporal lobe in subjects of age 20 to 100 years. J Neurochem 1994;63(5):1802–11.
[117] Han X, D MH, McKeel Jr DW, Kelley J, Morris JC. Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. J Neurochem 2002;82(4):809–18.
[118] Han X. Multi-dimensional mass spectrometry-based shotgun lipidomics and the altered lipids at the mild cognitive impairment stage of Alzheimer's disease. Biochim Biophys Acta 2010;1801(8):774–83.
[119] Filippov V, Song MA, Zhang K, Vinters HV, Tung S, Kirsch WM, et al. Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. J Alzheimers Dis 2012;29(3):537–47.
[120] Katsel P, Li C, Haroutunian V. Gene expression alterations in the sphingolipid metabolism pathways during progression of dementia and Alzheimer's disease: a shift toward ceramide accumulation at the earliest recognizable stages of Alzheimer's disease? Neurochem Res 2007;32(4–5):845–56.
[121] Abbott SK, Li H, Muñoz SS, Knoch B, Batterham M, Murphy KE, et al. Altered ceramide acyl chain length and ceramide synthase gene expression in Parkinson's disease. Mov Disord 2014;29(4):518–26.
[122] Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med 2009;361(17):1651–61.
[123] Mielke MM, Maetzler W, Haughey NJ, Bandaru VV, Savica R, Deuschle C, et al. Plasma ceramide and glucosylceramide metabolism is altered in sporadic Parkinson's disease and associated with cognitive impairment: a pilot study. PLoS One 2013;8(9):e73094.
[124] Karunakaran I, van Echten-Deckert G. Sphingosine 1-phosphate – A double edged sword in the brain. Biochim Biophys Acta Biomembr 2017;1859(9, Part B):1573–82.
[125] Pirhaji L, Milani P, Dalin S, Wassie BT, Dunn DE, Fenster RJ, et al. Identifying therapeutic targets by combining transcriptional data with ordinal clinical measurements. Nat Commun 2017;8(1):623.
[126] Maglione V, Marchi P, Di Pardo A, Lingrell S, Horkey M, Tidmarsh E, et al. Impaired ganglioside metabolism in Huntington's disease and neuroprotective role of GM1. J Neurosci 2010;30(11):4072–80.
[127] Di Pardo A, Amico E, Basit A, Armirotti A, Joshi P, Neely MD, et al. Defective Sphingosine-1-phosphate metabolism is a druggable target in Huntington's disease. Sci Rep 2017;7(1):5280.
[128] Di Pardo A, Basit A, Armirotti A, Amico E, Castaldo S, Pepe G, et al. De novo synthesis of sphingolipids is defective in experimental models of Huntington's disease. Front Neurosci 2017;11:698.
[129] Pepe G, Capocci L, Marracino F, Realini N, Lenzi P, Martinello K, et al. Treatment with THI, an inhibitor of sphingosine-1-phosphate lyase, modulates glycosphingolipid metabolism and results therapeutically effective in experimental models of Huntington's disease. Mol Ther 2022;31(1):282–99.
[130] Di Pardo A, Pepe G, Capocci L, Marracino F, Amico E, Del Vecchio L, et al. Treatment with K6PC-5, a selective stimulator of SPHK1, ameliorates intestinal homeostasis in an animal model of Huntington's disease. Neurobiol Dis 2020;143:105009.
[131] Denny CA, Desplats PA, Thomas EA, Seyfried TN. Cerebellar lipid differences between R6/1 transgenic mice and humans with Huntington's disease. J Neurochem 2010;115(3):748–58.
[132] Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. Neurobiol Dis 2007;27(3):265–77.
[133] Phillips GR, Saville JT, Hancock SE, Brown SHJ, Jenner AM, McLean C, et al. The long and the short of Huntington's disease: how the sphingolipid profile is shifted in the caudate of advanced clinical cases. Brain Commun 2022;4(1):fcab303.
[134] Pirhaji L, Milani P, Leidl M, Curran T, Avila-Pacheco J, Clish CB, et al. Revealing disease-associated pathways by network integration of untargeted metabolomics. Nat Methods 2016;13(9):770–6.
[135] Skene DJ, Middleton B, Fraser CK, Pennings JL, Kuchel TR, Rudiger SR, et al. Metabolic profiling of presymptomatic Huntington's disease sheep reveals novel biomarkers. Sci Rep 2017;7:43030.
[136] Di Pardo A, Amico E, Maglione V. Impaired levels of gangliosides in the corpus callosum of Huntington disease animal models. Front Neurosci 2016;10:457.
[137] Gizaw ST, Koda T, Amano M, Kamimura K, Ohashi T, Hinou H, et al. A comprehensive glycome profiling of Huntington's disease transgenic mice. Biochim Biophys Acta 2015;1850(9):1704–18.
[138] Higatsberger MR, Sperk G, Bernheimer H, Shannak KS, Hornykiewicz O. Striatal ganglioside levels in the rat following kainic acid lesions: comparison with Huntington's disease. Exp Brain Res 1981;44(1):93–6.
[139] Hunter M, Demarais NJ, Faull RLM, Grey AC, Curtis MA. An imaging mass spectrometry atlas of lipids in the human neurologically normal and Huntington's disease caudate nucleus. J Neurochem 2021;157(6):2158–72.
[140] Hunter M, Demarais NJ, Faull RLM, Grey AC, Curtis MA. Subventricular zone lipidomic architecture loss in Huntington's disease. J Neurochem 2018;146(5):613–30.
[141] Crivelli SM, van Kruining D, Luo Q, Stevens JAA, Giovagnoni C, Paulus A, et al. Ceramide analog [(18)F]F-HPA-12 detects sphingolipid disbalance in the brain of Alzheimer's disease transgenic mice by functioning as a metabolic probe. Sci Rep 2020;10(1):19354.
