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Review

Sphingomyelin Synthases in Neuropsychiatric Health and Disease

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Key Words
Sphingomyelin synthase • Neurological disease • Psychiatric disease • Brain • Central nervous system

Abstract
Sphingomyelin synthases (SMS) catalyze the conversion of ceramide and phosphatidylcholine to sphingomyelin and diacylglycerol and are thus crucial for the balance between synthesis and degradation of these structural and bioactive molecules. SMS thereby play an essential role in sphingolipid metabolism, cell signaling, proliferation and differentiation processes. Although tremendous progress has been made toward understanding the involvement of SMS in physiological and pathological processes, literature in the area of neuropsychiatry is still limited. In this review, we summarize the main features of SMS as well as the current methodologies and tools used for their study and provide an overview of SMS in the central nervous system and their implications in neurological as well as psychiatric disorders. This way, we aim at establishing a basis for future mechanistic as well as clinical investigations on SMS in neuropsychiatric health and diseases.

Introduction

Sphingolipids, named after the mythological sphinx, remain enigmatic because of their unprecedented complexity, with thousands of molecular species and functions both as membrane components and bioactive molecules. Based on exciting recent advances in techniques, there has been remarkable progress made in understanding the regulation of sphingolipids by a network of enzymes. Relative to a multitude of publications addressing sphingomyelinase, reports on sphingomyelin synthase (SMS) are fewer (by an order of magnitude) but slowly growing (mainly in the area of human malignancies). Implications of the regulation of SMS in somatic clinical disorders have been well summarized [1]. Therefore, in this review, we have focused on the portion of the SMS literature addressing the central nervous system as well as neurological and psychiatric health and diseases.

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Sphingomyelin synthase family

SMS activity and family members

Sphingomyelin (SM) is the most abundant eukaryotic sphingolipid and an essential component of cellular membranes. SM is present in a wide variety of organisms, from mammals to protozoa [2] with ubiquitous distribution within mammalian tissues, and particularly high levels in the central nervous system. The bulk of SM is produced in the Golgi lumen and is delivered by vesicular transport to the plasma membrane [3], where it accumulates in the outer leaflet, and it is an important constituent of lipid rafts [4, 5]. SM takes part in a multitude of cellular processes owing to its special ability to form extensive hydrogen bonds with other membrane molecules. Ceramide, the sphingolipid precursor of sphingomyelin, is a well-known lipid mediator that induces cell death, differentiation, senescence, autophagy, and migration [6]. For these reasons, the regulation of SM synthesis plays an important role in signal transduction and membrane trafficking, and although this topic has recently gained much research interest, many details remain cryptic [7, 8].

The most important cellular pathway of SM formation is *de novo* synthesis (Fig. 1). The last step of this multi-stage process is mediated by a phosphatidylcholine/ceramide cholinephosphotransferase known as SM synthase (SMS, EC 2.7.8.27) by transferring the phosphocholine from phosphatidylcholine onto the primary hydroxyl group of ceramide, generating SM and diacylglycerol (DAG) [9] [10]. However, the biological importance of SMS resides not only in the biosynthesis of SM but also in the regulation of the levels of ceramide and DAG as important bioactive lipids. There is also some indication of a reverse sphingomyelin-synthase activity, where sphingomyelin can be used as a source of phosphorylcholine for phosphatidylcholine synthesis [11]. Interestingly, while for the enzyme ceramide synthase, each of the six isoforms exhibits a restricted substrate specificity towards the chain length of the fatty acids to be linked to sphingosine, such selectivity has not been found for SMS [12].

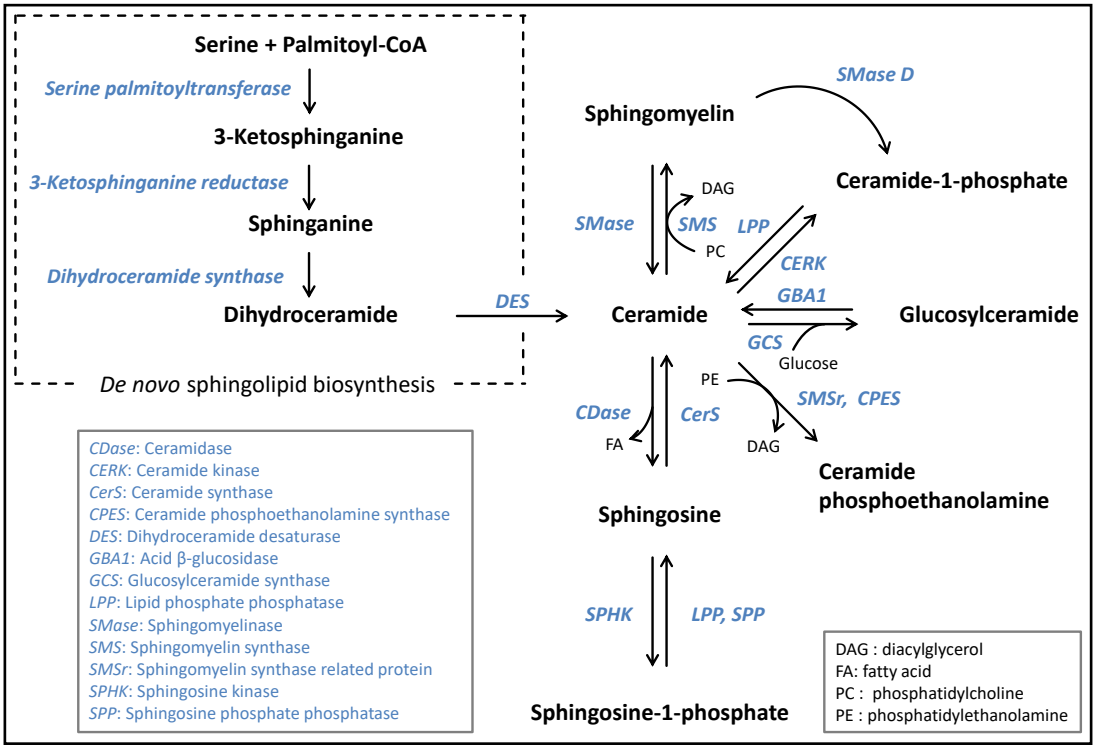

Fig. 1. Biochemical pathways of sphingolipids and catalyzing enzymes (in Italics).

