Elevation of plasma lysosphingomyelin-509 and urinary bile acid metabolite in Niemann-Pick disease type C-affected individuals

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ABSTRACT

Niemann-Pick disease type C (NPC) is a neurovisceral disorder associated with the accumulation of lipids such as cholesterol and sphingolipids. NPC is caused by either NPC1 or NPC2, which encode lysosomal proteins located at membraneous and soluble fractions, respectively. For the past decade, the oxidation products of cholesterol, such as cholestane-3β,5α,6β-triol and 7-ketocholesterol, have been considered selective biomarkers for NPC. However, recent evidence has indicated numerous novel biomarkers for NPC, which raises the possibility that the diagnosis of NPC might be associated with the elevation of multiple lipid biomarkers, rather than a single biomarker. Sphingosylphosphorylcholine (SPC) has been suggested to be one such biomarker for NPC, in which the level of sphingomyelin is increased in NPC in the liver and spleen. A recent study revealed the prevalence of classical NPC 1/89,229, while the incidence of late-onset NPC incompletely predicted 1/65,000. Several murine NPC models could offer a promising platform for the diagnosis of NPC.

1. Introduction

Niemann-Pick disease type C (NPC) is a neurovisceral disorder caused by a defective mutation in either the NPC1 (OMIM 607623) or the NPC2 (OMIM 601015) gene [1,2]. The impaired egress of cholesterol from the late endosome/lysosomal compartment has been suggested to be a relevant mechanism for the pathogenesis of NPC. Early evidence showed consistently that NPC was associated with the accumulation of various lipids, including cholesterol in humans [3]. These lipids include a variety of cholesterol metabolites such as oxysterol, including cholestane-3β,5α,6β-triol and 7-ketocholesterol [4–9], bile acids [10–14], and glucosylated cholesterol [15], respectively. The NPC1 protein is a membraneous protein in the lysosome, which facilitates the transportation of cholesterol from the lysosome to plasma membrane, whereas NPC2 is a soluble protein in the lysosome, which binds stoichiometrically to cholesterol. Based on these biochemical properties of NPC1 and NPC2, the mechanism(s) of NPC might be, at least partly, attributed to the failure of proper lipid trafficking in the cells [2]. This possibility was evidenced in several murine NPC models that showed positive therapeutic outcomes on the established NPC manifestations by treatment with cyclodextrin, a circular oligosaccharide that facilitates cholesterol transportation across the plasma membrane [16–18]. A recent study revealed the prevalence of classical NPC is 1/89,229, while the incidence of late-onset NPC incompletely predicted 1/65,000. Several murine NPC models could offer a promising platform for the diagnosis of NPC.

Both clinical and experimental evidence have indicated that the level of sphingomyelin is increased in NPC in the liver and spleen [2,3]. In mammals, the biosynthesis of sphingomyelin is initiated by serine phosphorylation of serine, which is then transferred to choline to form sphingosine, which is finally converted to sphingomyelin by sphingomyelin synthase [19]. Sphingomyelin is a major phospholipid that is present in the plasma membrane, where it serves as a major phospholipid, in addition to cholesterol, as a platform for the accumulation of other lipids and lipoproteins [20]. In addition, sphingomyelin is involved in the regulation of various cellular processes, including cell growth, cell differentiation, and cell death [21]. Therefore, the level of sphingomyelin is thought to be a potential biomarker for NPC, but its clinical usefulness has not been fully established.

A recent study revealed the prevalence of classical NPC is 1/89,229, while the incidence of late-onset NPC incompletely predicted 1/65,000. Several murine NPC models could offer a promising platform for the diagnosis of NPC.

