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Brain pathology in Niemann Pick disease type A: Insights from the acid sphingomyelinase knockout mice

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Abstract

Severe neurological involvement characterizes Niemann Pick disease (NPD) type A, an inherited disorder caused by loss of function mutations in the gene encoding acid sphingomyelinase (ASM). Mice lacking ASM (ASMko), which mimic NPD type A, have provided important insights into the aberrant brain phenotypes induced by ASM deficiency. For example, lipid alterations, including the accumulation of sphingolipids, affect the membranes of different subcellular compartments of neurons and glial cells, leading to anomalies in signalling pathways, neuronal polarization, calcium homeostasis, synaptic plasticity, myelin production or immune response. These findings contribute to our understanding of the overall role of sphingolipids and their metabolic enzymes in brain physiology, and pave the way to design and test new therapeutic strategies for type A NPD and other neurodegenerative disorders. Some of these have already been tested in ASMko mice with promising results.

Introduction

Mutations in the *SMPD1* gene encoding acid sphingomyelinase (ASM) cause Niemann Pick diseases (NPD) types A and B (Brady et al., 1966). Both forms of the disorder are characterized by progressive visceral organ abnormalities, including hepatosplenomegaly, pulmonary insufficiency and cardiovascular disease (Schuchman and Desnick., 2001). However, while NPD type B is a later-onset form in which patients exhibit little or no neurological involvement, NPD type A is the infantile form of ASM deficiency characterized by a rapidly progressive neurodegenerative course that leads to death in early childhood. The different clinical presentations of types A and B NPD are likely due to small differences in the amount of residual ASM activity. For example, while an effective *in situ* residual ASM activity of ~5% results in NPD type B, a further reduction to ~1-2% or less induces the severe type A phenotype (Graber et al., 1994). These observations highlight the fact that although low levels of ASM activity are sufficient to maintain intact neurological function, the absence of this activity has devastating consequences in the brain.

ASM is a lysosomal enzyme that converts sphingomyelin (SM) into ceramide and phosphorylcholine (Gatt, 1963; Fowler, 1969). Therefore, SM accumulation in lysosomes characterizes NPD patient cells, and both type A and B are classified as lysosomal storage

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disorders. It is assumed that lysosomal storage develops only when the residual activity of the lysosomal enzyme falls below a critical threshold and the substrate degradation rate is lower than the rate of influx (Conzelmann and Sandhoff, 1983). SM influx in neural cells is lower than in liver, spleen or lymph nodes, and therefore it has been proposed that the low levels of residual activity in NPD type B would be sufficient to avoid lysosomal accumulation in neurons (Graber et al., 1994), thus precluding neurological involvement.

Unfortunately, despite these correlations of residual enzymatic activity with phenotype, *in vitro* ASM activity assays are not suitable to reliably predict the onset and extent of brain involvement in NPD patients. Moreover, even if the intralysosomal accumulation of unmetabolized substrates has been considered the primary cause of NPD, the molecular mechanisms leading from this event to the pathology are still obscure. Very likely the primary enzymatic defect results in multiple secondary biochemical and cellular abnormalities that could indeed be major contributing factors, or even the main cause, of tissue damage and death. Sphingolipids, including SM, exert many of their complex biological functions at the plasma membrane by modulating the lateral organization and biophysical properties of the membrane and by affecting the function of membrane-associated proteins or signaling complexes (Lingwood and Simons, 2010). Thus, the lysosomal deficiency of ASM and resultant defects in lysosomal catabolism might directly lead to altered plasma membrane composition and function. On the other hand, the presence of an extralysosomal pool of ASM at the cell surface (Grassme et al., 2001; Gulbins, 2003) suggests that irrespective of the lysosomal defect, plasma membrane alterations might arise when the enzyme is deficient. Data showing the ability of ASM to degrade SM within LDL particles at physiologic pH (Schissel et al., 1998) and the possibility that acidified microenvironments may exist at the cell surface (Bourguignon et al., 2004; Steinert et al., 2008), support the notion that ASM deficiency at the plasma membrane may contribute directly to NPD pathology.

Irrespective of these hypotheses, use of mice lacking ASM activity (ASMko), which mimic NPD type A disease (Horinouchi et al., 1995; Otterbach and Stoffel, 1995), has led to the discovery of a number of anomalies in brain tissue and cells that could explain the severe mental retardation and neurodegeneration of NPD type A patients. It is the aim of this review to present and discuss these findings.

Lipid alterations in ASMko mouse brains

Sphingolipid metabolism is a complex network of interdependent events, and is tightly connected with the intracellular traffic of these lipids. Moreover, recycling of catabolic fragments originated in the lysosome for biosynthetic purposes is quantitatively relevant, and lysosomal membranes under certain conditions can directly contribute to the repair of plasma membrane. Thus, it can be expected that the blockade of proper sphingolipid catabolism at the lysosomal level would lead to the jamming of the overall flow of metabolites, with consequences on the sphingolipid composition in all cellular compartments, including the plasma membrane. It is also becoming clear that the *in vivo* mechanisms regulating sphingolipid levels in cells and tissue are multiple and complex. As a consequence, the loss of a single enzyme activity of sphingolipid metabolism can lead to highly unexpected effects. Several reports have analyzed the consequences of the absence of ASM on the lipid composition of brain cells. These are summarized in the next section.

Lipid changes in total brain and myelin from ASMko mice

The analysis of total brain extracts of ASMko mouse brains showed a 6-fold SM increase compared to wild type (wt) mice (Scandroglio et al., 2008; Galvan et al., 2008). Similar increase (6.9-fold) was observed in purified myelin from ASMko brains (Buccinna et al.,

2009). Interestingly, such increase was predominantly due to the accumulation of a single SM molecular species (d18:1/18:0) as determined by ESI-MS in purified myelin (Buccinna et al., 2009) (Figure 1) and by Thin Layer Chromatography in brain (unpublished results). The differential clustering of SM molecular species resulting in a differential accessibility to the activity of ASM and neutral sphingomyelinase could explain why SM containing stearic acid is preferentially accumulated.

