



SPHINGOLIPIDS AND NEURONAL DEGENERATION IN LYSOSOMAL STORAGE DISORDERS.

Journal:	<i>Journal of Neurochemistry</i>
Manuscript ID	JNC-2018-0257.R1
Manuscript Type:	Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Grassi, Sara; University of Milan, Department of Medical Biotechnology and Translational Medicine Chiricozzi, Elena; Università degli Studi di Milano, Department of Medical Biotechnology and Translational Medicine; Mauri, Laura; University of Milan, Department of Medical Biotechnology and Translational Medicine SONNINO, SANDRO; Università degli Studi di Milano, Department of Medical Biotechnology and Translational Medicine Prinetti, Alessandro; University of Milan, Department of Medical Biotechnology and Translational Medicine
Area/Section:	Molecular Basis of Disease
Keywords:	sphingolipids, gangliosides, lysosome, membrane fusion, sphingolipidoses, sphingomyelin

1
2
3 SPHINGOLIPIDS AND NEURONAL DEGENERATION IN LYSOSOMAL STORAGE
4 DISORDERS.

5
6 Sara Grassi, Elena Chiricozzi, Laura Mauri, Sandro Sonnino and Alessandro Prinetti

7 *Department of Medical Biotechnology and Translational Medicine, University of Milan,*
8 *Milan, Italy*

9
10 Correspondence to:

11
12 Alessandro Prinetti, Department of Medical Biotechnology and Translational Medicine, Via
13 Fratelli Cervi 93, 20090 Segrate (Milano), Italy. Phone: +390250330376; fax,
14 +390250330365; e-mail: alessandro.prinetti@unimi.it.
15
16
17

18
19
20 Running title: Sphingolipids in lysosomal storage disorders
21

22
23
24 Keywords: sphingolipids, gangliosides, sphingomyelin, sphingolipidoses, lysosome,
25 membrane fusion
26
27
28
29
30
31
32
33
34
35
36

37 Abbreviations: Ganglioside and glycosphingolipid nomenclature is in accordance with the
38 IUPAC-IUBMB recommendations (Chester 1998).
39

40 ASM, acid sphingomyelinase; CERT, ceramide transfer protein; ER, endoplasmic
41 reticulum; FAPP2, four-phosphate-adaptor protein 2; LSDs, lysosomal storage disorders;
42 LVs, intra-endolysosomal vesicles; MAMs, mitochondria-associated ER membranes;
43 MCSs, Membrane Contact Sites; ORMDL, orosomuroid-like proteins; PM, plasma
44 membrane; SL, sphingolipids; SM, sphingomyelin; SMase, sphingomyelinase; TFEB,
45 transcription factor EB.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

ABSTRACT

Ceramide, sphingomyelin, and glycosphingolipids (both neutral and acidic) are characterized by the presence in the lipid moiety of an aliphatic base known as sphingosine. Altogether, they are sphingolipids particularly abundant in neuronal plasma membranes, where, via interactions with the other membrane lipids and membrane proteins, they play a specific role in modulating the cell signalling processes. The metabolic pathways determining the plasma membrane sphingolipid composition are thus the key point for functional changes of the cell properties. Unnatural changes of the neuronal properties are observed in sphingolipidoses, lysosomal storage diseases occurring when a lysosomal sphingolipid hydrolase is not working, leading to the accumulation of the substrate and to its distribution to all the cell membranes interacting with lysosomes. Moreover, secondary accumulation of sphingolipids is a common trait of other lysosomal storage diseases.

In this review article, we report on the sphingolipid chemical and biochemical properties and discuss some hypotheses about the possible links between sphingolipid incorrect catabolism and neurodegeneration.

INTRODUCTION

Sphingolipids are amphiphilic membrane lipids characterized by the presence of sphingosine, an amino-and-hydroxy alkylic long chain. After more than a century of studies, we now know their structure, their physico-chemical properties, their distribution and content in cells and tissues of many animal species, including humans (Sonnino *et al.* 2006). The final cellular site for sphingolipids is the plasma membrane, however their complex metabolic pathway requires many intracellular processes, so that a not negligible portion of them is also found intracellularly (Schulze *et al.* 2009, Kolter & Sandhoff 2010, Sandhoff & Kolter 2003, Hannun & Obeid 2018). As amphiphilic compounds they contain a hydrophilic head group, for example a saccharide, an oligosaccharide, a phosphocholine, protruding into the cell aqueous environment, and a hydrophobic chain called ceramide, in which the sphingosine is linked with a fatty acid via an amidic bond, inserted into the outer layer of plasma membrane.

Sphingolipids are typically asymmetric components of the plasma membranes enriched in the outer leaflet (van Meer 2011, van Meer & Hoetzl 2010, Fujimoto & Parmryd 2016), however it cannot be excluded that a minor pool of them could be transiently inserted into the inner layer with the hydrophilic group facing the cytosol. The hydrophilic group, thanks to the presence of carbohydrates and/or ionic charges, attracts water and ions, forms hydrogen bonds and easily interacts with the group of membrane components protruding from the membrane itself. At the water-lipid interface, the sphingolipid ceramide amide linkage accepts hydrogen bonds on the carbonyl group and donates hydrogen bonds via the N-H group, allowing the formation of a double linkage, which works as a padlock within molecules. The lipid moiety inserted into the lipid layer interacts with the lipid portion of the cholesterol in the membrane and with dipalmitoylphosphocholine, leading to the formation of membrane domains with reduced fluidity, now worldwide known as lipid rafts (Sonnino *et al.* 2006, Sonnino & Prinetti 2013, Sonnino *et al.* 2007). Most of the available information on the composition and organization of lipid rafts relies on the preparation and analysis of low-density, detergent-resistant membrane (DRM) fractions from cells, tissues or even entire organisms. The notion that lipid-driven segregation of membrane components could be a major mechanism for the creation of lateral order in biological membranes was supported by at least a decade of studies on model membranes, before

1
2
3 the formulation of the “lipid raft hypothesis” (van Meer *et al.* 1987). However, the method
4 published by Brown and Rose describing the isolation of a putative lipid raft fraction based
5 on its resistance to solubilization by Triton X-100 under well defined experimental
6 conditions (Brown & Rose 1992) has hallmarked an explosion of investigations using this
7 method to study the biological roles of lipid rafts. DRM are highly enriched in sphingolipids
8 and cholesterol, and relatively void of bulky membrane glycerolipids (for a detailed
9 analysis of the lipid composition of DRM prepared from primary cultured neurons, see
10 (Prinetti *et al.* 2001)). The method, despite its enormous popularity, has elicited as well
11 fierce opposition, based on some pieces of evidence indicating that the fraction prepared
12 with this method might be artifactual and not reflecting the real lipid raft structure as it
13 occurs in living cells (for a detailed discussion about this issue, see our reviews (Sonnino
14 & Prinetti 2008, Sonnino & Prinetti 2013)). The evolution of different technologies enabling
15 to probe the heterogeneous organization of cellular membranes suggested that
16 “membrane domains” (among those, lipid-driven domains or “lipid rafts”) are a multiple,
17 heterogeneous, highly dynamic entities, part of a complex organization hierarchy
18 (Bernardino de la Serna *et al.* 2016, Lu & Fairn 2018, Sezgin *et al.* 2017, Santos & Preta
19 2018). Isolated DRM fractions capture a non-dynamic situation representing the average
20 or sum of different situations. In this sense, DRM are indeed an artifact (as almost any kind
21 of biochemical preparation, defining an experimental item that is not reflecting the complex
22 reality of the *in vivo* counterpart). On the other hand, a few diverse techniques enabling to
23 observe organized domains in intact cell membranes (immune-electron microscopy under
24 conditions respecting the lipid integrity of the membrane (Fujita *et al.* 2007), stimulated
25 emission depletion nanoscopy (Iwabuchi *et al.* 2012, Eggeling *et al.* 2009) and
26 fluorescence correlation spectroscopy (Sezgin *et al.* 2012)) have demonstrated the
27 clustering and confinement of different glycosphingolipids at the plasma membrane level.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

44
45 Sphingolipids are ubiquitous components of mammal cell membranes, which are
46 particularly abundant in the nervous system, and, within the nervous system, in neurons
47 (Hansson *et al.* 1977, Schnaar 2016). Thus, sphingolipids are relatively abundant
48 components of the outer layer of neuronal plasma membranes making their concentration
49 very high in neuronal lipid rafts (Prinetti *et al.* 2001). Remarkably, membrane segregation
50 of sphingolipids in neurons seems to be higher than in any other cell type investigated so
51 far. Lipid rafts contain a few proteins, involved in cell signaling processes, whose
52 functional features are modulated through direct specific glycosphingolipid-protein
53
54
55
56
57
58
59
60

1
2
3 interactions or through a sphingolipid-dependent dynamic membrane organization (Prinetti
4 *et al.* 2009). As a matter of fact, several experimental approaches, which eventually lead to
5 alterations in the organization of the plasma membrane due to changes in sphingolipid
6 content or pattern, have been proven to be very effective in modulating cell functions.
7
8
9

10 METABOLISM OF SPHINGOLIPIDS

11 The sphingolipid metabolism combines several metabolic pathways, correlated with each
12 other and aimed at obtaining and maintaining a defined cell- and stage-specific cellular
13 content and plasma membrane pattern (Sandhoff & Kolter 2003, Yu *et al.* 2009, Furukawa
14 *et al.* 2014). Figure 2 shows a schematic representation of the complex sphingolipid
15 metabolic pathway.
16
17
18

