Published in final edited form as:

Clin Lipidol. 2014 November 1; 9(5): 533–551. doi:10.2217/clp.14.47.

Harnessing the power of yeast to elucidate the role of sphingolipids in metabolic and signaling processes pertinent to psychiatric disorders

Shyamalagauri Jadhav¹ and Miriam L Greenberg^{*,1}

¹Department of Biological Sciences, Wayne State University, Detroit, MI 48202, USA

Abstract

The development of therapies for neuropsychiatric disorders is hampered by the lack of understanding of the mechanisms underlying their pathologies. While aberrant sphingolipid metabolism is associated with psychiatric illness, the role of sphingolipids in these disorders is not understood. The genetically tractable yeast model can be exploited in order to elucidate the cellular consequences of sphingolipid perturbation. Hypotheses generated from studies in yeast and tested in mammalian cells may contribute to our understanding of the role of sphingolipids in psychiatric disorders and to the development of new treatments. Here, we compare sphingolipid metabolism in yeast and mammalian cells, discuss studies implicating sphingolipids in psychiatric disorders and propose approaches that utilize yeast in order to elucidate sphingolipid function and identify drugs that target sphingolipid synthesis.

Keywords

ceramide; mammals; psychiatric disorders; sphingolipid; sphingosine-1-phosphate; yeast

Despite decades of studies, little is known about the molecular mechanisms underlying the neuropsychiatric disorders schizophrenia, depression, anxiety and bipolar disorder, which contribute to approximately 14% of the global burden of disease [1]. The development of effective treatments for these devastating illnesses is hampered by the failure so far to elucidate the molecular mechanisms underlying their pathologies. Recent findings suggest that sphingolipids may play an important role in the pathophysiology of psychiatric disorders. Because sphingolipids are abundant in the brain and are involved in the regulation of processes that are essential for normal brain function, sphingolipids and sphingolipid metabolites may be promising targets for new treatments of psychiatric disorders.

Lipids constitute approximately 50% of the dry weight of the brain, which has the second highest lipid composition after adipose tissue [2,3]. The mammalian nervous system is rich

© 2014 Future Medicine Ltd

*Author for correspondence: mgreenberg@wayne.edu.

Financial & competing interests disclosure: The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed

No writing assistance was utilized in the production of this manuscript

in sphingomyelin, glycosphingolipids and molecules with ceramide backbones [4,5]. Sphingolipids are a ubiquitous, highly conserved, diverse group of bioactive lipids comprising approximately 10–20% of cellular lipids [6]. Technical advances in lipidomics and mass spectrometry in the past two decades have greatly facilitated the quantification and characterization of sphingolipids [7,8]. The yeast model has been invaluable in identifying and characterizing the genes and enzymes that mediate sphingolipid metabolism [9] and elucidating the role of sphingolipids in the regulation of essential cellular functions, including growth, endocytosis, protein trafficking, calcium homeostasis, aging, nutrient uptake, maintenance of lipid rafts, regulation of heat stress, the actin cytoskeleton and cell wall synthesis and repair [10–13]. Several of these functions have been associated with psychiatric disorders, including cellular signaling, endocytosis, inflammation, secretion, apoptosis and proliferation [14]. The new lipidomic technologies, combined with the powerful yeast system, can be used to generate genetic and molecular models of sphingolipid functions that can be tested in relevant mammalian systems, which may offer promising new avenues for drug design.

We begin this article with a comparison of sphingolipid metabolism in yeast and mammals. The very high degree of conservation of sphingolipid biosynthetic enzymes and of the functions of individual sphingolipids and metabolites underscores the utility of yeast for elucidating the molecular mechanisms underlying sphingolipid function. We then discuss studies associating the perturbation of sphingolipids with schizophrenia, depression, anxiety and bipolar disorder. Finally, we suggest avenues whereby yeast can be exploited further in order to design drugs that may be used to modulate the sphingolipid targets that are implicated in these disorders.

Sphingolipid structure

Sphingolipids contain two hydrophobic tails, including a long-chain base (LCB) and a fatty acid chain, linked to a hydrophilic polar head group. The fatty acid is linked to the C-2 of the LCB via an amide bond, and the polar head group is linked to C-1 of the LCB via an OH group [15]. The basic sphingolipid structure is shown in Figure 1.

In the yeast *Saccharomyces cerevisiae*, LCBs are linear alkanes containing hydroxyl groups on C-1 and C-3 and an amino group on C-2. The two major LCBs found in yeast are dihydrosphingosine (DHS) and phytosphingosine (PHS). PHS contains an extra hydroxyl group on C-4 compared with DHS. DHS in yeast contains 16, 18 or 20 carbons, and PHS contains 18 or 20 carbons [16]. Sphingosine, the major LCB of mammals, is not found in yeast. Mammals also have minor amounts of DHS [9]. The mammalian LCB, sphingosine, contains a 4,5-*trans* double bond, which is not found in yeast [17].

The fatty acid linked to the LCB in yeast is generally a C-26 saturated fatty acid containing zero to three hydroxyl groups, whereas the fatty acids in mammalian sphingolipids vary in chain length, degree of hydroxylation and saturation [10]. The hydrophilic polar head group also varies. In yeast, this group contains myo-inositols, namely phosphorylinositol, mannosyl phosphorylinositol or mannosyl diphosphorylinositol. In mammals, the polar groups are mostly phosphorylcholine (sphingomyelin), galactose (galactosylceramide) or

glucose (glucosylceramide). Mammals contain more than 100 classes of complex sphingolipids, while yeasts contain only three [15].

Comparative sphingolipid metabolism in yeast & mammals

Sphingolipids have long been known to play a structural role in cell membranes [18]. In addition, studies in yeast and mammals have shown that sphingolipids act as secondary messengers in several signaling pathways [19]. The sphingolipid synthesis pathway in yeast and mammals is highly conserved through the synthesis of ceramide, with minor variations in chain length, hydroxylation and saturation [20]. Ceramide is synthesized in the endoplasmic reticulum (ER) and transported to the Golgi, where complex sphingolipids are synthesized in both yeast and mammals [14]. The sphingolipid biosynthetic pathways of yeast and mammals are shown in Figure 2.

Synthesis of LCBs

The synthesis of LCBs, the precursors of sphingolipids, is highly conserved from yeast to mammals. Sphingolipid synthesis begins in the ER with the condensation of l-serine with fatty acyl-coenzyme A (CoA), primarily palmitoyl-CoA (but also stearoyl-CoA), and is catalyzed by serine palmitoyl transferase (SPT), which produces 3-ketodihydrosphingosine [21–23]. The genes encoding these enzymes are shown in Table 1.

In the second reaction of sphingolipid synthesis, the short-lived product 3-ketodihydrosphingosine is reduced to DHS in an NADPH-dependent reaction catalyzed by 3-ketodihydrosphingosine reductase [24–26]. PHS is synthesized in yeast via hydroxylation of the C-4 of DHS, catalyzed by sphinganine C-4-hydroxylase [24]. The reverse reaction – conversion of PHS to DHS does not occur. PHS is also found in the skin epidermis of mammals, where it regulates important functions such as apoptosis, inflammation and differentiation [58].

Ceramide synthesis

Ceramide is the central intermediate of sphingolipid metabolism. Interestingly, altered ceramide levels have been associated with schizophrenia, depression and bipolar disorder, as discussed below [59,60]. Ceramide exists as dihydroceramide or phytoceramide. DHS is mostly linked to a C-26 fatty acid via an amide linkage at the C-2 position in order to form dihydroceramide. The ER-localized ceramide synthase catalyzes this reaction. In yeast, dihydroceramide is hydroxylated to phytoceramide by sphinganine C-4-hydroxylase. Phytoceramide can also be synthesized by hydroxylating DHS to PHS, which is amide-linked to a C-26 fatty acid [24]. The yeast ceramide synthases Lag1 and Lac1 have overlapping functions, as single mutants of Lag1 and Lac1 are viable, while the double mutant is not [27].

Mammals contain six ceramide synthases, CerS1–6, which exhibit specificity for fatty acyl-CoAs. CerS1 exhibits specificity for C-18, CerS2 and CerS4 for C-22 and C-24 and CerS5 and CerS6 for C-16, while CerS3 utilizes a broad range of substrates [28]. In mammals, dihydroceramide is desaturated by 4-desaturase between C-4 and C-5 to phytoceramide [29–31].

Fatty acid elongation pathway

The very-long-chain fatty acids that are required for ceramide synthesis are synthesized in the ER by the fatty acid elongation pathway. The fatty acid elongase complex adds two carbons to fatty acyl CoAs per cycle [32]. Each isoform of fatty acid elongase synthesizes a very-long-chain fatty acyl-CoA of a characteristic length [61]. The four steps of fatty acid elongation in yeast and mammals include condensation, reduction, dehydration and a second reduction, as shown in Figure 3. Malonyl-CoA is first condensed with acyl-CoA in order to synthesize 3-ketoacyl-CoA, which is catalyzed by the highly conserved fatty acid elongase [32]. Three elongases in yeast exhibit specificity for fatty acids of specific chain lengths (Table 1). Elo1 catalyzes the elongation of C-14 to C-16, Elo2/Fen1 catalyzes the elongation of C-20 to C-22 and C-22 to C-24 and Elo3/Sur4 catalyzes the elongation of C-24 to C-26 and, with a lower efficiency, C-22 to C-24 [62]. Mammalian fatty acid elongases are encoded by seven genes, *ELOVL1–7*, which also exhibit acyl chain specificity (Table 1). *ELOVL6* elongates saturated fatty acyl-CoA chains of C-16 or shorter. *ELOVL3* and *ELOVL7* elongate both saturated and unsaturated fatty acyl-CoAs of C-16 to C-22, but have highest activity for C-18 [33]. *ELOVL1* elongates saturated C-18–C-26 and monounsaturated C-20:1 n-9 and C-22:1 n-9 [33]. *ELOVL1* is the major elongase that is responsible for the synthesis of C-24-containing sphingolipids [33]. *ELOVL4* elongates very long saturated fatty acids or polyunsaturated fatty acids with chains longer than C-26. *ELOVL2* and *ELOVL5* exhibit specificity for polyunsaturated fatty acyl-CoAs. *ELOVL2* elongates polyunsaturated fatty acyl-CoAs with C-22 and *ELOVL5* with C-18, and both can elongate polyunsaturated fatty acyl-CoAs with C-20 [34]. 3-ketoacyl-CoA is reduced by 3-ketoacyl-CoA reductase to 3-hydroxyacyl-CoA, which is dehydrated to 2, 3-*trans*-enoyl-CoA by 3-hydroxyacyl-CoA dehydratase. 3-hydroxyacyl-CoA undergoes another round of reduction catalyzed by enoyl reductase in order to yield an acyl-CoA with two more carbon chains than the original acyl-CoA [34]. The steps in the fatty acid elongation pathway, as well as the fatty acid elongases, are conserved from yeast to mammals [34].

Complex sphingolipid synthesis

Complex sphingolipids differ from yeast to mammals primarily in their head groups. Mammals contain more than 100 different sphingolipid classes due to diverse head groups linked to ceramide, while yeasts contain only three types of complex sphingolipids. Yeast sphingolipids are mostly derived from phytoceramide and, to a lesser extent, dihydroceramide [63,64]. In yeast, ceramides are transported to the Golgi via vesicular or nonvesicular ATP-independent transport [11]. The ER and the Golgi membranes must be in contact with each other for transport to occur [11]. It is proposed that an ATP-independent translocation protein transports ceramide to the Golgi at the contact sites [11]. In the Golgi, a polar, hydrophilic head group is transferred to ceramide in order to form complex sphingolipids, which are transported to the plasma membrane, where they are abundant [11,13,24]. The three types of complex sphingolipids synthesized in the yeast Golgi are inositol phosphoceramide (IPC), mannose-inositol phosphoceramide (MIPC) and mannose (inositol-P)₂ceramide, (M(IP)₂C).

The synthesis of IPC, catalyzed by IPC synthase, occurs via the addition of phosphorylinositol to ceramide [11,13,24]. MIPC is synthesized via the addition of mannose

from guanosine diphosphate mannose to IPC via MIPC synthase. There are two MIPC synthase complexes in yeast, one containing Sur1p and Csg2p and the other composed of Csh1p and Csg2p [13,24]. The most abundant complex sphingolipid, M (IP)₂C, is formed via the addition of a second phosphatidylinositol to MIPC, catalyzed by inositolphosphotransferase. [13,24].