The reverse reaction to SMS – the hydrolysis of SM to ceramide and phosphorylcholine – is catalyzed by sphingomyelinases which have been implicated in the onset and progression of various pathologies including neuropsychiatric diseases [13, 14]. Sphingomyelinases (SMases) are classified according to the pH optima of their activity: alkaline, neutral (NSM), and acid (ASM) sphingomyelinase [15]. ASMs can be further subdivided into lysosomal or secretory forms with distinctive characteristics [16]. Presently, four mammalian isoforms encoded by distinct genes are known for NSM [17, 18]. Finally, alkaline sphingomyelinase is secreted in the gut and is thought to break down dietary sphingomyelin [19]. By controlling the levels of SM and ceramide species, SMS and sphingomyelinase enzymes maintain cellular homeostasis [20].

There are three genes within the SMS family, *SGMS1* (sphingomyelin synthase 1), *SGMS2*, and *SAMD8* (sterile alpha motif domain containing 8), encoding their respective proteins: SMS1, SMS2, and SMS related protein (SMSr). While there are a large number of genetic variations in the SMS family genes, there was no indication for the presence of mutations in human *SGMS1*, *SGMS2*, or *SAMD8* in any of the searched databases (Pubmed, Uniprot, publicly available Human Gene Mutation Database). Interestingly, both *SAMD8* and *SGMS1* are located on chromosome 10, with *SGMS1* in direct neighborhood of *ASAH2* which encodes neutral ceramidase, an enzyme catalyzing the degradation of ceramide at neutral pH and thus very closely involved in sphingolipid metabolism.

Despite its high homology to SMS1 and SMS2, SMSr does not have any SM synthase activity [10] but rather catalyzes the synthesis of the SM analog ceramide phosphoethanolamine (CPE) in the lumen of the endoplasmic reticulum (ER) by transferring a phosphoethanolamine head group from phosphoethanolamine to ceramide. However, due to the trace amounts of produced CPE and DAG, SMSr is thought to mostly act as a ceramide sensor to control ceramide homeostasis [21]. Notably, SMSr is the most conserved SMS family member with homologs in insects and various marine organisms that lack SM [21, 22]. Compared to monofunctional SMS1 and SMSr, SMS2 holds a dual activity and is also capable of synthesizing CPE [23]. However, a recent study suggests the presence of CPE synthase activity at least in all mouse SMS family members since CPE levels were not altered in Smsr/Sms2 double KO mouse macrophages and, moreover, plasma CPE levels were reduced in Sms1 KO mice [24].

SMS1 and SMS2 are crucial factors in the control of cellular SM and DAG with effects on the rate of apoptosis. This is demonstrated by the significant increase in intracellular levels of SM and DAG by stable overexpression of SMS1 or SMS2, resulting in an increased number of detergent-insoluble microdomains, increased tumor necrosis factor-alpha-mediated apoptosis, and enhanced lysenin-mediated lysis through the affinity of the lysenin protein with SM-rich microdomains. Conversely, reduction of SMS1 or SMS2 by short interfering RNA (siRNA) leads to reduced DAG and intracellular as well as plasma membrane SM levels and a decrease in lipopolysaccharide-mediated apoptosis [25].

Down-regulation of SMS1 and, to a lesser extent, of SMS2 decreases the formation of subcellular (but not total) pools of DAG at the Golgi apparatus and consequently reduces the localization of protein kinase D (PKD), a DAG-binding protein implicated in cellular secretion through the trans-Golgi network (TGN), to this compartment [26]. Both enzymes are, therefore, capable of regulating TGN-mediated protein trafficking and secretion (exemplarily illustrated by the reduction of insulin secretion by SMS inhibition in rat beta cells) [27]. SMS1 is also indispensable for transferrin internalization and cell proliferation and is capable of producing higher SM content than SMS2 [28].

SMS protein structure and localization

SMS genes and proteins are found in vertebrate mammalians, birds, amphibians, and fish, with a highly conserved homology between species [1]. Interestingly, *Drosophila melanogaster* lacks homologs for SMS1 and SMS2 and synthesizes CPE instead of SM [29], however, mainly by an enzyme different from SMSr [22]. All members, SMS1 (413 amino acids), SMS2 (365 amino acids), and SMSr (415 amino acids), share a similar membrane

topology with the lipid phosphate phosphatase (LPP) superfamily (Fig. 2): Hydrophobicity analysis predicts six membrane-spanning alpha-helices (transmembrane domains, TM), connected by hydrophilic regions forming extramembrane loops. The N- and C-termini are facing the cytosol [8]. Both SMS1 and SMS2 were shown to form homodimers mediated by the carboxyl-terminal tail, where the N- and/or C-terminal tails of one SMS molecule are in close proximity to those of another SMS in the homodimer [30]. A sterile alpha motif (SAM) – involved in protein-protein interactions of yet unknown identity – is present at the N-terminus of SMS1 and SMSr but not SMS2.

SMS proteins contain four highly conserved sequence motifs, designated D1, D2, D3, and D4 [10]. Motifs D3 (C-G-D-X_3-S-G-H-T) and D4 (H-Y-T-X-D-V-X_3-Y-X_6-F-X_2-Y-H) facing the exoplasmic leaflet are thought to be part of the catalytic site, given the similarity to the C2 and C3 motifs that form the catalytic site in LPPs [10]. SMS activity is not affected in C-terminal mutants of SMS1, but the truncation from TM4 to TM6 or mutagenesis of the two histidine or the aspartic acid residues (HHD motif underlined forming the catalytic triad) cause a loss of SM synthesis activity in both SMS1 and SMS2 [31]. A single residue N-terminally adjacent to the catalytic histidine (first H in D4) located in the third exoplasmic loop was found to profoundly influence head group selectivity of the enzymes: glutamic acid (E) permits the SMS-catalyzed production of CPE while aspartic acid (D) restricts the enzyme to produce SM [32]. On the other hand, motifs D1 and D2 are exclusive for SMS proteins and are placed in the first extra membrane loop and third transmembrane helix, respectively [10].