1.1. NPC1 and NPC2

NPC1 is a membraneous protein in the lysosome, which facilitates the transportation of cholesterol from the lysosome to plasma membrane, whereas NPC2 is a soluble protein in the lysosome, which binds stoichiometrically to cholesterol. Based on these biochemical properties of NPC1 and NPC2, the mechanism(s) of NPC might be, at least partly, attributed to the failure of proper lipid trafficking in the cells [2]. This possibility was evidenced in several murine NPC models that showed positive therapeutic outcomes on the established NPC manifestations by treatment with cyclodextrin, a circular oligosaccharide that facilitates cholesterol transportation across the plasma membrane [16–18]. A recent study revealed the prevalence of classical NPC is 1/89,229, while the incidence of late-onset NPC incompletely predicted 1/65,000. Several murine NPC models could offer a promising platform for the diagnosis of NPC.

1.2. Biomarkers for NPC

The most common biomarker for NPC is the level of sphingomyelin, which is increased in NPC in the liver and spleen. However, recent evidence has indicated numerous novel biomarkers for NPC, which raises the possibility that the diagnosis of NPC might be associated with the elevation of multiple lipid biomarkers, rather than a single biomarker. Sphingosylphosphorylcholine (SPC) has been suggested to be one such biomarker for NPC, in which the level of sphingomyelin is increased in NPC in the liver and spleen. A recent study revealed the prevalence of classical NPC 1/89,229, while the incidence of late-onset NPC incompletely predicted 1/65,000. Several murine NPC models could offer a promising platform for the diagnosis of NPC.
and palmitoly-CoA in the endoplasmic reticulum through the enzymatic action of serine: palmitoly-CoA transferase (EC 2.3.1.50) [29]. Sphingomyelin is a major sphingolipid that is located in the outer membrane of cells. Because sphingomyelin accumulates in NPC-affected individuals, the plasma concentration of lysosphingomyelin, also known as sphingosylphosphorylcholine (SPC), has been proposed as a biomarker for NPC [5,21,22]. An early study showed that the plasma concentration of SPC was correlated with that of cholestan-3β,5α,6β-triol and 7-ketocholesterol, both of which are widely accepted measures for the diagnosis of NPC [5]. A recent report documented that SPC was elevated in patients with acyl sphingomyelinase deficiency as well as in patients with NPC [21]. Interestingly, this study also found that the selective accumulation of SPC in NPC was detectable only in the plasma, not in the dried blood spots [21]. Furthermore, lysosphingomyelin-509 is a novel biomarker with uncharacterized chemical nature, accumulating in the plasma of NPC-affected individuals [21–23]. Based on these results, we sought to determine whether the level of plasma SPC and lysosphingomyelin-509 might be correlated with another established biomarker for NPC. Among several candidate compounds, we focused on the recently discovered bile acid metabolite 3β-sulfooxy-7β-N-acetylglucosaminyl-5-cholen-24-oic acid (SNAG-Δ⁵-CA) because its elevation in NPC has been established [11].

2. Experimental procedure

2.1. Reagents

D-erythro-sphingosylphosphorylcholine (synthetic SPC) was purchased from Toronto Research Chemicals (Ontario, Canada). Sphingosylphosphorylcholine (C17 base, ISSPC) was purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Acetonitrile and methanol were purchased from Fischer Scientific (Tokyo, Japan). An Oasis HLB 96-well plate was purchased from Waters (Milford, MA, USA). Ammonium acetate and formic acid were purchased from Kanto Chemical (Tokyo, Japan). Deionized water was obtained from a Milli-Q water system (Millipore, Milford, MA, USA). Ammonium acetate and formic acid were purchased from Kanto Chemical (Tokyo, Japan). An Oasis HLB 96-well plate was purchased from Waters (Milford, MA, USA). 3β-Sulfooxy-7β-hydroxy-23-nor-5-cholenoic acid was synthesized and used as an internal standard for SNAG-Δ⁵-CA (IS bile acid) as previously described [11,24]. The other reagents used in this study were of the highest grade commercially available. The chemical structures of the compounds used in this study are shown in Fig. 1.

2.2. Approval by institutional research ethics board

This study was approved by the Research Ethics Board of the National Center for Child Health and Development.

2.3. Patient specimens

All NPC-affected individuals were diagnosed by previously established method [11,14].

