Accumulation of Ordered Ceramide-Cholesterol Domains in Farber Disease Fibroblasts

Natalia Santos Ferreira • Michal Goldschmidt-Arzi •
Helena Sabanay • Judith Storch • Thierry Levade •
Maria Gil Ribeiro • Lia Addadi • Anthony H. Futerman

Received: 13 March 2013 / Revised: 19 May 2013 / Accepted: 30 May 2013 / Published online: 12 July 2013
© SSIEM and Springer-Verlag Berlin Heidelberg 2013

Abstract Farber disease is an inherited metabolic disorder caused by mutations in the acid ceramidase gene, which leads to ceramide accumulation in lysosomes. Farber

disease patients display a wide variety of symptoms with most patients eventually displaying signs of nervous system dysfunction. We now present a novel tool that could potentially be used to distinguish between the milder and more severe forms of the disease, namely, an antibody that recognizes a mixed monolayer or bilayer of cholesterol:C16-ceramide, but does not recognize either ceramide or cholesterol by themselves. This antibody has previously been used to detect cholesterol:C16-ceramide domains in a variety of cultured cells. We demonstrate that levels of cholesterol:C16-ceramide domains are significantly elevated in fibroblasts from types 4 and 7 Farber disease patients, and that levels of the domains can be modulated by either reducing ceramide or cholesterol levels. Moreover, these domains are located in membranes of the endomembrane system, and also in two unexpected locations, namely, the mitochondria and the plasma membrane. This study suggests that the ceramide that accumulates in severe forms of Farber disease cells is sequestered to distinct membrane subdomains, which may explain some of the cellular pathology observed in this devastating lysosomal storage disease.

Communicated by: Gregory M. Pastores, MD

Competing interests: None declared

Authors Natalia Santos Ferreira and Michal Goldschmidt-Arzi contributed equally to this study

N.S. Ferreira • M.G. Ribeiro

National Health Institute Doutor Ricardo Jorge, Genetic Department, Centre for Medical Genetics Jacinto de Magalhães, Porto, Portugal

N.S. Ferreira

Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

N.S. Ferreira • M. Goldschmidt-Arzi • A.H. Futerman (✉)

Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

e-mail: tony.futerman@weizmann.ac.il

M. Goldschmidt-Arzi • L. Addadi

Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel

H. Sabanay

Department of Chemical Research Support, Weizmann Institute of Science, Rehovot 76100, Israel

J. Storch

Department of Nutritional Sciences and Rutgers Center for Lipid Research, Rutgers University, Rutgers, NJ 08901, USA

T. Levade

Equipe Labellisée Ligue Contre le Cancer 2013, Centre de Recherches en Cancérologie de Toulouse (CRCT), Université de Toulouse, Toulouse, France

T. Levade

Laboratoire de Biochimie Métabolique, Institut Fédératif de Biologie, CHU Purpan, Toulouse, France

M.G. Ribeiro

Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal

Abbreviations

CD	2-hydroxypropyl- β -cyclodextrin
Cer/chol	ceramide/cholesterol
LSD	lysosomal storage disease
PBS	phosphate buffered saline
TEM	transmission electron microscopy
WT	wild type

Introduction

Farber disease (Farber lipogranulomatosis: OMIM # 228000) is a lysosomal storage disease (LSD) caused by mutations in the acid ceramidase (*ASAH1*) gene, which results in intracellular ceramide accumulation (Levade et al. 2009). As with most LSDs, patients display a wide variety of symptoms, with the most severely affected displaying central nervous system involvement (Levade et al. 2009). Seven clinical subtypes of the disease are known which are associated with 17 distinct mutations in the *ASAH1* gene (Zhang et al. 2000; Bär et al. 2001; Muramatsu et al. 2002; Devi et al. 2006). The subtypes are categorized according to the age of onset, symptom severity, and the tissue where lipid accumulation appears (Levade et al. 2009). Types 1 and 5 are the most common forms, with patients displaying nervous system dysfunction and an average age of death between 2 and 3 years (Eviatar et al. 1986; Scriver 1995). Types 2 and 3 are milder forms with death occurring in the second or third decade of life (Ehlert et al. 2007). Type 4 is a very severe form, with patients displaying neurological deterioration, hepatosplenomegaly at birth, and granulomatous infiltrations in the liver, spleen, lymphoid tissue, thymus, and lungs; death typically occurs in the first years of life (Schafer et al. 1996). Type 6 disease has only been described once (Fusch et al. 1989). Type 7 Farber disease, which displays a very severe phenotype (Levade et al. 2009), is not due to mutations in the *ASAH1* gene but rather due to mutations in the gene encoding prosaposin D, which may serve as an activator for *ASAH1* (Levade et al. 2009).