SMS1, SMS2, and SMSr genes are ubiquitously expressed in humans [10] [33]. SMS1 is located exclusively in the Golgi apparatus at a steady-state and is mainly responsible for *de novo* SM synthesis presenting 60-80% of total SMS activity limited by substrate availability [34]. In contrast, SMS2 resides primarily at the plasma membrane with its catalytic site oriented toward the outside of the cell, serving a role in regenerating SM from ceramides liberated by sphingomyelinases on the cell surface and thereby neutralizing these ceramides as a protective cellular mechanism [20]. However, SMS2 is also present in the Golgi apparatus [10]. These localization patterns observed early by biochemical fractionation techniques [35, 36] and confirmed in overexpression studies [10] are not determined by conventional sorting signals but by targeting signals for Golgi and plasma membrane at the C-terminus of SMS1 and SMS2, respectively [37]. SMS2, but not SMS1, is palmitoylated at a cytoplasmic COOH-terminal cluster of four cysteine residues, which is required for its localization in plasma membranes. Nevertheless, this posttranslational modification is dispensable for its *in vitro* enzyme activity [38]. Contrary to SMS1 and SMS2, SMSr localizes to the endoplasmic reticulum [21], where it undergoes homotypic oligomerization via its SAM domain into

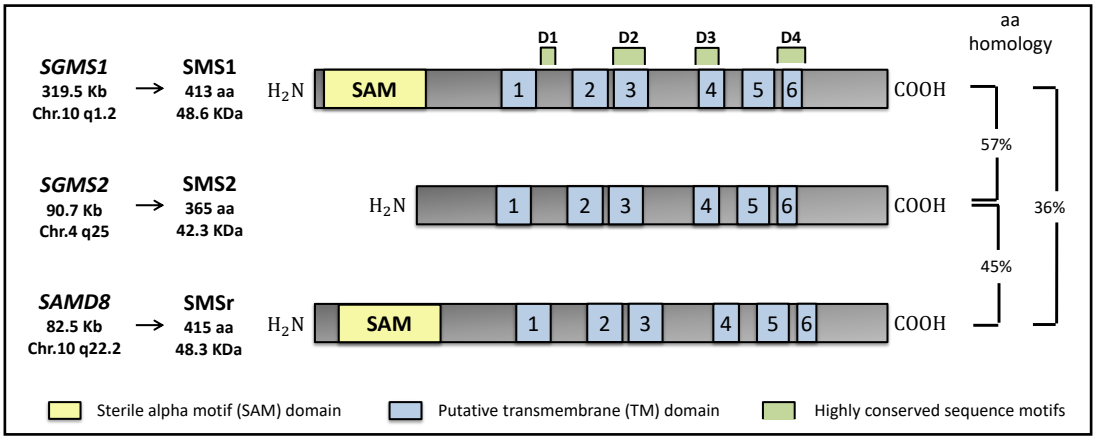

Fig. 2. Homologous protein structure of SMS family members with a sterile alpha motif (SAM) and putative transmembrane (1-6) domains and total amino acid number [1]. Information on genes, proteins and domains originates from GeneCards® Human Gene Database, UniProtKB [Q86VZ5 (SMS1_HUMAN); Q8NHU3 (SMS2_HUMAN); Q96LT4 (SAMD8_HUMAN)] and [10].

trimers and hexamers that resemble the helical oligomers formed by diacylglycerol kinase DGKdelta, a central regulator of lipid signaling at the plasma membrane [39].

Moreover, SMS activity has also been detected in the nuclear envelope and associated with the chromatin (where there was also evidence for NSM) with potentially opposite effects on regulation during proliferation and apoptosis [40].

Tools to study SMS function *in vitro* and *in vivo*

Following the relatively recent successful cloning of SMS genes [10, 41], studies overexpressing recombinant proteins or downregulating endogenous expression by siRNA as well as their detection at protein or expression level became feasible. First, initial expression studies to support the enzymatic activity of SMS1 and SMS2 were performed in yeast, which lacks endogenous SM synthase activity [8]. Epitope tagged SMS and fusion proteins with green fluorescent protein have been used to study localization patterns of these enzymes [37]. Knockout (KO) mouse models are available for *SGMS1* and *SGMS2* [42], including conditional KO animals [43, 44] with conditional KO cell lines [43], as well as for *SAMD8* [33]. Phenotypes of KO mice range from moderate neonatal lethality (*SGMS1*) to a milder (*SGMS2*) or no phenotype at all (*SAMD8*) (see [1] for details).

Classical methods for measuring SMS activity *in vitro* and *in situ* have used either fluorescently-labeled ceramide analogs or radiolabeled phosphatidylcholine or phosphocholine followed by thin-layer chromatography for separation and detection on a fluorescence detector of an image analyzer. The first quantitative measurement of SMS activities used a mass spectrometry (MS)-based method to characterize biochemical and cellular SMS activities, and demonstrated a similar time- and substrate-dependent SMS activity for both isoforms [45].

Despite a broad availability of inhibitors of sphingolipid metabolism to determine the physiological and pathophysiological role of involved enzymes, only a few small molecules have been reported to inhibit SMS activity, such as tricyclodecan-9-yl-xanthogenate (D-609). D609 has originally been identified as a competitive inhibitor for phosphatidyl choline-specific phospholipase and is known for its antiviral and antitumor properties [46]. The compound, with antioxidant properties *in vivo*, also suppresses LPS- and IFNγ-induced NO production and blocks oxidative glutamate toxicity in nerve cells. Although D609 has been used as a lipid-related enzyme inhibitor for decades [17], only Kato et al. elucidated the considerable differences in the inhibitory activity of the eight possible stereoisomers of this compound towards SMS and phosphatidylcholine-specific phospholipase C [47]. Since D609 is an unstable, insoluble, and easily oxidized chemical, a pivaloyloxynethyl analog was found to serve as a D609 prodrug [48]. However, the inhibition by D609 or its prodrug of SMS activity is not very efficient, with IC50s ranging from 177 μM [48] to 600 μM [49] for various cell lines.

Interestingly, SMS inhibitory activity has also been observed in naturally occurring compounds. A cyclic anhydrophytosphingosine isolated from the marine sponge, Jaspine B, was shown to trigger apoptosis in melanoma cells by inhibiting SMS activity [50]. Ginkgolic acid (15:1), the first naturally-occurring SMS inhibitor from a terrestrial plant extract library, inspired the concept of sphingolipid mimics based on physical properties that are similar to sphingosine and led to the development of a sphingosine-1-phosphate receptor agonist [51]. Different malabaricones isolated from the fruits of *Myristica cinnamomea* King from the nutmeg family have been characterized as inhibitors of SMS and could serve as candidates for alternative medicinal strategies for the treatment of metabolic syndrome (due to their non-toxic nature and oral efficacy in mouse models) [52].

Since SMS1 and SMS2 synthesize SM under similar conditions, their activities cannot be quantified separately. Zama et al. have, therefore, developed a sensitive, high-throughput method based on high-performance liquid chromatography for measuring specific enzyme activity by stable transfection of SMS-null cells with either SMS1 or SMS2 constructs. This

system allows screening for SMS-specific inhibitors using HPLC and fluorescent ceramide analogs [53]. Alternatively, purified recombinant SMS1 and SMS2 enzymes serve to determine specific inhibitory activities of drugs.