19 Differentiated neurons display the more complex sphingolipid pattern within all mammalian
20 cells (Svennerholm *et al.* 1994). In these neurons, sphingomyelin is the most abundant
21 sphingolipid. Gangliosides are distributed, all in similar quantity, among mono-, di-, tri- and
22 tetra-sialo compounds of the ganglio-4, Gg₄, series. The sphingolipid pattern changes
23 during the differentiation of the nervous system and parallels the expression of the
24 sphingolipid synthases (Yu *et al.* 2009, Prinetti *et al.* 2001, Riboni *et al.* 1990).
25 Sphingolipids are present in low amounts in the first stages of differentiation, then their
26 levels increase progressively as the differentiation process proceeds, before reaching a
27 phase of the process where they remain constant for many years. This phase is then
28 followed by a decrease in the sphingolipid content in very aged people. During the stages
29 of neural tube formation and neural stem cells proliferation, the ganglioside pattern is
30 characterized by the mono- and disialogangliosides of the Lac series, GM3 and GD3.
31 Then, these gangliosides become very scarce, giving way to the more complex
32 gangliosides of the Gg₄ series (GM1, GD1a, GT1b and GQ1b) as neurogenesis and
33 astrocytogenesis proceed. These gangliosides reach their highest quantity with
34 synaptogenesis and axonal/dendritic arborization (Schnaar 2016). At the onset of terminal
35 differentiation, synthesis of galactosylceramide and sulfatide in the brain is switched on,
36 then remains constant in mature oligodendrocytes (Norton & Poduslo 1973).
37 Sphingomyelin is also very scarce at the beginning of differentiation growing then
38 hyperbolically and reaching the maximum level in fully differentiated cells. In differentiated
39 rat cerebellar granule neurons, sphingomyelin content is 1.00 nmol/10⁶ cells, while
40 gangliosides account for 0.79 nmol/10⁶ cells, with a total glycerophospholipid content of
41 32.84 nmol/10⁶ cells (Prinetti *et al.* 2001).
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 The sphingolipid content is strictly related to the synthesis and content of sphinganine,
4 which displays a C18/C20 sphinganine ratio progressively reduced along differentiation.
5 The C20 species can hardly be detected in the first stages of differentiation. This is due to
6 a different activity of the palmitoyl- and stearoylCoA acyl-serine transferases along
7 neuronal differentiation (Chigorno *et al.* 1997), which results in the slow but continuous
8 increase in the content of gangliosides that contain C20-sphingosine during neuronal
9 maturation and aging (Valsecchi *et al.* 1993, Sonnino & Chigorno 2000).
10
11
12
13
14

15
16 *De novo* biosynthesis of sphingolipids starts with the synthesis of ceramide in the
17 endoplasmic reticulum and proceeds in the Golgi apparatus (see reference (Hannun &
18 Obeid 2018) for details). Neobiosynthesized sphingolipids reach the plasma membranes
19 *via* vesicular transport, becoming thus components of the external leaflet (Sandhoff &
20 Kolter 2003, van Meer & Hoetzl 2010).
21
22

23
24 Ceramide follows two paths to reach the Golgi, *via* the soluble transporter protein CERT to
25 become sphingomyelin in the luminal site, and *via* vesicle transport to become
26 glucosylceramide by glucosyltransferase on the cytosolic side of the membrane (van Meer
27 & Lisman 2002). Glucosylceramide can be translocated to the luminal side of the Golgi
28 membrane becoming substrate for the lactosylceramide synthases (D'Angelo *et al.* 2007).
29 A part of glucosylceramide is directly transported by a specific protein from the membrane
30 cytosolic layer of Golgi to the plasma membrane inner layer and to the outer by a flippase
31 (Warnock *et al.* 1994).
32
33
34
35
36
37

38
39 On the other hand, the biosynthetic activity at Golgi level is not the only event influencing
40 the final sphingolipid pattern associated with the plasma membrane. Indeed, the plasma
41 membrane itself is an active site for metabolic remodeling of the local sphingolipids. Both
42 hydrolytic and biosynthetic sphingolipid enzymes, associated to the plasma membranes,
43 have *cis* and *trans* activities (Preti *et al.* 1980, Saito *et al.* 1995, Aureli *et al.* 2014a, Aureli
44 *et al.* 2014b, Aureli *et al.* 2012, Aureli *et al.* 2011, Sonnino *et al.* 2010). Plasma membrane-
45 associated sphingomyelinases, sialidases, hexosaminidase, galactosidase, glucosidases,
46 sphingomyelin synthase, sialyltransferase have been characterized and studied (see
47 references (Schnaar 2016, Hannun & Obeid 2018) for details). Hydrolysis of SM by PM-
48 associated acid or neutral SMases, and synthesis of SM from ceramide by SMS2 likely
49 play an important role in regulating the formation, at the plasma membrane, of ceramide-
50 enriched domains acting as signaling platforms (Zhang *et al.* 2009) or initiating vesiculation
51
52
53
54
55
56
57
58
59
60

1
2
3 processes (Holopainen *et al.* 2000). Regulation of PM ceramide levels, or more complex
4 modulation of glycosphingolipid patterns and glycosphingolipid-dependent signaling can
5 also be achieved through the action of PM-associated glycohydrolases or
6 glycosyltransferases (Sonnino *et al.* 2010). Hydrolase activities are likely modulated by the
7 activity of proton pumps able to determine local acidification in proximity of the lipid raft
8 microenvironment, to obtain optimum acidic pH required for the enzyme activity (Aureli *et*
9 *al.* 2012).

10
11
12
13
14 Glycosylation/deglycosylation is not the only opportunity for the local modulation of PM
15 glycosphingolipid composition. The local increase in proton concentration in proximity of
16 glycosphingolipid-enriched membrane domains determined by the activity of the proton
17 pumps/antiporters mentioned above favors the formation of ganglioside lactones, in
18 particular GD1b lactones. The lactonization process deeply influences the conformational,
19 aggregational and biological properties of gangliosides (Acquotti *et al.* 1987, Bassi *et al.*
20 1991), even if its biological relevance is still poorly understood. However, in human brain
21 the content of GD1b-lactone, that is very small in young subjects, significantly increases
22 with the aging process (Riboni *et al.* 1986).

23
24
25
26
27
28
29 A further opportunity for the remodeling of the PM sphingolipid pattern is represented by
30 the release of sphingolipids or sphingolipid-rich vesicles from the plasma membrane into
31 the extracellular environment (at least, in cultured cells (Chigorno *et al.* 2006)). On the
32 other hand, sphingolipids present in the extracellular milieu are taken up, at least partially,
33 by cells (not necessarily by the same cells from which they have been released),
34 remaining associated with the cell membranes.

35
36
37
38
39
40 While the enzymology of sphingolipid *de novo* biosynthesis and remodeling at the PM level
41 has been unveiled in many of its details, still relatively little is known about its regulation,
42 even if the regulation of the biosynthetic pathway has been regarded for a long time as the
43 main mechanism responsible for the formation of a cell- and stage-specific sphingolipid
44 pattern.

45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Sphingolipid synthesis is mainly regulated at the transcriptional level through the control of
the expression levels of ceramide synthases, sphingomyelin synthases,
glycosyltransferases or transporter proteins. Indeed, the changes in cellular
glycosphingolipid patterns occurring in neurons along neurogenesis, axonal arborisation
and synaptogenesis, as well as in oligodendrocytes along differentiation and myelin
formation, are nicely paralleled by changes in the expression of the corresponding

glycosyltransferases (Ngamukote *et al.* 2007, Yu *et al.* 2009), and apparently histone acetylation of the glycosyltransferase genes represent an important regulatory in brain (Suzuki *et al.* 2011, Itokazu *et al.* 2017). An important regulation mechanism for the reaction catalyzed by the serine-palmitoylCoA transferase, which likely represents the rate-limiting step in the biosynthetic pathway, is represented by an inhibitory feedback controlled in mammals by ORMDL proteins (Gupta *et al.* 2015, Siow & Wattenberg 2012). In addition, it has been recently suggested that ceramide synthase activity could be relevantly regulated at the protein level by phosphorylation (Wegner *et al.* 2016).

However, the possibility that differential intracellular flows of different sphingolipids could influence the resulting sphingolipid patterns (independently from the expression levels of synthases) should not be neglected. In other words, the regulation of intracellular sphingolipid traffic might be of equal importance as the control of synthetic enzymes expression in determining the final sphingolipid composition of the plasma membrane. From this point of view, it is worth to recall that the mechanism of glucosylceramide delivery to the ganglioside biosynthetic machinery in the Golgi is likely not mediated by the transport protein FAPP2. It is generally assumed to be due to a vesicular transport, however this crucial point in the biosynthetic pathway of gangliosides remains elusive.

The degradation of plasma membrane sphingolipids takes place in the lysosomes, that are reached by the endocytic vesicular flow through the early and late endosomal compartment (Kolter & Sandhoff 2005). Along their route to the lysosomes, the plasma membrane sphingolipids can be diverted to the Golgi apparatus where they undergo direct glycosylation with the formation of more complex products, able in turn to reach again the plasma membrane. It has been suggested that this process might be quantitatively relevant at least for certain cell types, including neurons (Riboni *et al.* 1994), thus representing potential mechanism for the regulation of plasma membrane sphingolipid pattern at the level of intracellular traffic.