Ceramide accumulation is common to all Farber disease types. Surprisingly, despite wide interest in ceramide in both intracellular signaling pathways (Hannun and Obeid 2008) and as a structural component of membrane lipid microdomains/rafts (Schenck et al. 2007; Lingwood and Simons 2010), only a few studies have used Farber cells to examine the effect of ceramide accumulation on cell function (Levade et al. 1993; Tardy et al. 2004). We now take advantage of a newly developed tool, namely, a monoclonal antibody that was raised and selected to recognize a mixed monolayer phase composed of 60:40 mol% cholesterol: C16-ceramide, at which molar ratio, the two lipids form an ordered and homogeneous phase when deposited as a monolayer at the air-water interface and as a single hydrated bilayer (Ziblat et al. 2012). This anti-C16-ceramide/cholesterol (anti-C16-Cer/Chol) antibody interacts specifically with C16-ceramide/cholesterol and does not bind to pure cholesterol or ceramide monolayers (Scheffer et al. 2006). Using this antibody, we have previously demonstrated that C16-Cer/Chol domains are found at high levels in late endosomes in a variety of cultured cells (Goldschmidt-Arzi et al. 2011). We now use this antibody to determine the levels and localization of C16-Cer/Chol

domains in Farber disease patient fibroblasts, and demonstrate that types 4 and 7 fibroblasts display high levels of the domains, which can be abrogated either by reduction of ceramide or of cholesterol levels.

Materials and Methods

Reagents

Dulbecco's Modified Eagle medium (DMEM) was from Gibco Invitrogen (Carlsbad, CA, USA). The following antibodies were used: anti-voltage-dependent anion-selective channel protein 1 (VDAC1), anti-LAMP2a, anti-calnexin, and anti-Rab7 (Abcam, Cambridge, UK); anti-Rab5 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA); anti-Golga5 (Sigma-Aldrich, St Louis, Missouri, MO); and Cy2- and Cy3-labeled secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA). Wheat germ agglutinin (WGA), Texas Red, and 4',6-diamidino-2-phenylindole (DAPI) were from Molecular Probes (Eugene, OR, USA). An anti-mouse Fab fragment conjugated to 1.2 nm colloidal gold was from Nanoprobes (Yaphank, NY, USA). Bovine serum albumin, antibiotics for use in cell culture, fetal bovine serum, trypsin, and 2-hydroxypropyl- β -cyclodextrin (CD) were from Sigma-Aldrich (St Louis, Missouri, MO). All other reagents or solvents were of the highest grade.

Cell Culture

Farber patient cell lines were purchased from the NIGMS Human Genetic Cell Repository (see Table 1). The transformed uncorrected Farber Moh (pAS-Moh) and gene-corrected Moh (pAS-Moh-ACx5) cell lines were previously described (Medin et al. 1999). Normal human skin fibroblasts were provided by Dr. Lúcia Lacerda (National Health Institute Doutor Ricardo Jorge, Porto, Portugal). Cells were maintained in culture medium supplemented with 10% fetal bovine serum and antibiotics (100 IU/ml penicillin, 100 μ g/ml streptomycin, and 1 mg/ml fungizone) at 37°C in 5% CO₂.