First, structural variations of a lead compound resulted in the identification of derivatives with micromolar inhibitory activities against SMS2 and excellent isoform preferences over SMS1 [54]. Adachi et al. identified a 2-quinolone derivative as an SMS2 selective inhibitor that requires the amino acids S227 and H229 within the SMS2 catalytic domain to results in an IC50 of 950 nM and >100-fold selectivity for SMS2 over SMS1 [55]. Further advances led to improved highly selective SMS2 inhibitors with oral efficacy in mice [56]. Currently, Ly93 (belonging to the class of 2-benzyloxybenzamides) is one of the most potent inhibitors, with a selective ratio of over 1400 (IC50 values of 91 nM and 133.9 μM against purified SMS2 and SMS1, respectively) and dose-dependent pharmacological activity in mice [57].

Such selective inhibitors open up the possibility to differentiate between SMS1 and SMS2 activities during assays with samples of different origin and to perform mechanistic studies. However, to our knowledge, no specific SMS1 inhibitor has been reported so far which prevents the direct specific quantification of SMS2 activity in biological samples such a patients' material. Although a structure-based virtual screening on human SMS1 lead to the report on the discovery of a novel SMS1 inhibitor (IC50 value of 2.1 μM in enzymatic assays), its specificity for SMS1 over SMS2 was not proven yet [58].

Brain-specific SMS

SMS expression

Due to a differential regulation of sphingolipid metabolism, disturbances in sphingolipids or enzymes are not necessarily reflective of cerebral processes and circulating sphingolipids do not directly influence sphingolipid abundance in the brain. A comparative study of sphingolipid classes in the hippocampus, cerebral cortex, and plasma of male mice which were maintained on a low or saturated fat enriched diet, with, or without additional treatment with sphingolipid modulating agents, found both positive and negative correlations between concentrations of plasma and brain ceramide species [59]. Accordingly, a number of studies indicate a specific expression and role of SMS in the central nervous system compared to the periphery.

In primary rat astrocytes, a rapid activation of SM biosynthesis appears to be the major mechanism responsible for the marked decrease in cellular ceramide as an early response to the mitogenic activity of basic fibroblast growth factor in the signaling pathway leading to astrocyte proliferation. Enzymatic analysis revealed a marked increase in SMS activity without any alterations in other metabolic pathways involved in ceramide turnover such as SM degradation or ceramide biosynthesis which was inhibited by brefeldin A (a disruptor of the Golgi apparatus) and D609 [60].

In the embryonic rat brain, SMS mRNA expression varies in a developmental stage-specific manner [61]. In the adult rat brain, *SGMS1* is expressed at significantly higher levels than *SGMS2* [61]. In contrast to scarce data on the regulation of *SGMS2* expression, numerous studies indicate a complex pattern for *SGMS1* gene comprising at least 24 exons assumed to be regulated at the transcriptional, post-transcriptional and translational levels [62]. The levels of full-length and alternative SMS1 transcripts vary considerably between different human tissues and might be controlled by tissue-specific intron polyadenylation causing the appearance of truncated transcripts not involved in the synthesis of the full-length protein SMS1 [63]. Moreover, alternative promoters within *SGMS1* introns were found to participate in regulating SMS1 expression in human tissues [64].

RNA interference-mediated silencing of SMS1 in murine Neuro-2a cells with neuronal characteristics lead to a significant decrease in SM levels resulting in reduced proliferation, morphological changes including neurite-like outgrowth, cell cycle arrest and reduced migratory potential associated with decreased levels of matrix metalloproteinases [65].

SMS1 and SMS2 are differentially expressed in neuronal subtypes and play distinct roles in regulating local lysenin-binding SM clustering in their vicinity. Only SMS2 localizes to neurites of hippocampal neurons and is assumed to predominantly synthesize SM in the long neurites and growth cones at the tip. By inducing lysenin-binding SM clusters in its vicinity, SMS2 is thought to be involved in creating the micro-domains that anchor signal transduction proteins [66]. Moreover, SMS2 may affect the regulation of drug transporters in the brain and has therefore been suggested as a potential target for enhancing drug access to the brain. SMS2 deficiency in mice resulted in significantly reduced gene expression of *Mdr1* (multidrug resistance 1), but not seven other analyzed drug transporters, and downregulated function of Pgp (P-glycoprotein, encoded by *Mdr1*) in the brain. Moreover, suppressed expression of Pgp, ezrin and β-actin in both cortex and paraventricular areas was detected by immunohistochemistry in these animals [67].

In cultured mammalian and insect cells, acute disruption of SMSr catalytic activity leads to an accumulation of ceramides in the ER and consequently to a structural collapse of ER exit sites and induction of mitochondrial apoptosis [21, 34]. These effects are suppressed by inhibiting *de novo* ceramide synthesis, stimulating ER export of ceramides or targeting SMS1 to the ER, thus supporting a role of SMSr as critical regulator of ER ceramide levels. On the other hand, ubiquitous inactivation of SMSr catalytic activity in mice resulted primarily in disruption of CPE biosynthesis in the brain whereas there were no obvious alterations in steady state ceramide levels, cell integrity or survival [33] [24]. These contradictory outcomes could be explained by cell- or tissue-specific compensatory mechanisms that overcome a deregulation of ER ceramides over time in the animal model [32].

SMS circRNAs

So-called competing endogenous RNAs or commonly named “RNA sponges” present a relatively new concept of RNAs that share microRNA recognition elements which prevent those from binding their cognate target messenger RNAs [68, 69]. Reports on their involvement as post-transcriptional signals in diseases, mainly on cancer, are steadily increasing. Circular RNAs (circRNAs) belong to this class and are predominantly found in mammalian cells with tissue- and organ-specific expression, high abundance in the nervous system and upregulation during neuronal differentiation [70]. Particularly, *SGMS1* is one of the few eukaryotic genes with detailed studies on circRNA: 11 circRNAs have been discovered to originate from the characteristic multi-exon 5’ untranslated region (5’UTR) with a complex exon-intron structure including six (and additional alternative) exons, and introns which carry Alu repeats in an orientation that contributes to multi-step alternative splicing and the emergence of the circRNAs [71, 72]. These *SGMS1* circRNAs have been detected predominantly in different parts of the brain, with higher expression in rats than mice and even higher in humans [73]. Despite the high conservation of circRNAs in the mammalian brain, the content of circRNA in the human often exceeds that of the rodent brain [70]. This could result from the four-fold higher synaptic density in humans compared to mice [74] and the enrichment of circRNAs in synapses [70] or the larger diversity of inverted repeats such as Alu contributing to circRNA appearance in primates over rodents [75].