In mammals, the C-1 position of ceramide is linked to a hydrophilic group, such as phosphorylcholine, galactose or glucose, in order to form complex sphingolipids. Ceramide is primarily converted to sphingomyelin via the addition of a phosphorylcholine to the ceramide backbone. Sphingomyelin synthase (SMS1) catalyze the synthesis of sphingomyelin in the Golgi. SMS2 is mainly responsible for the synthesis of sphingomyelin in the plasma membrane. In addition to its activity in the plasma membrane, SMS2 functions redundantly with SMS1 in the Golgi [35]. Ceramide can also be galactosylated in the ER in order to produce galactosylceramide [65], the predominant glycosphingolipid (galactocerebroside) in the brain [66].

Ceramide transported via vesicular or nonvesicular transport to the Golgi is glucosylated to glucosylceramide [67,68]. Glucosylceramide and glycosylceramide act as precursors for the synthesis of glycosphingolipids, catalyzed by Golgi glycosyltransferases [69,70]. These enzymes transfer a carbohydrate from the sugar nucleotides UDP-glucose, UDP-galactose and CMP-sialic acid to ceramide or to the nonreducing end of a growing carbohydrate chain on a ceramide molecule. Glucosylceramide, is galactosylated in order to produce lactosylceramide by galactosyltransferase [71–73]. Lactosylceramide is a precursor for the synthesis of a number of complex glycosphingolipids, such as GA2 [74,75], GM3 [76], Gb3 [77] and Lc3 [70,78], which are themselves precursors for the synthesis of other complex glycosphingolipids [70]. In addition, galactosylceramide is sialylated in order to produce the ganglioside GM4 or sulfated in order to produce sulfatides [70]. Sulfate esters of galactosylceramide, or sulfatides, are thought to play a role in the function of myelin. Perturbation of complex sphingolipid synthesis (especially GM1 and sulfatides) has been described in schizophrenia, as discussed below.

LCB phosphate

In yeast, the LCBs DHS and PHS are phosphorylated to DHS phosphate (DHS1P) or PHS phosphate (PHS1P) by the sphingolipid base kinases Lcb4 and Lcb5 [79]. Lcb4 contributes approximately 97% of the kinase activity. Lcb5, which contributes approximately 3% of the activity, is important for phosphorylation during heat stress [79,80]. Although the double mutant lacking both kinases has an aberrant sphingolipid base content, it remains viable [27].

In mammals, sphingosine bases are phosphorylated at C-1 by two sphingosine kinases that are homologous to the yeast enzyme. Sphingosine-1-phosphate (S1P) is the major product of the kinase, but DHS1P also exists [15]. S1P is an important signaling molecule, as discussed later.

Ceramide-1-phosphate

In mammals, ceramides are phosphorylated at the C-1 position by the calcium-stimulated ceramide kinase in order to form ceramide-1-phosphate (C1P). C1P is a bioactive signaling molecule that is involved in macrophage migration, inflammation and vesicular transport [15,81,82]. Ceramide kinase and C1P are not found in yeast [37].

Catabolism of complex sphingolipids

Isc1, the only sphingomyelinase (SMase) identified in yeast, hydrolyzes all three complex sphingolipids to phytoceramide. Isc1 is homologous to mammalian neutral SMase (nSMase) [38]. In mammals, five types of SMases degrade complex sphingolipids to ceramide. These include acid SMase (aSMase), secretory SMase, alkaline SMase, Mg^{2+} -independent nSMase and Mg^{2+} -dependent nSMase [83]. A single gene, *ASM/SMPD1*, encodes both aSMase, which is targeted to the lysosomes, and sSMase, which is present in secretory vesicles [39,40]. Alkaline SMase is expressed in bile and the intestinal tract [39,40]. Very little is known about Mg^{2+} -independent nSMase, and the gene encoding this enzyme has not been identified. Three Mg^{2+} -dependent nSMases have been identified [41–43].

Catabolism of ceramides

Ceramide that is synthesized *de novo* from sphingolipid bases or produced via the hydrolysis of complex sphingolipids is cleaved by ceramidases to LCBs, which can be phosphorylated or recycled for the synthesis of ceramides [44,45]. Reacylation of the cleaved sphingolipid bases in order to form ceramide is reported to be less efficient than with the *de novo* synthesized sphingolipid bases, but it has been suggested that these cleaved bases can aid in the formation of ceramide with fatty acids of varying N-acyl chain lengths [27,44,45]. Yeasts contain two ceramidases, Ydc1 and Ypc1, while mammals contain three pH-dependent ceramidases. Acid ceramidases are located in the lysosomes [46,47]. Neutral ceramidases are located in the plasma membrane or secreted to the extracellular fluid following cleavage of the transmembrane region [48,49]. Two alkaline ceramidases have been identified: one in the ER/Golgi, which selectively degrades phytoceramides [49], and one in the ER, which degrades N-acylsphingosine [50].

Catabolism of LCB phosphates

In yeast, DHS1P and PHS1P is cleaved by the sphingolipid lyase Dpl1 in order to generate ethanolamine phosphate and fatty aldehydes [84]. This is the only known route whereby a sphingolipid can be converted to a nonsphingolipid. Ethanolamine phosphate is shuttled in order to synthesize phosphatidylethanolamine through the Kennedy pathway [52]. DHS1P and PHS1P are dephosphorylated to DHS and PHS. In mammals, S1P is catabolized to fatty aldehyde and phosphatidylethanolamine by S1P lyase encoded by SPL, which is homologous to yeast Dpl1 [15]. S1P regulates cell survival, cell proliferation and migration and neurotransmitter release [85].

Sphingolipid base transporter

In yeast, deletion of the sphingolipid lyase Dpl1 leads to the accumulation of sphingosine base phosphates and the inhibition of cell growth [51]. In a screen to identify suppressors of

the *dpl1* mutant growth defect caused by the accumulation of sphingosine phosphates, the ATP-dependent sphingosine transporter Rsb1p was identified [53]. Rsb1p is thought to be a transporter or flippase that transports sphingosine bases from the cytoplasmic side to the extracytoplasmic side of the membrane in order to maintain normal intracellular levels of LCBs and membrane composition [53]. Homologs of Rsb1p have not been found in mammals.

Sphingolipids regulate essential functions

Metabolites and intermediates of sphingolipid metabolism, including (but not limited to) LCBs, ceramide, S1P and C1P, are signaling molecules that regulate essential cellular processes that are required for the regulation for neuronal processes [86]. These include SNARE complex formation, synaptic vesicle exocytosis at nerve terminals, neurogenesis, neural tube closure, apoptosis, neurodegeneration and neurotransmitter release [85,87–91].

LCBs & sphingosine

LCBs regulate the actin cytoskeleton, heat stress and endocytosis [86]. Heat stress causes a transient two- to three-fold increase in the levels C-18 DHS and C-18 PHS and a 100-fold increase in the levels of C-20 DHS and C-20 PHS [92,93]. LCBs are required for proper actin cytoskeleton organization [94] and for the regulation of endocytosis [95]. LCBs also play a role in inhibiting the uptake of uracil and the amino acids tryptophan, leucine and histidine [96]. It is not understood how LCBs regulate amino acid transport, but PHS is important for the ubiquitin-mediated breakdown of the uracil transporter Fur4 [97]. Sphingosine activates synaptobrevin in synaptic vesicles, facilitating SNARE complex assembly, which is implicated in membrane fusion. In addition, sphingosine increases synaptic vesicle exocytosis in nerve terminals and neuromuscular junctions [91].

Sphingosine-1-phosphate

S1P regulates cell survival, cell proliferation and migration and neurotransmitter release [85,88], as well as rescuing cells from ceramide-induced apoptosis [98]. The effect of S1P depends on the level of neuronal differentiation. Thus, S1P induces proliferation in neural undifferentiated progenitor cells [99], but it increases apoptosis in well-differentiated neurons [100]. In addition to its role in cell proliferation, S1P is important for neural and vascular development [90]. Sphingosine kinase mutants (*SphK1* and *SphK2*) do not show any abnormalities, but the double mutant exhibits embryonic lethality, perturbed neurogenesis, neural tube closure and angiogenesis in mice [90].

Ceramide

Ceramides are antiproliferative and promote apoptosis [89]. Increased levels of ceramide cause cell death [101], while depletion of ceramide reduces the progression of apoptosis [102,103]. Altered ceramide levels are linked to cancer, neurodegeneration and inflammation [103]. Maintaining normal ceramide levels is crucial for brain function.

Ceramide is also known to regulate cell senescence. The genes *LAG1* and *LAC1*, which encode ceramide synthase, regulate the yeast lifespan [104]. The mammalian homolog of *LAG1* (*CerS1*) is highly expressed in the brain [105]. Overexpression of *CerS1* leads to

increased levels of C-18 ceramide and induces apoptosis [105]. CerS1-generated C-18 ceramide negatively regulates the human telomerase reverse transcriptase promoter [106,107]. Telomerase is expressed in neurons in the early stages of development in neural stem cells and progenitors, but is downregulated in developed cells. Telomerase protects postmitotic neurons from stress-induced apoptosis and is important for the survival of neurons in the developing brain. Therefore, C-18 ceramide very likely contributes to neuronal vulnerability in age-related neurodegenerative disorders [107].

Ceramide affects several signal transduction pathways. The signaling molecules Akt serine/threonine kinase and PP2A are known targets of ceramide [108–110]. Ceramide was shown to reduce Akt phosphorylation at Ser-473; this dephosphorylation was reversed after treatment with antidepressants [111]. Ceramide induces mitochondrial outer membrane permeabilization, which is important for the induction of apoptosis, by forming ceramide channels. In addition, ceramide enhances the autophosphorylation of the kinase suppressor of Ras, which activates the RAF1/MEK/ERK/MAPK cascade, thereby inducing apoptosis [112,113]. Several studies have demonstrated that ceramide activates the JNK pathway [114–116]. JNK activation regulates proteins of the Bcl-2 family, which are involved in apoptosis [117]. Ceramide also activates PKC- σ and inhibits PKC- α [118], phenotypes that are reversed by the antidepressants imipramine and amitriptyline [60]. These findings suggest that antidepressants may restore normal signaling that is perturbed by increased ceramide levels.

Ceramide-1-phosphate

Phosphorylation of ceramide produces C1P, which decreases apoptosis in several ways. C1P blocks ceramide synthesis by inhibiting aSMase [119] and SPT activity [120]. C1P also enhances the activity of the antiapoptotic protein kinase B and NF- κ B [120]. While the function of C1P has not been definitively elucidated, an early study proposed that it plays a role in synaptic vesicle functioning and the release of neurotransmitters [87].

Sphingolipid metabolism in psychiatric disorders

As discussed above, sphingolipids affect essential cellular processes that are crucial not only for processes of cellular homeostasis, such as apoptosis, cell survival and development, but also for neuronal functions. In particular, sphingosine is required for the activation of exocytosis of the synaptic vesicles at the nerve terminal. Ceramide, S1P and C1P regulate apoptosis, neurogenesis and neurotransmitter release. In view of the key role of sphingolipids in neurogenesis, neuronal cell death and neurotransmission, it is not surprising that perturbation of sphingolipid metabolism is observed in depression, anxiety, schizophrenia and bipolar disorder.

Depression

Depression is a chronic, severe, life-threatening disorder characterized by depressed mood, insomnia, weight loss, loss of interest or pleasure in normal activities, feelings of worthlessness and a high risk of suicide. The worldwide prevalence of depression is 10% [121]. Recent studies suggest that depression is linked to a change in the levels of

neurogenic and apoptotic factors in the hippocampus, leading to hippocampal atrophy and neurodegeneration [122,123].

A number of studies indicate that depression is associated with altered sphingolipid synthesis. A recent lipidomic analysis identified significant alterations in plasma sphingomyelin species in patients with depression [124]. Several studies, summarized below, report increased levels of ceramide and/or aSMase in depression and decreased levels of these components following antidepressant treatment.