After reaching reached the lysosomes, sphingolipids are catabolized to simple compounds (Kolter & Sandhoff 2005, Kolter & Sandhoff 2010), but some glycolipid intermediates escape from the lysosomes and can reach directly the Golgi entering into the biosynthetic pathway. This salvage pathways, related to gangliosides, in neurons are not negligible from the quantitative point of view (Riboni *et al.* 1996), but very little is known about the mechanisms of escape from the lysosome and the transfer of these intermediates to the Golgi or other cellular districts, nor on the regulation of these processes. The presence of

1
2
3 soluble ganglioside-protein complexes in the cytosol might reflect, at least in part, the
4 intracellular traffic linked to the recycle of these intermediates (Sonnino *et al.* 1979). On
5 the other hand, it is well known that lysosomes interact with many cellular organelles and
6 with the plasma membrane. Processes of membrane fusions could be responsible for the
7 shift of glycolipids from one cellular compartment to another (for details, see the following
8 section).
9
10
11
12
13

14 The final degradation products, sphingosine, fatty acids and sugars, leave the lysosomes
15 through specific membrane proteins and, in part, are recycled for the biosynthesis of new
16 sphingolipids (Sonderfeld *et al.* 1985, Tettamanti 2004, Riboni *et al.* 1996). In particular,
17 the recycling of sphingosine is very high and in some cells covers up to 80% the need of
18 the long chain base for sphingolipid biosynthesis (Riboni *et al.* 1999). However,
19 information on the intracellular trafficking of sphingosine, from lysosomes to the reticulum
20 is not available. Lysosome-originated sialic acid is also recycled at a great extent for the
21 biosynthesis of sialoglycoconjugates. Sialic acid escape from the lysosomes requires the
22 membrane protein sialin (Prolo *et al.* 2009). The lack of sialin leads to lysosomal
23 impairment and neurodegeneration in the pathology known as Salla's disease.
24
25
26
27
28
29

30 Sphingolipid lysosomal catabolism is not a simple process (Kolter & Sandhoff 2006, Kolter
31 & Sandhoff 2010). Sphingolipids are components of the external layer of plasma
32 membranes and upon membrane endocytosis they become components of the negatively
33 curved luminal layer of the limiting lysosomal membrane, which is covered by a thick and
34 complex glyocalix (Eskelinen *et al.* 2003). Thus, their accessibility to the lysosomal
35 hydrolytic enzymes and activator proteins would be limited, resulting in a slow and difficult
36 hydrolytic process. Small endo-lysosomal vesicles characterized by a high content of
37 bis(monoacylglycero)phosphate, with the sphingolipids in the outer layer facing the lysosol
38 with a topology more suitable to the enzymatic hydrolysis, are rapidly formed in the late
39 lysosomes (Schwarzmann *et al.* 2015). Thus, both lysosomal membrane sphingolipids and
40 endo-lysosomal vesicle membrane sphingolipids can be found in the lysosomes. The
41 implications of lysosome fusion with the PM will be discussed in the next section.
42
43
44
45
46
47
48
49
50

51 According to this scenario, it is clear that the sphingolipid plasma membrane composition
52 can be easily modified by different metabolic mechanisms. This is very relevant from the
53 functional point of view, considering the ability of sphingolipids to modulate the biological
54
55
56
57
58
59
60

1
2
3 functions of PM-associated proteins, the membrane lateral organization (Prinetti et al.
4 2009).

5
6 However, the processes of neobiosynthesis and subsequent transport to the plasma
7 membrane of the final compounds seem to require too much time to be used to modify the
8 plasma membrane sphingolipid concentration and pattern in response to intra- and
9 extracellular signals. In addition, it has been reported that the glycosphingolipid half life is
10 short in neurons (Riboni et al. 1990, Prinetti *et al.* 2000). On the other hand, as discussed
11 above, changes of sphingolipid pattern could be rapidly achieved upon activation of
12 plasma membrane-associated enzymes as a consequence of ligand-triggered interactions,
13 changes in their membrane lipid microenvironment or local proton concentration.
14 Nevertheless, great care must be devoted also to the intracellular sphingolipid trafficking
15 and to the recycling of partially degraded sphingolipids, which implies an important role of
16 lysosomal activity.

17
18 Any derailment from the correct metabolic/traffic process can lead to an abnormal
19 sphingolipid composition of the plasma membrane and, consequently, to an abnormal cell
20 function modulation.
21
22

23 24 25 26 27 28 29 30 SPHINGOLIPIDS AND LYSOSOMAL STORAGE DISORDERS (LSDs).

31
32 Lysosomal storage disorders are a wide group of genetic diseases due to reduced or
33 absent enzymatic activity of catabolic lysosomal enzymes.

34
35 Altogether, more than 70 different LSDs are known, with an incidence of 1:200,000 - 1:
36 350,000 each. This makes the overall LSDs incidence around 1:5,000. Within LSDs, we
37 count several sphingolipidoses due to genetic anomalies related to either the sphingolipid
38 hydrolytic enzyme or the activator protein necessary for the enzyme activity.
39 Glycohydrolases are exo-enzymes, and the incorrect catabolic process leads to the
40 cellular primary accumulation of the enzyme substrate followed by a secondary
41 accumulation of compounds likely due to lysosomal impairment (Fuller & Futerman 2018).
42 In fact, secondary cell sphingolipid accumulation occurs probably in any cell with badly
43 working lysosomes (see Table 1).
44
45

46
47 The main site of intracellular degradation of plasma membrane SL is represented by intra-
48 endolysosomal vesicles (LVs) inside the late endosomal and lysosomal compartment,
49 which are formed by an inward budding of the limiting endosomal membrane. Indirect
50 evidence suggests that SL are sorted from the limiting lysosomal membrane to the intra-
51 endolysosomal membranes: in fact, gangliosides artificially incorporated into the limiting
52 lysosomal membrane are protected from degradation, while on the other hand they are
53
54
55
56
57
58
59
60

1 promptly degraded at the lysosomal level upon normal plasma membrane turnover by
2 endocytosis (Henning & Stoffel 1973). However, it is not known whether a certain amount
3 of SL, after reaching the lysosome, remain associated with the limiting lysosomal
4 membrane, thus escaping degradation. In rat liver, GM1 and GD1a can be formed by
5 direct glycosylation of intravenously administered GM2 (Trinchera & Ghidoni 1990). In
6 cultured neuroblastoma cells and neurons, exogenously administered GM1 can be
7 converted into GD1a and GD1a into GT1b in the presence of chloroquine (Tettamanti
8 2004). These findings strongly suggest that a significant pool of ganglioside can escape
9 lysosomal degradation and reach the Golgi apparatus, possibly by avoiding the sorting to
10 degradation-prone LVs and remaining confined to the limiting lysosomal membrane. It is
11 reasonable to expect that the magnitude of this pool might increase when for any reason
12 sphingolipids are accumulated at the lysosomal level. An indirect support to this
13 hypothesis comes from the finding that SM levels at the plasma membrane (as judged by
14 lysenin staining) are 3.8 fold higher in cultured hippocampal ASM knock-out vs. wild type
15 mice (Ledesma *et al.* 2011, Galvan *et al.* 2008).

16 The complexity of LSD, at the phenotypic and molecular level, particularly considering the
17 aspects related to neuronal dysfunctions, clearly indicates that lysosomal impairment by
18 itself might not necessarily be the central event in the pathogenesis of these diseases.
19 Considering the importance of sphingolipid-dependent signal transduction and of
20 sphingolipid-driven membrane organization (Santos & Preta 2018) in the physiology of
21 neural cells, the obvious question is if and how primary or secondary accumulation of
22 undegraded sphingolipids at the endosomal/lysosomal level could affect the
23 composition/organization of the plasma membrane and of intracellular membranes limiting
24 different organelles, with consequences on the pathology at the level of the nervous
25 system.

26 Endosome/lysosomes can fuse with the plasma membrane. The exocytosis of
27 multivesicular endosomes is likely the main mechanism leading to the release of
28 exosomes, and has been extensively studied under this perspective (Harding *et al.* 2013),
29 while the contribution of this event to a possible remodeling of the PM composition has
30 received much less attention. On the other hand, it has been shown that lysosomes can
31 fuse with the PM in response to the increase in intracellular Ca^{2+} , behaving as ubiquitous
32 Ca^{2+} -regulated secretory vesicles (Rodriguez *et al.* 1997). Ca^{2+} -regulated exocytosis of
33 lysosomes has been proposed as a mechanism for restoring the PM integrity after injury
34 (Reddy *et al.* 2001) or for the insertion of pore-forming proteins such as certain bacterial
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 toxins (Andrews *et al.* 2014). This process has been shown in several cellular models;
4 however its relevance *in vivo* is still not confirmed, since only a few studies in tissues are
5 available (Cheng *et al.* 2015, Jimenez & Perez 2017, Krause *et al.* 1994). The Ca^{2+} -
6 sensing lysosomal protein synaptotagmin VII seems the crucial player for the regulation of
7 the fusion of lysosomes with the PM upon influx of extracellular Ca^{2+} as a consequence of
8 the loss of PM integrity (Reddy *et al.* 2001). In addition, PM repair by lysosomal fusion
9 requires the rapid mobilization of lysosomes from the perinuclear region to the proximity of
10 PM along microtubules via kinesin motors, or the transport of lysosomes, already present
11 in the proximity of the PM, to the PM itself via actin filaments, events regulated by small
12 GTPases, including Rab3a (Encarnacao *et al.* 2016) and Arl8b (Michelet *et al.* 2018). The
13 exact mechanism by which lysosomal exocytosis promotes PM repair is still not clearly
14 understood. The fusion could give origin to an intracellular “membrane patch” which could
15 directly fuse with the wounded membrane leading to PM resealing. However, it is quite
16 difficult to imagine a direct merge of the intracellular patch with the irregular wound. An
17 alternative explanation could be the spontaneous resealing of the bilayer due to the
18 reduction in membrane tension consequent to the exocytic process. A third, more complex
19 possibility implies the enzymatic remodeling of the PM lipid composition as a consequence
20 of the lysosomal fusion (reviewed in (Andrews *et al.* 2014)). Lysosomal acid
21 sphingomyelinase (ASM) is secreted in the extracellular space or translocated to the cell
22 surface upon membrane-damaging conditions. The hydrolytic activity of ASM on SM
23 present in the outer leaflet of the PM can lead to the production of ceramide and of
24 ceramide-enriched microdomains, triggering membrane dynamic remodeling and
25 membrane invagination with consequent endocytosis, which in turn contributes to the
26 removal of the lesion PM area. Lesion removal by ASM-regulated endocytosis would
27 explain why Ca^{2+} -dependent lysosomal exocytosis, also repairs PM lesions caused by the
28 insertion of pore-forming proteins. Besides ASM, other lysosomal hydrolytic enzymes
29 acting on glycosphingolipids (β -hexosaminidases, sialidases, β -galactosidases, β -
30 glucocerebrosidases) (Aureli *et al.* 2014a) can reach the PM after lysosomal exocytosis,
31 potentially leading to the local production of ceramide or to a remodeling of the
32 glycosphingolipid pattern of lipid raft/caveolar membrane domains. Indeed, glycolipid
33 remodeling in caveolar domains could play a relevant role in this scenario, since caveolar
34 endocytosis significantly contributes to PM lesion removal, as demonstrated by the
35 observation that depletion of caveolin-1 by siRNA inhibited PM repair (Corrotte *et al.*
36 2013).
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 All these proposed mechanisms imply the traffic of lysosomal components (lipids and
4 enzymes associated with the limiting lysosomal membrane or soluble material present in
5 the lysosomal lumen) to the cell surface. When considering the situation of LSD, this has
6 profound implications that could explain the diversity of PM-generated alterations observed
7 in these diseases (such as, merely as example, the ectopic dendritogenesis reported in
8 GM2 gangliosidoses (Purpura & Suzuki 1976), as well as in α -mannosidosis (Siegel &
9 Walkley 1994) and MPS type I (Walkley 2003), where secondary storage of GM2 is
10 present). Most genes involved in lysosomal biogenesis and in lysosomal functions
11 (including autophagy and lysosomal exocytosis) are under the control of the transcription
12 factor EB (TFEB). The aberrant storage of uncatabolized material, common to all LSDs,
13 induces the dephosphorylation of TFEB and its translocation to the nucleus, with the
14 consequent activation of its target genes, leading to increased lysosomal biogenesis (a
15 possible compensatory mechanism for the loss of lysosome functionality) (Sardiello *et al.*
16 2009). TFEB overexpression was sufficient to activate several key lysosomal genes and to
17 induce lysosomal biogenesis. TFEB pathway was activated upon lysosomal storage of
18 sucrose, a useful experimental model of lysosomal impairment, and TFEB activation was
19 demonstrated in fibroblasts from mouse models of three different LSDs,
20 Mucopolysaccharidoses types II (Muenzer *et al.* 2002) and IIIA (Hemsley & Hopwood
21 2005) (MPSII and MPSIIIA) and Multiple Sulfatase Deficiency (Settembre *et al.* 2007)
22 (MSD). In addition, increased lysosomal biogenesis (even if not necessarily linked only to
23 TFEB activation) was shown in several others LSDs or in their animal models
24 (summarized in Table 2). Our group recently showed that, in sucrose-loaded human
25 fibroblasts, reduced lysosomal activity led to the accumulation of undegraded sphingolipids
26 both intracellularly and at the PM level, with consequent arrest of cell proliferation.
27 Remarkably, this was accompanied by increased lysosomal biogenesis and lysosome
28 fusion with the PM (Samarani *et al.* 2018). Obviously, increased lysosomal biogenesis in
29 principle favors the traffic of lysosomal components to the cell surface with the above-
30 described mechanisms implying lysosomal fusion with the membrane. This would explain
31 the contribution of PM alterations to the pathogenesis of LSDs.