An active post-transcriptional regulation of SMS1 protein expression is also supported by the absence of a correlation between the amount of expressed full-length protein in different human tissues and the level of coding transcripts with putative truncated isoforms of SMS1 protein undetectable by immunoblotting [76].

SMS family genes in GWAS of neuropsychiatric health

A meta-analysis using phylogenetically controlled methods estimated the average heritability of human behavior to be 0.235; however, with considerable variation among behaviors [77]. Whereas no studies are available with respect to SMS enzymatic activity, protein levels, or expression connected to healthy behavior, we have identified several associations of SMS genetic variants with well-being, educational attainment, and mathematical ability based on genome-wide association studies (GWAS).

In a recent GWAS [78], the single nucleotide polymorphisms (SNPs) rs2574985 and rs2099527 within the *SGMS1* gene were found to be associated with subjective well-being or life satisfaction, respectively, in 298, 420 individuals assessed by survey questions on life satisfaction, positive affect, or happiness [78], and thus to contribute to the roughly 36% of the weighted average heritability of well-being based on a meta-analysis of twin studies [79]. Moreover, the rs2574985-A allele with a minor allele frequency (MAF) of 0.283 (www.ncbi.nlm.nih.gov/variation/tools/1000genomes/, CEU population) was tested positive for an enriched association with depressive symptoms [78].

Educational attainment serves as a "model phenotype" for several behavioral traits and correlates with many social, economic, and health outcomes. Educational attainment is moderately heritable and often measured as the number of years of schooling completed (EduYears) [80]. A GWAS of educational attainment in 1.1 million individuals of European ancestry identified 1,703 newly prioritized genes compared to an earlier study. These genes exhibited particularly high expression levels in the brain (both pre- and postnatally). Among these genes, *SAMD8* encoding SMSr was identified by the SNP rs2657283 with a risk allele frequency of 0.588 [81]. This SNP also reached genome-wide significance in the pooled-sex GWAS of EduYears [81].

Moreover, two SNPs among the 1, 271 independent genome-wide-significant SNPs were reported to be located within *SGMS1* (rs11006237 and rs7921226) in this large-scale GWAS of educational attainment. In a subsample of participants of the personal genomics company 23andMe who had provided information on their mathematical background (N = 564, 692), the minor alleles (MAF 0.202 for both) were found to be associated with higher self-reported mathematical ability [81].

Neurological diseases

A literature search for publications on neurological diseases associated with SMS family members resulted in only few articles predominantly reporting on SMS1 (Table 1).

Relation to neurological biomarkers

A study on 750 relatively healthy Scottish adults from the Lothian Birth Cohort 1936 on genetic factors influencing plasma levels of 92 protein markers of the Olink® neurology panel linked to neurobiological processes or neurological diseases identified the SNP rs146075547 (MAF 0.018) within the *SGMS1* gene as one of the significant SNPs associated with the concentration of neutral ceramidase [82]. Interestingly, this enzyme hydrolyses ceramide and is thus closely related to SMS in the sphingolipid metabolism. Moreover, it is encoded by *ASAH2* located in direct proximity to *SGMS1*.

Hearing impairments

Hearing examinations in SMS1- and SMS2-deficient mice showed that, whereas the hearing abilities were not affected in SMS2-deficient mice, SMS1 deficiency caused hearing loss [83]. In addition, the altered expression of the potassium channel alpha subunit *KCNQ1* in the stria vascularis of the inner ear of SMS1−/− mice with atrophy of this structure and a decrease in the endocochlear potential required for the high sensitivity of the inner ear to sound indicated that SMS1 is essential for normal inner ear function. Further characterization in human embryonic kidney 293T cells expressing the KCNQ1/KCNE1 channel [84] showed that inhibition of SMSs by the nonspecific SMS inhibitor D609 significantly reduced current density and altered channel voltage dependence. However, knockdown of SMS1 by a short hairpin RNA or application of protein kinase D inhibitors reduced current density without changing channel properties. On the other hand, overexpression of SMS1 increased the current density alone. These data suggest a positive regulation of KCNQ1/KCNE1 channel density by SMS1 in a protein kinase D-dependent manner with therapeutic potential in hearing, cardiac and metabolic disorders [84].

Table 1. Summary of literature on sphingomyelin synthase in neuropsychiatric diseases

Enzyme or gene	Association	Reference
Neurological diseases		
SMS1 / *SGMS1*	SMS1 deficiency causes hearing loss by the regulation of KCNQ1/KCNE1 channel density in a protein kinase D-dependent manner	[83] [84]
	SMS1 is involved in JEV infection and its associated pathologies, such as meningitis	[86]
	Expression of SMS1 rescues PSE phenotype in the Drosophila neuronal cortex caused by the absence of CPES	[87]
	SMS1 regulates ceramide levels in N2a cells and plays a potent protective role in oxidative stress-induced apoptosis, partly through the p38 pathway	[90]
	Expression of SMS1 is decreased by both ischemia and the associated surgical stress	[88]
	Expression of SMS1 is elevated in the hippocampus but not the cerebellum of post mortem brains in Alzheimer's disease patients	[92]
	Inhibition of SMS1 ameliorates Alzheimer-like pathology in APP/PS1 transgenic mice through promoting lysosomal degradation of BACE1.	[93]
SMS2 / *SGMS2*	Downregulation of SMS2 by siRNA modulates exosome secretion and promotes clearance of amyloid-β by microglia	[94]
SMSr / *SAMD8*	none	
SMS1 and SMS2	Studies in rats suggest neuroprotection after stroke by D609, a non-specific SMS inhibitor	[89]
	Hypoxia induces synthesis of S1P and multiple dihydrosphingolipids but it does not change the activity of sphingomyelinases or SMS in an *in vitro* stroke model	[91]
	Inhibition of SMS by D609 increase exosome release and inhibited NO production in rat midbrain slice cultures, but it does not affect dopaminergic neurodegeneration	[95]
Psychiatric diseases (without comorbidities / mechanisms)		
SMS1 / *SGMS1*	GWAS: SNPs rs2574985 and rs2099527 are associated with subjective well-being / life satisfaction	[78]
	GWAS: SNP rs2574985 is associated with depressive symptoms	[78]
	GWAS: SNPs rs11006237 and rs7921226 are associated with mathematical ability	[81]
	SMS1 knockdown suppresses development delay and lethality effects induced by clozapine in *C. elegans*	[98] [99]
SMS2 / *SGMS2*	Performance in Morris Water Maze is impaired and depression-like behavior is induced more easily in SMS2 KO mice	[123]
	SGMS2 expression is significantly increased in patients diagnosed with insomnia with improved sleep after treatment compared to insomnia patients with non-improved sleep after treatment	[134]
	GWAS: SNP rs72675567 is among the top 100 SNPs to be associated with nicotine withdrawal symptom	[104]
	Stronger alcohol-induced oxidative stress is present in in the hippocampal CA1 of SMS2 KO mice compared to controls	[116]
	SMS2−/− mice pups present a greater alcohol-induced compensatory long-term neural proliferation compared to WT pups	[118]
	Mossy cells undergo apoptosis in a more prominent way after *in utero* alcohol exposure in SMS2−/− offspring compared to WT	[119]
SMSr / *SAMD8*	GWAS: SNP rs2657283 is associated with educational attainment	[81]
	GWAS: SNP rs4746270 located in close proximity to *SAMD8* is associated with the age of initiation of regular smoking and with the binary phenotype of ever smoking regularly	[106]
SMS1 and SMS2	Pharmacological inhibition of SMS1 and SMS2 with D609 lead to a rapid ceramide accumulation in the ER, activation of autophagy and improvement of stress-induced depression-like behavior	[133]