The total brain ganglioside content also was slightly but significantly higher (1.2-fold) in ASMko compared to wt mice. However, the combined content of two minor monosialogangliosides, GM3 and GM2, was greatly increased (12-fold higher) (Scandroglio et al., 2008). Remarkably, a striking accumulation of GM3 and GM2 gangliosides was previously reported in the brain of a NPD type A patient (Rodríguez-Lafrasse et al., 1999). In addition, secondary accumulation of GM3 and/or GM2 has been observed in several other lysosomal storage disorders, including sphingolipidosis with or without primary defects in ganglioside catabolism, as well as lysosomal storage disorders not affecting sphingolipid degradation (α -mannosidosis and different types of mucopolysaccharidosis) (reviewed in Walkley, 2004) (Table 1). A remarkable example is Niemann Pick C disease, where GM3 and GM2 accumulate in neurons from human patients and animal models (Zervas et al., 2001). Moreover, brain accumulation of GM3 and GM2 has been also detected in neurodegenerative pathologies without a clear lysosomal involvement including Alzheimer's disease (Kracun et al., 1992; Barrier et al., 2007) and severe malignant autosomal recessive osteopetrosis (Prinetti et al., 2009). The literature is strangely silent on the mechanisms underlying GM3 and GM2 accumulation in these pathologies, however fragmentary evidence points out, again, that alterations in intracellular lipid trafficking, especially at the level of the endosomal system, with a consequent lack of feedback regulation of sphingolipid synthesis within the Golgi/TGN, might be responsible for these changes (Walkley, 2004).

Although ganglioside storage is, therefore, not exclusive of lysosomal storage disorders, and may represent a general feature of many neurological conditions, the findings in ASMko mice and other lysosomal diseases suggested that ganglioside reduction could be a unifying approach to treating their neurological symptoms. Indeed, this has been attempted by reducing the overall synthesis of glycosphingolipids via the use of synthetic enzyme inhibitors (i.e., substrate reduction therapy) (Andersson et al., 2004; Platt and Lachmann, 2009). While some clinical efficacy of this approach has been demonstrated in lysosomal storage disease animal models, leading to regulatory approval for certain diseases (e.g., NPD type C) (Lachmann et al., 2004), the effects are limited, suggesting that other molecular changes strongly contribute to the disease pathology.

In contrast to gangliosides, the myelin-enriched sphingolipids, galactocerebroside and galactosulfocerebroside, decreased in the brain of ASMko mice compared to wt, in parallel with the mRNA levels of the two transferases responsible for their synthesis (Buccinna et al., 2009). This is consistent with the defective myelin architecture and function reported in NPD type A patients (Landrieu and Said, 1984; Folkerth, 1999; Di Rocco et al., 2005).

As mentioned, the lack of ASM by itself is not sufficient to explain the unexpected changes in lipid composition observed in ASMko mice, i.e., the preferential accumulation of a single SM molecular species and the accumulation of GM3 and GM2 gangliosides. The diversity in the structure of the ceramide moiety of cellular sphingolipids is likely due to both specificity of ceramide synthesis (at least six different ceramide synthases contribute to the fatty acid heterogeneity of ceramide in sphingolipids (Pewzner-Jung et al., 2006)) and selectivity in the traffic of ceramides with different molecular structure and metabolic fate (at least two different transport mechanisms, a vesicular one and a non vesicular, CERT

mediated one, are responsible for the structure-selective delivery of ceramide from the sites of synthesis to the sites of its metabolic utilization (Bartke et al., 2009)). On the other hand, the *de novo* synthetic flow, the extent of recycling of catabolic fragments and the enzyme activities of both the biosynthetic and catabolic pathways of sphingolipids are likely subjected to a very complex regulation *in vivo*. The mechanisms underlying these differences still remain to be elucidated, but it is reasonable to predict that metabolic events apparently not related to the loss of ASM activity, and not necessarily restricted to the lysosome or to the catabolic pathway of sphingolipids, might be involved in the unexpected modifications of lipid levels and topology observed in ASMko mice brains.

Lipid changes in particular neuronal populations and subcellular compartments in the absence of ASM activity

In addition to the analysis of lipid alterations in total brain and myelin, efforts have also been directed to elucidate whether and how such alterations affect particular neuronal populations. High levels of SM were confirmed in both cultured primary hippocampal and cerebellar granule neurons from ASMko mice compared to wt. However, ganglioside increase was not evident in these neurons indicating that such accumulation is not a direct consequence of the enzyme defect (Scandroglio et al., 2008, Galvan et al., 2008).

Further efforts have been aimed to characterize the lipid alterations in subcellular compartments of neuronal cells and tissues. Isolation of Golgi-enriched, lysosomal-enriched and lysosomal-free fractions from ASMko and wt mice brains revealed two interesting facts: i) Golgi membranes are not significantly affected by SM accumulation in ASMko tissue, and ii) SM accumulation is not restricted to lysosomes when ASM is lacking; i.e., non-lysosomal membranes are also affected (6.1- and 4.8-fold higher SM content in ASMko vs wt lysosomal and non lysosomal membranes, respectively) (Galvan et al., 2008). ASMko non lysosomal membranes were also enriched in SM derivatives such as sphingosylphosphorylcholine (SPC) and sphingosine compared to wt membranes. However, no significant differences were found in the content of other lipids (i.e. cholesterol, triglycerides, phospholipids or ceramide) (Galvan et al., 2008). In contrast, increased cholesterol content as indicated by filipin staining, was found in lysosomes of neurons (Sarna et al., 2001; Passini et al., 2005). The above reported findings suggested the presence of high SM, but not cholesterol levels, at the plasma membrane. This was confirmed in cultured hippocampal neurons by staining of non-permeabilized cells from ASMko and wt mice with lysoenin or filipin, which respectively bind SM or cholesterol. A 3.8-fold increase in SM signal at the cell surface, but no significant changes in that of cholesterol, were evidenced in ASMko hippocampal neurons (Galvan et al., 2008).

Isolation of synaptosomes from ASMko and wt mice brains also led to the discovery that SM and sphingosine were increased (3- and 5-fold, respectively) in ASMko synaptic membranes (Camoletto et al., 2008). In recent years evidence has accumulated indicating that, due to their chemical affinity, sphingolipids and cholesterol form microdomains in cellular membranes, which play a key role in cellular signaling (Lingwood and Simons, 2010). Therefore, the lipid composition of detergent resistant membranes (DRMs), which are considered a biochemical correlate of such microdomains, has also been analyzed in ASMko total brain and cultured neurons. Increased sphingolipid content was found in DRMs from ASMko mice brains (Galvan et al., 2008, Scandroglio et al., 2008), and a higher detergent-to-protein ratio was needed to isolate them with respect to wt (Scandroglio et al., 2008), likely reflecting a reduced fluidity in restricted membrane areas.