Antibody Production, Screening, and Purification

The anti-C16-Cer/Chol antibody was produced as described (Scheffer et al. 2006). Briefly, antibodies were purified from ascites fluid by affinity chromatography using an immuno-Pure IgM purification kit (Thermo Scientific-Pierce, Epsom, UK). The purified antibody was extensively dialyzed against phosphate buffered saline (PBS) and stored at 4°C. Antibody concentrations were calculated using absorbance at 280 nm. The antibody was used within 7 days after purification.

Table 1 Labeling intensity of C16-Cer/Chol domains in Farber disease patient fibroblasts. The fibroblasts used in this study are shown along with information about their phenotypes. The labeling

intensity with the anti-C16-Cer/Chol antibody was integrated and normalized over the cell area; data are means $\pm$ s.e.m., $n = 50$ cells from two different cultures

NIGMS human genetic cell repository designation	Name used in this study	Age at death	Presumed Farber type	Fold-change of anti-C16-Cer/Chol labeling intensity
WT ^a	WT 1-4	20–32 years	None	1.00 $\pm$ 0.10
GM20018	Type 1 #1	22 months	1	0.42 $\pm$ 0.12
GM20015	Type 1 #2	24 months	1	1.19 $\pm$ 0.08
GM02314	Type 2	6 years	2–3	0.65 $\pm$ 0.13
GM02315	Type 3	30 years	3	1.22 $\pm$ 0.13
GM05752	Type 4 #1	6 months	4	4.75 $\pm$ 0.06
GM18313	Type 4 #2	11 months	4	5.08 $\pm$ 0.08
GM20017	Type 5	3 years	5	0.35 $\pm$ 0.12
GM20016	Type 6 ⁴	29 months	6	0.42 $\pm$ 0.12
Prosaposin-deficient ^b	Type 7	4 months	7	2.79 $\pm$ 0.12
GM02316	Carrier	37 years	None	0.86 $\pm$ 0.11
pAS-Moh ^c	Type 4 #3	3 days	4	6.94 $\pm$ 0.07
pAS-Moh-ACx5 ^c	Type 4 #3/ AC	3 days	4	0.95 $\pm$ 0.11

^a Provided by Dr. Lúcia Lacerda (National Health Institute Doutor Ricardo Jorge, Porto, Portugal)

^b See Chatelut et al. 1997; Medin et al. 1999

^c In addition to mutations in the *ASAH1* gene, this patient was also reported to carry mutations in the β -hexosaminidase gene (Fusch et al. 1989; Levade et al. 1995)

Immunofluorescence Microscopy

Cells were cultured on 12–13-mm glass coverslips to a confluency of ~70%. Cells were fixed by incubating with 3% paraformaldehyde in PBS at room temperature for 45 min, rinsed three times with PBS containing 0.03% bovine serum albumin for 4 min, prior to incubation with the anti-C16-Cer/Chol antibody (10 μ g/ml) for 1 h. The anti-Rab7 antibody (which labels late endosomes) was used at a dilution of 1:50, anti-Rab5 (early endosomes) at 1:50, anti-Golga5 (Golgi apparatus) at 1:100, anti-VDAC1 (mitochondria) at 1:180, anti-calnexin (endoplasmic reticulum) at 1:250, anti-Lamp 2a (lysosomes) at 1:180, and anti-WGA (plasma membrane) at a 1:200 dilution. Cultures were rinsed three times with PBS containing 0.03% bovine serum albumin and then incubated for 30 min with the appropriate secondary antibodies. Nuclei were stained using DAPI at a dilution of 1:1,000 (5 min). Cells were mounted in Gel Mount (Molecular Probes) and observed 24 h later. Acquisition was performed using a confocal FV1000 on an Olympus IX81 microscope, using a 1.35 numerical aperture UPLSAPO 60x oil objective, and image quantification was performed using software provided with the instrument. Intensity measurements were performed by integrating the intensity values and normalizing over the cell area for a minimum of 50 cells per analysis.