32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50 In addition to lysosome-mediated vesicular transport of lipids and lipid-specific enzymes,
51 another form of lipid traffic has received much attention in recent times, and could
52 contribute to alterations of lipid composition outside the lysosome in LSD, involving not
53 only the PM but also intracellular membranes. It is becoming evident that intracellular
54 organelles are physically and functionally interconnected by a complex network of contact
55
56
57
58
59
60

1
2
3 sites between the membranes of different organelles (Membrane Contact Sites, MCSs)
4 (reviewed in (Helle *et al.* 2013)). The endoplasmic reticulum (ER) establishes MCSs with
5 almost all other cellular membranes, including the PM, lysosomes, early endosomes,
6 endolysosomes and autophagic vacuoles, even if the best studied MCS is represented by
7 the mitochondria-associated ER membranes (MAMs).
8
9

10 In additions, MCSs have been detected between lysosomes and mitochondria, lysosomes
11 and the nuclear envelope, mitochondria and the Golgi membranes. The molecular
12 organization of MCSs is complex and not fully understood, however it requires the
13 tethering of both membrane protein complexes and lipids (likely organized as specialized
14 lipid rafts) allowing to maintain the juxtaposed membranes in strict contact without
15 undergoing fusion. Even if fusion does not occur, active exchange of lipids seems to take
16 place, and one of the main supposed functions of MCSs seems the interorganelle lipid
17 exchange. Since mitochondria are disconnected from vesicular traffic, exchange of lipids
18 between ER and mitochondria at MAMs is likely crucial for the correct shaping of
19 mitochondrial phospholipid pattern. Likely, lipid transport at MCSs involves lipid transport
20 proteins able to extract lipids from a membrane and deliver it to the adjacent one. In the
21 case of sphingolipids, at least two of such proteins, CERT (Kudo *et al.* 2008) and CLN8
22 (Winter & Ponting 2002), have been implicated as possible transporters of ceramide and
23 other sphingolipids at MCSs. Topology of sphingolipids requires further care and according
24 to their localization to one of the two membrane layers, together with the lipid transport
25 proteins, flippase could be also necessary, this making the process of their transport at
26 MCSs further complex. To date, still little is known about the contribution of altered lipid
27 traffic at MCSs in LSDs. The best-studied example is GM1-gangliosidosis (reviewed in
28 (Annunziata *et al.* 2018)). In the β -Gal^{-/-} mice, the animal model of GM1-gangliosidosis,
29 GM1 is massively accumulated in brain, and in particular in neurons, which undergo
30 significant degeneration and death followed by micro- and astrogliosis. In neurons from β -
31 Gal^{-/-} mice brain, GM1 accumulation at the lysosomal level is accompanied by its abnormal
32 redistribution from the lysosomes and the PM to the ER, where it accumulates prevalently
33 at the level of MAMs. Increased GM1 levels in MAMs lipid rafts trigger a complex series of
34 events leading to the formation and activation of a mitochondrial Ca²⁺ pore, and ultimately
35 to the activation of the mitochondrial intrinsic apoptotic pathway. Interestingly, it has been
36 shown that, on the other hand, changes in the structure of mitochondrial lipid rafts can play
37 roles in the protection from neurotoxic stimuli (Garofalo *et al.* 2018).
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Taken together, all these pieces of evidence suggest that the hypothesis we proposed,
4 that “the blockade of proper SL catabolism at the lysosomal level leads to the jamming of
5 the overall flow of metabolites, with consequences on the SL composition in all cellular
6 districts” (Piccinini *et al.* 2010) is still valid, however the “jamming” is likely much more
7 complex than we would have expected, and involves highly specific events. Accumulation
8 of SL in lysosomes in LSDs, either primary or secondary, eventually leads to a
9 redistribution of SL in other subcellular districts. Considering the incredible complexity of
10 the brain lipidome, the diversity of biological functions of different SL in different brain cell
11 populations, and the variety of mechanisms potentially driving SL from lysosomes to other
12 cellular membranes, with consequences of their organization, it is not surprising that
13 neurological manifestations encompass a wide spectrum in LSDs. Addressing this
14 diversity is one of the challenges of the basic research in this field.
15
16
17
18
19
20
21
22
23
24
25
26
27

28 ACKNOWLEDGEMENTS AND CONFLICT OF INTEREST DISCLOSURE

29
30 AP is an editor for the Journal of Neurochemistry and Council Member of the International
31 Society for Neurochemistry.
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Primary or secondary accumulation of (glyco)sphingolipids in LSDs ((Ballabio & Gieselmann 2009, Walkley & Vanier 2009, Hulkova *et al.* 2005, Piccinini *et al.* 2010) and references herein)

Disease	Primary biochemical defect	Primary storage material	Secondary GSL storage
<i>Mucopolysaccharidoses (MPS)</i>			
MPS I (Hurler and related)	α -iduronidase	dermatan sulphate, heparan sulphate	GM2, GM3
MPS II (Hunter)	iduronate-2-sulphatase	dermatan sulphate, heparan sulphate	GM2, GM3, LacCer
MPS IIIA (Sanfilippo)	heparan-N-sulphatase	heparan sulphate	GM2, GM3, GD2, LacCer
MPS IIIB (Sanfilippo)	N-acetyl- β -glucosaminidase	heparan sulphate	GM2, GM3, GD2
MPS IIIC (Sanfilippo)	acetylCoA: α -glucosamide N-acetyltransferase	heparan sulphate	GM2, GM3, GD2
MPS IIID (Sanfilippo)	N-acetylglucosamine-6-sulphatase	heparan sulphate	GM2, GM3, GD2
MPS VI (Maroteaux-Lamy)	arylsulphatase B	dermatan sulphate	GM2, GM3
MPS VII (Sly)	β -glucuronidase	heparan sulphate, dermatan sulphate, chondroitin sulphates	GM2, GM3
<i>Sphingolipidoses</i>			
Fabry	α -galactosidase A	Gb4Cer, Gb4-sphingosine	
Gaucher	β -glucosidase	GlcCer, GM1, GM2, GM3, GD3, Glc-sphingosine	
Globoid cell leukodystrophy (Krabbe)	galactocerebroside β -galactosidase	GalCer, Gal-sphingosine	LacCer, Gb3Cer, Gb4Cer,
Metachromatic leukodystrophy	arylsulphatase A	Sulfatide and other sulfolipids	GM2
Niemann-Pick A and B	sphingomyelinase	sphingomyelin	GM2, GM3, GlcCer, LacCer, Gb3Cer, Gb4Cer
GM1 gangliosidosis	β -galactosidase	GM1, GA1, lyso-GM1	GlcCer, LacCer, GM2, GM3, GD1a,
GM2 gangliosidosis (Tay-Sachs)	β -hexosaminidase A	GM2, GalNAcGD1a, GA2, lyso-GM2	
GM2 gangliosidosis (Sandhoff)	β -hexosaminidase A and B	GM2, GalNAcGD1a, globoside, GA2, lyso-GM2	
Salla	Membrane carrier mediating the egress of sialic acid from lysosomes		gangliosides
<i>Others</i>			
Niemann-Pick C	NPC1 and 2	cholesterol	LacCer
α -mannosidosis	α -mannosidase	mannose-containing oligosaccharides	GM2, GM3
Glycogenosis type II (Pompe)	α -glucosidase	glycogen	LacCer