Altogether, the above findings highlight the complexity of lipid abnormalities that ASM deficiency causes in the brain. They involve different kinds of neurons and glial cells and

affect distinct cellular compartments. Next we discuss current information regarding the functional consequences that such alterations have in ASMko brain cells.

Consequences of ASM deficiency in neurons

High susceptibility of Purkinje neurons in the cerebellum

The first observations of aberrant phenotypes in the brains of ASMko mice were those of the accumulation of distended lysosomes within the cytoplasm of neurons and the complete loss of the ganglionic cell layer of Purkinje cells, leading to severe impairment of neuromotor coordination (Horinouchi et al., 1995; Otterbach and Stoffel, 1995). In addition, it was shown that even as the ASMko mice age and the degree of storage pathology affected neurons in all brain regions (Macauley et al., 2008), a subset of Purkinje cells (those zebrin II-negative) was particularly and very early compromised (Sarna et al., 2001). Although the reason for the differential susceptibility, even between morphological identical cells (Purkinje neurons) but with different molecular phenotypes (zebrin positive or negative), is not completely elucidated, several possibilities have been postulated. For example, the topographical distribution of the small heat shock protein Hsp25 (Armstrong et al., 2000) and the p75 nerve growth factor receptor (Dusart et al., 1994) matched that of zebrin positive or negative Purkinje cells, respectively. Hence, it has been proposed that while the high expression of Hsp25 would protect zebrin II positive neurons, a possible disruption of the prosurvival p75 signalling in the zebrin negative neurons would trigger their preferential early death (Sarna et al., 2001). The ratio between ceramide and sphingomyelin has been suggested to be critical in regulating plasma membrane-dependent signaling events (Zhang et al., 2009), and in fact, ceramide and lipid microdomains have a prominent role in signalling via p75 (Brann et al., 1999). Therefore their alterations due to ASM deficiency could underlie p75 impaired signalling in ASMko zebrin negative Purkinje neurons. Further research is needed to confirm this point.

Defective calcium homeostasis

Alterations of calcium (Ca^{2+}) homeostasis are a feature in several sphingolipid storage diseases. Ginzburg and Futerman (2005) demonstrated that this is also the case in ASMko mice brains. These alterations seem to concern Ca^{2+} release, but not its uptake. Interestingly, Ca^{2+} abnormalities affected the cerebellum but not the cortex of ASMko mice. Such specificity correlated with the decreased expression of the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) and of the major Ca^{2+} release channel in the cerebellum IP3R1 (Ginzburg and Futerman, 2005). Importantly, the reduction in these Ca^{2+} related molecules was dramatic in the Purkinje cell layer, where they are particularly abundant, and preceded the loss of zebrin II expression. Therefore, alterations in Ca^{2+} homeostasis could be another reason for the preferential susceptibility of the cerebellum in NPD type A. How changes in this pathway affect neuronal viability is not clear. Altered intracellular Ca^{2+} concentrations may affect signal transduction pathways and the opening of ion channels at the plasma membrane (Berridge et al., 2003). On the other hand, high cytosolic Ca^{2+} can also lead to cell death due to induction of oxidative stress (Mattson and Chan, 2003). The observations that addition of exogenous SM did not affect Ca^{2+} modulation by SERCA or IP3R and that the levels of its lyso-derivative SPC needed to induce a small effect were significantly higher than those observed in NPD type A brains (Ginzburg and Futerman, 2005), made unlikely a direct effect of storage lipids on the proteins involved in regulating Ca^{2+} homeostasis. However, this needs to be more carefully evaluated.

Alterations in axonal polarity and impaired endocytosis

As mentioned above, the sphingolipid content of DRMs is elevated in ASMko brains (Galvan et al., 2008; Scandroglio et al., 2008), and given the crucial role of these lipid

domains in cell signalling, such alterations may underlie the aberrant neurological phenotypes in NPD type A. Some examples (i.e. p75 signalling) have been already discussed and further research is necessary to fully characterize these changes. In addition, DRMs and SM are involved in the establishment of neuronal polarity by contributing to the sorting of DRM enriched molecules to the axons (Ledesma et al., 1998, 1999). Among such molecules are the ganglioside GM1 and GPI- anchored proteins like the Prion protein (PrPc) (Galvan et al., 2005). Analysis of the distribution of these molecules in ASMko cultured hippocampal neurons revealed their presence both in axons and dendrites, different from their almost exclusive axonal distribution in wt neurons (Galvan et al., 2008). The aberrant distribution of PrPc was also observed *in situ* in the hippocampus of ASMko mice, strongly suggesting that similar alterations might also exist in the neurons of NPD type A patients. The molecular mechanism underlying these alterations implied the impaired endocytosis from dendrites due to deficient membrane attachment and activation of the small GTPase RhoA (Galvan et al., 2008). In this case, a direct effect of SM accumulation on the aberrant phenotype was demonstrated. Hence, addition of SM to cultured wt hippocampal neurons diminished RhoA membrane attachment and impaired DRM molecule endocytosis and polarized axonal distribution. Consistent with this observation, addition of exogenous sphingomyelinase to ASMko neuronal cultures rescued the aberrant phenotypes (Galvan et al., 2008). It is likely that altered polarization of DRM molecules will affect their function, and more work is needed to fully clarify this issue.

Altered presynaptic plasticity

It has been shown that sphingolipid accumulation also affects synaptic membranes from ASMko mice (Camoletto et al., 2009). This observation opened the possibility that synaptic alterations might occur in NPD type A. Supporting this view, a careful analysis on the time course of the disease progression in ASMko mice brains revealed that axonal synaptic terminals are the first parts of the neuronal cells to show signs of degeneration (Macauley et al., 2008). Electrophysiological recordings in ASMko and wt hippocampal slices showed enhanced paired-pulse facilitation and post-tetanic potentiation in the former, whereas basal synaptic transmission and synaptic depression were not significantly changed (Camoletto et al., 2009). This was consistent with a decreased probability of neurotransmitter release. Electron microscopy analysis of ASMko and wt CA1 hippocampal areas indeed revealed a reduced number of docked vesicles in ASMko presynaptic terminals, which is an anatomical correlate of neurotransmitter release in the hippocampus (Camoletto et al., 2009). The molecular mechanism underlying this aberrant phenotype involved the altered interaction of the cytosolic factor Munc 18 and the SNARE syntaxin1, which form a platform for vesicular docking (Toonen and Verhage., 2007). This alteration was due, in turn, to the conformational change promoted in syntaxin1 by sphingosine accumulation. Exogenous addition of this SM derivative to wt cultured hippocampal neurons or slices was indeed capable of altering Munc18-syntaxin1 interaction, of impairing synaptic vesicle release, and of enhancing paired-pulse facilitation (Camoletto et al., 2009). These evidences in ASMko mice strongly indicate that synaptic alterations occur in NPD type A. In addition, the fact that not only the presynaptic, but also the postsynaptic, termini are significantly smaller in ASMko mice compared to wt (Camoletto et al., 2009) opens the perspective that dendritic spines, structures that are key in learning and memory processes, could also be affected.