Transmission Electron Microscopy

Fibroblasts grown on tissue culture plates were gently removed by washing with medium. Fixation and labeling were performed as above except that gold-conjugated (1.2 nm) secondary antibodies were used. Enhancement to a size of 10–30 nm was performed using a Gold Enhancement kit (Nanoprobes, Yaphank, NY). The pellet was left overnight in 2% glutaraldehyde. Prior to freezing, the pellets were washed by centrifugation ($290 \times g_{av}$, 4 min). The Tokuyasu method was used for immunolabeling (Tokuyasu 1973). Samples were infiltrated, cooled, and embedded in gelatin and fixed with 2% glutaraldehyde on ice, cryo-protected in sucrose, and vitrified by plunging in liquid N₂. The frozen samples were cryo-sectioned using a Leica EM FC6 cryomicrotome, and then transferred to bare 200-mesh nickel or Formvar-coated transmission electron microscopy (TEM) grids. Sucrose was removed and grids were stained with uranyl acetate and embedded in methylcellulose. Samples were viewed on a TEM, T12-Technai TEM microscope operating at 120 kV. Images were recorded on an Eagle 2 K₂ K FEI camera (Eindhoven, Netherlands).

Fig. 1 Immunofluorescence using the anti-C16-Cer/Chol antibody. C16-Cer/Chol domains were labeled using the anti-C16-Cer/Chol antibody in control fibroblasts (WT-2) and in a type 4 Farber disease patient (Type 4#1). The upper panels show immunofluorescence and the lower panels are phase contrast. Scale bar = 10 μ m

Results

Upon incubating fibroblasts with the anti-C16-Cer/Chol antibody, some labeling could be detected in WT fibroblasts, with a similar distribution to that observed in a number of other cell types (Goldschmidt-Arzi et al. 2011), i.e., in a perinuclear region indicative of late endosomes and the trans-Golgi network (Fig. 1; see also Fig. 4). In contrast, substantially more intense labeling was observed in fibroblasts derived from a type 4 Farber disease patient (Type 4 #1, Table 1 and Fig. 1), suggesting that C16-Cer/Chol domains accumulate in Farber disease fibroblasts.

Levels of C16-Cer/Chol domains were subsequently quantified in fibroblasts from Farber disease patients of different severity. The most severe patients, namely, the three type 4s and one type 7, displayed the highest level of labeling (Table 1), which was ~three- to fivefold higher than in control fibroblasts. Fibroblasts derived from patients who displayed a less severe disease, or a patient who was a Farber disease carrier, did not show elevated levels of C16-Cer/Chol domains (Table 1).

We next determined whether levels of C16-Cer/Chol domains could be altered either by modifying ceramide or cholesterol levels. For the former, we used human simian virus 40 (SV40) large T-transformed skin fibroblasts derived from a patient with type 4 Farber disease (type 4 #3), which had been transduced with a recombinant

Fig. 2 C16-Cer/Chol domains in Farber disease patient fibroblasts after acid ceramidase transduction. The intensity of the C16-Cer/Chol domains was measured in fibroblasts from a type 4 Farber patient (Type 4 #3, see Table 1) that had been transduced with acid ceramidase (Type 4 #3/AC). Panel A shows the quantification (data are means $\pm$ s.e.m, $n = 50$) and representative images are shown in panel B. The upper panels show immunofluorescence and the lower panels are phase contrast. Scale bar = 10 μ m

retroviral vector containing the *ASAH1* cDNA (type 4 #3/AC) (Medin et al. 1999). As expected, the untreated cell line showed high levels of C16-Cer/Chol domains (Fig. 2 and Table 1), whereas levels of the domains were reduced by ~sevenfold in the acid ceramidase-expressing cells (Fig. 2). Likewise, treatment of Type 4#1 Farber fibroblasts with CD, which reduces cholesterol levels in normal and

Fig. 3 Effect of CD treatment on anti-C16-Cer/Chol labeling. Domain intensity was measured in WT-2 and Type 4#1 fibroblasts after treatment with or without 100 μ M CD for 48h. Values are means $\pm$ s.e.m., $n = 2$

a significant increase in domain labeling was also observed in both the mitochondria and in the plasma membrane of type 4 Farber disease fibroblasts (Fig. 4). This localization was confirmed by immunogold labeling and TEM, in which significant labeling was detected in the mitochondria and in the plasma membrane (Fig. 5).