Increased ceramide & aSMase in depression

Several studies suggest that increased ceramide levels are associated with depression. Increased aSMase activity was observed in the peripheral blood mononuclear cells of patients suffering from major depressive disorder who were free of therapy for at least 10 days [60]. As discussed above, aSMase converts sphingomyelin to ceramide and phosphorylcholine [111,125,126]. Therefore, this finding suggests that increased levels of ceramide may be associated with depression. Consistent with this possibility, increasing the levels of ceramide by injecting C-16 ceramide directly in the hippocampus or by administering D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (a glycosyltransferase inhibitor that increases ceramide levels by inhibiting glycosylation of ceramide) induced depressive disorder-like symptoms in mice [111]. Plasma ceramide levels were analyzed in Alzheimer's disease patients with recent depression, past depression and no depression. Interestingly, ceramide levels were elevated in patients with recent depression compared with patients with past or no depression [127]. Indirect evidence associating ceramide with depression comes from a report of increased activity of phospholipase A₂, which is stimulated by ceramide, in patients with major depression [128].

Antidepressants inhibit aSMase activity & decrease ceramide levels

If ceramide plays a causal role in depression, as suggested by the correlational studies cited above, antidepressants may exert their therapeutic effects by inhibiting aSMase, thus decreasing the release of ceramide from sphingomyelin. Consistent with this, treatment with the antidepressants imipramine and amitriptyline caused a rapid reduction of aSMase activity in the peripheral blood mononuclear cells of depressive patients [60]. In addition, a therapeutic concentration of the antidepressants amitriptyline and fluoxetine inhibited aSMase activity in cultured neurons [111]. Wild-type mice treated with the antidepressants amitriptyline (a tricyclic drug) or fluoxetine (a serotonin reuptake inhibitor) exhibited decreased aSMase protein levels and activity [111].

In order to study the effect of antidepressants on ceramide levels, ceramidase-heterozygous mice overexpressing aSMase were generated. These mice exhibited reduced neurogenesis and maturation due to increased ceramide levels. When treated with antidepressants, ceramide levels decreased. In addition, antidepressant treatment increased neurogenesis, neuronal maturation and survival. This suggests that antidepressants decrease the levels of ceramide due to the inhibition of aSMase activity, which normalizes the reduced neurogenesis and neuronal maturation [111].

A proposed mechanism underlying antidepressant-mediated decreased aSMase activity is as follows: the inner leaflet of the lysosomal membrane is negatively charged due to the presence of the anionic lipid bis(monoacylglycero)phosphate [129]. Positively charged aSMase adheres to the inner lysosomal membrane leaflet electrostatically [130]. Lipophilic compounds and weak bases such as antidepressants in the neutral form can passively traverse through the cell and lysosomal membranes and accumulate in lysosomes. The lysosomal concentration of the drug increases when protonated, trapping it in the organelle [131,132]. The lipophilic part of the drug adheres to the membrane, while the positively charged moiety faces the lumen. Accumulation of the drug thus prevents the aSMase from adhering to the lysosomal membrane [130]. This results in proteolytic cleavage and, consequently, decreased activity of the enzyme [133]. In summary, this mechanism suggests that antidepressants bring about the degradation of aSMase, resulting in decreased levels of ceramide, thereby reversing the perturbation of neurogenesis and neuronal maturation caused by increased ceramide levels. This suggests that antidepressants decrease the levels of ceramide due to inhibition of aSMase activity via degradation of the aSMase protein, which normalizes the reduced neurogenesis and neuronal maturation caused by increased ceramide levels.

Interestingly, amitriptyline and fluoxetine increased neurogenesis, neuronal maturation and survival in wild-type mice, but not in aSMase-deficient mice, suggesting that the mechanism of action of the drugs is by affecting aSMase. Treatment with the antidepressants amitriptyline and fluoxetine or the aSMase inhibitor fendiline attenuated corticosterone-induced depressive-like behavior in wild-type mice, but had no effect on corticosterone-treated aSMase-deficient mice [111]. These studies suggest that increased levels of ceramide may play a causal role in the pathophysiology of depression and that a potential therapeutic mechanism underlying antidepressant efficacy is the reduction of ceramide levels via the inhibition of aSMase activity. Reducing ceramide levels could be a goal for the development of new antidepressants.

Anxiety

Anxiety disorders are characterized by severe and prolonged distress and fear with physiological symptoms, which are manifested early and recur throughout life [134]. They are the sixth-leading cause of disability in both low- and high-income countries [134]. Several studies suggest that sphingolipids may play a role in the pathophysiology of anxiety disorder. In particular, increased levels of S1P are associated with anxiety-like behavior, while increased levels of GM1 may reduce anxiety and cause neuroprotection.

Increased levels of S1P

Chronic stress is known to cause anxiety-like behavior. In several models of stress-induced anxiety in rats, including daily immobilization for 3 weeks or electric shock followed by immobilization, serum S1P levels were increased [135]. In the former model, increased expression of protein markers of neurodegeneration were also observed, including inducible nitric oxide synthase, glial fibrillary acidic protein and the N-methyl-D-aspartate receptor channel. Infusion of S1P into the lateral cerebroventricle also led to increased expression of these protein markers, suggesting that stress-induced anxiety causes increased S1P, which

leads to degeneration [136]. However, an alternative possibility comes from a study using an elevated plus maze model of anxiety, in which S1P-treated animals spent significantly less time in the open arms of the maze, suggesting that S1P induces anxiety [135].

Perturbation of glycosphingolipid levels

Precocious weaning is known to induce anxiety-like behavior in male mice, which is more pronounced than that observed in precociously weaned female or normally weaned mice [137]. Interestingly, precociously weaned male mice exhibited an accumulation of galactosylceramide in the amygdala (but not in the hippocampus or prefrontal cortex), along with an increase in number and reduction in diameter of myelinated axons at 5 weeks. Galactosylceramide and sulfatide contribute approximately 30% of the lipid content of the myelin sheath [138]. The developmental time of myelin formation differs for each brain region [139]. In normally weaned male mice, galactosylceramide increases linearly until 8 weeks of age in the amygdala and saturates by 5 weeks in the prefrontal cortex and hippocampus. Therefore, early weaning may induce precocious myelination, which may account for the decreased axonal diameter observed in early weaning. This can cause changes in axonal structure and neural circuits in the basolateral amygdala, a site controlling anxiety-like behavior [140–142]. Therefore, early myelination of the amygdala in precociously weaned male mice might affect the development of an anxiety-related network and thereby increase anxiety-related behavior.

GM1 is a neuroprotective sialic acid-containing glycosphingolipid [143,144]. An early study suggested that GM1 improved memory, but not anxiety. GM1 was administered to male rats containing electrolytic lesions in the left cortex (electrolytic lesions produce transient behavioral deficits [145,146]). Working memory was measured in a Morris water maze and anxiety levels were tested in a plus maze. GM1 treatment led to improvements in working memory, but not in anxiety levels [147]. However, GM1 was effective in blocking anxiety-related responses to ethanol withdrawal, including hypolocomotion, tremors and other anxiety-like behaviors [144]. Pretreatment with GM1 or its constituent sialic acid was also effective in terms of reducing the effects of ethanol intoxication, such as nose-poke exploration, decreased locomotion and anxiety [148]. Pretreatment with ganglioside or sialic acid also improved staircase climbing by 22%, which is consistent with lower anxiety [148].

These studies suggest that cerebrosides may play a role in anxiety, as galactosylceramide accumulates in at least one model of anxiety. Galactosylceramide and GM1 are required for the proper myelination of the axon, thus exerting a protective role.

Schizophrenia

Schizophrenia is a debilitating mental disorder with drastic psychosocial consequences. It is characterized by defects in synaptic connectivity in the white matter tracts, leading to cognitive abnormalities [149]. Schizophrenic patients exhibit both positive symptoms (delusions and hallucinations) and negative symptoms (social withdrawal and loss of motivation) [150]. Schizophrenia is known to affect 1% of the world population [151,152]. Several neurochemical and neuropathological theories have been proposed in order to explain the pathology of schizophrenia, including perturbations in GABAergic [153], dopaminergic

[154] and glutamatergic signaling [155,156], as well as aberrations in neurodevelopment [157], prefrontal cortex function [158,159], and inflammatory responses [160,161].

Perturbation of complex sphingolipids

Complex sphingolipids are required for the myelination of neurons [162]. They play a role in carbohydrate–carbohydrate interactions between opposing membranes, cell–cell adhesion and transmembrane signaling, as well as communication between the axon and myelin sheath, which affects axonal and myelin functions and neurodegeneration [163].

Decreased levels of complex sphingolipids, including sulfatides and cerebroside, were first observed in a post-mortem schizophrenic brain in 1969 [164]. Subsequently, decreased levels of sphingomyelin and galactocerebroside, which are required for myelin synthesis in the brain, were observed in post-mortem brain samples of chronic schizophrenic patients [165]. Consistent with decreased sphingomyelin, increased levels of phosphatidylserine, an activator of nSMase, were observed in post-mortem schizophrenic samples [165]. Decreased levels of glycosphingolipids could result in decreased myelination and altered cell–cell adhesion and transmembrane signaling, leading to neurodegeneration. However, this conclusion is obscured by studies reporting increased levels of complex sphingolipids in schizophrenia, including glycosphingolipids and the gangliosides GM3 and GD3 [166].

In a microarray analysis comparing postmortem brain samples of the prefrontal cortex from schizophrenic patients and healthy controls, the expression of a number of genes that encode enzymes of sphingolipid metabolism was observed. Changes were most notable in the early stages of the disorder (within 5 years of diagnosis) [57]. A significant reduction was observed in the expression of seven genes related to sphingolipid metabolism, including *UGT8*, *GAL3ST1*, *SPTLC2*, *SGPPI*, *GALC*, *SPTLC2* and *ASAHI* [57]. *UGT8* encodes UDP glycosyltransferase, which converts ceramide to galactosylceramide. The *GAL3ST1*-encoded enzyme, galactose-3-O-sulfotransferase, catalyzes the synthesis of sulfatides. Sulfatides and galactosylceramide are components of the myelin sheath, and altered levels of these lipids may lead to impaired myelination. Post-mortem studies of schizophrenic patients have revealed abnormalities in the myelination of oligodendrocytes [167]. Consistent with the hypothesis of impaired myelination, oligodendrocyte dysfunction was observed in schizophrenic patients [168]. However, chronic treatment with haloperidol was associated with the decreased expression of myelin oligodendrocyte-related genes in the white matter of mouse brain samples [169]. Therefore, the role of abnormal myelination in schizophrenia remains to be elucidated.

Aberrant ceramide levels

Several reports indicate that ceramide levels are altered in schizophrenia, although a consistent pattern of alteration is not apparent. In a study comparing epidermal lipid profiles of drug-naïve (first-episode) schizophrenics and healthy controls, several classes of ceramide were altered [170]. A significant increase of α -hydroxy and 6-hydroxy-sphingosine with C-26 or C-28 fatty acid chains and nonhydroxy-6-hydroxysphingosine/ α -hydroxysphingosine with C-26 or C-28 fatty acid chains, as well as a decrease in ceramide-containing ω -hydroxylated fatty acids with greater than C-30 fatty acid chains esterified to

linoleic acid and nonhydroxy-PHS with C-26 or C-28 fatty acid chains, were observed in patients compared with the healthy controls [170]. Results of a high-throughput analysis of lipids from schizophrenic and normal controls suggest that ceramide levels are increased in the white matter of schizophrenic samples [59].

The microarray analysis of post-mortem brain samples of schizophrenic patients discussed in the previous section identified the altered expression of genes that metabolize ceramide [57]. Interestingly, the genes encoding enzymes that decrease ceramide levels were downregulated in chronic schizophrenia, including *UGT8*, *GAL3ST1* and *ASAH1*. *ASAH1* was also found to have strong linkage to schizophrenia in a genome-wide metascan study [171]. In addition, two single-nucleotide polymorphisms in *ASAH1* were found to be associated with schizophrenia [172]. In summary, altered ceramide levels have been associated with schizophrenia, but a causal role for these lipids in the etiology of the disorder has not been determined.

Bipolar disorder

Bipolar disorder, which is characterized by depression and mania, is a severe mood disorder that affects 1–2% of the world's population [173]. A high-throughput lipidomics study of post-mortem brain samples from bipolar patients and normal controls revealed statistically significantly increased levels of ceramide in the white matter of bipolar samples, not accompanied by decreased levels of phosphatidylcholine, suggesting that the increase in ceramide results from the increased synthesis rather than the increased breakdown of sphingomyelin [59]. While these studies are very preliminary, they suggest that bipolar disorder may be associated with altered ceramide levels and ceramide signaling. However, whether ceramide plays a role in the development and progression of bipolar disorder or whether it is simply an outcome of the disease remains unknown.