Consequences of ASM deficiency in glial cells

Deficient myelination of the central nervous system (CNS) has been observed in NPD type A patients (Landrieu and Said, 1984; Folkerth, 1999; Di Rocco et al., 2005). This, together with the neuronal anomalies described above, could contribute to explain the severe mental retardation in the disease. Hence, the analysis of myelin producing and other glial cells appears to be key to fully understand brain pathology in the absence of ASM activity.

Reduced expression of myelin specific proteins

Oligodendrocytes are the myelin producing cells in the CNS. Myelination is a two-step process in which first oligodendrocyte precursors proliferate and differentiate until they are mature enough to then acquire the capacity of producing the myelin sheath (Folkerth, 1999). A delay or failure in the myelination procedure may thus result from deficiency in the differentiation process, leading to reduced numbers of mature oligodendrocytes, or from changes in myelin sheath assembly and maintenance arising from metabolic alterations in mature oligodendrocytes. Buccinna et al (2009) offered evidence to discriminate between these two alternatives by analyzing time dependent protein and mRNA expression of oligodendrocyte specific proteins involved in myelin architecture and function (MBP, MAG, CNP and PLP-DM20). They observed that such expression did not change between ASMko and wt mice at birth, but became significantly reduced at later postnatal times in ASMko conditions. These findings suggested that oligodendrocyte differentiation during embryogenesis would be insensitive to ASM deficiency. This is consistent with the fact that neurological function of the ASMko mice appears normal at birth, as does that of the type A NPD children, but rapidly progresses postnatally. Although further evaluation of the number of oligodendrocyte precursors in ASMko brains would be necessary to confirm this hypothesis, the fact that ASMko brains express normal levels of the transcription factors involved in oligodendrocyte differentiation (Buccinna et al., 2009), makes unlikely that there is a loss of oligodendrocyte during myelination. Instead, the findings described above pave the way to investigate metabolic alterations of mature ASMko oligodendrocytes that could explain hypomyelination in NPD type A patients.

Impaired microparticle release from ASMko astrocytes

Microglia have been considered the immune cells of the CNS, which release microparticles from the plasma membrane containing crucial cytokines (IL-1 β) in CNS inflammatory events. However, it has been recently shown that such microparticles are also released by astrocytes in an ASM dependent manner (Bianco et al., 2009). Accordingly, a complete blockade of microparticle shedding and IL-1 β release was observed in ASMko astrocytes (Bianco et al., 2009). This is consistent with the reduced levels of IL-1 β found in the brain of ASMko mice (Ng and Griffin, 2006). These observations led to the suggestion that the increased frequency of infections observed in NPD type A patients (Minai et al., 2000) might be related with the capability of ASM to control cytokine release and therefore to regulate the immune responses.

Towards a therapy

NPD type A is a devastating disease that always results in early childhood death, usually by 2-3 years of age. At the present time there is no treatment. Early attempts at BMT, amniotic cell, and liver transplantation did not impact the neurological disease course in type A NPD patients, and new approaches are clearly needed. When considering these approaches several factors must be taken into account. First, this is a very early onset disease. While patients are generally born without evidence of neurological dysfunction, by 3-6 months this is clearly evident. Since it is unlikely that damage incurred by ASM deficiency will be reversible, any successful therapies must therefore be initiated at birth or shortly thereafter. Secondly, therapies must be carefully judged with regard to significant improvement of quality of life. This is particularly important when translating results obtained in the ASMko mouse model (below) to patients. For example, in the mouse even modest improvements in neurological function may be considered an experimental “success”; however, similar results in patients might ultimately lead to more pain and suffering for families by slowing (but not preventing) the rate of neurological decline, ultimately prolonging the disease. On the positive side, as discussed above, for ASM deficiency it is known that very low levels of

residual enzymatic activity (~5%) can recover neurological function. In fact, recent studies using mutation specific mouse models (Jones et al. 2009) have shown that as little as 8% ASM activity can completely prevent the occurrence of neurological disease. Thus, the therapeutic threshold is low, provided that the enzyme can be delivered globally throughout the CNS to the proper sites of pathology, and that the therapy can be initiated prior to the time when irreversible damage occurs.

Enzyme replacement therapy (ERT), administered by intravenous infusion of the recombinant enzyme into deficient patients, is a successful strategy for several lysosomal storage diseases. However, the presence of the brain blood barrier generally renders the brain unresponsive to this form of therapy. This was confirmed in the ASMko mice in which intravenous ASM replacement therapy (at doses up to 10mg/Kg) resulted in the improvement of visceral but not brain pathology (Miranda et al., 2000). Thus, while ERT is currently being evaluated in NPD type B patients (non neurological), and it may alter the visceral disease in type A NPD, it is likely not to affect the severe neurological course in these patients.

The fact that NPD type A is a single-gene disorder also opened the possibility for gene replacement by intracranial injection of viral vectors. Adeno-associated virus (AAV) is particularly suited for brain applications since besides being nontoxic and neurotropic, it has the potential to sustain long-term expression in the CNS. Several AAV serotypes and different brain injection sites have been tested in ASMko mice to induce the expression of human ASM and alter the course of the mouse disease (Passini et al., 2005; Dodge et al., 2005). Such studies showed that wide-spread enzyme distribution could be obtained throughout the mouse CNS, leading to alleviation of storage pathology, rescue of Purkinje cells and correction of behavioural deficits. Thus, enzyme replacement by gene therapy is envisioned as candidate for future clinical trials. One challenge, however, is to scale up this approach from the mouse to primate brain in order to achieve similar biodistribution of the virus and ASM expression.