Table 2. LSDs with increased lysosomal biogenesis

LSDs	References
MPSI	(Ohmi <i>et al.</i> 2003)
MPSII	(Muenzer <i>et al.</i> 2002, Karageorgos <i>et al.</i> 1997)
MPSIIIA	(Hemsley & Hopwood 2005)
MPSIIIB	(Ohmi <i>et al.</i> 2003)
MPSVI	(Karageorgos <i>et al.</i> 1997)
MSD	(Settembre <i>et al.</i> 2007)
MLIV	(Venugopal <i>et al.</i> 2009)
Neuronal ceroid lipofuscinosis 3	(Pohl <i>et al.</i> 2007)
I-cell disease	(Kawashima <i>et al.</i> 2007)
Fabry	(Pereira <i>et al.</i> 2014)
Niemann-Pick A	(Gabande-Rodriguez <i>et al.</i> 2014)
Niemann-Pick C	(Kwiatkowska <i>et al.</i> 2014)
Sialidosis	(Wu <i>et al.</i> 2010)
Salla	(Karageorgos <i>et al.</i> 1997)
Fucosidosis	(Kondagari <i>et al.</i> 2015)
Pompe	(Karageorgos <i>et al.</i> 1997)

1
2
3
4 LEGEND TO FIGURES
5
6

7 **Figure 1.** Comparison of the volume occupied by ceramide and by the hydrophilic head groups of selected
8 sphingolipids.
9

10 Minimum energy structure of ceramide, of phosphocholine (head group of sphingomyelin), galactose (head
11 group of galactosylceramide, cerebroside), $\text{II}^3\text{Neu5Ac-Gg}_4$ (head group of ganglioside GM1),
12 $\text{IV}^3\text{Neu5Ac,II}^3\text{Neu5Ac}_2\text{-Gg}_4$ (head group of ganglioside GT1b) and $\text{IV}^4\text{GalNAc,IV}^3\text{Neu5Ac,II}^3\text{Neu5Ac-Gg}_4$
13 (head group of ganglioside GalNAc-GD1a). Oligosaccharide structures are aligned on the first sugar.
14
15
16
17

18 **Figure 2.** Metabolic pathways of sphingolipids. Representation of the main processes determining the
19 membrane sphingolipid composition.
20

21 Ceramide is synthesized in the ER, transported to the Golgi where it is converted into SM and
22 glycosphingolipids. Sphingolipids reach the plasma membrane by vesicular fusion and in very minor part
23 transported by specific soluble proteins. Cell surface local structural changes can occur due to
24 lactonization and to the enzymatic remodeling by cell surface associated hydrolases or synthases.
25 Shedding of sphingolipids (Chigorno *et al.* 2005) as well as their uptake by the membranes were described
26 (Saqr *et al.* 1993). The sphingolipid catabolism mainly occurs in lysosomes, from which sphingosine and
27 some sugars can escape to be recycled (Ghidoni *et al.* 1987). Some primary and secondary lysosomes
28 fuse with other membranes or exchange their membrane components (Trinchera *et al.* 1990).
29
30
31
32
33
34

35 REFERENCES
36

- 37 Acquotti, D., Fronza, G., Riboni, L., Sonnino, S. and Tettamanti, G. (1987) Ganglioside Lactones - H-1-Nmr
38 Determination of the Inner Ester Position of Gd1b-Ganglioside Lactone Naturally-Occurring in Human-Brain
39 or Produced by Chemical Synthesis. *Glycoconjugate J*, **4**, 119-127.
40 Andrews, N. W., Almeida, P. E. and Corrotte, M. (2014) Damage control: cellular mechanisms of plasma membrane
41 repair. *Trends in cell biology*, **24**, 734-742.
42 Annunziata, I., Sano, R. and d'Azzo, A. (2018) Mitochondria-associated ER membranes (MAMs) and lysosomal
43 storage diseases. *Cell death & disease*, **9**, 328.
44 Aureli, M., Loberto, N., Bassi, R., Ferraretto, A., Perego, S., Lanteri, P., Chigorno, V., Sonnino, S. and Prinetti, A.
45 (2012) Plasma membrane-associated glycohydrolases activation by extracellular acidification due to proton
46 exchangers. *Neurochemical research*, **37**, 1296-1307.
47 Aureli, M., Loberto, N., Lanteri, P., Chigorno, V., Prinetti, A. and Sonnino, S. (2011) Cell surface sphingolipid
48 glycohydrolases in neuronal differentiation and aging in culture. *Journal of neurochemistry*, **116**, 891-899.
49 Aureli, M., Samarani, M., Loberto, N., Bassi, R., Murdica, V., Prioni, S., Prinetti, A. and Sonnino, S. (2014a) The
50 glycosphingolipid hydrolases in the central nervous system. *Molecular neurobiology*, **50**, 76-87.
51 Aureli, M., Samarani, M., Murdica, V., Mauri, L., Loberto, N., Bassi, R., Prinetti, A. and Sonnino, S. (2014b)
52 Gangliosides and cell surface ganglioside glycohydrolases in the nervous system. *Advances in neurobiology*,
53 **9**, 223-244.
54 Ballabio, A. and Gieselmann, V. (2009) Lysosomal disorders: from storage to cellular damage. *Biochimica et*
55 *biophysica acta*, **1793**, 684-696.
56 Bassi, R., Chigorno, V., Fiorilli, A., Sonnino, S. and Tettamanti, G. (1991) Exogenous gangliosides GD1b and GD1b-
57 lactone, stably associated to rat brain P2 subcellular fraction, modulate differently the process of protein
58 phosphorylation. *Journal of neurochemistry*, **57**, 1207-1211.
59 Bernardino de la Serna, J., Schutz, G. J., Eggeling, C. and Cebecauer, M. (2016) There Is No Simple Model of the
60 Plasma Membrane Organization. *Frontiers in cell and developmental biology*, **4**, 106.

- 1
2 Brown, D. A. and Rose, J. K. (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains
3 during transport to the apical cell surface. *Cell*, **68**, 533-544.
- 4 Cheng, X., Zhang, X., Yu, L. and Xu, H. (2015) Calcium signaling in membrane repair. *Seminars in cell &*
5 *developmental biology*, **45**, 24-31.
- 6 Chester, M. A. (1998) IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of
7 glycolipids--recommendations 1997. *European journal of biochemistry*, **257**, 293-298.
- 8 Chigorno, V., Giannotta, C., Ottico, E., Sciannamblo, M., Mikulak, J., Prinetti, A. and Sonnino, S. (2005) Sphingolipid
9 uptake by cultured cells: complex aggregates of cell sphingolipids with serum proteins and lipoproteins are
10 rapidly catabolized. *The Journal of biological chemistry*, **280**, 2668-2675.
- 11 Chigorno, V., Negroni, E., Nicolini, M. and Sonnino, S. (1997) Activity of 3-ketosphinganine synthase during
12 differentiation and aging of neuronal cells in culture. *J Lipid Res*, **38**, 1163-1169.
- 13 Chigorno, V., Sciannamblo, M., Mikulak, J., Prinetti, A. and Sonnino, S. (2006) Efflux of sphingolipids metabolically
14 labeled with [1-3H]sphingosine, L-[3- 3H]serine and [9,10- 3H]palmitic acid from normal cells in culture.
15 *Glycoconj J*, **23**, 159-165.
- 16 Corrotte, M., Almeida, P. E., Tam, C. et al. (2013) Caveolae internalization repairs wounded cells and muscle fibers.
17 *eLife*, **2**, e00926.
- 18 D'Angelo, G., Polishchuk, E., Di Tullio, G. et al. (2007) Glycosphingolipid synthesis requires FAPP2 transfer of
19 glucosylceramide. *Nature*, **449**, 62-67.
- 20 Eggeling, C., Ringemann, C., Medda, R. et al. (2009) Direct observation of the nanoscale dynamics of membrane
21 lipids in a living cell. *Nature*, **457**, 1159-1162.
- 22 Encarnacao, M., Espada, L., Escrevente, C. et al. (2016) A Rab3a-dependent complex essential for lysosome
23 positioning and plasma membrane repair. *Journal of Cell Biology*, **213**, 631-640.
- 24 Eskelinen, E. L., Tanaka, Y. and Saftig, P. (2003) At the acidic edge: emerging functions for lysosomal membrane
25 proteins. *Trends in cell biology*, **13**, 137-145.
- 26 Fujimoto, T. and Parmryd, I. (2016) Interleaflet Coupling, Pinning, and Leaflet Asymmetry-Major Players in Plasma
27 Membrane Nanodomain Formation. *Frontiers in cell and developmental biology*, **4**, 155.
- 28 Fujita, A., Cheng, J., Hirakawa, M., Furukawa, K., Kusunoki, S. and Fujimoto, T. (2007) Gangliosides GM1 and GM3
29 in the living cell membrane form clusters susceptible to cholesterol depletion and chilling. *Molecular biology*
30 *of the cell*, **18**, 2112-2122.
- 31 Fuller, M. and Futerman, A. H. (2018) The brain lipidome in neurodegenerative lysosomal storage disorders.
32 *Biochemical and biophysical research communications*.
- 33 Furukawa, K., Ohmi, Y., Ohkawa, Y., Tajima, O. and Furukawa, K. (2014) Glycosphingolipids in the regulation of the
34 nervous system. *Advances in neurobiology*, **9**, 307-320.
- 35 Gabande-Rodriguez, E., Boya, P., Labrador, V., Dotti, C. G. and Ledesma, M. D. (2014) High sphingomyelin levels
36 induce lysosomal damage and autophagy dysfunction in Niemann Pick disease type A. *Cell death and*
37 *differentiation*, **21**, 864-875.
- 38 Galvan, C., Camoletto, P. G., Cristofani, F., Van Veldhoven, P. P. and Ledesma, M. D. (2008) Anomalous surface
39 distribution of glycosyl phosphatidyl inositol-anchored proteins in neurons lacking acid sphingomyelinase.
40 *Molecular biology of the cell*, **19**, 509-522.
- 41 Garofalo, T., Ferri, A., Sorice, M., Azmoon, P., Grasso, M., Mattei, V., Capozzi, A., Manganelli, V. and Misasi, R.
42 (2018) Neuroglobin overexpression plays a pivotal role in neuroprotection through mitochondrial raft-like
43 microdomains in neuroblastoma SK-N-BE2 cells. *Molecular and cellular neurosciences*, **88**, 167-176.
- 44 Ghidoni, R., Trinchera, M., Sonnino, S., Chigorno, V. and Tettamanti, G. (1987) The sialic acid residue of exogenous
45 GM1 ganglioside is recycled for biosynthesis of sialoglycoconjugates in rat liver. *The Biochemical journal*,
46 **247**, 157-164.
- 47 Gupta, S. D., Gable, K., Alexaki, A., Chandris, P., Proia, R. L., Dunn, T. M. and Harmon, J. M. (2015) Expression of
48 the ORMDLS, modulators of serine palmitoyltransferase, is regulated by sphingolipids in mammalian cells.
49 *The Journal of biological chemistry*, **290**, 90-98.
- 50 Hannun, Y. A. and Obeid, L. M. (2018) Sphingolipids and their metabolism in physiology and disease. *Nature*
51 *reviews. Molecular cell biology*, **19**, 175-191.
- 52 Hansson, H. A., Holmgren, J. and Svennerholm, L. (1977) Ultrastructural localization of cell membrane GM1
53 ganglioside by cholera toxin. *Proceedings of the National Academy of Sciences of the United States of*
54 *America*, **74**, 3782-3786.
- 55 Harding, C. V., Heuser, J. E. and Stahl, P. D. (2013) Exosomes: looking back three decades and into the future. *The*
56 *Journal of cell biology*, **200**, 367-371.
- 57 Helle, S. C., Kanfer, G., Kolar, K., Lang, A., Michel, A. H. and Kornmann, B. (2013) Organization and function of
58 membrane contact sites. *Biochimica et biophysica acta*, **1833**, 2526-2541.
- 59 Hemsley, K. M. and Hopwood, J. J. (2005) Development of motor deficits in a murine model of
60 mucopolysaccharidosis type IIIA (MPS-III A). *Behavioural brain research*, **158**, 191-199.
- Henning, R. and Stoffel, W. (1973) Glycosphingolipids in lysosomal membranes. *Hoppe-Seyler's Zeitschrift fur*