2.4. Solid-phase extraction

The preparation and analysis of plasma samples by LC-MS/MS assay with slight modifications was previously reported [5]. In brief, an aliquot of plasma (100 μL) was mixed with Solution 1 (900 μL, 75% water, 25% methanol, and 0.1% H₃PO₄), which contained 2.169 pmol ISSPC. This suspension then was loaded onto the Oasis HLB 96-well plate (30 μm, 30 mg, Waters), prewashed with hexane (1 mL) and methanol (1 mL), followed by equilibration with Solution 1 (1 mL × 2). After the sample was loaded, the column was washed with Solution 1 (1 mL) and Solution 2 (75% water and 25% methanol, 1 mL). Finally, the mixture of SPC and ISSPC was eluted with Solution 3 (0.01% NH₃ in methanol, 0.4 mL × 3). After the removal of solvent under N₂ using a dry gas generator (Nihonseiki Co., Ltd., Osaka, Japan), the residue was
reconstituted using Solution 4 (90% methanol, 10% water, and 0.1% formic acid, 60 μL).

2.5. Quantification of plasma SPC and lysosphingomyelin-509

An aliquot (1 μL) was injected onto a Nexara UPLC system linked to an LCMS8030plus mass spectrometer (Shimadzu, Kyoto, Japan). SPC, lysosphingomyelin-509 and IS_{SPC} were chromatographed on an InertSustainSwift C18 column (2.1 × 30 mm, 3 μm, GL Sciences, Tokyo, Japan) over 3 min using mobile phase A (5 mM ammonium acetate in methanol/water = 5/95) and mobile phase B (0.1% formic acid in methanol) at a flow rate of 0.5 mL/min. The temperature of the analytical column was maintained at 50 °C using a column oven CTO-10 (Shimadzu). The column was initially maintained using 60% mobile phase B for 0.5 min followed by a linear gradient using 60–100% mobile phase B at 0.5–1.5 min. Then the column was washed using 100% mobile phase B for 1.5–2.0 min followed by equilibration using 60% mobile phase B for 2.0–3.0 min. The data were acquired by multiple reaction monitoring (MRM) and the electrospray (ESI) positive mode. The chromatographic data were collected using software LabSolutions (Shimadzu). The instrumentation is described in detail in Supplementary Tables 1–3.

2.6. Quantification of a urinary bile acid metabolite SNAG-Δ⁵-CA

The concentration of SNAG-Δ⁵-CA was quantified as reported previously using an automated LC-MS/MS system with online-based sample purification [11]. In brief, an aliquot (50 μL) of a diluted urinary sample with the internal standard (100 μL of urine +100 μL of 2 μM IS_{bile acid}) was injected into a trapping column (Shim-pack MAYI-C8, 5 μm, 4.6 × 10 mm, Shimadzu, Kyoto, Japan) using binary mobile phases comprising aqueous 20 mM ammonium acetate (pH 5.5) and an LC-MS8030plus mass spectrometer (Shimadzu, Kyoto, Japan). SPC, lysosphingomyelin-509 and IS_{SPC} were chromatographed on an InertSustainSwift C18 column (2.1 × 30 mm, 3 μm, GL Sciences, Tokyo, Japan) over 3 min using mobile phase A (5 mM ammonium acetate in methanol/water = 5/95) and mobile phase B (0.1% formic acid in methanol) at a flow rate of 0.5 mL/min. The temperature of the analytical column was maintained at 50 °C using a column oven CTO-10 (Shimadzu). The column was initially maintained using 60% mobile phase B for 0.5 min followed by a linear gradient using 60–100% mobile phase B at 0.5–1.5 min. Then the column was washed using 100% mobile phase B for 1.5–2.0 min followed by equilibration using 60% mobile phase B for 2.0–3.0 min. The data were acquired by multiple reaction monitoring (MRM) and the electrospray (ESI) positive mode. The chromatographic data were collected using software LabSolutions (Shimadzu). The instrumentation is described in detail in Supplementary Tables 1–3.