Intracerebral transplantation of bone marrow-derived mesenchymal stem cells (MSCs) (Jin et al., 2002) or adult mouse neural progenitor cells (NPCs) (Shihabuddin et al., 2004) expressing the human ASM also has been assessed in the ASMko mice. These studies demonstrated that genetically engineered cells can serve as a vehicle for enzymatic cross-correction and reversal of storage pathology of host brain cells in the mouse model. Hence, human ASM-encoding MSCs injected in the hippocampus and cerebellum migrated away from the injection sites and survived at least 6 months after transplant. Greater survival times and significantly delayed Purkinje cell loss were observed in the transplanted animals (Jin et al., 2002). Also promising were the results obtained upon NPC transplantation. Transplanted, ASM expressing NPCs survived, migrated and showed region-specific differentiation in the host brain. Although the levels of hASM were barely detectable by immunostaining they were sufficient for uptake and cross-correction of host cells leading to reversal of distended lysosomal pathology and regional clearance of SM and cholesterol storage (Shihabuddin et al., 2004).

Future approaches for type A NPD that could be evaluated in the mouse models also include small molecule approaches aimed at enzyme enhancement (e.g., chaperone) or substrate reduction, as well as enhanced methods to deliver recombinant ASM or gene therapy vectors across the blood brain barrier. Clearly, for all of these approaches research on the aberrant neurological phenotypes in ASMko mice will provide read out systems that can be used to test their efficacy, and will hopefully reveal new targets for genetic and/or pharmacological intervention.

Concluding remarks

Observations on type A NPD, dating back nearly 100 years, have highlighted the important and essential role of ASM in the brain, and studies in the ASMko mouse model are beginning to elucidate the molecular mechanisms leading to severe neurological dysfunction in this disorder. The combined efforts of different disciplines, ranging from protein and lipid biochemistry and biophysics to molecular biology, has begun to unravel the complex biology underlying the brain abnormalities in these patients. Abnormalities involve neurons and glial cells, as well as lysosomal and non lysosomal membranes. In this regard, the results discussed in this review open the question on whether type A NPD should be still considered a lysosomal storage disorder. Lysosomal dysfunction due to SM accumulation will certainly contribute to the pathology and the lack of ASM at the lysosomal level is surely responsible, at least in part, for its onset. However, the recent demonstrations of dramatic changes in the lipid content of the plasma and synaptic membranes, which could explain many of the aberrant brain phenotypes reported and are likely independent of the lysosomes, point to non-lysosomal events as the main and/or initiating cause for this neurological disease. Such findings are valuable tools for the design and testing of new therapeutic strategies for type A NPD, and some of these have already shown promising results in the mouse model, opening new opportunities for the treatment of this devastating disease. Moreover, since patients with several common neurological disorders (e.g., Alzheimer's disease) also exhibit abnormalities in the sphingolipid pathway, including alterations in ASM activity, it is likely that findings in the ASMko mice will influence these diseases as well.

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References

- Andersson U, Smith D, Jeyakumar M, Butters TD, Borja MC, Dwek RA, Platt FM. Improved outcome of N-butyldeoxygalactonojirimycin-mediated substrate reduction therapy in a mouse model of Sandhoff disease. *Neurobiol. Dis.* 2004; 16:506–515. [PubMed: 15262262]
- Ando S, Toyoda Y, Nagai Y, Ikuta F. Alterations in brain gangliosides and other lipids of patients with Creutzfeldt-Jakob disease and subacute sclerosing panencephalitis (SSPE). *Jpn. J. Exp. Med.* 1984; 54:229–234. [PubMed: 6399079]
- Armstrong CL, Krueger-Naug AM, Currie RW, Hawkes R. Constitutive expression of heat shock protein HSP25 in the central nervous system of the developing and adult mouse. *J. Comp. Neurol.* 2001; 434:262–274. [PubMed: 11331528]
- Barrier L, Ingrand S, Damjanac M, Rioux Bilan A, Hugon J, Page G. Genotype-related changes of ganglioside composition in brain regions of transgenic mouse models of Alzheimer's disease. *Neurobiol Aging.* 2007; 28:1863–1872. [PubMed: 17007963]
- Bartke N, Hannun YA. Bioactive sphingolipids: metabolism and function. *J. Lipid Res.* 2009; 50(Suppl):S91–96. [PubMed: 19017611]
- Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* 2003; 4:517–529. [PubMed: 12838335]
- Bianco F, Perrotta C, Novellino L, Francolini M, Riganti L, Menna E, Saglietti L, Schuchman EH, Furlan R, Clementi E, Matteoli M, Verderio C. Acid sphingomyelinase activity triggers microparticle release from glial cells. *EMBO J.* 2009; 28:1043–1054. [PubMed: 19300439]
- Bourguignon LY, Singleton PA, Diedrich F, Stern R, Gilad E. CD44 interaction with Na⁺-H⁺ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B