Conclusion

There is a profound need to elucidate the molecular mechanisms underlying psychiatric disorders and to develop effective therapies in order to treat them. While many studies associate aberrant sphingolipid metabolism with the pathophysiology of psychiatric illnesses, the role of sphingolipids in the etiology of these disorders has not been established. The yeast model could be exploited in order to characterize the regulation of sphingolipid metabolism, the roles of specific sphingolipids in essential cell functions and the global cellular consequences of the perturbation of their synthesis. Because of the very high degree of conservation of function of these lipids between yeast and human cells, the data from studies in yeast can be used in order to generate testable hypotheses that can be experimentally addressed in appropriate mammalian systems. This knowledge has the potential to contribute to our understanding of the mechanisms underlying the pathophysiology of psychiatric disorders and to the development of new drugs that can be used in their treatment.

Future perspective: exploiting yeast to elucidate sphingolipid functions & develop sphingolipid-modifying therapeutics

While the studies summarized above suggest that sphingolipid metabolism is perturbed in psychiatric disorders, the fundamental question – does perturbation of sphingolipid metabolism play a causal role or is it a secondary effect of the disorder? – remains to be elucidated. The complexity of mammalian systems and the lack of suitable animal models that accurately reflect human psychiatric illnesses complicate the ability to effectively address this question. How, then, can a unicellular eukaryote shed light on these complex processes? The genetic tractability of yeast offers powerful advantages that can be exploited in order to elucidate the role of sphingolipids in essential and highly conserved cellular processes as follows: first, all of the genes encoding the enzymes of the sphingolipid metabolism in yeast are well characterized, and most of the enzymes of sphingolipid metabolism have homologs or orthologs in mammals, as discussed above. The homology of yeast and mammalian genes has been exploited in order to clone mammalian sphingolipid genes by the complementation of yeast mutants [10,174,175]. The signaling pathways and cellular processes that are controlled by sphingolipids are also conserved. Conservation of sphingolipid synthesis and function suggests that knowledge gleaned from the yeast system can shed light on similar processes in human cells. Second, a wealth of well-characterized yeast mutants can be exploited in *in vivo* experiments in order to address the role of specific sphingolipids in essential cellular processes and to ascertain the global effects of targeted sphingolipid perturbations. Third, yeast bioassays utilizing sphingolipid mutants can be developed in order to screen for drugs that target specific sphingolipid components. Fourth, yeast genetic and metabolic databases can be utilized for *in vivo* and *in silico* experiments in order to determine the effects of sphingolipid perturbation on cell function.

Identifying inhibitors of ceramide & S1P synthesis

Increased levels of ceramide in depression, schizophrenia and bipolar disorder suggest that ceramide synthesis may be a potential therapeutic target. As discussed above, mammals have six different ceramide synthase enzymes that exhibit tissue specificity as well as specificity for fatty acyl-CoAs [176]. Creating and analyzing the effects of ceramide synthase knockouts would help to elucidate the roles of specific ceramide species. Several knockout mice have already been described [177,178]. CerS6-knockout mice exhibited a decrease in C-16:0-containing ceramide species accompanied by behavioral abnormalities, such as habituation deficits and clasping abnormalities of their hind limbs [177]. CerS2-deficient mice exhibit myelin sheath defects, cerebellar degeneration and hepatocarcinomas [178]. Creating other CerS knockouts will enable the study of the functional roles of specific ceramide synthases and ceramide species.

The available ceramide synthase inhibitors (fumonisins B and austalifugin) target the enzymes nonspecifically, but specific inhibitors would be useful in elucidating the functions of each enzyme. Yeast ceramide synthase mutants (*lag1* and *lac1*) exhibit growth defects that can be functionally complemented with mammalian homologs [179]. Therefore, inhibitors of specific ceramide synthases could be identified by inhibiting the growth of yeast *lag1* or *lac1* mutants expressing the mammalian enzyme.

A similar approach could be used in order to identify inhibitors of S1P, the levels of which appear to be increased in anxiety. Currently, there are no drugs that target S1P for the treatment of psychiatric disorders. However, S1P-targeting drugs are used for the treatment of multiple sclerosis. These include the S1P mimic, FTY720 [180], and the S1P receptor (S1P1) agonist, siponimod [181]. Similarly, inhibitors of sphingosine kinases and sphingosine phosphatases could be developed for the treatment of psychiatric disorders associated with increased S1P levels. Yeast may be a powerful tool for developing these inhibitors. Yeast *lcb4* and *lcb5* mutants, which completely lack sphingosine kinase activity, can be engineered in order to express a single mammalian homolog. The levels of sphingosine and S1P in these cells can thus be attributed to the mammalian enzyme. These levels can be easily monitored and the effects of potential inhibitors can be determined.

Inhibitors of these specific steps in sphingolipid metabolism would be powerful tools for elucidating the cellular roles of ceramide and S1P, just as studies with the SPT and the ceramide synthase inhibitors myriocin and fumonisins B1 have contributed greatly to our understanding of the roles of sphingolipids in autophagy, heat stress, lifespan and apoptosis [14,182,183].

Mathematical modeling of the effects of the perturbation of ceramide & S1P on yeast cell homeostasis

Mathematical modeling is a powerful tool for organizing and analyzing high-throughput data pertaining to biochemical pathways [184]. Mathematical modeling has been exploited in order to generate complex network maps of sphingolipid metabolism in yeast [184–186]. For example, a recent study simulated the action of drugs affecting sphingolipid metabolism in the ER in order to determine the effects of the drugs on ergosterol in the plasma membrane [187]. Simulation of the inhibition of IPC synthase, a step inhibited by aerobasidin, suggested that vesicular cotransport of ergosterol and complex sphingolipids is impaired, resulting in the redistribution of ergosterol from the outer to the inner leaflet of the plasma membrane. Similarly, simulations with drugs such as myriocin, an inhibitor of SPT, suggested a decrease in vesicular ergosterol transport flux and an increase of the nonvesicular ergosterol flux. Mathematical modeling can be used in order to simulate the perturbation of ceramide and S1P levels, generating *in silico* disease models in yeast. The predictions of these models can be validated easily in yeast and then translated to complex mammalian systems [188].

Elucidating the global *in vivo* effects of the perturbation of ceramide & S1P levels

Altered levels of ceramide or S1P in yeast can be achieved by genetic manipulation using mutants of ceramide synthase (*LAG1* and *LAC1*), sphingosine base kinases (*LCB4* and *LCB5*) and sphingosine base phosphate (*LCB3* and *YSR3*). The effects of this perturbation can be analyzed in the context of the available yeast metabolome, transcriptome, proteome and interactome data [189–193]. Lipidomic analyses may identify specific (and unexpected) metabolites that are important in maintaining homeostasis and that may be altered in disease conditions [188]. Recent advances with mass spectrometric tools in the field of lipidomics have made it possible to identify and quantify many species of sphingolipids. In addition, shotgun lipidomics enables the identification and quantification of individual lipid species

directly from lipid extracts of biological samples [194]. Alterations in specific sphingolipid species may provide insights into their role in pathogenesis [195]. The results from these studies can be investigated and validated in mammalian systems.

Acknowledgments

The ML Greenberg laboratory acknowledges support from NIH grant R01 DK 081367.

References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest

1. Prince M, Patel V, Saxena S, et al. No health without mental health. *Lancet*. 2007; 370(9590):859–877. [PubMed: 17804063]
2. Watkins BA, Li Y, Seifert MF. Lipids as modulators of bone remodelling. *Curr Opin Clin Nutr Metab Care*. 2001; 4(2):105–110. [PubMed: 11224653]
3. Morell P, Toews AD. Schwann cells as targets for neurotoxicants. *Neurotoxicology*. 1996; 17(3–4):685–695. [PubMed: 9086490]
4. Cutler RG, Mattson MP. Sphingomyelin and ceramide as regulators of development and lifespan. *Mech Ageing Dev*. 2001; 122(9):895–908. [PubMed: 11348657]
5. Tettamanti G. Ganglioside/glycosphingolipid turnover: new concepts. *Glycoconj J*. 2004; 20(5):301–317. [PubMed: 15229395]
6. Van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol*. 2008; 9(2):112–124. [PubMed: 18216768]
7. Ejsing CS, Sampaio JL, Surendranath V, et al. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc Natl Acad Sci USA*. 2009; 106(7):2136–2141. [PubMed: 19174513]
8. Shevchenko A, Simons K. Lipidomics: coming to grips with lipid diversity. *Nat Rev Mol Cell Biol*. 2010; 11(8):593–598. [PubMed: 20606693]
9. Dickson RC, Lester RL. Sphingolipid functions in *Saccharomyces cerevisiae*. *Biochim Biophys Acta*. 2002; 1583(1):13–25. [PubMed: 12069845]
10. Dickson RC, Sumanasekera C, Lester RL. Functions and metabolism of sphingolipids in *Saccharomyces cerevisiae*. *Prog Lipid Res*. 2006; 45(6):447–465. [PubMed: 16730802]
11. Funato K, Vallee B, Riezman H. Biosynthesis and trafficking of sphingolipids in the yeast *Saccharomyces cerevisiae*. *Biochemistry*. 2002; 41(51):15105–15114. [PubMed: 12484746]
12. Cowart LA, Hannun YA. Using genomic and lipidomic strategies to investigate sphingolipid function in the yeast heat-stress response. *Biochem Soc Trans*. 2005; 33(Pt 5):1166–1169.
13. Sims KJ, Spassieva SD, Voit EO, Obeid LM. Yeast sphingolipid metabolism: clues and connections. *Biochem Cell Biol*. 2004; 82(1):45–61. [PubMed: 15052327]
14. Breslow DK. Sphingolipid homeostasis in the endoplasmic reticulum and beyond. *Cold Spring Harb Perspect Biol*. 2013; 5(4):a013326. [PubMed: 23545423]
15. Kihara A, Mitsutake S, Mizutani Y, Igarashi Y. Metabolism and biological functions of two phosphorylated sphingolipids, sphingosine 1-phosphate and ceramide 1-phosphate. *Prog Lipid Res*. 2007; 46(2):126–144. [PubMed: 17449104]
16. Lester RL, Dickson RC. High-performance liquid chromatography analysis of molecular species of sphingolipid-related long-chain bases and long-chain base phosphates in *Saccharomyces cerevisiae* after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. *Anal Biochem*. 2001; 298(2):283–292. [PubMed: 11700984]
17. Dickson RC, Lester RL. Metabolism and selected functions of sphingolipids in the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta*. 1999; 1438(3):305–321. [PubMed: 10366774]