Table 3

| Investigator       | Plasma SPC (nM) | Cutoff (nM) | Increase (fold) | n  | Extraction | Country/area | Ref          |
|--------------------|----------------|-------------|-----------------|----|------------|--------------|--------------|
| Welford RW et al.  | 7.23–69.73     | Not determined | 2.8             | 57 | SPE        | Europe and Brazil | [5]          |
| Kucher L et al.    | 16–111         | Not determined | 3.3             | 15 | Butanol    | Not described            | [21]          |
| Polo G et al.      | Approx 20–80    | 16.8        | 3.4             | 11 | N/A        | Italy            | [22]          |
| Mashima R et al.   | 5.1–11.7       | Not determined | 2.6             | 7  | SPE        | Japan           | This study   |

SPE, solid-phase extraction.
methanol (9/1, v/v) at a flow rate of 1.0 mL/min. After 3 min, \( \Delta^\alpha \)-CA was delivered to a YMC-Pack Pro C18 (5 \( \mu \)m, 2.0 × 150 mm, YMC, Kyoto) using 20 mM ammonium acetate (pH 5.5) and methanol (5/5, v/v) at a flow rate of 0.2 mL/min. The temperature of the columns was maintained at 40 °C.

An API 5000 mass spectrometer (AB Sciex, Framingham, MA) was used for the detection of SNAG-\( \Delta^\alpha \)-CA. For selected reaction monitoring mode, we chose the combination of m/z 672.3 and m/z 97.0 for Q1 and Q3, respectively. The dwell time and collision energy were set at 250 ms and −70 V, respectively. The data were analyzed using the Analyst 1.4.1 software (AB Sciex). The instrumentation is described in detail in Supplementary Tables 4–6.

### 2.7. Statistical analysis

The data were expressed as mean ± SD. The mean values of the two groups were compared using a Student’s t-test. The difference was considered statistically significant at \( p < 0.05 \).

### 3. Results

#### 3.1. Assay validation

To quantify the plasma SPC and lysosphingomyelin-509 concentration with the highest possible sensitivity and precision, we selected SPC as the surrogate biomarker for lysosphingomyelin-509 using reversed-phase chromatography with MS/MS-based detection. Under our assay conditions, both SPC and ISSPC showed linear responses ranging from 1 to 200 fmol (Supplementary Fig. 1). In this case, the intraday CV (%) was 7%, 6%, 6% for 216 nM, 108 nM, and 21.6 nM, respectively (\( n = 5 \)). To examine the recovery of SPC in the biological samples, we first investigated the recovery of SPC in PBS as the blank matrix. Spiked SPC (11 nM) was detected in PBS with a recovery of 119% (Supplementary Fig. 2 and Table 1). Similarly, the recovery of spiked SPC (11 nM) in plasma was 120% under this assay condition. The interday CV (%) of the spiked SPC in plasma was 19% in 108 nM SPC, 6% in 11 nM, and 11% in 1 nM SPC (Table 2).

#### 3.2. Plasma SPC and lysosphingomyelin-509 concentration in NPC-affected individuals

Based on the results of this validation study, we first examined the plasma SPC concentrations in the NPC-affected individuals. As shown in Fig. 2, the peak of SPC, which migrated at 1.5 min, was elevated in all NPC-affected individuals compared to the controls (Fig. 2, top), whereas the amount of ISSPC remained unaltered (Fig. 2, bottom). In this study, the averaged plasma SPC concentrations in the NPC-affected individuals were \( 8.2 \pm 2.8 \) nM (mean ± SD; median, 7.0 nM; max, 11.7 nM; min, 5.1 nM; \( n = 5 \)), whereas in the controls, the averaged plasma SPC concentrations were \( 3.1 \pm 1.4 \) nM (median, 2.9 nM; max, 4.8 nM; min, 1.5 nM; \( n = 7 \)) (Fig. 3A). Thus, the increase in the averaged SPC concentration in the NPC-affected individuals was 2.6-fold higher than in the controls (Table 3). Similarly, the median value of multiple of median of plasma lysosphingomyelin-509 in NPC-affected individuals was 65.2-fold (max, 73.2; min, 26.7; \( n = 5 \)) (Fig. 3B). To further ensure that the accumulation of these two biomarkers were consistent with previously characterized biomarker for NPC, we sought to determine whether SNAG-\( \Delta^\alpha \)-CA, an established biomarker for NPC in urine, might be elevated in NPC-affected individuals. Thus, we quantified urinary SNAG-\( \Delta^\alpha \)-CA concentrations in samples taken from the same individuals using 3β-sulfooxy-7β-hydroxy-23-nor-5-cholenoic acid as IS\(_{\text{Bile acid}}\). The LC-MS/MS assay revealed that the SNAG-\( \Delta^\alpha \)-CA concentrations in the NPC-affected samples were 2477 ± 2968 ng/mL (median, 1332 ng/mL; max, 7731 ng/mL; min, 449 ng/mL; \( n = 5 \)), whereas those in the control samples were 39 ± 73 ng/mL (mean ± SD; median, 18 ng/mL; max, 204 ng/mL; min, 0 ng/mL; \( n = 7 \)) (Fig. 3C).