[142] Yue X, Jin H, Liu H, Rosenberg AJ, Klein RS, Tu Z. A potent and selective C-11 labeled PET tracer for imaging sphingosine-1-phosphate receptor 2 in the CNS demonstrates sexually dimorphic expression. Org Biomol Chem 2015;13(29):7928–39.
[143] Di Pardo A, Pepe G, Castaldo S, Marracino F, Capocci L, Amico E, et al. Stimulation of sphingosine Kinase 1 (SPHK1) is beneficial in a Huntington's disease pre-clinical model. Front Mol Neurosci 2019;12:100.
[144] Di Pardo A, Castaldo S, Amico E, Pepe G, Marracino F, Capocci L, et al. Stimulation of S1PR5 with A-971432, a selective agonist, preserves blood-brain barrier integrity and exerts therapeutic effect in an animal model of Huntington's disease. Hum Mol Genet 2018;27(14):2490–501.
[145] Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G, et al. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. Nat Rev Drug Discov 2010;9(11):883–97.
[146] Huwiler A, Zangemeister-Wittke U. The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: Recent findings and new perspectives. Pharmacol Ther 2018;185:34–49.
[147] Spiegel S, Milstien S. The outs and the ins of sphingosine-1-phosphate in immunity. Nat Rev Immunol 2011;11(6):403–15.
[148] Manchon JFM, Uzor N-E, Dabaghian Y, Furr-Stimming EE, Finkbeiner S, Tsvetkov AS. Cytoplasmic sphingosine-1-phosphate pathway modulates neuronal autophagy. Sci Rep 2015;5:15213.
[149] Moruno-Manchon JF, Uzor NE, Blasco-Conesa MP, Mannuru S, Putluri N, Furr-Stimming EE, et al. Inhibiting sphingosine kinase 2 mitigates mutant Huntingtin-induced neurodegeneration in neuron models of Huntington disease. Hum Mol Genet 2017;26(7):1305–17.
[150] Proia RL. Glycosphingolipid functions: insights from engineered mouse models. Philos Trans R Soc Lond Ser B Biol Sci 2003;358(1433):879–83.
[151] Chiavegatto S, Sun J, Nelson RJ, Schnaar RL. A functional role for complex gangliosides: motor deficits in GM2/GD2 synthase knockout mice. Exp Neurol 2000;166(2):227–34.
[152] Sha S, Zhou L, Yin J, Takamiya K, Furukawa K, Furukawa K, et al. Deficits in cognitive function and hippocampal plasticity in GM2/GD2 synthase knockout mice. Hippocampus 2014;24(4):369–82.
[154] Alpaugh M, Galleguillos D, Forero J, Morales LC, Lackey SW, Kar P, et al. Disease-modifying effects of ganglioside GM1 in Huntington's disease models. EMBO Mol Med 2017;9(11):1537–57.
[155] Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proc Natl Acad Sci U S A 2012;109(9):3528–33.
[156] Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, et al. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron 2009;64(6):828–40.
[157] Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol 2011;7(7):453–60.
[158] Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. J Cell Biol 2009;187(7):1083–99.
[159] Ledeen RW, Wu G. The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends Biochem Sci 2015;40(7):407–18.
[160] Hwang J, Lee S, Lee JT, Kwon TK, Kim DR, Kim H, et al. Gangliosides induce autophagic cell death in astrocytes. Br J Pharmacol 2010;159(3):586–603.
[161] Mocchetti I. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cell Mol Life Sci 2005;62(19–20):2283–94.
[162] Schneider JS, Roeltgen DP, Rothblat DS, Chapas-Crilly J, Seraydarian L, Rao J. GM1 ganglioside treatment of Parkinson's disease: an open pilot study of safety and efficacy. Neurology 1995;45(6):1149–54.

[163] Schneider JS, Roeltgen DP, Mancall EL, Chapas-Crilly J, Rothblat DS, Tatarian GT. Parkinson's disease: improved function with GM1 ganglioside treatment in a randomized placebo-controlled study. Neurology 1998;50(6):1630–6.
[164] Schneider JS, Sendek S, Daskalakis C, Cambi F. GM1 ganglioside in Parkinson's disease: Results of a five year open study. J Neurol Sci 2010;292(1-2):45–51.
[165] Schneider JS, Gollomp SM, Sendek S, Colcher A, Cambi F, Du W. A randomized, controlled, delayed start trial of GM1 ganglioside in treated Parkinson's disease patients. J Neurol Sci 2013;324(1–2):140–8.
[166] Svennerholm L, Bråne G, Karlsson I, Lekman A, Ramström I, Wikkelsö C. Alzheimer disease - effect of continuous intracerebroventricular treatment with GM1 ganglioside and a systematic activation programme. Dement Geriatr Cogn Disord 2002;14(3):128–36.
[167] Di Biase E, Lunghi G, Maggioni M, Fazzari M, Pomè DY, Loberto N, et al. GM1 oligosaccharide crosses the human blood-brain barrier in vitro by a paracellular route. Int J Mol Sci 2020;21(8).
[168] Pelled D, Lloyd-Evans E, Riebeling C, Jeyakumar M, Platt FM, Futerman AH. Inhibition of calcium uptake via the sarco/endoplasmic reticulum Ca2+-ATPase in a mouse model of Sandhoff disease and prevention by treatment with N-butyldeoxynojirimycin. J Biol Chem 2003;278(32):29496–501.