- 1 *physiologische Chemie*, **354**, 760-770.
- 2
- 3 Holopainen, J. M., Angelova, M. I. and Kinnunen, P. K. (2000) Vectorial budding of vesicles by asymmetrical
4 enzymatic formation of ceramide in giant liposomes. *Biophysical Journal*, **78**, 830-838.
- 5 Hulkova, H., Ledvinova, J., Asfaw, B., Koubek, K., Kopriva, K. and Elleder, M. (2005) Lactosylceramide in lysosomal
6 storage disorders: a comparative immunohistochemical and biochemical study. *Virchows Archiv : an*
7 *international journal of pathology*, **447**, 31-44.
- 8 Itokazu, Y., Tsai, Y. T. and Yu, R. K. (2017) Epigenetic regulation of ganglioside expression in neural stem cells and
9 neuronal cells. *Glycoconj J*, **34**, 749-756.
- 10 Iwabuchi, K., Nakayama, H., Masuda, H., Kina, K., Ogawa, H. and Takamori, K. (2012) Membrane microdomains in
11 immunity: glycosphingolipid-enriched domain-mediated innate immune responses. *BioFactors*, **38**, 275-283.
- 12 Jimenez, A. J. and Perez, F. (2017) Plasma membrane repair: the adaptable cell life-insurance. *Current opinion in*
13 *cell biology*, **47**, 99-107.
- 14 Karageorgos, L. E., Isaac, E. L., Brooks, D. A., Ravenscroft, E. M., Davey, R., Hopwood, J. J. and Meikle, P. J.
15 (1997) Lysosomal biogenesis in lysosomal storage disorders. *Experimental cell research*, **234**, 85-97.
- 16 Kawashima, I., Ohsawa, M., Fukushige, T. et al. (2007) Cytochemical analysis of storage materials in cultured skin
17 fibroblasts from patients with I-cell disease. *Clinica chimica acta; international journal of clinical chemistry*,
18 **378**, 142-146.
- 19 Kolter, T. and Sandhoff, K. (2005) Principles of lysosomal membrane digestion: stimulation of sphingolipid
20 degradation by sphingolipid activator proteins and anionic lysosomal lipids. *Annual review of cell and*
21 *developmental biology*, **21**, 81-103.
- 22 Kolter, T. and Sandhoff, K. (2006) Sphingolipid metabolism diseases. *Biochimica et biophysica acta*, **1758**, 2057-
23 2079.
- 24 Kolter, T. and Sandhoff, K. (2010) Lysosomal degradation of membrane lipids. *FEBS letters*, **584**, 1700-1712.
- 25 Kondagari, G. S., Fletcher, J. L., Cruz, R., Williamson, P., Hopwood, J. J. and Taylor, R. M. (2015) The effects of
26 intracisternal enzyme replacement versus sham treatment on central neuropathology in preclinical canine
27 fucosidosis. *Orphanet journal of rare diseases*, **10**, 143.
- 28 Krause, T. L., Fishman, H. M., Ballinger, M. L. and Bittner, G. D. (1994) Extent and mechanism of sealing in
29 transected giant axons of squid and earthworms. *The Journal of neuroscience : the official journal of the*
30 *Society for Neuroscience*, **14**, 6638-6651.
- 31 Kudo, N., Kumagai, K., Tomishige, N., Yamaji, T., Wakatsuki, S., Nishijima, M., Hanada, K. and Kato, R. (2008)
32 Structural basis for specific lipid recognition by CERT responsible for nonvesicular trafficking of ceramide.
33 *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 488-493.
- 34 Kwiatkowska, K., Marszalek-Sadowska, E., Traczyk, G., Koprowski, P., Musielak, M., Lugowska, A., Kulma, M.,
35 Grzelczyk, A. and Sobota, A. (2014) Visualization of cholesterol deposits in lysosomes of Niemann-Pick type
36 C fibroblasts using recombinant perfringolysin O. *Orphanet journal of rare diseases*, **9**, 64.
- 37 Ledesma, M. D., Prinetti, A., Sonnino, S. and Schuchman, E. H. (2011) Brain pathology in Niemann Pick disease
38 type A: insights from the acid sphingomyelinase knockout mice. *Journal of neurochemistry*, **116**, 779-788.
- 39 Lu, S. M. and Fairn, G. D. (2018) Mesoscale organization of domains in the plasma membrane - beyond the lipid
40 raft. *Critical reviews in biochemistry and molecular biology*, **53**, 192-207.
- 41 Michelet, X., Tuli, A., Gan, H., Geadas, C., Sharma, M., Remold, H. G. and Brenner, M. B. (2018) Lysosome-
42 Mediated Plasma Membrane Repair Is Dependent on the Small GTPase Arl8b and Determines Cell Death
43 Type in Mycobacterium tuberculosis Infection. *Journal of immunology*.
- 44 Muenzer, J., Lamsa, J. C., Garcia, A., Dacosta, J., Garcia, J. and Treco, D. A. (2002) Enzyme replacement therapy
45 in mucopolysaccharidosis type II (Hunter syndrome): a preliminary report. *Acta paediatrica*, **91**, 98-99.
- 46 Ngamukote, S., Yanagisawa, M., Ariga, T., Ando, S. and Yu, R. K. (2007) Developmental changes of
47 glycosphingolipids and expression of glycogenes in mouse brains. *Journal of neurochemistry*, **103**, 2327-
48 2341.
- 49 Norton, W. T. and Poduslo, S. E. (1973) Myelination in rat brain: changes in myelin composition during brain
50 maturation. *Journal of neurochemistry*, **21**, 759-773.
- 51 Ohmi, K., Greenberg, D. S., Rajavel, K. S., Ryazantsev, S., Li, H. H. and Neufeld, E. F. (2003) Activated microglia in
52 cortex of mouse models of mucopolysaccharidoses I and IIIB. *Proceedings of the National Academy of*
53 *Sciences of the United States of America*, **100**, 1902-1907.
- 54 Pereira, E. M., do Monte, S. J., do Nascimento, F. F. et al. (2014) Lysosome-associated protein 1 (LAMP-1) and
55 lysosome-associated protein 2 (LAMP-2) in a larger family carrier of Fabry disease. *Gene*, **536**, 118-122.
- 56 Piccinini, M., Scandroglio, F., Prioni, S. et al. (2010) Deregulated sphingolipid metabolism and membrane
57 organization in neurodegenerative disorders. *Molecular neurobiology*, **41**, 314-340.
- 58 Pohl, S., Mitchison, H. M., Kohlschutter, A., van Diggelen, O., Braulke, T. and Storch, S. (2007) Increased
59 expression of lysosomal acid phosphatase in CLN3-defective cells and mouse brain tissue. *Journal of*
60 *neurochemistry*, **103**, 2177-2188.
- Preti, A., Fiorilli, A., Lombardo, A., Caimi, L. and Tettamanti, G. (1980) Occurrence of sialyltransferase activity in the