| Investigator    | Year | Biomarkers                        | Correlation | Ref |
|-----------------|------|-----------------------------------|-------------|-----|
| Porter FD et al.| 2010 | Plasma cholestane-3β,5α,6β-triol vs plasma 7-ketocholesterol | Yes         | [25]|
| Welford RW et al.| 2014 | Plasma SPC vs plasma cholestane-3β,5α,6β-triol | Yes         | [5] |
|                  | 2014 | Plasma SPC vs plasma glucosylphosphingosine | No          | [5] |
| Jiang X et al.  | 2016 | Plasma bile acid A vs plasma bile acid B | Yes         | [13]|
|                  | 2016 | Plasma cholestane-3β,5α,6β-triol vs IS\(_{\text{Bile acid}}\) | Yes         | [13]|
| Mashima R et al.| 2018 | Plasma SPC vs urinary bile acid metabolite SNAG-\( \Delta^\alpha \)-CA | Yes         | This study |
|                  | 2018 | Plasma lysosphingomyelin-509 vs urinary bile acid metabolite SNAG-\( \Delta^\alpha \)-CA | Yes         | This study |

Bile acid A, 5α-cholanic acid-3β,5α,6β-triol; bile acid B, 5α-cholanic acid-3β,5α,6β-triol N-(carboxymethyl)-amide.
3.3. The correlation between SPC and lysosphingomyelin-509 and a bile acid metabolite SNAG-$\Delta^5$-CA

Fig. 4A shows a summary of the correlation between plasma SPC and urinary bile acid metabolite SNAG-$\Delta^5$-CA. As shown, all NPC-affected individuals examined in this study exhibited higher concentrations of both SPC and SNAG-$\Delta^5$-CA. These results demonstrated that the combined quantification of plasma SPC and urinary bile acid metabolite SNAG-$\Delta^5$-CA using LC-MS/MS provided a more solid diagnostic basis for NPC than the single biomarkers did. Similarly, the correlation of plasma lysosphingomyelin-509 and urinary SNAG-$\Delta^5$-CA concentrations was clearly demonstrated (Fig. 4B). Within our examination, the sensitivity and specificity of plasma biomarkers, such as SPC and lysosphingomyelin-509, and of urine metabolites in NPC was 1 and 1, respectively.

4. Discussion

NPC is a lysosomal storage disorder caused by the pathogenic deficiency of either the NPC1 or NPC2 gene. Although the precise mechanism of the occurrence and development of NPC induced by these two genes remains uncertain, the accumulation of cholesterol in late endosome/lysosome has been suggested to be the precondition of this disorder. The hallmark of NPC involves the accumulation of cholesterol and its oxidation products. In support, previous studies showed that the elevation of plasma oysterols, such as cholestane-$\Delta^5$,5a,6b-triol and 7-ketocholesterol, were accepted as the diagnostic measure of NPC. In fact, the specificity of plasma cholestane-$\Delta^5$,5a,6b-triol and 7-ketocholesterol for NPC were 1.0 and 0.9984, respectively [25]. The overall correlation between previously proposed biomarkers for NPC was summarized in Table 4.