[169] Ginzburg L, Li SC, Li YT, Futerman AH. An exposed carboxyl group on sialic acid is essential for gangliosides to inhibit calcium uptake via the sarco/endoplasmic reticulum Ca2+-ATPase: relevance to gangliosidoses. J Neurochem 2008;104(1):140–6.
[170] Korkotian E, Schwarz A, Pelled D, Schwarzmann G, Segal M, Futerman AH. Elevation of intracellular glucosylceramide levels results in an increase in endoplasmic reticulum density and in functional calcium stores in cultured neurons. J Biol Chem 1999;274(31):21673–8.
[171] Raas-Rothschild A, Pankova-Kholmyansky I, Kacher Y, Futerman AH. Glycosphingolipidoses: beyond the enzymatic defect. Glycoconj J 2004;21(6):295–304.
[172] Simpson MA, Cross H, Proukakis C, Priestman DA, Neville DC, Reinkensmeier G, et al. Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. Nat Genet 2004;36(11):1225–9.
[173] Burtscher J, Romani M, Bernardo G, Popa T, Ziviani E, Hummel FC, et al. Boosting mitochondrial health to counteract neurodegeneration. Prog Neurobiol 2022;215:102289.
[174] Sánchez I, Mahlke C, Yuan J. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. Nature 2003;421(6921):373–9.
[175] Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 1998;95(1):55–66.
[176] Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 2004;431(7010):805–10.
[177] Miller J, Arrasate M, Shaby BA, Mitra S, Masliah E, Finkbeiner S. Quantitative relationships between huntingtin levels, polyglutamine length, inclusion body formation, and neuronal death provide novel insight into huntington's disease molecular pathogenesis. J Neurosci 2010;30(31):10541–50.
[178] Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 2011;10(1):83–98.
[179] Eshraghi M, Karunadharma PP, Blin J, Shahani N, Ricci EP, Michel A, et al. Mutant Huntingtin stalls ribosomes and represses protein synthesis in a cellular model of Huntington disease. Nat Commun 2021;12(1):1461.
[180] Burke KA, Kauffman KJ, Umbaugh CS, Frey SL, Legleiter J. The interaction of polyglutamine peptides with lipid membranes is regulated by flanking sequences associated with huntingtin. J Biol Chem 2013;288(21):14993–5005.
[181] Marquette A, Aisenbrey C, Bechinger B. Membrane interactions accelerate the self-aggregation of Huntingtin exon 1 fragments in a polyglutamine length-dependent manner. Int J Mol Sci 2021;22(13).
[182] Liu KY, Shyu YC, Barbaro BA, Lin YT, Chern Y, Thompson LM, et al. Disruption of the nuclear membrane by perinuclear inclusions of mutant huntingtin causes cell-cycle re-entry and striatal cell death in mouse and cell models of Huntington's disease. Hum Mol Genet 2015;24(6):1602–16.
[183] Burke KA, Hensal KM, Umbaugh CS, Chaibva M, Legleiter J. Huntingtin disrupts lipid bilayers in a polyQ-length dependent manner. Biochim Biophys Acta 2013;1828(8):1953–61.
[184] Burke KA, Yates EA, Legleiter J. Biophysical insights into how surfaces, including lipid membranes, modulate protein aggregation related to neurodegeneration. Front Neurol 2013;4:17.
[185] Riguet N, Mahul-Mellier AL, Maharjan N, Burtscher J, Croisier M, Knott G, et al. Nuclear and cytoplasmic huntingtin inclusions exhibit distinct biochemical composition, interactome and ultrastructural properties. Nat Commun 2021;12(1):6579.
[186] Bäuerlein FJB, Saha I, Mishra A, Kalemanov M, Martínez-Sánchez A, Klein R, et al. In situ architecture and cellular interactions of PolyQ inclusions. Cell 2017;171(1):179–187.e10.
[187] Ueda M, Li S, Itoh M, Wang MX, Hayakawa M, Islam S, et al. Expanded polyglutamine embedded in the endoplasmic reticulum causes membrane distortion and coincides with Bax insertion. Biochem Biophys Res Commun 2016;474(2):259–63.
[188] Choo YS, Johnson GV, MacDonald M, Detloff PJ, Lesort M. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet 2004;13(14):1407–20.
[189] Beasley M, Groover S, Valentine SJ, Legleiter J. Lipid headgroups alter huntingtin aggregation on membranes. Biochim Biophys Acta Biomembr 2021;1863(1):183497.
[190] Dubinsky JM. Towards an understanding of energy impairment in Huntington's Disease brain. J Huntingtons Dis 2017;6(4):267–302.
[191] Burtscher J, Maglione V, Di Pardo A, Millet GP, Schwarzer C, Zangrandi L. A rationale for hypoxic and chemical conditioning in Huntington's disease. Int J Mol Sci 2021;22(2).
[192] Mazziotta JC, Phelps ME, Pahl JJ, Huang SC, Baxter LR, Riege WH, et al. Reduced cerebral glucose metabolism in asymptomatic subjects at risk for Huntington's disease. N Engl J Med 1987;316(7):357–62.
[193] Powers WJ, Videen TO, Markham J, McGee-Minnich L, Antenor-Dorsey JV, Hershey T, et al. Selective defect of in vivo glycolysis in early Huntington's disease striatum. Proc Natl Acad Sci U S A 2007;104(8):2945–9.
[194] Mochel F, Durant B, Meng X, O'Callaghan J, Yu H, Brouillet E, et al. Early alterations of brain cellular energy homeostasis in Huntington disease models. J Biol Chem 2012;287(2):1361–70.
[195] Adanyeguh IM, Rinaldi D, Henry PG, Caillet S, Valabregue R, Durr A, et al. Triheptanoin improves brain energy metabolism in patients with Huntington disease. Neurology 2015;84(5):490–5.