Multiple sclerosis

Although abnormalities in sphingolipid metabolism including altered activity of serine-palmitoyltransferase within the ceramide *de novo* pathway were reported for multiple sclerosis and indicate that targeting ceramide biosynthesis could present a unique therapeutic strategy [85], no data are available on SMS activity for this disease so far.

Meningitis and Encephalitis

Recent insights using SMS1/SMS2 double KO mice have shown that SM generated by SMS1, but not SMS2, at the plasma membrane is involved in Japanese encephalitis virus (JEV) attachment and subsequent cell entry [86]. Compared with wild-type mice, SMS1-deficient mice showed an obvious decrease of JEV infection and its associated pathologies, such as meningitis, lymphocyte infiltration, and elevation of the cytokine interleukin 6.

Epilepsy

In the *Drosophila* model, the absence of ceramide phosphoethanolamine synthase (CPES, Fig. 1), which produces phosphoethanolamine, closely related to sphingomyelin generated by SMS, lead to failure of cortical glial cells to encapsulate the neuronal cell bodies and predisposed the flies to photosensitive epilepsy, a common type of epilepsy in humans with seizures induced by visual stimulation [87]. This phenotype was rescued by the expression of SMS1, highlighting the role of these evolutionarily conserved lipids in cortical glial membranes.

Stroke and ischemia/hypoxia events

First changes in SMS1 expression were observed in rats after focal cerebral ischemia. Levels of SMS1 transcripts initially decreased in the ischemic cortex but returned to control levels within three days. Interestingly, SMS1 mRNA expression was reduced in the subcortex of both rats with occlusion and sham-operated animals for at least three days suggesting a long-term effect of surgical stress itself [88]. Studies in rats demonstrated a neuroprotective effect of the nonspecific SMS inhibitor D609 after stroke possibly by preventing mature neurons from entering the cell cycle at the early post-stroke reperfusion stage without interfering with protective factors of later microglia/macrophage proliferation. The inhibition of SMS lead to an increased ceramide level, and induction of cell-cycle arrest by up-regulating the cyclin-dependent kinase (Cdk) inhibitor p21 and causing hypophosphorylation of the retinoblastoma (pRb) protein, a cell cycle inhibitor [89].

A major mechanism of neuronal injury after cerebral ischemia/reperfusion involves oxidative stress. In mouse neuroblastoma Neuro-2A (N2a) cells, H_2O_2, one of the main forms of reactive oxygen species, upregulated the expression of SMS1, increased SMS activity and intracellular ceramide levels, and resulted in apoptosis. Moreover, inhibition of SMS1 prior to H_2O_2 exposure by D609 or silencing RNA further increased ceramide levels and potentiated apoptosis, which indicates a protective role of SMS1 in this oxidative stress-induced apoptosis [90]. Although in *in vitro* stroke models, hypoxia induced the synthesis of sphingosine-1-phosphate and multiple dihydrosphingolipids, which might be involved in ameliorating the effects of stroke, there were no observed changes in the activity of sphingomyelinases or SMS [91].

Neurodegenerative diseases

SMS seems to play an important role in multiple neurodegenerative diseases. Expression of SMS1 has been found to be elevated in the hippocampus but not the cerebellum of *post mortem* brains in Alzheimer's disease patients [92]. Inhibition of SMS1 activity significantly reduced the level of amyloid-beta peptide (Aβ), attenuated Alzheimer's disease pathology in APP/PS1 transgenic mice and promoted BACE1 translocation to the lysosome for degradation [93].

The role of SMS in neurodegenerative diseases might be linked to exosomes. Downregulation of SMS2 by siRNA caused an up-regulation of exosome secretion, of Aβ

uptake by microglia, and a reduction of extracellular Aβ [94]. Moreover, it was shown that inhibition of SMSs by D609 increased exosome release and inhibited NO production in rat midbrain slice cultures, although it did not affect dopaminergic neurodegeneration [95].

Increasing genetic and biochemical evidence supports the role of ceramide homeostasis in neurodegenerative diseases, including Parkinson's disease (PD) [96]. In *post mortem* brain tissues, total ceramide and sphingomyelin levels were reduced in the anterior cingulate cortex of PD samples compared to controls, and a significant shift in the ceramide acyl chain composition toward shorter length in PD was observed. These changes were attributed to the detected upregulation of ceramide synthase-1 gene expression as a response to reduced ceramide levels but could also result from altered SMS gene expression, which has not been analyzed in this study (which was restricted to assessing mRNA expression of the major ceramide synthase genes) [97].

Psychiatric diseases

A search for data on psychiatric diseases associated with SMS family members revealed relatively few publications and GWAS entries (Table 1).

Schizophrenia

Clozapine is the first developed atypical antipsychotic drug and unusually effective for a broad range of syndromes, including treatment-resistant schizophrenia, but it also causes multiple side effects. Using *Caenorhabditis elegans* as a physiological and genetic model, a whole-genome wide RNA screen for suppressors of clozapine-induced development delay and lethality identified 40 genes with relation to clozapine effects [98]. Among them, *SGMS1* was found to be a strong suppressor: RNAi application to knockdown the gene largely relieved the developmental phenotype, particularly the clozapine-induced inhibition of pharyngeal pumping, in the worm [99].

For humans, in a most recent presentation of whole-exome sequencing results from 24,000 schizophrenia cases and 97,000 controls, no SMS-related gene was among the newly identified ten genes with ultra-rare variants implicated in schizophrenia development [100].