Discussion

Ceramide accumulation is the defining feature of Farber disease. In this study, we demonstrate that the ceramide that accumulates in the most severe forms of Farber disease, namely, types 4 and 7, co-localizes in structured domains with cholesterol in cell membranes. Moreover, these domains are not only found in late endosomes and lysosomes as might be expected, but also accumulate in mitochondria and the plasma membrane. The latter results are consistent with the

Fig. 4 Co-localization of C16-Cer/Chol domains. The extent of co-localization between the anti-C16-Cer/Chol antibody and each of the markers was determined in WT-2 and Type 4 #1 fibroblasts. Values

represent the extent of co-localization within one pair, such that the numbers are not additive. Data are means $\pm$ s.e.m., $n = 2$ ($*p < 0.01$, $**p < 0.0001$)

Niemann-Pick C disease cells (Kilsdonk et al. 1995; Rosenbaum et al. 2010; McCauliff et al. 2011) significantly decreased the intensity of the C16-Cer/Chol domains by ~sixfold, with no effect on levels of the domains in WT fibroblasts (Fig. 3).

The intracellular location of the C16-Cer/Chol domains was further examined by co-localization with a number of organelle markers. As observed in earlier studies (Goldschmidt-Arzi et al. 2011), significant co-localization was observed in membranes of the endocytic pathway, including early and late endosomes and lysosomes but, unexpectedly,

notion that extra-lysosomal substrates accumulate in a variety of lysosomal storage diseases (Korkotian et al. 1999; Futerman and van Meer 2004).

Ceramide and cholesterol are major components of lipid microdomains/rafts (Lingwood and Simons 2010). Each of these lipids, either together or separately, affects the biophysical properties of membrane bilayers. While ceramide is known to accumulate in Farber disease, there is no evidence that cholesterol accumulates. Our study, using an anti-C16-Cer/Chol antibody, which binds to C16-ceramide/cholesterol monolayers and not to pure

Fig. 5 Detection of C16-Cer/Chol domains by TEM. WT-2 and type 4 #1 fibroblasts were labeled using the anti-C16-Cer/Chol antibody and a gold-conjugated secondary antibody, and examined by TEM. The

images are inverted (gold particles are indicated as magnified *black dots*). The plasma membrane (PM) was identified via its invaginations, and the mitochondria via their cisternae. Scale bar = 100 nm

cholesterol or ceramide, suggests that ceramide sequesters cholesterol in microdomains upon its accumulation in Farber disease. This may be of relevance for understanding the intracellular signaling pathways that are altered in Farber disease, since many signaling pathways are modulated by the membrane lipid composition (Foster et al. 2003). This is particularly true since significant levels of C16-Cer/Chol domains were detected in the plasma membrane, which is the main interface via which cells sense their environment. Interestingly, cholesterol depletion by CD decreased levels of the domains. Whether cholesterol depletion might be a therapeutic option in Farber disease remains to be explored, it should be noted that we have not detected elevated cholesterol in Farber disease, but rather elevated sequestration of accumulated ceramide in ceramide/cholesterol domains.

The accumulation of C16-Cer/Chol domains in mitochondria was unexpected, although a significant body of data has been generated in the past few years demonstrating that mitochondria are a key hub in ceramide biology (Birbes et al. 2002). Indeed, ceramide levels in mitochondria are elevated as a response to stress (Birbes et al. 2001; Siskind et al. 2010), suggesting that Farber cells might be in a constant state of cellular stress. The accumulation of C16-Cer/Chol domains in the plasma membrane is easier to explain, since the plasma membrane is both enriched in cholesterol and part of the endomembrane system, and perhaps once lysosomes become saturated with ceramide, it could overflow into other interconnected compartments.