- activation and breast tumor cell invasion. *J. Biol. Chem.* 2004; 279:26991–27007. [PubMed: 15090545]
- Brady RO, Kanfer JN, Mock MB, Fredrickson DS. The metabolism of sphingomyelin. II. Evidence of an enzymatic deficiency in Niemann-Pick disease. *Proc. Natl. Acad. Sci. U S A.* 1966; 55:366–369. [PubMed: 5220952]
- Brann AB, Scott R, Neuberger Y, Abulafia D, Boldin S, Fainzilber M, Futeran AH. Ceramide signaling downstream of the p75 neurotrophin receptor mediates the effects of nerve growth factor on outgrowth of cultured hippocampal neurons. *J. Neurosci.* 1999; 19:8199–8206. [PubMed: 10493721]
- Brooksbank BW, McGovern J. Gangliosides in the brain in adult Down's syndrome and Alzheimer's disease. *Mol. Chem. Neuropathol.* 1989; 11:143–156. [PubMed: 2534985]
- Buccinnà B, Piccinini M, Prinetti A, Scandroglio F, Prioni S, Valsecchi M, Votta B, Grifoni S, Lupino E, Ramondetti C, Schuchman EH, Giordana MT, Sonnino S, Rinaudo MT. Alterations of myelin-specific proteins and sphingolipids characterize the brains of acid sphingomyelinase-deficient mice, an animal model of Niemann-Pick disease type A. *J Neurochem.* 2009; 109:105–115. [PubMed: 19187445]
- Camoletto PG, Vara H, Morando L, Connell E, Marletto FP, Giustetto M, Sassoè-Pognetto M, Van Veldhoven PP, Ledesma MD. Synaptic vesicle docking: sphingosine regulates syntaxin1 interaction with Munc18. *PLoS One.* 2009; 4:e5310. [PubMed: 19390577]
- Constantopoulos G, Dekaban AS. Neurochemistry of the mucopolysaccharidoses: brain lipids and lysosomal enzymes in patients with four types of mucopolysaccharidosis and in normal controls. *J. Neurochem.* 1978; 30:965–973. [PubMed: 96217]
- Constantopoulos G, Eiben RM, Schafer IA. Neurochemistry of the mucopolysaccharidoses: brain glycosaminoglycans, lipids and lysosomal enzymes in mucopolysaccharidosis type III B (alpha-N-acetylglucosaminidase deficiency). *J. Neurochem.* 1978; 31:1215–1222. [PubMed: 100580]
- Constantopoulos G, Iqbal K, Dekaban AS. Mucopolysaccharidosis types IH, IS, II, and IIIA: glycosaminoglycans and lipids of isolated brain cells and other fractions from autopsied tissues. *J. Neurochem.* 1980; 34:1399–1411. [PubMed: 6770045]
- Conzelmann E, Sandhoff K. Partial enzyme deficiencies: residual activities and the development of neurological disorders. *Dev. Neurosci.* 1983; 6:58–71. [PubMed: 6421563]
- Crino PB, Ullman MD, Vogt BA, Bird ED, Volicer L. Brain gangliosides in dementia of the Alzheimer type. *Arch. Neurol.* 1989; 46:398–401. [PubMed: 2705899]
- Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, Seyfried TN, Thomas EA. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. *Neurobiol. Dis.* 2007; 27:265–277. [PubMed: 17600724]
- Di Martino A, Safar J, Callegaro L, Salem N Jr. Gibbs CJ Jr. Ganglioside composition changes in spongiform encephalopathies: analyses of 263K scrapie-infected hamster brains. *Neurochem. Res.* 1993; 18:907–913. [PubMed: 8371832]
- Di Rocco M, Rossi A, Parenti G, Allegri AE, Filocamo M, Pessagno A, Tortori-Donati P, Minetti C, Biancheri R. Different molecular mechanisms leading to white matter hypomyelination in infantile onset lysosomal disorders. *Neuropediatrics.* 2005; 36:265–269. [PubMed: 16138252]
- Dodge JC, Clarke J, Song A, Bu J, Yang W, Taksir TV, Griffiths D, Zhao MA, Schuchman EH, Cheng SH, O'Riordan CR, Shihabuddin LS, Passini MA, Stewart GR. Gene transfer of human acid sphingomyelinase corrects neuropathology and motor deficits in a mouse model of Niemann-Pick type A disease. *Proc. Natl. Acad. Sci. U S A.* 2005; 102:17822–17827. [PubMed: 16301517]
- Dusart I, Morel MP, Sotelo C. Parasagittal compartmentation of adult rat Purkinje cells expressing the low-affinity nerve growth factor receptor: changes of pattern expression after a traumatic lesion. *Neuroscience.* 1994; 63:351–356. [PubMed: 7891850]
- Folkerth RD. Abnormalities of developing white matter in lysosomal storage diseases. *J. Neuropathol. Exp. Neurol.* 1999; 58:887–902. [PubMed: 10499433]
- Fowler S. Lysosomal localization of sphingomyelinase in rat liver. *Biochim. Biophys. Acta.* 1969; 191:481–484. [PubMed: 4311152]

- Galvan C, Camoletto PG, Dotti CG, Aguzzi A, Ledesma MD. Proper axonal distribution of PrP(C) depends on cholesterol-sphingomyelin-enriched membrane domains and is developmentally regulated in hippocampal neurons. *Mol. Cell. Neurosci.* 2005; 30:304–315. [PubMed: 16139509]
- Galvan C, Camoletto PG, Cristofani F, Van Veldhoven PP, Ledesma MD. Anomalous surface distribution of glycosyl phosphatidyl inositol-anchored proteins in neurons lacking acid sphingomyelinase. *Mol. Biol. Cell.* 2008; 19:509–522. [PubMed: 18032586]
- Gatt S. Enzymic hydrolysis and synthesis of ceramides. *J. Biol. Chem.* 1963; 238:3131–3133. [PubMed: 14081938]
- Ginzburg L, Futerman AH. Defective calcium homeostasis in the cerebellum in a mouse model of Niemann-Pick A disease. *J. Neurochem.* 2005; 95:1619–1628. [PubMed: 16277603]
- Goodman LA, Livingston PO, Walkley SU. Ectopic dendrites occur only on cortical pyramidal cells containing elevated GM2 ganglioside in alpha-mannosidosis. *Proc. Natl. Acad. Sci. U S A.* 1991; 88:11330–11334. [PubMed: 1763046]
- Graber D, Salvayre R, Levade T. Accurate differentiation of neuronopathic and nonneuronopathic forms of Niemann-Pick disease by evaluation of the effective residual lysosomal sphingomyelinase activity in intact cells. *J. Neurochem.* 1994; 63:1060–1068. [PubMed: 8051547]
- Grassmé H, Schwarz H, Gulbins E. Molecular mechanisms of ceramide-mediated CD95 clustering. *Biochem. Biophys. Res. Commun.* 2001; 284:1016–1030. [PubMed: 11409897]
- Gulbins E. Regulation of death receptor signaling and apoptosis by ceramide. *Pharmacol. Res.* 2003; 47:393–399. [PubMed: 12676513]
- Higatsberger MR, Sperk G, Bernheimer H, Shannak KS, Hornykiewicz O. Striatal ganglioside levels in the rat following kainic acid lesions: comparison with Huntington's disease. *Exp. Brain Res.* 1981; 44:93–96. [PubMed: 6456150]
- Horinouchi K, Erlich S, Perl DP, Ferlinz K, Bisgaier CL, Sandhoff K, Desnick RJ, Stewart CL, Schuchman EH. Acid sphingomyelinase deficient mice: a model of types A and B Niemann-Pick disease. *Nat. Genet.* 1995; 10:288–293. [PubMed: 7670466]
- Jones I, He X, Katouzian F, Darroch PI, Schuchman EH. Characterization of common SMPD1 mutations causing types A and B Niemann-Pick disease and generation of mutation-specific mouse models. *Mol. Genet. Metab.* 2008; 95:152–162. [PubMed: 18815062]
- Kalanj S, Kracun I, Rosner H, Cosović C. Regional distribution of brain gangliosides in Alzheimer's disease. *Neurol. Croat.* 1991; 40:269–281. [PubMed: 1751644]
- Kracun I, Rosner H, Drnovsek V, Heffer-Laue M, Cosović C, Laue G. Human brain gangliosides in development, aging and disease. *Int. J. Dev. Biol.* 1991; 35:289–295. [PubMed: 1814411]
- Kracun I, Kalanj S, Talan-Hranilovic J, Cosovic C. Cortical distribution of gangliosides in Alzheimer's disease. *Neurochem. Int.* 1992; 20:433–438. [PubMed: 1304338]
- Lachmann RH, te Vrugte D, Lloyd-Evans E, Reinkensmeier G, Sillence DJ, Fernandez-Guillen L, Dwek RA, Butters TD, Cox TM, Platt FM. Treatment with miglustat reverses the lipid-trafficking defect in Niemann-Pick disease type C. *Neurobiol. Dis.* 2004; 16:654–658. [PubMed: 15262277]
- Landrieu P, Saïd G. Peripheral neuropathy in type A Niemann-Pick disease. A morphological study. *Acta Neuropathol.* 1984; 63:66–71. [PubMed: 6328834]
- Ledesma MD, Simons K, Dotti CG. Neuronal polarity: essential role of protein-lipid complexes in axonal sorting. *Proc. Natl. Acad. Sci. U S A.* 1998; 95:3966–3971. [PubMed: 9520476]
- Ledesma MD, Brügger B, Bünning C, Wieland FT, Dotti CG. Maturation of the axonal plasma membrane requires upregulation of sphingomyelin synthesis and formation of protein-lipid complexes. *EMBO J.* 1999; 18:1761–1771. [PubMed: 10202140]
- Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science.* 2010; 327:46–50. [PubMed: 20044567]
- Liour SS, Jones MZ, Suzuki M, Bieberich E, Yu RK. Metabolic studies of glycosphingolipid accumulation in mucopolysaccharidosis IIID. *Mol. Genet. Metab.* 2001; 72:239–247. [PubMed: 11243730]
- Macauley SL, Sidman RL, Schuchman EH, Taksir T, Stewart GR. Neuropathology of the acid sphingomyelinase knockout mouse model of Niemann-Pick A disease including structure-function studies associated with cerebellar Purkinje cell degeneration. *Exp. Neurol.* 2008; 214:181–192. [PubMed: 18778708]