Substance use disorders – nicotine

As a major risk factor for non-communicable diseases such as cardiovascular diseases, cancers and chronic obstructive pulmonary disease, smoking contributes to over 5 million preventable deaths per year [101]. Nicotine dependence causes most smokers to continue their tobacco use to avoid nicotine withdrawal symptoms [102]. Heritability of nicotine dependence is estimated to range between 40 and 75% with distinct genetic factors for smoking initiation, intensity and cessation [103]. A GWAS in 1,715 participants from the Finnish twin family study investigated the impact of common and low-frequency variants on three measures of nicotine addiction, namely smoking quantity, nicotine dependence and nicotine withdrawal based on DSM-IV. The SNP rs72675567 (MAF 0.086) within *SGMS2* was identified among the top 100 SNPs to be associated with the nicotine withdrawal symptom count [104]. These symptoms peak during the first week of a quit attempt and are also a strong predictors of relapse [105].

Moreover, the SNP rs4746270 (located close to *SAMD8* encoding SMSr) was identified among 1,193 independent, genome-wide significantly associated common variants in a GWAS of up to 1.2 million individuals investigating multiple stages of tobacco use (initiation, cessation, and heaviness). The risk allele A (MAF 0.4 in the study sample) was associated with the age of initiation of regular smoking and also with the binary phenotype of ever smoking regularly [106]. Interestingly, in the multivariate analysis of pleiotropy, every locus associated with the age of initiation of smoking was found to be pleiotropic for other phenotypes, such as the number of cigarettes per day or – with respect to alcohol use – drinks per week [106].

Substance use disorders – alcohol

Like tobacco use, alcohol use is a leading cause of mortality that influences the risk of many complex diseases. Despite its heritability [107] and etiological connection to nicotine dependence [108] resulting in a high comorbidity rate, alcohol addiction has also been largely resistant to gene discovery efforts [109], and according to our literature search, no direct association with SMS family genes has been reported so far.

Excessive alcohol consumption provokes an array of degenerative pathologies, but the detailed underlying mechanisms are poorly understood [110]. Sphingolipids and associated enzymes were involved in mediating the effects of ethanol exposure in a series of animal experiments and human studies [14, 111, 112]. Particularly, the lysosomal and secretory forms of ASM catalyzing the reverse reaction of SMS (i.e., the hydrolysis of sphingomyelin to ceramide and phosphorylcholine) are increased in alcohol-dependent patients [113-115]. However, data on SMS itself in the field of alcohol addiction is scarce and limited to animal models.

Although chronic alcohol exposure led to hepatocytic steatosis and hepatic fibrosis in both wild-type and SMS2−/− mice, the increase in the number of positive c-Fos and NF-κB cells in the hippocampal CA1 area of KO animals was significantly higher than in wild-type mice, as assessed both by immunohistochemistry and immunoblotting. The stronger alcohol-induced oxidative stress was attributed to alterations in ceramide levels [116].

Further evidence is provided by a mouse model of prenatal alcohol exposure to study alcohol-induced neuroapoptosis. Since ethanol – as a well-established teratogen – can cross the brain-blood-barrier, it may directly cause neurotoxicity to the central nervous system leading to neuronal apoptosis and decreased synapse density [117]. Daily intragastric administration of ethanol to the pregnant mother led to reduced blood SM levels in wild-type and SMS2−/− pups [118]. Neural cell proliferation and the number of new neurons in the hippocampal dentate gyrus were dose-dependently increased in both genotypes but to a greater extent in the KO animals, suggesting that the accumulation of ceramide upregulated alcohol-induced compensatory long-term neural proliferation. While the number of newborn (doublecortin positive) neurons in the dentate gyrus decreased with age, SMS2−/− pups generally showed a significantly higher number than age-matched wild-type animals. The increase in the expression level of the important activator protein of the ceramide/ceramide-1-phosphate pathway, protein kinase C alpha, was less prominent in the hippocampus of SMS−/− compared to wild-type offspring [118].

In a similar subsequent study of *in utero* alcohol exposure, SM levels were also down-regulated in a dose-dependent manner, both in wild-type and SMS2−/− offspring, and again significantly lower in the KO pups compared to wild-type [119]. Furthermore, the number of apoptotic mossy cells in the hippocampus was dose-dependently increased in both groups but to a higher degree in the SMS−/− offspring. The expressions of activated Caspase 8 and activated Caspase 3 were increased after prenatal alcohol exposure in hippocampal tissues of both genotypes of pups [119]. Thus, pharmacological and toxicological mechanisms of ethanol are related to the effects of the ceramide pathway, which seems to influence the degree of alcohol-induced neuronal proliferation and apoptosis. This is particularly relevant to the field of fetal alcohol spectrum disorder with a high prevalence and long-lasting growth and developmental deficits resulting from maternal alcohol consumption during pregnancy [120] since ethanol diffuses through the placenta and distributes rapidly in the fetal organism. The considerably slower rate of elimination (3–4% of the maternal rate) leads to further accumulation of exposure to ethanol in the amniotic fluid and even greater fetal exposure [121].

Learning under stress conditions

A sphingolipid mechanism has been shown to be involved in extinction of behavior, which involves active re-learning accompanied by emotional behaviors of mild depression [122]. In a cognitive behavior characterization of SMS2 KO mice, no difference in learning ability in the context-dependent fear learning and novel object recognition test was observed

compared to wild-type mice [123]. However, Morris water maze test performance was impaired in SMS2 KO mice. The authors concluded that the more stressful conditions due to the wet environment compromise the learning ability of SMS2 KO mice. In agreement, depression-like behavior was induced more easily in SMS2 KO mice by forced swimming [123]. These results support the proposal that altered synthesis of ceramide is associated with a depression-like tendency in animal models and depressive disorder in humans.

Depression

In fact, alterations in ceramide-sphingomyelin metabolism have been linked to major depression disorder (MDD). Brain membrane lipids are crucially involved in the induction of depression- and anxiety-related behaviors [124] and peripheral serum lipids have been found to be related to depression severity and its prospective course [125]. The enzyme catalyzing the reverse reaction of SMS, ASM, has been implicated in depression in animal and clinical studies at the protein activity level [126, 127] as well as via alternative splicing regulation [128, 129].