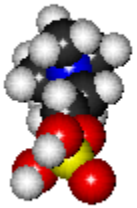
- synaptosomal membranes prepared from calf brain cortex. *Journal of neurochemistry*, **35**, 281-296.
- Prinetti, A., Chigorno, V., Prioni, S., Loberto, N., Marano, N., Tettamanti, G. and Sonnino, S. (2001) Changes in the lipid turnover, composition, and organization, as sphingolipid-enriched membrane domains, in rat cerebellar granule cells developing in vitro. *The Journal of biological chemistry*, **276**, 21136-21145.
- Prinetti, A., Chigorno, V., Tettamanti, G. and Sonnino, S. (2000) Sphingolipid-enriched membrane domains from rat cerebellar granule cells differentiated in culture. A compositional study. *The Journal of biological chemistry*, **275**, 11658-11665.
- Prinetti, A., Loberto, N., Chigorno, V. and Sonnino, S. (2009) Glycosphingolipid behaviour in complex membranes. *Biochimica et biophysica acta*, **1788**, 184-193.
- Prolo, L. M., Vogel, H. and Reimer, R. J. (2009) The lysosomal sialic acid transporter sialin is required for normal CNS myelination. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **29**, 15355-15365.
- Purpura, D. P. and Suzuki, K. (1976) Distortion of neuronal geometry and formation of aberrant synapses in neuronal storage disease. *Brain research*, **116**, 1-21.
- Reddy, A., Caler, E. V. and Andrews, N. W. (2001) Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes. *Cell*, **106**, 157-169.
- Riboni, L., Bassi, R., Prinetti, A. and Tettamanti, G. (1996) Salvage of catabolic products in ganglioside metabolism: a study on rat cerebellar granule cells in culture. *FEBS letters*, **391**, 336-340.
- Riboni, L., Bassi, R., Prinetti, A., Viani, P. and Tettamanti, G. (1999) Predominance of the acylation route in the metabolic processing of exogenous sphingosine in neural and extraneural cells in culture. *The Biochemical journal*, **338 (Pt 1)**, 147-151.
- Riboni, L., Bassi, R. and Tettamanti, G. (1994) Effect of brefeldin A on ganglioside metabolism in cultured neurons: implications for the intracellular traffic of gangliosides. *Journal of biochemistry*, **116**, 140-146.
- Riboni, L., Prinetti, A., Pitto, M. and Tettamanti, G. (1990) Patterns of endogenous gangliosides and metabolic processing of exogenous gangliosides in cerebellar granule cells during differentiation in culture. *Neurochemical research*, **15**, 1175-1183.
- Riboni, L., Sonnino, S., Acquotti, D., Malesci, A., Ghidoni, R., Egge, H., Mingrino, S. and Tettamanti, G. (1986) Natural occurrence of ganglioside lactones. Isolation and characterization of GD1b inner ester from adult human brain. *The Journal of biological chemistry*, **261**, 8514-8519.
- Rodriguez, A., Webster, P., Ortego, J. and Andrews, N. W. (1997) Lysosomes behave as Ca²⁺-regulated exocytic vesicles in fibroblasts and epithelial cells. *The Journal of cell biology*, **137**, 93-104.
- Saito, M., Tanaka, Y., Tang, C. P., Yu, R. K. and Ando, S. (1995) Characterization of sialidase activity in mouse synaptic plasma membranes and its age-related changes. *Journal of neuroscience research*, **40**, 401-406.
- Samarani, M., Loberto, N., Soldà, G. et al. (2018) A lysosome-plasma membrane-sphingolipid axis linking lysosomal storage to cell growth arrest. *FASEB J*.
- Sandhoff, K. and Kolter, T. (2003) Biosynthesis and degradation of mammalian glycosphingolipids. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **358**, 847-861.
- Santos, A. L. and Preta, G. (2018) Lipids in the cell: organisation regulates function. *Cellular and molecular life sciences : CMLS*, **75**, 1909-1927.
- Saqr, H. E., Pearl, D. K. and Yates, A. J. (1993) A review and predictive models of ganglioside uptake by biological membranes. *Journal of neurochemistry*, **61**, 395-411.
- Sardiello, M., Palmieri, M., di Ronza, A. et al. (2009) A gene network regulating lysosomal biogenesis and function. *Science*, **325**, 473-477.
- Schnaar, R. L. (2016) Gangliosides of the Vertebrate Nervous System. *Journal of molecular biology*, **428**, 3325-3336.
- Schulze, H., Kolter, T. and Sandhoff, K. (2009) Principles of lysosomal membrane degradation: Cellular topology and biochemistry of lysosomal lipid degradation. *Biochimica et biophysica acta*, **1793**, 674-683.
- Schwarzmann, G., Breiden, B. and Sandhoff, K. (2015) Membrane-spanning lipids for an uncompromised monitoring of membrane fusion and intermembrane lipid transfer. *J Lipid Res*, **56**, 1861-1879.
- Settembre, C., Annunziata, I., Spampinato, C. et al. (2007) Systemic inflammation and neurodegeneration in a mouse model of multiple sulfatase deficiency. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 4506-4511.
- Sezgin, E., Levental, I., Grzybek, M. et al. (2012) Partitioning, diffusion, and ligand binding of raft lipid analogs in model and cellular plasma membranes. *Biochimica et biophysica acta*, **1818**, 1777-1784.
- Sezgin, E., Levental, I., Mayor, S. and Eggeling, C. (2017) The mystery of membrane organization: composition, regulation and roles of lipid rafts. *Nature reviews. Molecular cell biology*, **18**, 361-374.
- Siegel, D. A. and Walkley, S. U. (1994) Growth of ectopic dendrites on cortical pyramidal neurons in neuronal storage diseases correlates with abnormal accumulation of GM2 ganglioside. *Journal of neurochemistry*, **62**, 1852-1862.
- Siow, D. L. and Wattenberg, B. W. (2012) Mammalian ORMDL proteins mediate the feedback response in ceramide

- 1 biosynthesis. *The Journal of biological chemistry*, **287**, 40198-40204.
- 2 Sonderfeld, S., Conzelmann, E., Schwarzmann, G., Burg, J., Hinrichs, U. and Sandhoff, K. (1985) Incorporation and
3 metabolism of ganglioside GM2 in skin fibroblasts from normal and GM2 gangliosidosis subjects. *European*
4 *journal of biochemistry*, **149**, 247-255.
- 5
6 Sonnino, S., Aureli, M., Loberto, N., Chigorno, V. and Prinetti, A. (2010) Fine tuning of cell functions through
7 remodeling of glycosphingolipids by plasma membrane-associated glycohydrolases. *FEBS letters*, **584**,
8 1914-1922.
- 9 Sonnino, S. and Chigorno, V. (2000) Ganglioside molecular species containing C18- and C20-sphingosine in
10 mammalian nervous tissues and neuronal cell cultures. *Biochimica et biophysica acta*, **1469**, 63-77.
- 11 Sonnino, S., Ghidoni, R., Marchesini, S. and Tettamanti, G. (1979) Cytosolic gangliosides: occurrence in calf brain
12 as ganglioside--protein complexes. *Journal of neurochemistry*, **33**, 117-121.
- 13 Sonnino, S., Mauri, L., Chigorno, V. and Prinetti, A. (2007) Gangliosides as components of lipid membrane domains.
14 *Glycobiology*, **17**, 1R-13R.
- 15 Sonnino, S. and Prinetti, A. (2008) Membrane lipid domains and membrane lipid domain preparations: are they the
16 same thing? *Trends Glycosci Glyc*, **20**, 315-340.
- 17 Sonnino, S. and Prinetti, A. (2013) Membrane domains and the "lipid raft" concept. *Current medicinal chemistry*, **20**,
18 4-21.
- 19 Sonnino, S., Prinetti, A., Mauri, L., Chigorno, V. and Tettamanti, G. (2006) Dynamic and structural properties of
20 sphingolipids as driving forces for the formation of membrane domains. *Chemical reviews*, **106**, 2111-2125.
- 21 Suzuki, Y., Yanagisawa, M., Ariga, T. and Yu, R. K. (2011) Histone acetylation-mediated glycosyltransferase gene
22 regulation in mouse brain during development. *Journal of neurochemistry*, **116**, 874-880.
- 23 Svennerholm, L., Bostrom, K., Jungbjer, B. and Olsson, L. (1994) Membrane lipids of adult human brain: lipid
24 composition of frontal and temporal lobe in subjects of age 20 to 100 years. *Journal of neurochemistry*, **63**,
25 1802-1811.
- 26 Tettamanti, G. (2004) Ganglioside/glycosphingolipid turnover: new concepts. *Glycoconj J*, **20**, 301-317.
- 27 Trinchera, M. and Ghidoni, R. (1990) Precursor-product relationship between GM1 and GD1a biosynthesized from
28 exogenous GM2 ganglioside in rat liver. *Journal of biochemistry*, **107**, 619-623.
- 29 Trinchera, M., Ghidoni, R., Sonnino, S. and Tettamanti, G. (1990) Recycling of glucosylceramide and sphingosine
30 for the biosynthesis of gangliosides and sphingomyelin in rat liver. *The Biochemical journal*, **270**, 815-820.
- 31 Valsecchi, M., Palestini, P., Chigorno, V., Sonnino, S. and Tettamanti, G. (1993) Changes in the ganglioside long-
32 chain base composition of rat cerebellar granule cells during differentiation and aging in culture. *Journal of*
33 *neurochemistry*, **60**, 193-196.
- 34 van Meer, G. (2011) Dynamic transbilayer lipid asymmetry. *Cold Spring Harbor perspectives in biology*, **3**.
- 35 van Meer, G. and Hoetzel, S. (2010) Sphingolipid topology and the dynamic organization and function of membrane
36 proteins. *FEBS letters*, **584**, 1800-1805.
- 37 van Meer, G. and Lisman, Q. (2002) Sphingolipid transport: rafts and translocators. *The Journal of biological*
38 *chemistry*, **277**, 25855-25858.
- 39 van Meer, G., Stelzer, E. H., Wijnaendts-van-Resandt, R. W. and Simons, K. (1987) Sorting of sphingolipids in
40 epithelial (Madin-Darby canine kidney) cells. *The Journal of cell biology*, **105**, 1623-1635.
- 41 Venugopal, B., Mesires, N. T., Kennedy, J. C., Curcio-Morelli, C., Laplante, J. M., Dice, J. F. and Slaugenhaupt, S.
42 A. (2009) Chaperone-mediated autophagy is defective in mucopolidosis type IV. *Journal of cellular*
43 *physiology*, **219**, 344-353.
- 44 Walkley, S. U. (2003) Neurobiology and cellular pathogenesis of glycolipid storage diseases. *Philosophical*
45 *transactions of the Royal Society of London. Series B, Biological sciences*, **358**, 893-904.
- 46 Walkley, S. U. and Vanier, M. T. (2009) Secondary lipid accumulation in lysosomal disease. *Biochimica et*
47 *biophysica acta*, **1793**, 726-736.
- 48 Warnock, D. E., Lutz, M. S., Blackburn, W. A., Young, W. W., Jr. and Baenziger, J. U. (1994) Transport of newly
49 synthesized glucosylceramide to the plasma membrane by a non-Golgi pathway. *Proceedings of the National*
50 *Academy of Sciences of the United States of America*, **91**, 2708-2712.
- 51 Wegner, M. S., Schiffmann, S., Parnham, M. J., Geisslinger, G. and Grosch, S. (2016) The enigma of ceramide
52 synthase regulation in mammalian cells. *Progress in lipid research*, **63**, 93-119.
- 53 Winter, E. and Ponting, C. P. (2002) TRAM, LAG1 and CLN8: members of a novel family of lipid-sensing domains?
54 *Trends in biochemical sciences*, **27**, 381-383.
- 55 Wu, X., Steigelman, K. A., Bonten, E., Hu, H., He, W., Ren, T., Zuo, J. and d'Azzo, A. (2010) Vacuolization and
56 alterations of lysosomal membrane proteins in cochlear marginal cells contribute to hearing loss in
57 neuraminidase 1-deficient mice. *Biochimica et biophysica acta*, **1802**, 259-268.
- 58 Yu, R. K., Nakatani, Y. and Yanagisawa, M. (2009) The role of glycosphingolipid metabolism in the developing brain.
59 *Journal of lipid research*, **50 Suppl**, S440-445.
- 60 Zhang, Y., Li, X., Becker, K. A. and Gulbins, E. (2009) Ceramide-enriched membrane domains--structure and
function. *Biochimica et biophysica acta*, **1788**, 178-183.

