Mutations in the Leucine Zipper Motif and Sterol-sensing Domain Inactivate the Niemann-Pick C1 Glycoprotein

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Niemann-Pick type C (NPC) disease, characterized by accumulation of low density lipoprotein-derived free cholesterol in lysosomes, is caused by mutations in the NPC1 gene. We examined the ability of wild-type NPC1 and NPC1 mutants to correct the NPC sterol trafficking defect and their subcellular localization in CT60 cells. Cells transfected with wild-type NPC1 expressed 170- and 190-kDa proteins. Tunicamycin treatment resulted in a 140-kDa protein, the deduced size of NPC1, suggesting that NPC1 is N-glycosylated. Mutation of all four asparagines in potential N-terminal N-glycosylation sites to glutamines resulted in a 20-kDa reduction of the expressed protein. Proteins with a single N-glycosylation site mutation localized to late endosomal lysosomal compartments, as did wild-type NPC1, and each corrected the cholesterol trafficking defect. However, mutation of all four potential N-glycosylation sites reduced ability to correct the NPC phenotype commensurately with reduced expression of the protein. Mutations in the putative sterol-sensing domain resulted in inactive proteins targeted to lysosomal membranes encircling cholesterol-laden cores. N-terminal leucine zipper motif mutants could not correct the NPC defect, although they accumulated in lysosomal membranes. We conclude that NPC1 is a glycoprotein that must have an intact sterol-sensing domain and leucine zipper motif for cholesterol-mobilizing activity.

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The abbreviations used are: NPC, Niemann-Pick type C; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SCAP, sterol response element-binding protein cleavage-activating protein; EGFP, enhanced green fluorescent protein.

EXPERIMENTAL PROCEDURES
Production of Rabbit Antihuman NPC1 N Terminus Polyclonal Antibodies—A cDNA encoding NPC1 sequences from amino acid residues 25–266 was cloned into the pQE-30 vector (Qiagen) that places a six-histidine tag at the N-terminus of the encoded protein. The six-histidine-tagged recombinant protein was purified on nickel-nitriolate triacetic acid resin (Qiagen). The eluted protein was dialyzed against...
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phosphate-buffered saline containing 0.1 mmol/liter phenylmethylsulfonyl fluoride, 1 mmol/liter dithiothreitol, and 10% glycerol. The diazylated protein was used to inoculate rabbits (Rockland, Gilbertsville, PA) to generate polyclonal antisera.

NPC1 cDNA Expression Constructs—The N70Q, L73P/L80F, D76A/L80F, L507F/L94P, N122Q, N185Q, N222Q, N70Q/N122Q/N185Q/N222Q, Y634C, Y634S, and P691S mutations were produced by site-directed mutagenesis as described previously (7). Each construct was sequenced to confirm that the desired mutation(s) had been introduced into the cDNA.

Cell Culture and Transfection—The Chinese hamster ovary CT60 cells, generously provided by T.-Y. Chang (Dartmouth College School of Medicine, Hanover, NH), and monkey kidney COS-1 cells were cultured as described previously (7, 17). Cells were cultured in six-well plastic culture plates at 50–80% confluence and were transfected using LipofectAMINE PLUS reagent (Life Technologies, Inc.) with either empty pSV-SPORT-1, the wild-type, or the mutant NPC1 cDNAs in pSVSPORT-1 with or without the plasmid expressing enhanced green fluorescent protein carrying a nuclear targeting sequence (pEGFP) (7). pEGFP was used to identify transfected cells for analysis of filipin staining. In some experiments, the control plasmid pCH110, which expresses β-galactosidase, was included to assess transfection efficiency. After transfection, cells were cultured in medium supplemented with lipoprotein-deficient serum for 24 h and then changed into medium supplemented with 5% lipoprotein-deficient serum and 50 µg/ml human low density lipoprotein for an additional 24 h of culture. Cultures were then terminated for Western blot analysis, filipin staining, and immunocytochemistry. In some experiments, cultures were pretreated with tunicamycin (10 µg/ml) for 24 h prior to termination.

Western Blot Analysis—Cells were scraped from culture dishes into a lysis buffer consisting of 100 mM Tris-HCl, pH 8.0, containing 1% Nonidet P-40, 150 mM NaCl, 0.2% sodium azide, 100 µg/ml phenylmethylsulfonyl fluoride, and 1 µg/ml aprotinin and centrifuged at 10,000 × g for 2 min at 4 °C. Supernatants were subjected to SDS-polyacrylamide gel electrophoresis and then Western blotting. Anti-NPC1 N terminus polyclonal antiserum was used to detect NPC1.