18. Obeid LM, Okamoto Y, Mao C. Yeast sphingolipids: metabolism and biology. *Biochim Biophys Acta*. 2002; 1585(2–3):163–171. [PubMed: 12531550]
19. Dickson RC. Sphingolipid functions in *Saccharomyces cerevisiae*: comparison to mammals. *Annu Rev Biochem*. 1998; 67:27–48. Comprehensive review comparing sphingolipid functions in yeast and mammals. [PubMed: 9759481]
20. Epstein S, Riezman H. Sphingolipid signaling in yeast: potential implications for understanding disease. *Front Biosci (Elite Ed)*. 2013; 5:97–108. [PubMed: 23276973]
21. Gable K, Han G, Monaghan E, et al. Mutations in the yeast LCB1 and LCB2 genes, including those corresponding to the hereditary sensory neuropathy type I mutations, dominantly inactivate serine palmitoyltransferase. *J Biol Chem*. 2002; 277(12):10194–10200. [PubMed: 11781309]
22. Hanada K. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim Biophys Acta*. 2003; 1632(1–3):16–30. [PubMed: 12782147]
23. Lowther J, Naismith JH, Dunn TM, Campopiano DJ. Structural, mechanistic and regulatory studies of serine palmitoyltransferase. *Biochem Soc Trans*. 2012; 40(3):547–554.
24. Dickson RC. Thematic review series: sphingolipids new insights into sphingolipid metabolism and function in budding yeast. *J Lipid Res*. 2008; 49(5):909–921. [PubMed: 18296751]
25. Beeler T, Bacikova D, Gable K, et al. The *Saccharomyces cerevisiae* TSC10/YBR265w gene encoding 3-ketosphinganine reductase is identified in a screen for temperature-sensitive suppressors of the Ca^{2+} -sensitive csg2Delta mutant. *J Biol Chem*. 1998; 273(46):30688–30694. [PubMed: 9804843]
26. Kihara A, Igarashi Y. FVT-1 is a mammalian 3-ketodihydrosphingosine reductase with an active site that faces the cytosolic side of the endoplasmic reticulum membrane. *J Biol Chem*. 2004; 279(47):49243–49250. [PubMed: 15328338]
27. Cowart LA, Obeid LM. Yeast sphingolipids: recent developments in understanding biosynthesis, regulation, and function. *Biochim Biophys Acta*. 2007; 1771(3):421–431. [PubMed: 16997623]
28. Mizutani Y, Kihara A, Igarashi Y. LASS3 (longevity assurance homologue 3) is a mainly testis-specific (dihydro)ceramide synthase with relatively broad substrate specificity. *Biochem J*. 2006; 398(3):531–538. [PubMed: 16753040]
29. Ternes P, Franke S, Zahringer U, Sperling P, Heinz E. Identification and characterization of a sphingolipid delta 4-desaturase family. *J Biol Chem*. 2002; 277(28):25512–25518. [PubMed: 11937514]
30. Mizutani Y, Kihara A, Igarashi Y. Identification of the human sphingolipid C4-hydroxylase, hDES2, and its up-regulation during keratinocyte differentiation. *FEBS Lett*. 2004; 563(1–3):93–97. [PubMed: 15063729]
31. Omae F, Miyazaki M, Enomoto A, Suzuki M, Suzuki Y, Suzuki A. DES2 protein is responsible for phytoceramide biosynthesis in the mouse small intestine. *Biochem J*. 2004; 379(Pt 3):687–695. [PubMed: 14731113]
32. Jakobsson A, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lipid Res*. 2006; 45(3):237–249. [PubMed: 16564093]
33. Ohno Y, Suto S, Yamanaka M, et al. ELOVL1 production of C24 acyl-CoAs is linked to C24 sphingolipid synthesis. *Proc Natl Acad Sci USA*. 2010; 107(43):18439–18444. [PubMed: 20937905]
34. Kihara A. Very long-chain fatty acids: elongation, physiology and related disorders. *J Biochem*. 2012; 152(5):387–395. Excellent review of fatty acid elongation and its role in disorders. [PubMed: 22984005]
35. Hanada K, Kumagai K, Tomishige N, Kawano M. CERT and intracellular trafficking of ceramide. *Biochim Biophys Acta*. 2007; 1771(6):644–653. [PubMed: 17314061]
36. Schulte S, Stoffel W. Ceramide UDPgalactosyltransferase from myelinating rat brain: purification, cloning, and expression. *Proc Natl Acad Sci USA*. 1993; 90(21):10265–10269. [PubMed: 7694285]
37. Sugiura M, Kono K, Liu H, et al. Ceramide kinase, a novel lipid kinase. Molecular cloning and functional characterization. *J Biol Chem*. 2002; 277(26):23294–23300. [PubMed: 11956206]

38. Sawai H, Okamoto Y, Luberto C, et al. Identification of ISC1 (YER019w) as inositol phosphosphingolipid phospholipase C in *Saccharomyces cerevisiae*. *J Biol Chem*. 2000; 275(50): 39793–39798. [PubMed: 11006294]
39. Wu J, Hansen GH, Nilsson A, Duan RD. Functional studies of human intestinal alkaline sphingomyelinase by deglycosylation and mutagenesis. *Biochem J*. 2005; 386(Pt 1):153–160. [PubMed: 15458386]
40. Duan RD. Alkaline sphingomyelinase: an old enzyme with novel implications. *Biochim Biophys Acta*. 2006; 1761(3):281–291. [PubMed: 16631405]
41. Tomiuk S, Hofmann K, Nix M, Zumbansen M, Stoffel W. Cloned mammalian neutral sphingomyelinase: functions in sphingolipid signaling? *Proc Natl Acad Sci USA*. 1998; 95(7): 3638–3643. [PubMed: 9520418]
42. Hofmann K, Tomiuk S, Wolff G, Stoffel W. Cloning and characterization of the mammalian brain-specific, Mg^{2+} -dependent neutral sphingomyelinase. *Proc Natl Acad Sci USA*. 2000; 97(11): 5895–5900. [PubMed: 10823942]
43. Krut O, Wiegmann K, Kashkar H, Yazdanpanah B, Kronke M. Novel tumor necrosis factor-responsive mammalian neutral sphingomyelinase-3 is a C-tail-anchored protein. *J Biol Chem*. 2006; 281(19):13784–13793. [PubMed: 16517606]
44. Ogretmen B, Pettus BJ, Rossi MJ, et al. Biochemical mechanisms of the generation of endogenous long chain ceramide in response to exogenous short chain ceramide in the A549 human lung adenocarcinoma cell line Role for endogenous ceramide in mediating the action of exogenous ceramide. *J Biol Chem*. 2002; 277(15):12960–12969. [PubMed: 11815611]
45. Sultan I, Senkal CE, Ponnusamy S, et al. Regulation of the sphingosine-recycling pathway for ceramide generation by oxidative stress, and its role in controlling c-Myc/Max function. *Biochem J*. 2006; 393(Pt 2):513–521. [PubMed: 16201965]
46. Koch J, Gartner S, Li CM, et al. Molecular cloning and characterization of a full-length complementary DNA encoding human acid ceramidase. Identification of the first molecular lesion causing Farber disease. *J Biol Chem*. 1996; 271(51):33110–33115. [PubMed: 8955159]
47. Ferlinz K, Kopal G, Bernardo K, et al. Human acid ceramidase: processing, glycosylation, and lysosomal targeting. *J Biol Chem*. 2001; 276(38):35352–35360. [PubMed: 11451951]
48. Tani M, Iida H, Ito M. O-glycosylation of mucin-like domain retains the neutral ceramidase on the plasma membranes as a type II integral membrane protein. *J Biol Chem*. 2003; 278(12):10523–10530. [PubMed: 12499379]
49. Mao C, Xu R, Szulc ZM, Bielawska A, Galadari SH, Obeid LM. Cloning and characterization of a novel human alkaline ceramidase. A mammalian enzyme that hydrolyzes phytoceramide. *J Biol Chem*. 2001; 276(28):26577–26588. [PubMed: 11356846]
50. Mao C, Xu R, Szulc ZM, et al. Cloning and characterization of a mouse endoplasmic reticulum alkaline ceramidase: an enzyme that preferentially regulates metabolism of very long chain ceramides. *J Biol Chem*. 2003; 278(33):31184–31191. [PubMed: 12783875]
51. Kim S, Fyrst H, Saba J. Accumulation of phosphorylated sphingoid long-chain bases results in cell growth inhibition in *Saccharomyces cerevisiae*. *Genetics*. 2000; 156(4):1519–1529. [PubMed: 11102354]
52. Panwar SL, Moyer-Rowley WS. Long-chain base tolerance in *Saccharomyces cerevisiae* is induced by retrograde signals from the mitochondria. *J Biol Chem*. 2006; 281(10):6376–6384. [PubMed: 16407254]
53. Kihara A, Igarashi Y. Identification and characterization of a *Saccharomyces cerevisiae* gene, RSB1, involved in sphingoid long-chain base release. *J Biol Chem*. 2002; 277(33):30048–30054. [PubMed: 12034738]
54. Nowak M, Dziegiel P, Madej J, Ugorski M. Ceramide galactosyltransferase (UGT8) as a molecular marker of canine mammary tumor malignancy. *Folia Histochem Cytobiol*. 2013; 51(2):164–167. [PubMed: 23907947]
55. Huang WC, Nagahashi M, Terracina KP, Takabe K. Emerging role of sphingosine-1-phosphate in inflammation, cancer, and lymphangiogenesis. *Biomolecules*. 2013 Epub ahead of print. 10.3390/biom3030408

56. Hill CH, Graham SC, Read RJ, Deane JE. Structural snapshots illustrate the catalytic cycle of beta-galactocerebrosidase, the defective enzyme in Krabbe disease. *Proc Natl Acad Sci USA*. 2013; 110(51):20479–20484. [PubMed: 24297913]
57. Narayan S, Head SR, Gilmartin TJ, Dean B, Thomas EA. Evidence for disruption of sphingolipid metabolism in schizophrenia. *J Neurosci Res*. 2009; 87(1):278–288. Perturbation of sphingolipid metabolism leads to pathologies associated with schizophrenia, such as deficits in myelination and oligodendrocyte function. [PubMed: 18683247]
58. Kim BH, Lee JM, Jung YG, Kim S, Kim TY. Phytosphingosine derivatives ameliorate skin inflammation by inhibiting NF-kappaB and JAK/STAT signaling in keratinocytes and mice. *J Invest Dermatol*. 2014; 134(4):1023–1032. [PubMed: 24177187]
59. Schwarz E, Prabakaran S, Whitfield P, et al. High throughput lipidomic profiling of schizophrenia and bipolar disorder brain tissue reveals alterations of free fatty acids, phosphatidylcholines, and ceramides. *J Proteome Res*. 2008; 7(10):4266–4277. [PubMed: 18778095]
60. Kornhuber J, Medlin A, Bleich S, et al. High activity of acid sphingomyelinase in major depression. *J Neural Transm*. 2005; 112(11):1583–1590. [PubMed: 16245071]
61. Denic V, Weissman JS. A molecular caliper mechanism for determining very long-chain fatty acid length. *Cell*. 2007; 130(4):663–677. [PubMed: 17719544]
62. Oh CS, Toke DA, Mandala S, Martin CE. ELO2 and ELO3, homologues of the *Saccharomyces cerevisiae* ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation. *J Biol Chem*. 1997; 272(28):17376–17384. [PubMed: 9211877]
63. Perry RJ, Ridgway ND. Molecular mechanisms and regulation of ceramide transport. *Biochim Biophys Acta*. 2005; 1734(3):220–234. [PubMed: 15907394]
64. Vallee B, Riezman H. Lip1p: a novel subunit of acyl-CoA ceramide synthase. *EMBO J*. 2005; 24(4):730–741. [PubMed: 15692566]
65. Holthuis JC, Pomorski T, Raggars RJ, Sprong H, Van Meer G. The organizing potential of sphingolipids in intracellular membrane transport. *Physiol Rev*. 2001; 81(4):1689–1723. [PubMed: 11581500]
66. Svennerholm L, Stallberg-Stenhagen S. Changes in the fatty acid composition of cerebrosides and sulfatides of human nervous tissue with age. *J Lipid Res*. 1968; 9(2):215–225. [PubMed: 5640502]
67. Hanada K, Kumagai K, Yasuda S, et al. Molecular machinery for non-vesicular trafficking of ceramide. *Nature*. 2003; 426(6968):803–809. [PubMed: 14685229]
68. Funakoshi T, Yasuda S, Fukasawa M, Nishijima M, Hanada K. Reconstitution of ATP- and cytosol-dependent transport of *de novo* synthesized ceramide to the site of sphingomyelin synthesis in semi-intact cells. *J Biol Chem*. 2000; 275(39):29938–29945. [PubMed: 10882735]
69. Maccioni HJ, Quiroga R, Ferrari ML. Cellular and molecular biology of glycosphingolipid glycosylation. *J Neurochem*. 2011; 117(4):589–602. [PubMed: 21371037]
70. Merrill AH Jr. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chem Rev*. 2011; 111(10):6387–6422. [PubMed: 21942574]
71. Nishie T, Hikimochi Y, Zama K, et al. Beta4-galactosyltransferase-5 is a lactosylceramide synthase essential for mouse extra-embryonic development. *Glycobiology*. 2010; 20(10):1311–1322. [PubMed: 20574042]
72. Nomura T, Takizawa M, Aoki J, et al. Purification, cDNA cloning, and expression of UDP-Gal: glucosylceramide beta-1,4-galactosyltransferase from rat brain. *J Biol Chem*. 1998; 273(22):13570–13577. [PubMed: 9593693]
73. Kumagai T, Sato T, Natsuka S, et al. Involvement of murine beta-1,4-galactosyltransferase V in lactosylceramide biosynthesis. *Glycoconj J*. 2010; 27(7–9):685–695. [PubMed: 21057870]
74. Nagata Y, Yamashiro S, Yodoi J, Lloyd KO, Shiku H, Furukawa K. Expression cloning of beta 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. *J Biol Chem*. 1992; 267(17):12082–12089. [PubMed: 1601877]
75. Hidari JK, Ichikawa S, Furukawa K, Yamasaki M, Hirabayashi Y. Beta 1–4N-acetylgalactosaminyltransferase can synthesize both asialoglycosphingolipid GM2 and glycosphingolipid GM2 *in vitro* and *in vivo*: isolation and characterization of a beta 1–4N-acetylgalactosaminyltransferase cDNA clone from rat ascites hepatoma cell line AH7974F. *Biochem J*. 1994; 303(Pt 3):957–965. [PubMed: 7980468]