[196] Lopes C, Tang Y, Anjo SI, Manadas B, Onofre I, de Almeida LP, et al. Mitochondrial and redox modifications in Huntington Disease induced pluripotent stem cells rescued by CRISPR/Cas9 CAGs targeting. Front Cell Dev Biol 2020;8:576592.
[197] Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH. Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 1996;39(3):385–9.
[198] Brennan Jr WA, Bird ED, Aprille JR. Regional mitochondrial respiratory activity in Huntington's disease brain. J Neurochem 1985;44(6):1948–50.
[199] Boos F, Labbadia J, Herrmann JM. How the mitoprotein-induced stress response safeguards the cytosol: a unified view. Trends Cell Biol 2020;30(3):241–54.
[200] Beal MF, Brouillet E, Jenkins BG, Ferrante RJ, Kowall NW, Miller J, et al. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci 1993;13(10):4181–92.
[201] Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, et al. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol 1997;41(5):646–53.
[202] Tabrizi SJ, Cleeter MW, Xuereb J, Taanman JW, Cooper JM, Schapira AH. Biochemical abnormalities and excitotoxicity in Huntington's disease brain. Ann Neurol 1999;45(1):25–32.
[203] Naseri NN, Bonica J, Xu H, Park LC, Arjomand J, Chen Z, et al. Novel metabolic abnormalities in the tricarboxylic acid cycle in peripheral cells from Huntington's disease patients. PLoS One 2016;11(9):e0160384.
[204] Naseri NN, Xu H, Bonica J, Vonsattel JP, Cortes EP, Park LC, et al. Abnormalities in the tricarboxylic Acid cycle in Huntington disease and in a Huntington disease mouse model. J Neuropathol Exp Neurol 2015;74(6):527–37.
[205] Gardiner SL, Milanese C, Boogaard MW, Buijsen RAM, Hogenboom M, Roos RAC, et al. Bioenergetics in fibroblasts of patients with Huntington disease are associated with age at onset. Neurol Genet 2018;4(5):e275.
[206] Mehrotra A, Sood A, Sandhir R. Mitochondrial modulators improve lipid composition and attenuate memory deficits in experimental model of Huntington's disease. Mol Cell Biochem 2015;410(1–2):281–92.
[207] Lee J, Kosaras B, Del Signore SJ, Cormier K, McKee A, Ratan RR, et al. Modulation of lipid peroxidation and mitochondrial function improves neuropathology in Huntington's disease mice. Acta Neuropathol 2011;121(4):487–98.
[208] Wiedemann N, Pfanner N. Mitochondrial machineries for protein import and assembly. Annu Rev Biochem 2017;86:685–714.
[209] D'Amico D, Sorrentino V, Auwerx J. Cytosolic proteostasis networks of the mitochondrial stress Response. Trends Biochem Sci 2017;42(9):712–25.
[210] Yano H, Baranov SV, Baranova OV, Kim J, Pan Y, Yablonska S, et al. Inhibition of mitochondrial protein import by mutant huntingtin. Nat Neurosci 2014;17(6):822–31.
[211] Yablonska S, Ganesan V, Ferrando LM, Kim J, Pyzel A, Baranova OV, et al. Mutant huntingtin disrupts mitochondrial proteostasis by interacting with TIM23. Proc Natl Acad Sci 2019;116(33):16593–602.
[212] McQuade LR, Balachandran A, Scott HA, Khaira S, Baker MS, Schmidt U. Proteomics of Huntington's disease-affected human embryonic stem cells reveals an evolving pathology involving mitochondrial dysfunction and metabolic disturbances. J Proteome Res 2014;13(12):5648–59.
[213] Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, et al. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci 2002;5(8):731–6.
[214] Adegbuyiro A, Sedighi F, Jain P, Pinti MV, Siriwardhana C, Hollander JM, et al. Mitochondrial membranes modify mutant huntingtin aggregation. Biochim Biophys Acta Biomembr 2021;1863(10):183663.
[215] Orr AL, Li S, Wang CE, Li H, Wang J, Rong J, et al. N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. J Neurosci 2008;28(11):2783–92.
[216] Shirendeb U, Reddy AP, Manczak M, Calkins MJ, Mao P, Tagle DA, et al. Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. Hum Mol Genet 2011;20(7):1438–55.
[217] Schrepfer E, Scorrano L. Mitofusins, from Mitochondria to Metabolism. Mol Cell 2016;61(5):683–94.

[218] Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 2006;127(1):59–69.
[219] Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, et al. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell 2004;119(1):121–35.
[220] Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, et al. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci 2010;13(5):567–76.
[221] Hamilton J, Brustovetsky T, Sridhar A, Pan Y, Cummins TR, Meyer JS, et al. Energy metabolism and mitochondrial superoxide anion production in pre-symptomatic striatal neurons derived from human-induced pluripotent stem cells expressing mutant Huntingtin. Mol Neurobiol 2020;57(2):668–84.
[222] Burtscher J, Di Pardo A, Maglione V, Schwarzer C, Squitieri F. Mitochondrial respiration changes in R6/2 Huntington's Disease Model mice during aging in a brain region specific manner. Int J Mol Sci 2020;21(15):5412.
[223] Guidetti P, Charles V, Chen EY, Reddy PH, Kordower JH, Whetsell Jr WO, et al. Early degenerative changes in transgenic mice expressing mutant huntingtin involve dendritic abnormalities but no impairment of mitochondrial energy production. Exp Neurol 2001;169(2):340–50.
[224] Lou S, Lepak VC, Eberly LE, Roth B, Cui W, Zhu X-H, et al. Oxygen consumption deficit in Huntington disease mouse brain under metabolic stress. Hum Mol Genet 2016;25(13):2813–26.