- Mattson MP, Chan SL. Calcium orchestrates apoptosis. *Nat. Cell Biol.* 2003; 5:1041–1043. [PubMed: 14647298]
- Minai OA, Sullivan EJ, Stoller JK. Pulmonary involvement in Niemann-Pick disease: case report and literature review. *Respir Med.* 2000; 94:1241–1251. [PubMed: 11192962]
- Miranda SR, He X, Simonaro CM, Gatt S, Dagan A, Desnick RJ, Schuchman EH. Infusion of recombinant human acid sphingomyelinase into niemann-pick disease mice leads to visceral, but not neurological, correction of the pathophysiology. *FASEB J.* 2000; 14:1988–1995. [PubMed: 11023983]
- Ng CG, Griffin DE. Acid sphingomyelinase deficiency increases susceptibility to fatal alphavirus encephalomyelitis. *J. Virol.* 2006; 80:10989–10999. [PubMed: 16943298]
- Ohtani Y, Tamai Y, Ohnuki Y, Miura S. Ganglioside alterations in the central and peripheral nervous systems of patients with Creutzfeldt-Jakob disease. *Neurodegeneration.* 1996; 5:331–338. [PubMed: 9117545]
- Otterbach B, Stoffel W. Acid sphingomyelinase-deficient mice mimic the neurovisceral form of human lysosomal storage disease (Niemann-Pick disease). *Cell.* 1995; 81:1053–1061. [PubMed: 7600574]
- Passini MA, Macauley SL, Huff MR, Taksir TV, Bu J, Wu IH, Piepenhagen PA, Dodge JC, Shihabuddin LS, O’Riordan CR, Schuchman EH, Stewart GR. AAV vector-mediated correction of brain pathology in a mouse model of Niemann-Pick A disease. *Mol. Ther.* 2005; 11:754–762. [PubMed: 15851014]
- Pewzner-Jung Y, Ben-Dor S, Futerman AH. When do Lasses (longevity assurance genes) become CerS (ceramide synthases)? Insights into the regulation of ceramide synthesis. *J. Biol. Chem.* 2006; 281:25001–25005. [PubMed: 16793762]
- Platt FM, Lachmann RH. Treating lysosomal storage disorders: current practice and future prospects. *Biochim. Biophys. Acta.* 2009; 1793:737–745. [PubMed: 18824038]
- Prinetti A, Rocchetta F, Costantino E, Frattini A, Caldana E, Rucci F, Bettiga A, Poliani PL, Chigorno V, Sonnino S. Brain lipid composition in grey-lethal mutant mouse characterized by severe malignant osteopetrosis. *Glycoconj J.* 2009; 26:623–633. [PubMed: 18781385]
- Rodriguez-Lafrasse C, Vanier MT. Sphingosylphosphorylcholine in Niemann-Pick disease brain: accumulation in type A but not in type B. *Neurochem. Res.* 1999; 24:199–205. [PubMed: 9972865]
- Sarna J, Miranda SR, Schuchman EH, Hawkes R. Patterned cerebellar Purkinje cell death in a transgenic mouse model of Niemann Pick type A/B disease. *Eur. J. Neurosci.* 2001; 13:1873–1880. [PubMed: 11403680]
- Scandroglio F, Venkata JK, Loberto N, Prioni S, Schuchman EH, Chigorno V, Prinetti A, Sonnino S. Lipid content of brain, brain membrane lipid domains, and neurons from acid sphingomyelinase deficient mice. *J. Neurochem.* 2008; 107:329–338. [PubMed: 18673449]
- Schissel SL, Jiang X, Tweedie-Hardman J, Jeong T, Camejo EH, Najib J, Rapp JH, Williams KJ, Tabas I. Secretory sphingomyelinase, a product of the acid sphingomyelinase gene, can hydrolyze atherogenic lipoproteins at neutral pH. Implications for atherosclerotic lesion development. *J. Biol. Chem.* 1998; 273:2738–2746. [PubMed: 9446580]
- Schuchman, EH.; Desnick, RJ. Niemann Pick disease types A and B: acid sphingomyelinase deficiencias. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. *the Metabolic and Molecular Bases of Inherited Disease*. 8th edn.. McGraw-Hill; New York: p. 3589
- Shihabuddin LS, Numan S, Huff MR, Dodge JC, Clarke J, Macauley SL, Yang W, Taksir TV, Parsons G, Passini MA, Gage FH, Stewart GR. Intracerebral transplantation of adult mouse neural progenitor cells into the Niemann-Pick-A mouse leads to a marked decrease in lysosomal storage pathology. *J. Neurosci.* 2004; 24:10642–10651. [PubMed: 15564580]
- Steinert S, Lee E, Tresset G, Zhang D, Hortsch R, Wetzel R, Hebbar S, Sundram JR, Kesavapany S, Boschke E, Kraut R. A fluorescent glycolipid-binding peptide probe traces cholesterol dependent microdomain-derived trafficking pathways. *PLoS One.* 2008; 3:e2933. [PubMed: 18716682]
- Svennerholm L, Gottfries CG. Membrane lipids, selectively diminished in Alzheimer brains, suggest synapse loss as a primary event in early-onset form (type I) and demyelination in late-onset form (type II). *J. Neurochem.* 1994; 62:1039–1047. [PubMed: 8113790]