Interestingly, increased levels of domains were only observed in the most severe forms of the disease, suggesting that a threshold level of ceramide accumulation is required before it can sequester cholesterol and thus be detected by the anti-C16-Cer/Chol antibody.

Whether the anti-C16-Cer/Chol antibody might be used as a diagnostic tool for the most severe forms of Farber disease is open for discussion. Once a diagnosis of Farber disease has been made in the clinic (Dulaney et al. 1976) either by enzymatic or genetic analysis, the question of disease severity becomes relevant. The anti-C16-Cer/Chol antibody is able to distinguish the most severe forms of the disease in a relatively quick and inexpensive manner, but the few surviving patients with the severe forms of the disease are not conducive to widespread use of the antibody for diagnosis of severity. Moreover, the use of this antibody to demonstrate that ceramide is sequestered into domains containing cholesterol suggests that previously unexpected cellular pathways might be involved in Farber disease pathology, which could lead to novel modes of therapeutic intervention.

Acknowledgments N.S. Ferreira is a recipient of a PhD fellowship from the Fundação para a Ciência e Tecnologia (SFRH/BD/40319/2007). Electron microscopy was performed at the Irving and Cherna Moskowitz Center for Nano and Bio-Nano Imaging at the Weizmann Institute. We thank Dr. Lúcia Lacerda for providing cells and Vladimir Kiss for help with confocal microscopy. A.H. Futerman is the Joseph Meyerhoff Professor of Biochemistry and Lia Addadi is the incumbent of the Dorothy-and-Patrick-Gorman Professorial Chair of Biological Ultrastructure at the Weizmann Institute of Science.