76. Ishii A, Ohta M, Watanabe Y, et al. Expression cloning and functional characterization of human cDNA for ganglioside GM3 synthase. *J Biol Chem.* 1998; 273(48):31652–31655. [PubMed: 9822625]
77. Kojima Y, Fukumoto S, Furukawa K, et al. Molecular cloning of globotriaosylceramide/CD77 synthase, a glycosyltransferase that initiates the synthesis of globo series glycosphingolipids. *J Biol Chem.* 2000; 275(20):15152–15156. [PubMed: 10748143]
78. Biellmann F, Hulsmeier AJ, Zhou D, Cinelli P, Hennot T. The Lc3-synthase gene B3gnt5 is essential to pre-implantation development of the murine embryo. *BMC Dev Biol.* 2008; 8:109. [PubMed: 19014510]
79. Nagiec MM, Skrzypek M, Nagiec EE, Lester RL, Dickson RC. The LCB4 (YOR171c) and LCB5 (YLR260w) genes of *Saccharomyces* encode sphingoid long-chain base kinases. *J Biol Chem.* 1998; 273(31):19437–19442. [PubMed: 9677363]
80. Ferguson-Yankey SR, Skrzypek MS, Lester RL, Dickson RC. Mutant analysis reveals complex regulation of sphingolipid long-chain base phosphates and long-chain bases during heat stress in yeast. *Yeast.* 2002; 19(7):573–586. [PubMed: 11967828]
81. Chalfant CE, Spiegel S. Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling. *J Cell Sci.* 2005; 118(Pt 20):4605–4612. [PubMed: 16219683]
82. Mitsutake S, Igarashi Y. Calmodulin is involved in the Ca²⁺-dependent activation of ceramide kinase as a calcium sensor. *J Biol Chem.* 2005; 280(49):40436–40441. [PubMed: 16203736]
83. Samet D, Barenholz Y. Characterization of acidic and neutral sphingomyelinase activities in crude extracts of HL-60 cells. *Chem Phys Lipids.* 1999; 102(1–2):65–77. [PubMed: 11001562]
84. Saba JD, Nara F, Bielawska A, Garrett S, Hannun YA. The BST1 gene of *Saccharomyces cerevisiae* is the sphingosine-1-phosphate lyase. *J Biol Chem.* 1997; 272(42):26087–26090. [PubMed: 9334171]
85. Okada T, Kajimoto T, Jahangeer S, Nakamura S. Sphingosine kinase/sphingosine 1-phosphate signalling in central nervous system. *Cell Signal.* 2009; 21(1):7–13. [PubMed: 18694820]
86. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol.* 2008; 9(2):139–150. [PubMed: 18216770]
87. Shinghal R, Scheller RH, Bajjalieh SM. Ceramide 1-phosphate phosphatase activity in brain. *J Neurochem.* 1993; 61(6):2279–2285. [PubMed: 8245978]
88. Kajimoto T, Okada T, Yu H, Goparaju SK, Jahangeer S, Nakamura S. Involvement of sphingosine-1-phosphate in glutamate secretion in hippocampal neurons. *Mol Cell Biol.* 2007; 27(9):3429–3440. [PubMed: 17325039]
89. Van Brocklyn JR, Williams JB. The control of the balance between ceramide and sphingosine-1-phosphate by sphingosine kinase: oxidative stress and the seesaw of cell survival and death. *Comp Biochem Physiol B Biochem Mol Biol.* 2012; 163(1):26–36. [PubMed: 22613819]
90. Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL. Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol.* 2005; 25(24):11113–11121. [PubMed: 16314531]
91. Darios F, Wasser C, Shakirzyanova A, et al. Sphingosine facilitates SNARE complex assembly and activates synaptic vesicle exocytosis. *Neuron.* 2009; 62(5):683–694. [PubMed: 19524527]
92. Dickson RC, Nagiec EE, Skrzypek M, Tillman P, Wells GB, Lester RL. Sphingolipids are potential heat stress signals in *Saccharomyces*. *J Biol Chem.* 1997; 272(48):30196–30200. [PubMed: 9374502]
93. Jenkins GM, Richards A, Wahl T, Mao C, Obeid L, Hannun Y. Involvement of yeast sphingolipids in the heat stress response of *Saccharomyces cerevisiae*. *J Biol Chem.* 1997; 272(51):32566–32572. [PubMed: 9405471]
94. Schmelzle T, Helliwell SB, Hall MN. Yeast protein kinases and the RHO1 exchange factor TUS1 are novel components of the cell integrity pathway in yeast. *Mol Cell Biol.* 2002; 22(5):1329–1339. [PubMed: 11839800]
95. Dehart AK, Schnell JD, Allen DA, Hicke L. The conserved Pkh-Ypk kinase cascade is required for endocytosis in yeast. *J Cell Biol.* 2002; 156(2):241–248. [PubMed: 11807089]

96. Chung N, Mao C, Heitman J, Hannun YA, Obeid LM. Phytosphingosine as a specific inhibitor of growth and nutrient import in *Saccharomyces cerevisiae*. *J Biol Chem*. 2001; 276(38):35614–35621. [PubMed: 11468289]
97. Chung N, Jenkins G, Hannun YA, Heitman J, Obeid LM. Sphingolipids signal heat stress-induced ubiquitin-dependent proteolysis. *J Biol Chem*. 2000; 275(23):17229–17232. [PubMed: 10764732]
98. Cuvillier O, Pirianov G, Kleuser B, et al. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature*. 1996; 381(6585):800–803. [PubMed: 8657285]
99. Harada J, Foley M, Moskowitz MA, Waeber C. Sphingosine-1-phosphate induces proliferation and morphological changes of neural progenitor cells. *J Neurochem*. 2004; 88(4):1026–1039. [PubMed: 14756825]
100. Moore AN, Kampfl AW, Zhao X, Hayes RL, Dash PK. Sphingosine-1-phosphate induces apoptosis of cultured hippocampal neurons that requires protein phosphatases and activator protein-1 complexes. *Neuroscience*. 1999; 94(2):405–415. [PubMed: 10579204]
101. Obeid LM, Linardic CM, Karolak LA, Hannun YA. Programmed cell death induced by ceramide. *Science*. 1993; 259(5102):1769–1771. [PubMed: 8456305]
102. Santana P, Llanes L, Hernandez I, et al. Interleukin-1 beta stimulates sphingomyelin hydrolysis in cultured granulosa cells: evidence for a regulatory role of ceramide on progesterone and prostaglandin biosynthesis. *Endocrinology*. 1996; 137(6):2480–2489. [PubMed: 8641202]
103. Van Echten-Deckert G, Herget T. Sphingolipid metabolism in neural cells. *Biochim Biophys Acta*. 2006; 1758(12):1978–1994. [PubMed: 16843432]
104. Schorling S, Vallee B, Barz WP, Riezman H, Oesterhelt D. Lag1p and Lac1p are essential for the Acyl-CoA-dependent ceramide synthase reaction in *Saccharomyces cerevisiae*. *Mol Biol Cell*. 2001; 12(11):3417–3427. [PubMed: 11694577]
105. Pewzner-Jung Y, Ben-Dor S, Futerman AH. When do Lasses (longevity assurance genes) become CerS (ceramide synthases)? Insights into the regulation of ceramide synthesis. *J Biol Chem*. 2006; 281(35):25001–25005. [PubMed: 16793762]
106. Blasco MA, Funk W, Villeponteau B, Greider CW. Functional characterization and developmental regulation of mouse telomerase RNA. *Science*. 1995; 269(5228):1267–1270. [PubMed: 7544492]
107. 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. [PubMed: 22729185]
108. Mora A, Sabio G, Risco AM, et al. Lithium blocks the PKB and GSK3 dephosphorylation induced by ceramide through protein phosphatase-2A. *Cell Signal*. 2002; 14(6):557–562. [PubMed: 11897496]
109. Qin J, Berdyshev E, Poirer C, Schwartz NB, Dawson G. Neutral sphingomyelinase 2 deficiency increases hyaluronan synthesis by up-regulation of hyaluronan synthase 2 through decreased ceramide production and activation of Akt. *J Biol Chem*. 2012; 287(17):13620–13632. [PubMed: 22383528]
110. Wolff RA, Dobrowsky RT, Bielawska A, Obeid LM, Hannun YA. Role of ceramide-activated protein phosphatase in ceramide-mediated signal transduction. *J Biol Chem*. 1994; 269(30):19605–19609. [PubMed: 8034729]
111. Gulbins E, Palmada M, Reichel M, et al. Acid sphingomyelinase–ceramide system mediates effects of antidepressant drugs. *Nat Med*. 2013; 19(7):934–938. [PubMed: 23770692]
112. Zhang Y, Yao B, Delikat S, et al. Kinase suppressor of Ras is ceramide-activated protein kinase. *Cell*. 1997; 89(1):63–72. [PubMed: 9094715]
113. Basu S, Bayoumy S, Zhang Y, Lozano J, Kolesnick R. BAD enables ceramide to signal apoptosis via Ras and Raf-1. *J Biol Chem*. 1998; 273(46):30419–30426. [PubMed: 9804808]
114. Westwick JK, Bielawska AE, Dbaibo G, Hannun YA, Brenner DA. Ceramide activates the stress-activated protein kinases. *J Biol Chem*. 1995; 270(39):22689–22692. [PubMed: 7559390]
115. Verheij M, Bose R, Lin XH, et al. Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. *Nature*. 1996; 380(6569):75–79. [PubMed: 8598911]
116. Nica AF, Tsao CC, Watt JC, et al. Ceramide promotes apoptosis in chronic myelogenous leukemia-derived K562 cells by a mechanism involving caspase-8 and JNK. *Cell Cycle*. 2008; 7(21):3362–3370. [PubMed: 18948750]

117. Bogoyevitch MA, Kobe B. Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. *Microbiol Mol Biol Rev.* 2006; 70(4):1061–1095. [PubMed: 17158707]
118. Muller G, Ayoub M, Storz P, Rennecke J, Fabbro D, Pfizenmaier K. PKC zeta is a molecular switch in signal transduction of TNF-alpha, bifunctionally regulated by ceramide and arachidonic acid. *EMBO J.* 1995; 14(9):1961–1969. [PubMed: 7744003]
119. Gomez-Munoz A, Kong JY, Salh B, Steinbrecher UP. Ceramide-1-phosphate blocks apoptosis through inhibition of acid sphingomyelinase in macrophages. *J Lipid Res.* 2004; 45(1):99–105. [PubMed: 14523050]
120. Granado MH, Gangoiti P, Ouro A, Arana L, Gomez-Munoz A. Ceramide 1-phosphate inhibits serine palmitoyltransferase and blocks apoptosis in alveolar macrophages. *Biochim Biophys Acta.* 2009; 1791(4):263–272. [PubMed: 19416641]
121. Belmaker RH, Agam G. Major depressive disorder. *N Engl J Med.* 2008; 358(1):55–68. [PubMed: 18172175]
122. Santarelli L, Saxe M, Gross C, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science.* 2003; 301(5634):805–809. [PubMed: 12907793]
123. David DJ, Samuels BA, Rainer Q, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron.* 2009; 62(4):479–493. [PubMed: 19477151]
124. Demirkan A, Isaacs A, Ugocsai P, et al. Plasma phosphatidylcholine and sphingomyelin concentrations are associated with depression and anxiety symptoms in a Dutch family-based lipidomics study. *J Psychiatr Res.* 2013; 47(3):357–362. [PubMed: 23207112]
125. Gulbins E, Kolesnick R. Raft ceramide in molecular medicine. *Oncogene.* 2003; 22(45):7070–7077. [PubMed: 14557812]
126. Perrotta C, Bizzozero L, Cazzato D, et al. Syntaxin 4 is required for acid sphingomyelinase activity and apoptotic function. *J Biol Chem.* 2010; 285(51):40240–40251. [PubMed: 20956541]
127. Gracia-Garcia P, Rao V, Haughey NJ, et al. Elevated plasma ceramides in depression. *J Neuropsychiatry Clin Neurosci.* 2011; 23(2):215–218. [PubMed: 21677254]
128. Noponen M, Sanfilippo M, Samanich K, et al. Elevated PLA2 activity in schizophrenics and other psychiatric patients. *Biol Psychiatry.* 1993; 34(9):641–649. [PubMed: 8292693]
129. Schulze H, Kolter T, Sandhoff K. Principles of lysosomal membrane degradation: cellular topology and biochemistry of lysosomal lipid degradation. *Biochim Biophys Acta.* 2009; 1793(4):674–683. [PubMed: 19014978]
130. Kolzer M, Werth N, Sandhoff K. Interactions of acid sphingomyelinase and lipid bilayers in the presence of the tricyclic antidepressant desipramine. *FEBS Lett.* 2004; 559(1–3):96–98. [PubMed: 14960314]
131. De Duve C, De Barsey T, Poole B, Trouet A, Tulkens P, Van Hoof F. Commentary. Lysosomotropic agents. *Biochem Pharmacol.* 1974; 23(18):2495–2531. [PubMed: 4606365]
132. Trapp S, Rosania GR, Horobin RW, Kornhuber J. Quantitative modeling of selective lysosomal targeting for drug design. *Eur Biophys J.* 2008; 37(8):1317–1328. [PubMed: 18504571]
133. Hurwitz R, Ferlinz K, Sandhoff K. The tricyclic antidepressant desipramine causes proteolytic degradation of lysosomal sphingomyelinase in human fibroblasts. *Biol Chem Hoppe Seyler.* 1994; 375(7):447–450. [PubMed: 7945993]
134. Baxter AJ, Vos T, Scott KM, Ferrari AJ, Whiteford HA. The global burden of anxiety disorders in 2010. *Psychol Med.* 2014; 44(11):2363–2374.
135. Jang S, Kim D, Lee Y, Moon S, Oh S. Modulation of sphingosine 1-phosphate and tyrosine hydroxylase in the stress-induced anxiety. *Neurochem Res.* 2011; 36(2):258–267. [PubMed: 21076868]
136. Jang S, Suh SH, Yoo HS, Lee YM, Oh S. Changes in iNOS, GFAP and NR1 expression in various brain regions and elevation of sphingosine-1-phosphate in serum after immobilized stress. *Neurochem Res.* 2008; 33(5):842–851. Describes the increased expression of neurodegeneration markers and sphingosine-1-phosphate associated with stress. [PubMed: 17940874]