[225] van Diemen MPJ, Hart EP, Abbruscato A, Mead L, van Beelen I, Bergheanu SC, et al. Safety, pharmacokinetics and pharmacodynamics of SBT-020 in patients with early stage Huntington's disease, a 2-part study. Br J Clin Pharmacol 2021;87(5):2290–302.
[226] Brustovetsky N. Mutant Huntingtin and elusive defects in oxidative metabolism and mitochondrial calcium handling. Mol Neurobiol 2016;53(5):2944–53.
[227] Oliveira JM, Jekabsons MB, Chen S, Lin A, Rego AC, Gonçalves J, et al. Mitochondrial dysfunction in Huntington's disease: the bioenergetics of isolated and in situ mitochondria from transgenic mice. J Neurochem 2007;101(1):241–9.
[228] Graham SF, Pan X, Yilmaz A, Macias S, Robinson A, Mann D, et al. Targeted biochemical profiling of brain from Huntington's disease patients reveals novel metabolic pathways of interest. Biochim Biophys Acta Mol basis Dis 2018;1864(7):2430–7.
[229] Jain IH, Calvo SE, Markhard AL, Skinner OS, To T-L, Ast T, et al. Genetic screen for cell fitness in high or low oxygen highlights mitochondrial and lipid metabolism. Cell 2020;181(3):716–27.
[230] Baik AH, Jain IH. Turning the oxygen dial: balancing the highs and lows. Trends Cell Biol 2020;30(7):516–36.
[231] Alcalá-Vida R, Seguin J, Lotz C, Molitor AM, Irastorza-Azcarate I, Awada A, et al. Age-related and disease locus-specific mechanisms contribute to early remodelling of chromatin structure in Huntington's disease mice. Nat Commun 2021;12(1):364.
[232] Rozales K, Younis A, Saida N, Meller A, Goldman H, Kellerman L, et al. Differential roles for DNAJ isoforms in HTT-polyQ and FUS aggregation modulation revealed by chaperone screens. Nat Commun 2022;13(1):516.
[233] Langfelder P, Cantle JP, Chatzopoulou D, Wang N, Gao F, Al-Ramahi I, et al. Integrated genomics and proteomics define huntingtin CAG length-dependent networks in mice. Nat Neurosci 2016;19(4):623–33.
[234] Merienne N, Meunier C, Schneider A, Seguin J, Nair SS, Rocher AB, et al. Cell-type-specific gene expression profiling in adult mouse brain reveals normal and disease-state signatures. Cell Rep 2019;26(9):2477–2493.e9.
[235] Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, et al. Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 2006;15(6):965–77.
[236] Vashishtha M, Ng CW, Yildirim F, Gipson TA, Kratter IH, Bodai L, et al. Targeting H3K4 trimethylation in Huntington disease. Proc Natl Acad Sci U S A 2013;110(32):E3027–36.
[237] Bai G, Cheung I, Shulha HP, Coelho JE, Li P, Dong X, et al. Epigenetic dysregulation of hairy and enhancer of split 4 (HES4) is associated with striatal degeneration in postmortem Huntington brains. Hum Mol Genet 2015;24(5):1441–56.
[238] Achour M, Le Gras S, Keime C, Parmentier F, Lejeune FX, Boutillier AL, et al. Neuronal identity genes regulated by super-enhancers are preferentially down-regulated in the striatum of Huntington's disease mice. Hum Mol Genet 2015;24(12):3481–96.
[239] Valor LM, Guiretti D, Lopez-Atalaya JP, Barco A. Genomic landscape of transcriptional and epigenetic dysregulation in early onset polyglutamine disease. J Neurosci 2013;33(25):10471–82.
[240] Pinho BR, Almeida LM, Duchen MR, Oliveira JMA. Allosteric activation of Hsp70 reduces mutant huntingtin levels, the clustering of N-terminal fragments, and their nuclear accumulation. Life Sci 2021;285:120009.
[241] Atwal RS, Xia J, Pinchev D, Taylor J, Epand RM, Truant R. Huntingtin has a membrane association signal that can modulate huntingtin aggregation, nuclear entry and toxicity. Hum Mol Genet 2007;16(21):2600–15.
[242] Jimenez-Sanchez M, Licitra F, Underwood BR, Rubinsztein DC. Huntington's Disease: mechanisms of pathogenesis and therapeutic strategies. Cold Spring Harb Perspect Med 2017;7(7).
[243] Groover SE, Beasley M, Ramamurthy V, Legleiter J. Phosphomimetic mutations impact huntingtin aggregation in the presence of a variety of lipid systems. Biochemistry 2020;59(49):4681–93.
[244] Palladino END, Bernas T, Green CD, Weigel C, Singh SK, Senkal CE, et al. Sphingosine kinases regulate ER contacts with late endocytic organelles and cholesterol trafficking. Proc Natl Acad Sci U S A 2022;119(39):e2204396119.
[245] Gao X, Campbell WAT, Chaibva M, Jain P, Leslie AE, Frey SL, et al. Cholesterol modifies Huntingtin binding to, disruption of, and aggregation on lipid membranes. Biochemistry 2016;55(1):92–102.
[246] Horvath SE, Daum G. Lipids of mitochondria. Prog Lipid Res 2013;52(4):590–614.
[247] Tserng KY, Griffin R. Quantitation and molecular species determination of diacylglycerols, phosphatidylcholines, ceramides, and sphingomyelins with gas chromatography. Anal Biochem 2003;323(1):84–93.
[248] Funai K, Summers SA, Rutter J. Reign in the membrane: how common lipids govern mitochondrial function. Curr Opin Cell Biol 2020;63:162–73.