References

- Bär J, Linke T, Ferlinz K, Neumann U, Schuchman EH, Sandhoff K (2001) Molecular analysis of acid ceramidase deficiency in patients with Farber disease. *Hum Mutat* 17:199–209
- Birbes H, El Bawab S, Hannun YA, Obeid LM (2001) Selective hydrolysis of a mitochondrial pool of sphingomyelin induces apoptosis. *FASEB J* 15:2669–2679
- Birbes H, El Bawab S, Obeid LM, Hannun YA (2002) Mitochondria and ceramide: intertwined roles in regulation of apoptosis. *Adv Enzyme Regul* 42:113–129
- Chatelut M, Harzer K, Christomanou H et al (1997) Model SV40-transformed fibroblast lines for metabolic studies of human prosaposin and acid ceramidase deficiencies. *Clinica chimica acta. Int J Clin Chem* 262:61–76
- Devi ARR, Gopikrishna M, Ratheesh R, Savithri G, Swarnalata G, Bashyam M (2006) Farber lipogranulomatosis: clinical and molecular genetic analysis reveals a novel mutation in an Indian family. *J Hum Genet* 51:811–814
- Dulaney JT, Milunsky A, Sidbury JB, Hobolth N, Moser HW (1976) Diagnosis of lipogranulomatosis (Farber disease) by use of cultured fibroblasts. *J Pediatr* 89:59–61
- Ehlert K, Frosch M, Fehse N, Zander A, Roth J, Vormoor J (2007) Farber disease: clinical presentation, pathogenesis and a new approach to treatment. *Pediatric Rheumatol Online J* 5:15
- Eviatar L, Sklower SL, Wisniewski K, Feldman RS, Gochoco A (1986) Farber lipogranulomatosis: an unusual presentation in a black child. *Pediatr Neurol* 2:371–374
- Foster LJ, De Hoog CL, Mann M (2003) Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. *Proc Natl Acad Sci USA* 100:5813–5818
- Fusch C, Huenges R, Moser HW et al (1989) A case of combined Farber and Sandhoff disease. *Eur J Pediatr* 148:558–562
- Futerman AH, van Meer G (2004) The cell biology of lysosomal storage disorders. *Nat Rev Mol Cell Bio* 5:554–565
- Goldschmidt-Arzi M, Shimoni E, Sabanay H, Futerman AH, Addadi L (2011) Intracellular localization of organized lipid domains of C16-ceramide/cholesterol. *J Struct Biol* 175:21–30
- Hannun YA, Obeid LM (2008) Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Bio* 9:139–150
- Kilsdonk EP, Yancey PG, Stoudt GW et al (1995) Cellular cholesterol efflux mediated by cyclodextrins. *J Biol Chem* 270:17250–17256
- Korkotian E, Schwarz A, Pelled D, Schwarzmans G, Segal M, Futerman AH (1999) Elevation of intracellular glucosylceramide levels results in an increase in endoplasmic reticulum density and in functional calcium stores in cultured neurons. *J Biol Chem* 274:21673–21678
- Levade T, Tempesta MC, Salvayre R (1993) The in situ degradation of ceramide, a potential lipid mediator, is not completely impaired in Farber disease. *FEBS Lett* 329:306–312
- Levade T, Enders H, Schliephacke M, Harzer K (1995) A family with combined Farber and Sandhoff, isolated Sandhoff and isolated fetal Farber disease: postnatal exclusion and prenatal diagnosis of Farber disease using lipid loading tests on intact cultured cells. *Eur J Pediatr* 154:643–648
- Levade T, Sandhoff K, Schulze H, Medin JA (2009) Acid ceramidase deficiency: Farber lipogranulomatosis. In: Valle D et al (eds) *Scriver's OMMBID (Online Metabolic and Molecular Bases of Inherited Disease)*. McGraw-Hill, New York. <http://www.ommbid.com>
- Lingwood D, Simons K (2010) Lipid rafts as a membrane-organizing principle. *Science* 327:46–50
- McCauliff LA, Xu Z, Storch J (2011) Sterol transfer between cyclodextrin and membranes: similar but not identical mechanism to NPC2-mediated cholesterol transfer. *Biochemistry* 50:7341–7349
- Medin JA, Takenaka T, Carpentier S et al (1999) Retrovirus-mediated correction of the metabolic defect in cultured Farber disease cells. *Hum Gene Ther* 10:1321–1329
- Muramatsu T, Sakai N, Yanagihara I et al (2002) Mutation analysis of the acid ceramidase gene in Japanese patients with Farber disease. *J Inher Metab Dis* 25:585–592
- Rosenbaum AI, Zhang G, Warren JD, Maxfield FR (2010) Endocytosis of beta-cyclodextrins is responsible for cholesterol reduction in Niemann-Pick type C mutant cells. *Proc Natl Acad Sci* 107:5477–5482
- Schafer A, Harzer K, Kattner E, Schafer HJ, Stoltenburg G, Lietz H (1996) Disseminated lipogranulomatosis (Farber disease) with hydrops fetalis. *Pathologie* 17:145–149
- Scheffer L, Fargion I, Addadi L (2006) Structural recognition of cholesterol-ceramide monolayers by a specific monoclonal antibody. *Chembiochem* 7:1680–1682
- Schenck M, Carpinteiro A, Grassme H, Lang F, Gulbins E (2007) Ceramide: physiological and pathophysiological aspects. *Arch Biochem Biophys* 462:171–175
- Scriver CR (1995) *The metabolic and molecular bases of inherited disease*. McGraw-Hill Health Professions Division, New York
- Siskind LJ, Mullen TD, Romero Rosales K et al (2010) The BCL-2 protein BAK is required for long-chain ceramide generation during apoptosis. *J Biol Chem* 285:11818–11826
- Tardy C, Andrieu-Abadie N, Salvayre R, Levade T (2004) Lysosomal storage diseases: is impaired apoptosis a pathogenic mechanism? *Neurochem Res* 29:871–880
- Zhang Z, Mandal AK, Mital A et al (2000) Human acid ceramidase gene: novel mutations in Farber disease. *Mol Genet Metab* 70:301–309
- Ziblat R, Fargion I, Leiserowitz L, Addadi L (2012) Spontaneous formation of two-dimensional and three-dimensional cholesterol crystals in single hydrated lipid bilayers. *Biophys J* 103:255–264