137. Ono M, Kikusui T, Sasaki N, Ichikawa M, Mori Y, Murakami-Murofushi K. Early weaning induces anxiety and precocious myelination in the anterior part of the basolateral amygdala of male Balb/c mice. *Neuroscience*. 2008; 156(4):1103–1110. [PubMed: 18790016]
138. Norton WT. Recent advances in myelin biochemistry. *Ann NY Acad Sci*. 1984; 436:5–10. [PubMed: 6398023]
139. Akiyama K, Ichinose S, Omori A, Sakurai Y, Asou H. Study of expression of myelin basic proteins (MBPs) in developing rat brain using a novel antibody reacting with four major isoforms of MBP. *J Neurosci Res*. 2002; 68(1):19–28. [PubMed: 11933045]
140. Ledoux JE. Emotional memory systems in the brain. *Behav Brain Res*. 1993; 58(1–2):69–79. [PubMed: 8136051]
141. Schafe GE, Nader K, Blair HT, Ledoux JE. Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. *Trends Neurosci*. 2001; 24(9):540–546. [PubMed: 11506888]
142. Spiga F, Lightman SL, Shekhar A, Lowry CA. Injections of urocortin 1 into the basolateral amygdala induce anxiety-like behavior and c-Fos expression in brainstem serotonergic neurons. *Neuroscience*. 2006; 138(4):1265–1276. [PubMed: 16488545]
143. Ariga T, Suetake K, Nakane M, et al. Glycosphingolipid antigens in neural tumor cell lines and anti-glycosphingolipid antibodies in sera of patients with neural tumors. *Neurosignals*. 2008; 16(2–3):226–234. [PubMed: 18253060]
144. Wallis CJ, Rezazadeh SM, Lal H. GM1 ganglioside reduces ethanol intoxication and the development of ethanol dependence. *Alcohol*. 1995; 12(6):573–580. [PubMed: 8590622]
145. Reeves TM, Smith DC. Reinnervation of the dentate gyrus and recovery of alternation behavior following entorhinal cortex lesions. *Behav Neurosci*. 1987; 101(2):179–186. [PubMed: 3580120]
146. Loesche J, Steward O. Behavioral correlates of denervation and reinnervation of the hippocampal formation of the rat: recovery of alternation performance following unilateral entorhinal cortex lesions. *Brain Res Bull*. 1977; 2(1):31–39. [PubMed: 861769]
147. Glasier MM, Sutton RL, Stein DG. Effects of unilateral entorhinal cortex lesion and ganglioside GM1 treatment on performance in a novel water maze task. *Neurobiol Learn Mem*. 1995; 64(3):203–214. [PubMed: 8564374]
148. Gilmore N, Cheria L, Klemm WR. Ganglioside or sialic acid attenuates ethanol-induced decrements in locomotion, nose-poke exploration, and anxiety, but not body temperature. *Prog Neuropsychopharmacol Biol Psychiatry*. 1991; 15(1):91–104. [PubMed: 1672567]
149. Fitzsimmons J, Kubicki M, Shenton ME. Review of functional and anatomical brain connectivity findings in schizophrenia. *Curr Opin Psychiatry*. 2013; 26(2):172–187. [PubMed: 23324948]
150. Liddle PF. Schizophrenic syndromes, cognitive performance and neurological dysfunction. *Psychol Med*. 1987; 17(1):49–57. [PubMed: 3575577]
151. Capuano B, Crosby IT, Lloyd EJ. Schizophrenia: genesis, receptorology and current therapeutics. *Curr Med Chem*. 2002; 9(5):521–548. [PubMed: 11945123]
152. Merikangas KR, Kalaydjian A. Magnitude and impact of comorbidity of mental disorders from epidemiologic surveys. *Curr Opin Psychiatry*. 2007; 20(4):353–358. [PubMed: 17551350]
153. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*. 2005; 6(4):312–324. [PubMed: 15803162]
154. Carlsson A, Carlsson ML. A dopaminergic deficit hypothesis of schizophrenia: the path to discovery. *Dialogues Clin Neurosci*. 2006; 8(1):137–142. [PubMed: 16640125]
155. Kim JS, Kornhuber HH, Holzmüller B, Schmid-Burgk W, Mergner T, Krzepinski G. Reduction of cerebrospinal fluid glutamic acid in Huntington's chorea and in schizophrenic patients. *Arch Psychiatr Nervenkr*. 1980; 228(1):7–10. [PubMed: 6104477]
156. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry*. 1995; 52(12):998–1007. [PubMed: 7492260]
157. Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry*. 1987; 44(7):660–669. [PubMed: 3606332]
158. Andreasen N, Nasrallah HA, Dunn V, et al. Structural abnormalities in the frontal system in schizophrenia. A magnetic resonance imaging study. *Arch Gen Psychiatry*. 1986; 43(2):136–144. [PubMed: 3947208]

159. Ingvar DH, Franzen G. Abnormalities of cerebral blood flow distribution in patients with chronic schizophrenia. *Acta Psychiatr Scand.* 1974; 50(4):425–462. [PubMed: 4423855]
160. Lin A, Kenis G, Bignotti S, et al. The inflammatory response system in treatment-resistant schizophrenia: increased serum interleukin-6. *Schizophr Res.* 1998; 32(1):9–15. [PubMed: 9690329]
161. Rothermundt M, Missler U, Arolt V, et al. Increased S100B blood levels in unmedicated and treated schizophrenic patients are correlated with negative symptomatology. *Mol Psychiatry.* 2001; 6(4):445–449. [PubMed: 11443531]
162. Boggs JM, Gao W, Zhao J, Park HJ, Liu Y, Basu A. Participation of galactosylceramide and sulfatide in glycosynapses between oligodendrocyte or myelin membranes. *FEBS Lett.* 2010; 584(9):1771–1778. [PubMed: 19941861]
163. Boggs JM, Gao W, Hirahara Y. Signal transduction pathways involved in interaction of galactosylceramide/sulfatide-containing liposomes with cultured oligodendrocytes and requirement for myelin basic protein and glycosphingolipids. *J Neurosci Res.* 2008; 86(7):1448–1458. [PubMed: 18189317]
164. Cherayil GD. Estimation of glycolipids in four selected lobes of human brain in neurological diseases. *J Neurochem.* 1969; 16(3):913–920. [PubMed: 5822632]
165. Schmitt A, Wilczek K, Blennow K, et al. Altered thalamic membrane phospholipids in schizophrenia: a postmortem study. *Biol Psychiatry.* 2004; 56(1):41–45. [PubMed: 15219471]
166. Haselhorst U, Schenk H, Beyer I, Uebelhack R, Franke E, Kielstein V. Abnormality of gangliosides in erythrocyte membranes of schizophrenic patients. *Clin Physiol Biochem.* 1988; 6(5):281–284. [PubMed: 3229070]
167. Davis KL, Stewart DG, Friedman JI, et al. White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry.* 2003; 60(5):443–456. [PubMed: 12742865]
168. Kubicki M, McCarley RW, Shenton ME. Evidence for white matter abnormalities in schizophrenia. *Curr Opin Psychiatry.* 2005; 18(2):121–134. [PubMed: 16639164]
169. Narayan S, Kass KE, Thomas EA. Chronic haloperidol treatment results in a decrease in the expression of myelin/oligodendrocyte-related genes in the mouse brain. *J Neurosci Res.* 2007; 85(4):757–765. [PubMed: 17177202]
170. Smesny S, Schmelzer CE, Hinder A, et al. Skin ceramide alterations in first-episode schizophrenia indicate abnormal sphingolipid metabolism. *Schizophr Bull.* 2013; 39(4):933–941. [PubMed: 22589371]
171. Lewis CM, Levinson DF, Wise LH, et al. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II. Schizophrenia. *Am J Hum Genet.* 2003; 73(1):34–48. [PubMed: 12802786]
172. Zhang H, Li D, Su Y, et al. Identification of the N-acylsphingosine amidohydrolase 1 gene (ASAH1) for susceptibility to schizophrenia in a Han Chinese population. *World J Biol Psychiatry.* 2012; 13(2):106–113. [PubMed: 21375364]
173. Weissman MM, Warner V, John K, et al. Delusional depression and bipolar spectrum: evidence for a possible association from a family study of children. *Neuropsychopharmacology.* 1988; 1(4):257–264. [PubMed: 3251505]
174. 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):25847–25850. [PubMed: 12011103]
175. Modrak DE, Gold DV, Goldenberg DM. Sphingolipid targets in cancer therapy. *Mol Cancer Ther.* 2006; 5(2):200–208. [PubMed: 16505092]
176. Levy M, Futerman AH. Mammalian ceramide synthases. *IUBMB Life.* 2010; 62(5):347–356. [PubMed: 20222015]
177. Ebel P, Vom Dorp K, Petrasch-Parwez E, et al. Inactivation of ceramide synthase 6 in mice results in an altered sphingolipid metabolism and behavioral abnormalities. *J Biol Chem.* 2013; 288(29):21433–21447. [PubMed: 23760501]
178. Imgrund S, Hartmann D, Farwanah H, et al. Adult ceramide synthase 2 (CERS2)-deficient mice exhibit myelin sheath defects, cerebellar degeneration, and hepatocarcinomas. *J Biol Chem.* 2009; 284(48):33549–33560. Analysis of the biological effects of *CERS2* knockout in mice