[249] Ardail D, Popa I, Alcantara K, Pons A, Zanetta JP, Louisot P, et al. Occurrence of ceramides and neutral glycolipids with unusual long-chain base composition in purified rat liver mitochondria. FEBS Lett 2001;488(3):160–4.
[250] Yu J, Novgorodov SA, Chudakova D, Zhu H, Bielawska A, Bielawski J, et al. JNK3 signaling pathway activates ceramide synthase leading to mitochondrial dysfunction. J Biol Chem 2007;282(35):25940–9.
[251] Novgorodov SA, Chudakova DA, Wheeler BW, Bielawski J, Kindy MS, Obeid LM, et al. Developmentally regulated ceramide synthase 6 increases mitochondrial Ca2+ loading capacity and promotes apoptosis. J Biol Chem 2011;286(6):4644–58.
[252] Yabu T, Shimuzu A, Yamashita M. A novel mitochondrial sphingomyelinase in zebrafish cells. J Biol Chem 2009;284(30):20349–63.
[253] Wu BX, Rajagopalan V, Roddy PL, Clarke CJ, Hannun YA. Identification and characterization of murine mitochondria-associated neutral sphingomyelinase (MA-nSMase), the mammalian sphingomyelin phosphodiesterase 5. J Biol Chem 2010;285(23):17993–8002.
[254] Rajagopalan V, Canals D, Luberto C, Snider J, Voelkel-Johnson C, Obeid LM, et al. Critical determinants of mitochondria-associated neutral sphingomyelinase (MA-nSMase) for mitochondrial localization. Biochim Biophys Acta 2015;1850(4):628–39.
[255] Novgorodov SA, Wu BX, Gudz TI, Bielawski J, Ovchinnikova TV, Hannun YA, et al. Novel pathway of ceramide production in mitochondria: thioesterase and neutral ceramidase produce ceramide from sphingosine and acyl-CoA. J Biol Chem 2011;286(28):25352–62.
[256] Choi RH, Tatum SM, Symons JD, Summers SA, Holland WL. Ceramides and other sphingolipids as drivers of cardiovascular disease. Nat Rev Cardiol 2021;18(10):701–11.
[257] Hammerschmidt P, Ostkotte D, Nolte H, Gerl MJ, Jais A, Brunner HL, et al. CerS6-derived sphingolipids interact with Mff and promote mitochondrial fragmentation in obesity. Cell 2019;177(6):1536–1552.e23.
[258] Birbes H, Luberto C, Hsu YT, El Bawab S, Hannun YA, Obeid LM. A mitochondrial pool of sphingomyelin is involved in TNFalpha-induced Bax translocation to mitochondria. Biochem J 2005;386(Pt 3):445–51.
[259] Pratley RE, Salbe AD, Ravussin E, Caviness JN. Higher sedentary energy expenditure in patients with Huntington's disease. Ann Neurol 2000;47(1):64–70.
[260] Trejo A, Tarrats RM, Alonso ME, Boll MC, Ochoa A, Velásquez L. Assessment of the nutrition status of patients with Huntington's disease. Nutrition 2004;20(2):192–6.
[261] Burtscher J, Mallet RT, Pialoux V, Millet GP, Burtscher M. Adaptive responses to hypoxia and/or hyperoxia in humans. Antioxid Redox Signal 2020;37(13–15):887–912.
[262] Morin R, Goulet N, Mauger JF, Imbeault P. Physiological responses to hypoxia on triglyceride levels. Front Physiol 2021;12:730935.
[263] Choudhary R, Kumar M, Katyal A. 12/15-Lipoxygenase debilitates mitochondrial health in intermittent hypobaric hypoxia induced neuronal damage: an in vivo study. Redox Biol 2022;49:102228.
[264] Peña LA, Fuks Z, Kolesnick R. Stress-induced apoptosis and the sphingomyelin pathway. Biochem Pharmacol 1997;53(5):615–21.
[265] Obeid LM, Hannun YA. Ceramide: a stress signal and mediator of growth suppression and apoptosis. J Cell Biochem 1995;58(2):191–8.
[266] Hannun YA. Functions of ceramide in coordinating cellular responses to stress. Science 1996;274(5294):1855–9.
[267] Zhang Y, Li X, Becker KA, Gulbins E. Ceramide-enriched membrane domains–structure and function. Biochim Biophys Acta 2009;1788(1):178–83.
[268] Zhou DR, Eid R, Miller KA, Boucher E, Mandato CA, Greenwood MT. Intracellular second messengers mediate stress inducible hormesis and programmed cell death: a review. Biochim Biophys Acta, Mol Cell Res 2019;1866(5):773–92.
[269] Verheij M, Bose R, Lin XH, Yao B, Jarvis WD, Grant S, et al. Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. Nature 1996;380(6569):75–9.
[270] Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, et al. The stress-activated protein kinase subfamily of c-Jun kinases. Nature 1994;369(6476):156–60.
[271] Shamseddine AA, Clarke CJ, Carroll B, Airola MV, Mohammed S, Rella A, et al. P53-dependent upregulation of neutral sphingomyelinase-2: role in doxorubicin-induced growth arrest. Cell Death Dis 2015;6(10):e1947.
[272] Clarke CJ, Shamseddine AA, Jacob JJ, Khalife G, Burns TA, Hannun YA. ATRA transcriptionally induces nSMase2 through CBP/p300-mediated histone acetylation. J Lipid Res 2016;57(5):868–81.
[273] Filosto S, Ashfaq M, Chung S, Fry W, Goldkorn T. Neutral sphingomyelinase 2 activity and protein stability are modulated by phosphorylation of five conserved serines. J Biol Chem 2012;287(1):514–22.