Mice overexpressing ASM exhibit constitutively depression-like behavior [130]. Widely used antidepressants, such as amitriptyline and fluoxetine, acted as functional inhibitors of ASM [131, 132] and were found to decrease ASM activity in the hippocampus at therapeutic concentrations. This resulted in increased neuronal proliferation, maturation and survival of hippocampal neurons, and improved stress-related depression-like behavior in mice. Genetic deficiency of ASM abrogated the effects of these antidepressants on neurogenesis and behavior [130]. Moreover, antidepressant treatment for 14 days (but not 5 days) resulted in an increase of sphingomyelin abundance in lysosomes and Golgi membranes and, finally, in an increase of ceramide concentrations in the ER, which triggers autophagy via a cascaded activation of phosphatase 2A, Ulk, and phosphorylation and activation of Beclin and Vps34. Pharmacological inhibition of SMS1 and SMS2 with D609 lead to a rapid (within 3 days) ceramide accumulation in the ER, activation of autophagy, and improvement of stress-induced depression-like behavior [133]. This suggests SMSs as new therapeutic targets for the development of novel, fast-acting antidepressants.

Due to exposure to stressful conditions and irregular sleeping schedules, military personnel are at increased risk for insomnia, which is frequently comorbid with post-traumatic stress disorder or depression. Microarray whole-genome expression analysis was performed in 68 (mainly male) active duty US military personnel diagnosed with insomnia subdivided (based on their change in the Pittsburgh sleep quality index after 3 months of standard treatment for insomnia) into improved or non-improved sleep [134]. While gene-expression patterns did not differ at study inclusion, 217 coding genes were differentially expressed at follow-up compared to their baseline in the participants with improved sleep, who also showed decreased scores on the quick inventory of depressive symptomatology. Among the top 50 genes with increased expression, including inflammation, inflammatory response-associated and stress-related genes, *SGMS2* expression was found to be increased 1.8-fold in the improved sleep group. This preliminary study provides further support for a relationship between depression and SMS function.

Somatic diseases related to depression

Patients with depression frequently present somatic comorbidities, such as cardiovascular diseases, chronic obstructive pulmonary disease, inflammation, diabetes, cancer, asthma, chronic musculoskeletal disorders, stomach and duodenal ulcer, osteoporosis, renal diseases and allergies [135-137]. The comorbidity of mental disorders and physical diseases has been linked to decreased quality of life, increased mortality rates, and poorer health care outcomes, compared to patients with physical diseases without comorbid mental disorders [138]. In the case of depression, several of these comorbid somatic diseases have been associated to SMS affections.

Cardiovascular disease

SM cycle disturbances have been linked to a higher risk of cardiovascular diseases and atherosclerosis, which are physical diseases commonly associated to depression [135]. Several studies have shown a decrease of atherosclerosis in the absence of SMS in mice [139-141]. Moreover, the overexpression of SMS2 promotes intracellular cholesterol accumulation in an *in vitro* model of human umbilical vein endothelial cells [142].

Diabetes

Sphingolipid imbalances have also been associated with insulin resistance and diabetes, and ceramide is thought to be an important contributor to insulin resistance [143]. SMS also seems to play a role in this pathogenic condition since severe deficiencies in insulin secretion dependent on glucose stimuli were observed in SMS1 KO mice [42]. Moreover, islet cells of SMS1 KO mice showed mitochondrial abnormalities and could not up-regulate ATP production in response to glucose, suggesting that SMS1 is needed for normal mitochondrial function and insulin secretion in pancreatic β-cells.

Cancer

SMS activity has been linked to multiple cancer types, both with pro- and anti-tumorigenic effects. An increase of SMS was observed in mononuclear cells of leukemia patients as well as in drug-resistant HL-60/ ADR cells compared to drug-sensitive HL-60 cells [144]. On the other hand, SMS1 is frequently downregulated in melanoma, and low SMS1 expression has been related to a worse prognosis in metastatic melanoma patients [145]. In addition, SM was found to be decreased in glioma cells compared to nontumor (MRC-5) cells, and treatment with a potent antitumor compound (2-hydroxyoleic acid, 2OHOA) enhanced SM production by the activation of SMS [146].

Inflammation

During the last years, the inflammatory activation of the immune system is gaining importance to understand the mechanisms underlying depression [147]. SM levels, both in plasma and in cell membranes, seem to play an essential role in inflammation, and SMS activity is likely to be involved. In SMS2 KO mice, a reduction of very-long-chain SM (SM (d18:1/22:0)) has been observed, together with an increase in very-long-chain ceramides (Cer (d18:1/24:0 or d18:0/24:1) in the plasma, compared to WT mice [148]. Moreover, exogenously added very-long-chain SM strongly upregulated several macrophage activation markers, leading to inflammation and, therefore, suggesting that the very-long-chain SM produced by SMS2 is involved in inflammatory responses. In another study, treatment with Dy105, a strong inhibitor of SMS activity, reduced the activation of NFkB and p38 (a well-known MAP kinase mediating inflammation) in bone marrow-derived macrophages [149]. Thus, there seems to be a clear link between SMS activity and inflammation.

Conclusion

SMS is a central enzyme in the sphingolipids metabolism and is involved in diverse cell functions through the regulation of structural and bioactive lipids SM and ceramide, as well as PC and DAG. Given its biological importance, interest in SMS has grown in the last years, but lags considerably behind enzymes such as sphingomyelinases catalyzing the reverse reaction, which is certainly due to the late discovery and cloning of the SMS enzyme as well as the late discovery of specific inhibitors. The interest in SMS is reflected in the development of new methodologies to measure its activity and in the emergence of new improved and more selective SMS inhibitors. Nevertheless, surprisingly, there is no proven specific SMS1 inhibitor available, which would be essential for the *in vitro* study of SMS1 versus SMS2 activity.

Numerous diseases, such as cancer, cardiovascular diseases, atherosclerosis, diabetes, inflammation, osteoporosis and epidermal conditions, have been linked to alterations in SMS activity, suggesting that these enzymes represent relevant pharmacological targets. However, there is scarce literature of SMS related to neuropsychiatric diseases, even though the search performed for this review was systematically based on the categories provided by the ICD-10 F and G sections, comorbid or mechanistically associated disorders and GWAS databases. Although there are no known mutations in the SMS gene family, GWAS studies have associated polymorphisms in SMS to different healthy and diseased psychological conditions. Moreover, several studies indicate an involvement of SMS in different neurological and psychiatric diseases. Understanding the role that SMS play in the regulation of sphingolipid metabolism might be essential to elucidate the molecular mechanisms of this set of diseases and to develop preventive or therapeutic approaches. Thus, advancement in tools and cellular, animal as well as human studies is urgently required to further elucidate the involvement of SMS in neuropsychiatric diseases.

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C.M. has drafted the manuscript, C.M. and R.D.B.C. have performed the searches and written the text, R.D.B.C: has prepared the figures with support of C.M., J.K. has provided intellectual input and commented on the manuscript. All authors have revised the manuscript.

Disclosure Statement

The authors have no conflicts of interest to declare.

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