Bile acid metabolites, which are a series of compounds that originate in bile acids, have received much attention as biomarkers for NPC [10–14,26,27]. A milestone study was performed by Alvelius et al., which showed that bile acid metabolites in NPC-affected individuals were elevated by GC-MS [10]. Most bile acids produced in the body are excreted, whereas a small portion is known to circulate in the blood [28]. A recent study reported the correlation between cholestane-$\Delta^5$,5a,6b-triol in the plasma and the bile acid metabolite 5a-cholanic acid-$\Delta^5$,5a,6b-triol N-(carboxymethyl)-amide in a dried blood spot, which suggested the potential of circulating bile acid metabolites to be a novel biomarker for NPC [13]. The present study demonstrated a positive correlation between plasma biomarkers, such as SPC and/or lysosphingomyelin-509, and the urinary bile acid metabolite SNAG-$\Delta^5$-CA, which suggests that separate biological specimens (i.e., the plasma and urine) from one individual allowed for double-checking the results of diagnosis using a biochemical platform. It is generally anticipated that the prevalence of NPC is lower than other lysosomal-storage diseases such as Pompe disease and Fabry disease. This could be inversely related to the prevalence of NPC and the diagnostic platform. It is generally anticipated that the accumulation of SPC at least in part might be linked to sphingomyelin, which is a well-established biomarker for NPC [2]. In fact, to date, only one study performed by Welford et al. has demonstrated that plasma SPC concentration was correlated with plasma cholestane-3\beta,5a,6b-triol but not with glucosylsphingosine in NPC-affected individuals (Table 4) [5]. At this stage, while the mechanism of transformation of lysosphingomyelin-509 has attracted attention due to the higher specificity of diagnosis for NPC, the details of this awaits further investigation.

In summary, the present study provided evidence that plasma SPC was elevated in NPC-affected individuals using LC-MS/MS. More strikingly, plasma lysosphingomyelin-509 concentration in the NPC-affected individuals was strongly increased in NPC-affected individuals. This concentration was correlated with the urinary bile acid metabolite SNAG-$\Delta^5$-CA, thus, a combination of biomarkers from two separate biological sources, such as plasma and urine may provide a more solid biochemical basis for the diagnosis of NPC.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (16K08958) to RM and a grant-in-aid from the Japan Agency for Medical Research and Development to TO (15Ake0109050s0302).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jygmr.2018.03.005.