[274] Wang Y, Zhang C, Jin Y, Wang Q, He Z, Liu Q, et al. Alkaline ceramidase 2 is a novel direct target of p53 and induces autophagy and apoptosis through ROS generation. Sci Rep 2017;7:44573.

[275] Jenkins RW, Clarke CJ, Canals D, Snider AJ, Gault CR, Heffernan-Stroud L, et al. Regulation of CC ligand 5/RANTES by acid sphingomyelinase and acid ceramidase. J Biol Chem 2011;286(15):13292–303.
[276] Nikolova-Karakashian MN, Rozenova KA. Ceramide in stress response. Adv Exp Med Biol 2010;688:86–108.
[277] Futerman AH. Intracellular trafficking of sphingolipids: relationship to biosynthesis. Biochim Biophys Acta 2006;1758(12):1885–92.
[278] Ouro A, Correa-Paz C, Maqueda E, Custodia A, Aramburu-Núñez M, Romaus-Sanjurjo D, et al. Involvement of ceramide metabolism in cerebral ischemia. Front Mol Biosci 2022;9:864618.
[279] He Z, Ning N, Zhou Q, Khoshnam SE, Farzaneh M. Mitochondria as a therapeutic target for ischemic stroke. Free Radic Biol Med 2020;146:45–58.
[280] Novgorodov SA, Gudz TI. Ceramide and mitochondria in ischemia/reperfusion. J Cardiovasc Pharmacol 2009;53(3):198.
[281] Zhang YP, Huang QL, Zhao CM, Tang JL, Wang YL. GM1 improves neurofascin155 association with lipid rafts and prevents rat brain myelin injury after hypoxia-ischemia. Braz J Med Biol Res 2011;44(6):553–61.
[282] Maceyka M, Payne SG, Milstien S, Spiegel S. Sphingosine kinase, sphingosine-1-phosphate, and apoptosis. Biochim Biophys Acta 2002;1585(2-3):193–201.
[283] Zhang QJ, Holland WL, Wilson L, Tanner JM, Kearns D, Cahoon JM, et al. Ceramide mediates vascular dysfunction in diet-induced obesity by PP2A-mediated dephosphorylation of the eNOS-Akt complex. Diabetes 2012;61(7):1848–59.
[284] Bharath LP, Ruan T, Li Y, Ravindran A, Wan X, Nhan JK, et al. Ceramide-initiated protein phosphatase 2A activation contributes to arterial dysfunction in vivo. Diabetes 2015;64(11):3914–26.
[285] Schwalm S, Döll F, Römer I, Bubnova S, Pfeilschifter J, Huwiler A. Sphingosine kinase-1 is a hypoxia-regulated gene that stimulates migration of human endothelial cells. Biochem Biophys Res Commun 2008;368(4):1020–5.
[286] Ahmad M, Long JS, Pyne NJ, Pyne S. The effect of hypoxia on lipid phosphate receptor and sphingosine kinase expression and mitogen-activated protein kinase signaling in human pulmonary smooth muscle cells. Prostaglandins Other Lipid Mediat 2006;79(3–4):278–86.
[287] Dehne N, Brüne B. HIF-1 in the inflammatory microenvironment. Exp Cell Res 2009;315(11):1791–7.
[288] Ader I, Malavaud B, Cuvillier O. When the sphingosine kinase 1/sphingosine 1-phosphate pathway meets hypoxia signaling: new targets for cancer therapy. Cancer Res 2009;69(9):3723–6.
[289] Michaud MD, Robitaille GVA, Gratton J-P, Richard DE. Sphingosine-1-phosphate: a novel nonhypoxic activator of hypoxia-inducible factor-1 in vascular cells. Arterioscler Thromb Vasc Biol 2009;29(6):902–8.
[290] Heo K, Park K-A, Kim Y-H, Kim S-H, Oh Y-S, Kim I-H, et al. Sphingosine 1-phosphate induces vesicular endothelial growth factor expression in endothelial cells. BMB Rep 2009;42(10):685–90.
[291] Anelli V, Gault CR, Cheng AB, Obeid LM. Sphingosine Kinase 1 is up-regulated during hypoxia in U87MG glioma cells role of hypoxia-inducible factors 1 and 2. J Biol Chem 2008;283(6):3365–75.
[292] Ader I, Brizuela L, Bouquerel P, Malavaud B, Cuvillier O. Sphingosine kinase 1: a new modulator of hypoxia inducible factor 1alpha during hypoxia in human cancer cells. Cancer Res 2008;68(20):8635–42.
[293] Karliner JS, Honbo N, Summers K, Gray MO, Goetzl EJ. The lysophospholipids sphingosine-1-phosphate and lysophosphatidic acid enhance survival during hypoxia in neonatal rat cardiac myocytes. J Mol Cell Cardiol 2001;33(9):1713–7.
[294] Jin ZQ, Goetzl EJ, Karliner JS. Sphingosine kinase activation mediates ischemic preconditioning in murine heart. Circulation 2004;110(14):1980–9.
[295] Pchejetski D, Kunduzova O, Dayon A, Calise D, Seguelas MH, Leducq N, et al. Oxidative stress-dependent sphingosine kinase-1 inhibition mediates monoamine oxidase A-associated cardiac cell apoptosis. Circ Res 2007;100(1):41–9.
[296] Tao R, Zhang J, Vessey DA, Honbo N, Karliner JS. Deletion of the Sphingosine Kinase-1 gene influences cell fate during hypoxia and glucose deprivation in adult mouse cardiomyocytes. Cardiovasc Res 2007;74(1):56–63.