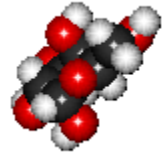
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

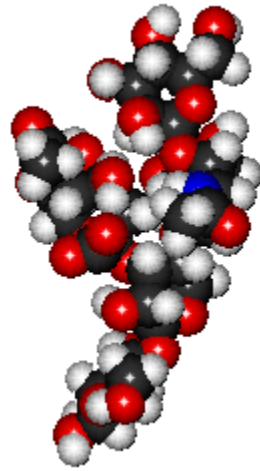
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41



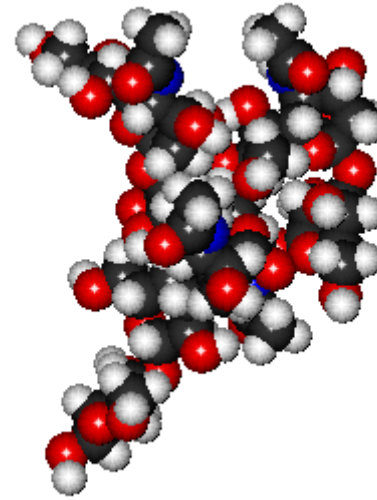
phosphocholine



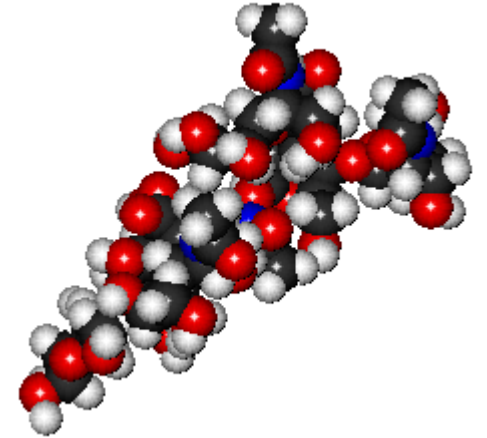
galactose



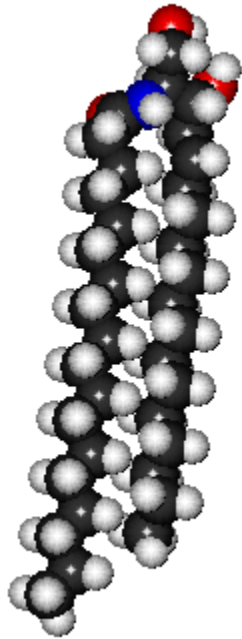
GM1



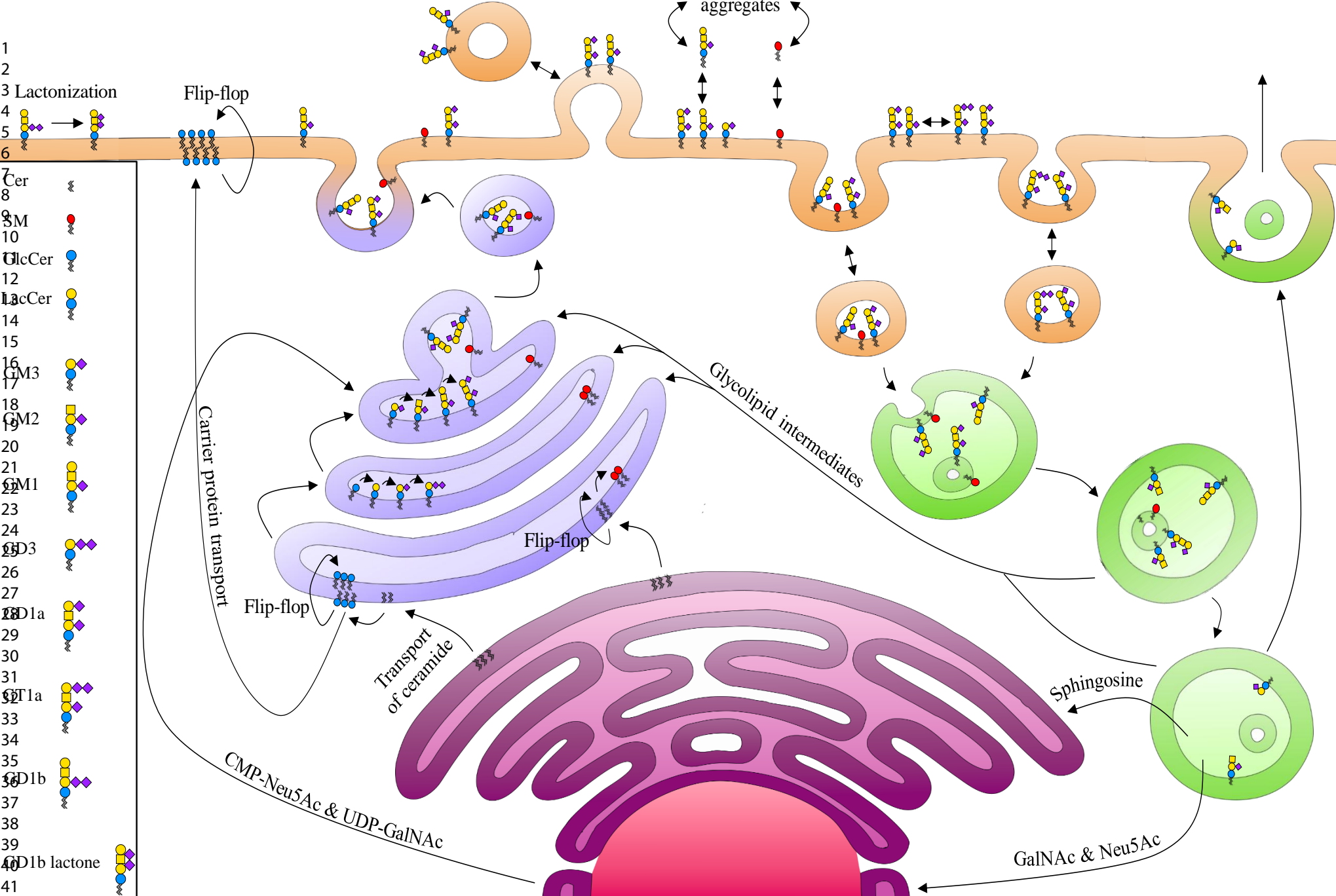
GT1b



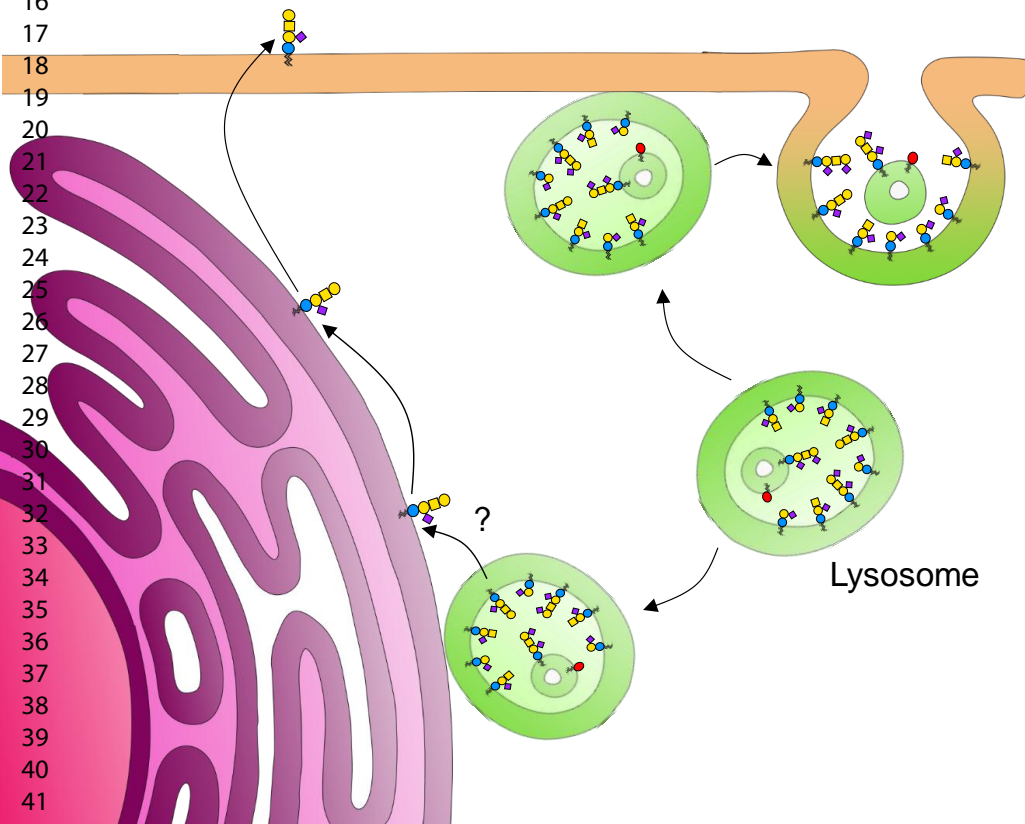
GaINAcGD1a



ceramide



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41



In this review article, we report on the sphingolipid chemical and biochemical properties and discuss some hypotheses about the possible links between sphingolipid incorrect catabolism and neurodegeneration.