References

[1] M.T. Vanier, Niemann-Pick disease type C, Orphanet J. Rare Dis. 5 (2010) 16.
[2] M.T. Vanier, Complex lipid trafficking in Niemann-Pick disease type C, J. Inherit. Metab. Dis. 38 (2015) 187–199.
[3] S.U. Walkley, M.T. Vanier, Secondary lipid accumulation in lysosomal disease, Biochim. Biophys. Acta 1793 (2009) 726–736.
[4] N. Lin, H. Zhang, W. Qiu, J. Ye, L. Han, Y. Wang, X. Gu, Determination of 7-ketocholesterol in plasma by LC-MS for rapid diagnosis of acid SMase-deficient Niemann-Pick disease, J. Lipid Res. 55 (2014) 338–343.
[5] R.W. Welford, M. Garcia-Sanchez, M. Vecchi, R. Girolami, E. Mengel, T. Marquardt, J. Reunert, Y. Amraoui, S.A. Kolb, O. Morand, P. Groenen, Plasma lysosphingomyelin demonstrates great potential as a diagnostic biomarker for Niemann-Pick disease type C in a retrospective study, PLoS One 9 (2014) e114669.
[6] J. Reunert, M. Fokker, F. Kannenberg, J. Du Chesne, M. Plate, J. Wellhausen, S. Rust, T. Marquardt, Rapid diagnosis of 83 patients with Niemann pick type C disease and related cholesterol transport disorders by Cholestatriol screening, ElBioMedicine 4 (2016) 170–175.
[7] J. Reunert, A.S. Lotz-Havla, G. Polo, F. Kannenberg, M. Fokker, M. Greise, E. Mengel, A.C. Muntz, F. Schnabel, O. Sommerburg, I. Borggraefe, A. Dardis, A.P. Burlina, M.A. Mall, G. Cianna, B. Bembi, A.B. Burlina, T. Marquardt, Niemann-Pick Type C2 Disease: Identification by Analysis of Plasma Cholestane-3Beta,Sulpha,6Beta-Triol and Further Insight into the Clinical Phenotype, JIMD Rep. 23 (2015) 17–26.
[8] X. Jiang, R. Sidhu, F.D. Porter, N.M. Yanjanin, A.O. Speak, D.T. te Vrucht, F.M. Platt, H. Fujiiwara, D.E. Scherrer, J. Zhang, D.J. Dietzen, J.E. Schaffer, D.S. Ory, A sensitive and specific LC/MS/MS method for rapid diagnosis of Niemann-Pick C disease from human plasma, J. Lipid Res. 52 (2011) 1435–1445.
[9] S. Pujares, A. Arias, J. Garcia-Villoria, J. Macias-Vidal, E. Ros, J. de las Heras, M. Giro, M.J. Coll, A. Ribes, Cholestane-3Beta,Sulpha,6Beta-triol: high levels in Niemann-Pick type C, cerebrotendinous xanthomatosis, and lysosomal acid lipase deficiency, J. Lipid Res. 56 (2015) 1926–1935.
[10] G. Alvelius, O. Hjalmarson, W.J. Griffiths, I. Bjorkhem, J. Sjovall, Identification of unusual 7-oxidized bile acid sulfates in a patient with Niemann-Pick disease, type C, J. Lipid Res. 42 (2001) 1571–1577.
[11] M. Markawa, O. Miyazawa, A. Sotoura, H. Yamaguchi, M. Togawa, K. Ohno, H. Nittono, G. Kakiyama, T. Iida, A.F. Hofmann, J. Goto, M. Shimada, N. Mano, LC/ESI-MS/MS analysis of urinary 3Beta-sulfoxy-7Beta,6Beta-triol: high levels in Niemann-Pick type C, cerebrotendinous xanthomatosis, and lysosomal acid lipase deficiency, J. Lipid Res. 56 (2015) 1926–1935.
[12] M. Markawa, M. Shimada, T. Iida, J. Goto, N. Mano, Tandem mass spectrometric characterization of bile acids and steroid conjugates based on low-energy collision-induced dissociation, Steroids 80 (2014) 80–91.
[13] X. Jiang, R. Sidhu, L. Mydock-McGrane, F.F. Hsu, D.F. Covey, D.E. Scherrer,
B. Earley, S.E. Gale, N.V. Farhat, F.D. Porter, D.J. Dietzen, J.J. Orsini, E. Berry-Kravis, X. Zhang, J. Reunert, T. Marquardt, H. Runz, R. Grigioni, J.E. Schaffer, D.S. Ory, Development of a bile acid-based newborn screen for Niemann-Pick disease type C, Sci. Transl. Med. 8 (2016) 337ra363.

F. Marzucchi, P. Mills, K. Mills, S. Camuzeaux, P. Gissen, E.R. Colico, C. Wassif, D. Te Vruchte, F.D. Porter, M. Maekawa, N. Mano, T. Iida, F. Platt, P.T. Clayton, Identification of novel bile acids as biomarkers for the early diagnosis of Niemann-Pick C disease, FEBS Lett. 590 (2016) 1651–1662.

C.D. Davidson, N.F. Ali, M.C. Micsenyi, G. Stepney, S. Renault, K. Dobrenis, D.S. Ory, M.T. Vanier, S.U. Walkley, Chronic cyclodextrin treatment of murine Niemann-Pick C disease ameliorates neuronal cholesterol and glycosphingolipid storage and disease progression, PLoS One 4 (2009) e6951.