[297] Schnitzer SE, Weigert A, Zhou J, Brüne B. Hypoxia enhances sphingosine kinase 2 activity and provokes sphingosine-1-phosphate-mediated chemoresistance in A549 lung cancer cells. Mol Cancer Res 2009;7(3):393–401.
[298] Wacker BK, Perfater JL, Gidday JM. Hypoxic preconditioning induces stroke tolerance in mice via a cascading HIF, sphingosine kinase, and CCL 2 signaling pathway. J Neurochem 2012;123(6):954–62.
[299] Wacker BK, Park TS, Gidday JM. Hypoxic preconditioning-induced cerebral ischemic tolerance: role of microvascular sphingosine kinase 2. Stroke 2009;40(10):3342–8.
[300] Pitson SM, Moretti PA, Zebol JR, Lynn HE, Xia P, Vadas MA, et al. Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. EMBO J 2003;22(20):5491–500.
[301] Hait NC, Bellamy A, Milstien S, Kordula T, Spiegel S. Sphingosine kinase type 2 activation by ERK-mediated phosphorylation. J Biol Chem 2007;282(16):12058–65.
[302] Bowles KR, Jones L. Kinase signalling in Huntington's disease. J Huntingtons Dis 2014;3(2):89–123.
[303] Truman JP, Ruiz CF, Trayssac M, Mao C, Hannun YA, Obeid LM. Sphingosine kinase 1 downregulation is required for adaptation to serine deprivation. FASEB J 2021;35(2):e21284.
[304] Pitson SM. Regulation of sphingosine kinase and sphingolipid signaling. Trends Biochem Sci 2011;36(2):97–107.
[305] Zuchner T, Brundin P. Mutant huntingtin can paradoxically protect neurons from death. Cell Death Differ 2008;15(3):435–42.
[306] Calabrese EJ, Bhatia TN, Calabrese V, Dhawan G, Giordano J, Hanekamp YN, et al. Cytotoxicity models of Huntington's disease and relevance of hormetic mechanisms: A critical assessment of experimental approaches and strategies. Pharmacol Res 2019;150:104371.
[307] Hansson O, Petersén A, Leist M, Nicotera P, Castilho RF, Brundin P. Transgenic mice expressing a Huntington's disease mutation are resistant to quinolinic acid-induced striatal excitotoxicity. Proc Natl Acad Sci U S A 1999;96(15):8727–32.
[308] Hansson O, Guatteo E, Mercuri NB, Bernardi G, Li XJ, Castilho RF, et al. Resistance to NMDA toxicity correlates with appearance of nuclear inclusions, behavioural deficits and changes in calcium homeostasis in mice transgenic for exon 1 of the huntington gene. Eur J Neurosci 2001;14(9):1492–504.
[309] Schiefer J, Alberty A, Dose T, Oliva S, Noth J, Kosinski CM. Huntington's disease transgenic mice are resistant to global cerebral ischemia. Neurosci Lett 2002;334(2):99–102.
[310] Skillings EA, Morton AJ. Delayed onset and reduced cognitive deficits through pre-conditioning with 3-nitropropionic acid is dependent on sex and CAG repeat length in the R6/2 mouse model of Huntington's Disease. J Huntington's Dis 2016;5(1):19–32.
[311] Joshi T, Kumar V, Kaznacheyeva EV, Jana NR. Withaferin A induces heat shock response and ameliorates disease progression in a mouse model of Huntington's disease. Mol Neurobiol 2021;58(8):3992–4006.
[312] Ferreira IL, Nascimento MV, Ribeiro M, Almeida S, Cardoso SM, Grazina M, et al. Mitochondrial-dependent apoptosis in Huntington's disease human cybrids. Exp Neurol 2010;222(2):243–55.
[313] Pellman JJ, Hamilton J, Brustovetsky T, Brustovetsky N. Ca(2+) handling in isolated brain mitochondria and cultured neurons derived from the YAC128 mouse model of Huntington's disease. J Neurochem 2015;134(4):652–67.
[314] Brustovetsky N, LaFrance R, Purl KJ, Brustovetsky T, Keene CD, Low WC, et al. Age-dependent changes in the calcium sensitivity of striatal mitochondria in mouse models of Huntington's Disease. J Neurochem 2005;93(6):1361–70.
[315] Butt UJ, Steixner-Kumar AA, Depp C, Sun T, Hassouna I, Wüstefeld L, et al. Hippocampal neurons respond to brain activity with functional hypoxia. Mol Psychiatry 2021;26(6):1790–807.
[316] Tang K, Xia FC, Wagner PD, Breen EC. Exercise-induced VEGF transcriptional activation in brain, lung and skeletal muscle. Respir Physiol Neurobiol 2010;170(1):16–22.
[317] Prados ME, Correa-Sáez A, Unciti-Broceta JD, Garrido-Rodríguez M, Jimenez-Jimenez C, Mazzone M, et al. Betulinic acid hydroxamate is neuroprotective and induces protein phosphatase 2A-dependent HIF-1α stabilization and post-transcriptional dephosphorylation of prolyl hydrolase 2. Neurotherapeutics 2021;18(3):1849–61.
[318] Polyzos AA, Wood NI, Williams P, Wipf P, Morton AJ, McMurray CT. XJB-5-131-mediated improvement in physiology and behaviour of the R6/2 mouse model of Huntington's disease is age- and sex- dependent. PLoS One 2018;13(4):e0194580.
[319] Moretti D, Tambone S, Cerretani M, Fezzardi P, Missineo A, Sherman LT, et al. NRF2 activation by reversible KEAP1 binding induces the antioxidant response in primary neurons and astrocytes of a Huntington's disease mouse model. Free Radic Biol Med 2021;162:243–54.