- suggesting that *CESR2* activity is required for the maintenance of myelin, stabilization of cerebellar and renal histology and protection against hepatocarcinomas. [PubMed: 19801672]
179. Guillas I, Jiang JC, Vionnet C, et al. Human homologues of LAG1 reconstitute acyl-CoA-dependent ceramide synthesis in yeast. *J Biol Chem*. 2003; 278(39):37083–37091. [PubMed: 12869556]
180. Cohen JA, Barkhof F, Comi G, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med*. 2010; 362(5):402–415. [PubMed: 20089954]
181. Pan S, Gray NS, Gao W, et al. Discovery of BAF312 (siponimod), a potent and selective S1P receptor modulator. *ACS Med Chem Lett*. 2013; 4(3):333–337. [PubMed: 24900670]
182. Daquinag A, Fadri M, Jung SY, Qin J, Kunz J. The yeast PH domain proteins Slm1 and Slm2 are targets of sphingolipid signaling during the response to heat stress. *Mol Cell Biol*. 2007; 27(2): 633–650. [PubMed: 17101780]
183. Huang X, Withers BR, Dickson RC. Sphingolipids and lifespan regulation. *Biochim Biophys Acta*. 2014; 1841(5):657–664. [PubMed: 23954556]
184. Alvarez-Vasquez F, Sims KJ, Cowart LA, Okamoto Y, Voit EO, Hannun YA. Simulation and validation of modelled sphingolipid metabolism in *Saccharomyces cerevisiae*. *Nature*. 2005; 433(7024):425–430. Describes mathematical modeling in relation to sphingolipid metabolism in yeast. [PubMed: 15674294]
185. Alvarez-Vasquez F, Sims KJ, Hannun YA, Voit EO. Integration of kinetic information on yeast sphingolipid metabolism in dynamical pathway models. *J Theor Biol*. 2004; 226(3):265–291. [PubMed: 14643642]
186. Alvarez-Vasquez F, Sims KJ, Voit EO, Hannun YA. Coordination of the dynamics of yeast sphingolipid metabolism during the diauxic shift. *Theor Biol Med Model*. 2007; 4:42. [PubMed: 17974024]
187. Alvarez-Vasquez F, Riezman H, Hannun YA, Voit EO. Mathematical modeling and validation of the ergosterol pathway in *Saccharomyces cerevisiae*. *PLoS ONE*. 2011; 6(12):e28344. [PubMed: 22194828]
188. Kemmer D, McHardy LM, Hoon S, et al. Combining chemical genomics screens in yeast to reveal spectrum of effects of chemical inhibition of sphingolipid biosynthesis. *BMC Microbiol*. 2009; 9:9. [PubMed: 19144191]
189. Ge H, Liu Z, Church GM, Vidal M. Correlation between transcriptome and interactome mapping data from *Saccharomyces cerevisiae*. *Nat Genet*. 2001; 29(4):482–486. [PubMed: 11694880]
190. Humston EM, Dombek KM, Tu BP, Young ET, Synovec RE. Toward a global analysis of metabolites in regulatory mutants of yeast. *Anal Bioanal Chem*. 2011; 401(8):2387–2402. [PubMed: 21416166]
191. Ptacek J, Devgan G, Michaud G, et al. Global analysis of protein phosphorylation in yeast. *Nature*. 2005; 438(7068):679–684. [PubMed: 16319894]
192. Schwikowski B, Uetz P, Fields S. A network of protein–protein interactions in yeast. *Nat Biotechnol*. 2000; 18(12):1257–1261. [PubMed: 11101803]
193. Velculescu VE, Zhang L, Zhou W, et al. Characterization of the yeast transcriptome. *Cell*. 1997; 88(2):243–251. [PubMed: 9008165]
194. Blanksby SJ, Mitchell TW. Advances in mass spectrometry for lipidomics. *Annu Rev Anal Chem (Palo Alto Calif)*. 2010; 3:433–465. Excellent review of the advances in the field of lipidomics techniques that enable tracking of changes in the levels of lipids found in very low abundance. [PubMed: 20636050]
195. Han X. Potential mechanisms contributing to sulfatide depletion at the earliest clinically recognizable stage of Alzheimer's disease: a tale of shotgun lipidomics. *J Neurochem*. 2007; 103(Suppl. 1):171–179. [PubMed: 17986152]

Executive Summary

Epidemiology

- Psychiatric disorders are severe debilitating illnesses that contribute to approximately 14% of the global burden of disease.

Role of sphingolipids in psychiatric disorders

- Many studies associate aberrant sphingolipid metabolism with the pathophysiology of psychiatric illnesses. However, their role in the etiology of these disorders has not been established.

Sphingolipid structure

- Sphingolipids contain two hydrophobic tails, including a long-chain base (LCB) and a fatty acid chain, linked to a hydrophilic polar head group. The fatty acid is linked to the C-2 of the LCB via an amide bond, and the polar head group is linked to the C-1 of the LCB via an OH group.

Sphingolipid synthesis in yeast & mammals

- Synthesis of ceramide is conserved from yeast to mammalian cells. Sphingolipid synthesis begins in the endoplasmic reticulum with the condensation of serine- and palmitoyl-CoA in order to produce a LCB. LCB is then linked to a fatty acid in order to form ceramide, which is transported to the Golgi. In the Golgi, ceramide is modified via the attachment of sugars in order to form complex sphingolipids, which vary in yeast and mammals.

Importance of sphingolipid metabolism in depression, anxiety, schizophrenia & bipolar disorder

- Sphingolipids affect essential cellular processes that are crucial for neurogenesis, neuronal cell death and neurotransmission.
- In particular, sphingosine is required for the activation of exocytosis of the synaptic vesicles, while ceramide, sphingosine-1-phosphate and ceramide-1-phosphate regulate apoptosis, neurogenesis and neurotransmitter release.
- Altered levels of ceramide are implicated in depression and bipolar disorder.
- Perturbation of sphingosine-1-phosphate and GM1 have been described in anxiety.
- Schizophrenia is associated with altered levels of ceramide and complex sphingolipids.
- A causal role for these in the etiology of psychiatric disorders has not been established.

The yeast model for elucidating sphingolipid function

- The conservation of the synthesis and function of sphingolipids has been demonstrated between yeast and human cells.

- The yeast model offers powerful advantages that can be exploited in order to elucidate the role of sphingolipids in cell functions.
- Well-characterized yeast mutants can be exploited in *in vivo* studies of sphingolipid function and in studies of the global effects of targeted sphingolipid perturbations. These mutants can be exploited in bioassays for drugs that target specific sphingolipid components. Genetic and metabolic databases can be utilized for *in vivo* and *in silico* experiments in order to examine the effects of sphingolipid perturbation on cell function.
- Testable hypotheses derived from experiments in yeast can be experimentally addressed in appropriate mammalian systems.
- This knowledge has the potential to contribute to our understanding of the mechanisms underlying the pathophysiology of psychiatric disorders and to the development of new drugs that can be used in their treatment.

Figure 1. Sphingolipid structure

The sphingolipid backbone consists of a long-chain base linked to a fatty acid chain via an amide link. A polar head group R is linked to the OH of C-1 of the long-chain base via a OH bond. R = H in a ceramide molecule; R = phosphocholine in sphingomyelin; R = sugar in glycosphingolipids. \\ represents the number of carbons in the fatty acid attached.

Figure 2. Synthesis and metabolism of sphingolipids in yeast and mammals

Common synthesis pathway: sphingolipid synthesis begins in the endoplasmic reticulum in both yeast and mammals. Acyl-CoA is condensed with serine to form 3-ketohydrosphingosine, which is further reduced to DHS. C-26 fatty acid is transferred to DHS in order to yield dihydroceramide. In yeast, DHS is hydroxylated to PHS, which is attached to a C-26 fatty acid in order to synthesize phytoceramide. Dihydroceramide can also be hydroxylated to phytoceramide. Phosphorylinositol is transferred to phytoceramide in order to produce IPC, which is mannosylated to MIPC. Another phosphorylinositol group is added to MIPC in order to yield $M(IP)_2C$. In mammals, DHS is N-acetylated to dihydroceramide, which is converted to ceramide. Different polar groups are added to the ceramide backbone in order to synthesize complex sphingolipids, including phosphorylcholine for producing sphingomyelin and galactose and glucose for synthesizing galactosylceramide and glucosylceramide, respectively. Glucosylceramide and galactosylceramide can be modified by the addition of sugars such as fucose, galactose, sialic acid, N-acetylglucosamine and N-acetylgalactosamine in order to produce several glycosphingolipids. Common cleavage pathway: complex sphingolipids are catabolized to ceramide, which is cleaved to LCBs. LCBs are phosphorylated in order to form long-chain base phosphates, which are dephosphorylated to LCBs or further cleaved to fatty aldehyde and phosphatidylethanolamine. In mammals, ceramide is phosphorylated to C1P. In yeast, Rsb1 transports sphingosine bases from the cytoplasmic side to the extracytoplasmic side of the membrane.

C1P: Ceramide-1-phosphate; CoA: Coenzyme A; DHS: Dihydrosphingosine; IPC: Inositol phosphorylceramide; LCB: Long-chain base; $M(IP)_2C$: Mannosyl(inositolphosphoryl)₂ ceramide; MIPC: Mannoyl-inositol phosphorylceramide; PHS: Phytosphingosine.

Figure 3. Fatty acid elongation pathway

Step 1: malonyl-CoA is condensed with acyl-CoA in order to synthesize 3-ketoacyl-CoA.

Step 2: 3-ketoacyl-CoA is reduced by 3-ketoacyl-CoA reductase to 3-hydroxyacyl-CoA.

Step 3: 3-hydroxyacyl-CoA is dehydrated to 2,3-*trans*-enoyl-CoA by 3-hydroxyacyl-CoA

dehydratase. Step 4: 3-hydroxyacyl-CoA undergoes a round of reduction catalyzed by enoyl reductase in order to yield an acyl-CoA with two more carbon chains than the original acyl-CoA.

CoA: Coenzyme A; NADPH: Nicotinamide adenine dinucleotide phosphate.

Table 1
Yeast and mammalian genes involved in sphingolipid metabolism

Enzymes	Genes		Ref.
	Yeast	Mammals	
Serine palmitoyl transferase	<i>LCB1, LCB2, LCB3</i>	<i>SPTLC1, SPTLC2, SPTLC3</i>	[21–23]
3-ketodihydrosphingosine reductase	<i>TSC10</i>	<i>FVT-1</i>	[24–26]
Sphinganine C-4-hydroxylase	<i>SUR2</i>	–	[24]
Ceramide synthase	<i>LAG1, LAC1, LIP1</i>	<i>CERS1–6</i>	[27,28]
Dihydroceramide desaturase	–	<i>DES1, DES2</i>	[29–31]
Fatty acid elongases	<i>ELO1, ELO2/FEN1, ELO3, SUR4</i>	<i>ELOVL1–7</i>	[32–34]
3-ketoacyl-CoA reductase	<i>Ybr159w, AYR1</i>	<i>KAR</i>	[34]
3-hydroxyacyl-CoA dehydratase	<i>PHS1</i>	<i>HACD1–4</i>	[34]
Enoyl reductase	<i>TSC13</i>	<i>TER</i>	[34]
IPC synthase	<i>AUR1</i>	–	[11,13,24]
Sphingolipid α -hydroxylase	<i>SCS7</i>	–	[11,13,24]
MIPC synthase	<i>SUR1, CSH1, CSG2</i>	–	[13,24]
Inositol phosphotransferase	<i>IPT1</i>	–	[13,24]
Sphingomyelin synthase	–	<i>SMS1, SMS2</i>	[35]
Glucosylceramide synthase	–	<i>GlcT-1</i>	[36]
Galactosyltransferase	–	<i>CGT</i>	[36]
Long-chain base kinase	<i>LCB4, LCB5</i>	<i>SPHK1, SPKH2</i>	[15]
Ceramide kinase	–	<i>CERK</i>	[37]
Sphingomyelinase	<i>ISC1</i>	aSMase, sSMase; <i>ASM/SMPD1</i> , alk-SMase; <i>NPP7</i> , Mg^{2+} -dependent nSMase; <i>SMPD3, SMPD4, SMPD5</i> , Mg^{2+} -independent nSMase; not known	[38–43]
Ceramidase	<i>YDC1, YPC1</i>	Acidic: <i>ASAH1/AC</i> ; neutral: <i>ASAH2/NCDase</i> ; alkaline: <i>PHC, CER1/AAH3</i>	[44–50]
Sphingolipid-1-phosphate lyase	<i>DPL1</i>	<i>SPL</i>	[15,51]
Long-chain base phosphatase	<i>LCB3, YSR3</i>	<i>SPP1, SPP2, LPP1–3</i>	[15,52]
Sphingolipid transporter	<i>RSB1</i>	–	[53]
UDP glycosyltransferase 8	–	<i>UGT8</i>	[54]
Sphingosine-1-phosphate phosphatase 1	–	<i>SGPP1</i>	[55]
Galactosylceramidase	–	<i>GALC</i>	[56]
Galactose-3-O-sulfotransferase 1	–	<i>GAL3ST1</i>	[57]

alk-SMase: Alkaline sphingomyelinase; aSMase: Acid sphingomyelinase; CoA: Coenzyme A; IPC: Inositol phosphorylceramide; MIPC: Mannoyl-inositol phosphorylceramide; nSMase: Neutral sphingomyelinase; sSMase: Secretory sphingomyelinase; UDP: Uridine diphosphate.