C.M. Ramirez, B. Liu, A.M. Taylor, J.J. Repa, D.K. Burns, A.G. Weinberg, S.D. Turley, J.M. Dietzchy, Weekly cyclodextrin administration normalizes cholesterol metabolism in nearly every organ of the Niemann-Pick type C1 mouse and markedly prolongs life, Pediatr. Res. 68 (2010) 309–315.

C.A. Wassif, J.L. Cross, J. Ledvinova, Quantitation of plasmatic lysosphingomyelin-509 for differential screening of Niemann-Pick A/B and C diseases, Anal. Biochem. 525 (2017) 73–77.

G. Polo, A.F. Burlina, T.B. Kolamunnage, M. Zampieri, C. Dionisi-Vici, P. Strisciuglio, M. Zaninotto, M. Pibiani, A.B. Burlina, Diagnosis of sphingolipidoses: a new simultaneous measurement of lysosphingolipids by LC-MS/MS, Clin. Chem. Lab. Med. 55 (2017) 403–414.

A.K. Giese, H. Maser, U. Gritter, S. Eichler, G. Kramp, J. Lukas, D. te Vruchte, N. Al Eisa, M. Cortina-Borja, F.D. Porter, F.M. Platt, A. Rolfs, A novel, highly sensitive and specific biomarker for Niemann-Pick disease type C1 disease, Orphanet J. Rare Dis. 10 (2015) 78.

T. Iida, G. Kakiyama, Y. Hibuya, S. Miyata, T. Inoue, K. Ohno, T. Goto, N. Mano, J. Goto, T. Nambara, A.F. Hofmann, Chemical synthesis of the 3-sulfooxy-7-N-acetylgalactosaminyl24-amidated conjugates of 3beta,7beta-dihydroxy-5-cholen-24-oic acid, and related compounds: unusual, major metabolites of bile acid in a patient with Niemann-Pick disease type C1, Steroids 71 (2006) 18–29.

F.D. Porter, D.E. Scherrer, M.H. Lainier, S.J. Langmade, V. Molugu, S.E. Gale, D. Ottesen, R. Sidhu, D.J. Dietzen, R. Fu, C.A. Wassif, N.M. Vanann, S.P. Marso, J. House, C. Vite, J.E. Schaffer, D.S. Ory, Cholesterol oxidation products are sensitive and specific blood-based biomarkers for Niemann-Pick C1 disease, Sci. Transl. Med. 2 (2010) 56ra81.

M. Maekawa, M. Shimada, K. Ohno, M. Togawa, H. Nittou, T. Iida, A.F. Hofmann, J. Goto, H. Yamaguchi, N. Mano, Focused metabolomics using liquid chromatography/electrospray ionization tandem mass spectrometry for analysis of urinary conjugated cholesterol metabolites from patients with Niemann-Pick disease type C and 3beta-hydroxy-5-cholesten-3-one dehydrogenase deficiency, Ann. Clin. Biochem. 52 (2015) 576–587.

M. Maekawa, K. Omura, S. Sekiguchi, T. Iida, D. Saigusa, H. Yamaguchi, N. Mano, Identification of two sulfated cholesterol metabolites found in the urine of a patient with Niemann-Pick disease type C as novel candidate diagnostic markers, Mass Spectrom. (Tokyo) 5 (2016) S0053.

J.Y. Chiang, Bile acids: regulation of synthesis, J. Lipid Res. 50 (2009) 1955–1966.

N. El-Najjar, E. Orso, S. Wallner, G. Liebisch, G. Schmitz, Increased levels of Sphingosylphosphorylcholine (SPC) in plasma of metabolic syndrome patients, PLoS One 10 (2015) e0140683.

G. Imokawa, Y. Takagi, K. Higuchi, H. Arai, Biochemical and molecular characterization of a novel choline-specific glycosphingolipid, Anal. Biochem. 525 (2017) 73–77.

D.S. Ory, M.T. Vanier, J.E. Bailey-Wilson, L.G. Biesecker, F.D. Porter, High incidence of unrecognized visceral/ neurological late-onset Niemann-Pick disease, Anal. Biochem. 525 (2017) 73–77.