- Tamai Y, Ohtani Y, Miura S, Narita Y, Iwata T, Kaiya H, Namba M. Creutzfeldt-Jakob disease--alteration in ganglioside sphingosine in the brain of a patient. *Neurosci. Lett.* 1979; 11:81–86. [PubMed: 372860]
- Toonen RF, Verhage M. Munc18-1 in secretion: lonely Munc joins SNARE team and takes control. *Trends Neurosci.* 2007; 30:564–572. [PubMed: 17956762]
- Walkley SU. Secondary accumulation of gangliosides in lysosomal storage disorders. *Semin. Cell. Dev. Biol.* 2004; 15:433–444. [PubMed: 15207833]
- Wherrett JR, Brown BL. Erythrocyte glycolipids in Huntington's chorea. *Neurology.* 1969; 19:489–493. [PubMed: 4239722]
- Yu RK, Ledeen RW, Gajdusek DC, Gibbs CJ. Ganglioside changes in slow virus diseases: analyses of chimpanzee brains infected with kuru and Creutzfeldt-Jakob agents. *Brain Res.* 1974; 70:103–112. [PubMed: 4207047]
- Zervas M, Dobrenis K, Walkley SU. Neurons in Niemann-Pick disease type C accumulate gangliosides as well as unesterified cholesterol and undergo dendritic and axonal alterations. *J. Neuropathol. Exp. Neurol.* 2001; 60:49–64. [PubMed: 11202175]
- Zhang Y, Li X, Becker KA, Gulbins E. Ceramide-enriched membrane domains--structure and function. *Biochim. Biophys. Acta.* 2009; 1788:178–183. [PubMed: 18786504]

Figure 1. Specific increase of a single SM molecular specie in ASMko mice brains

Analysis of SM in purified myelin from wt and ASMko mice was carried out by high performance liquid chromatography/Electrospray ionization mass spectrometry as described in Valsecchi et al., 2007.

Table 1
Secondary accumulation of gangliosides in diseases with neurological impairment

Table 1 summarizes the alterations in glycosphingolipid metabolism leading to secondary ganglioside accumulation in several diseases characterized by neurological impairment, with or without impairment of lysosomal function.

Disease	Lysosomal involvement	Effect on gangliosides	References
Niemann Pick type A	yes	GM3 and GM2 accumulation	Rodriguez-Lafrasse et al., 1999; Scandroglio et al., 2008; Buccinna et al., 2009
Niemann Pick type C	yes	GM3 and GM2 accumulation	Siegel and Walkley, 1994; Sleat et al., 2004
Mucopolysaccharidosis	yes	GM3 and/or GM2 accumulation	Siegel and Walkley, 1994; Constantopoulos and Dekaban, 1977; Constantopoulos et al., 1978; Constantopoulos et al., 1980; Liour et al., 2001
α -mannosidosis	yes	GM3 and GM2 accumulation	Siegel and Walkley, 1994, Goodman et al., 1991
Alzheimer's disease	no	Reduced ganglioside concentration in several brain areas and altered ratios of a-series to b-series gangliosides Elevated levels of simpler gangliosides (GM3 and GM2)	Brooksbank and McGovern, 1989; Crino et al., 1989; Kalanj et al., 1991; Kracun et al., 1991; Kracun et al., 1992; Svennerholm et al., 1994 Kracun et al., 1992; Barrier et al., 2007)
Huntington's disease	no	Reduced ganglioside concentration in erythrocytes, striatum and caudate Abnormal expression of glycosyltransferase genes Increased GD3 levels	Wherrett and Brown, 1969; Higatsberger et al., 1981; Desplats et al., 2007 Desplats et al., 2007 Desplats et al., 2007
Prion diseases	no	Reduced ganglioside content with a shift from complex to simpler species (GM3, GD3, GD2) Alterations in the long-chain base composition of gangliosides	Yu et al., 1974; Tamai et al., 1979; Ando et al., 1984; Di Martino et al., 1993; Ohtani et al., 1996 Di Martino et al., 1993
Severe malignant autosomal recessive osteopetrosis	uncertain	Accumulation of GM3 and GM2, no changes in lysosomal glycohydrolases	Prinetti et al., 2009

Table 2
Aberrant phenotypes in ASMko brain cells

The table summarizes the aberrant phenotypes found until now in neurons, oligodendrocytes and astrocytes of ASMko mice brains. Corresponding references are also indicated.

Brain cell type	Aberrant phenotype	References
NEURONS		
	Deficient Ca ²⁺ homeostasis	Ginzburg and Futerman, 2005
	Altered molecular polarization	Galvan et al., 2008
	Impaired endocytosis	Galvan et al., 2008
	Smaller synapses	Camoletto et al., 2009
	Altered presynaptic plasticity	Camoletto et al., 2009
	Complete loss of Purkinje neurons	Horinouchi et al., 2005; Otterbach and Stoffel., 2005;Sarna et al., 2001; Macauley et al., 2008
	Altered lipid composition	Scandroglio et al., 2008; Galvan et al., 2008
OLIGODENDROCYTES		
	Decreased expression of myelin proteins	Buccinna et al., 2009
	Altered lipid composition	Buccinna et al., 2009
ASTROCYTES		
	Complete blockade of microparticle shedding and IL-1 β release	Bianco et al., 2009