Trypsin and N-Glycosidase F Treatment—CT60 cells transfected with wild-type NPC1 or N-glycosylation site mutant plasmids were scraped from dishes, centrifuged at 1000 × g for 5 min at 4 °C, resuspended in 1 ml of phosphate-buffered saline, and centrifuged as above. The cell pellet was resuspended in 0.4 ml of sucrose-containing buffer (10 mM Hepes-KOH, pH 7.4, 10 mM KCl, 1.5 mM MgCl₂, 5 mM sodium EDTA, 5 mM sodium EGTA, 250 mM sucrose), passed through a 22 gauge needle 10 times, and centrifuged at 1000 × g for 5 min at 4 °C. The supernatant was centrifuged at 1500 × g for 10 min at 4 °C. The resulting pellet was resuspended in 30 μl of sucrose-containing buffer plus 0.1 mM NaCl. Aliquots were treated with 1 μg of trypsin (Sigma) at 25 °C for 30 min. The reaction was stopped by the addition of 400 units of soybean trypsin inhibitor (Sigma). To determine whether the wild-type or N-glycosylation site mutant plasmids were transfected with or without N-glycosidase F using the N-glycosidase F deglycosylation kit (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer’s instructions. The mixtures were heated at 100 °C for 5 min and subjected to SDS-polyacrylamide gel electrophoresis and Western blotting as described above.

Immunochemistry and Filippin Staining—CT60 cells grown on uncoated glass coverslips were transfected as described above. At the end of the culture period, cells were fixed with 3% paraformaldehyde in phosphate-buffered saline for 30 min. Immunostaining for NPC1 was performed with rabbit affinity purified anti-NPC1-C antibodies, and for lysosomal membrane glycoprotein, mouse monoclonal anti-lgp95 was used as described previously (7, 15, 18). Stained cells were examined with a Zeiss LSM 410 confocal microscope equipped with a UV laser.

Filipin staining was performed as described previously (7). For quantitation of fluorescence, 100 EGFP-positive cells were examined. Intense filipin fluorescence staining of cholesterol in large perinuclear granules is characteristic of the lysosomal accumulation in NPC cells (7, 15). Cells with markedly reduced filipin fluorescence were scored as corrected. Intermediate phototypes were scored as “uncorrected” so that our analysis underestimates the action of biologically active NPC1. Values presented are means ± S.E. from three separate experiments. Analysis of variance and the Tukey-Kramer test were used to determine significant differences between correction rates for empty vector control, wild-type, or mutant NPC1 proteins. p < 0.05 was used as the level of significance.

RESULTS

NPC1 Is a Glycoprotein with N-Glycosylation Sites in the N Terminus—Our antiserum generated against the NPC1 N-terminal domains specifically recognized 190- and 170-kDa immunoreactive bands in extracts of CT60 cells transfected with wild-type NPC1 (Fig. 1A). The same proteins were recognized by an antibody against a synthetic peptide representing sequences in the NPC1 C terminus (7, 18). The fact that the sizes of the detected proteins (170 and 190 kDa) were larger than the molecular mass of 142 kDa estimated from the deduced amino acid sequence suggests that NPC1 protein is posttranslationally modified. Because one of the most common types of protein modifications is N-linked glycosylation and there are 14 potential N-glycosylation sites conserved in human and mouse NPC1 proteins, we incubated CT60 cells transfected with wild-type NPC1 in the absence or presence of tunicamycin, an inhibitor of posttranslational N-glycosylation for 24 h. As shown in Fig. 1A, cells treated with tunicamycin express an NPC1 protein with an apparent molecular mass of 140 kDa, indicating that wild-type NPC1 is indeed N-glycosylated.

To confirm these results, membrane fractions isolated from COS-1 cells transfected with wild-type NPC1 were digested with trypsin and incubated with or without N-glycosidase F. The digests were then subjected to Western blotting using the polyclonal anti-N-terminal antibody. The trypsin digests not exposed to N-glycosidase F yielded a 56-kDa immunoreactive band, whereas the digest treated with N-glycosidase F gave a 36-kDa band (Fig. 1B). These observations are consistent with N-glycosylation of the NPC1 N terminus. The approximately 20-kDa difference in molecular mass predicted from this study indicates that other domains of the NPC1 protein lying downstream of the N terminus must also be glycosylated to account for the approximately 50-kDa size difference between the predicted mass of the nonglycosylated protein (142 kDa) and the largest species of the protein detected (190 kDa).

Four of the 14 potential N-glycosylation sites conserved in human and mouse NPC1 are located in the N terminus. We mutated each potential N-glycosylation site asparagine (Asn-70, Asn-122, Asn-185, and Asn-222) in the N terminus to a glutamine residue. Introduction of the individual N70Q, N122Q, N185Q, and N222Q mutations resulted in a modest reduction in the electrophoretic mobility of the proteins, indicating a 4–5-kDa change in molecular mass (Fig. 2A). A quadruple mutant (N70Q/N122Q/N185Q/N222Q, called 4N-Q) yielded proteins with lower molecular masses, amounting to an approximate 20-kDa reduction in mass compared with...
wild-type NPC1. The 4N-Q proteins were consistently present at about 50% of the level of expression of the wild-type and single N-glycosylation site mutants, suggesting that the quadrupler mutant is either synthesized at a lower rate or more rapidly degraded. Trypsin digestion of membranes isolated from CT60 cells transfected with the N-glycosylation mutants followed by Western blot analysis with the anti-NPC1 N-terminal antibody confirmed the modest reduction in molecular mass associated with single N-glycosylation site mutants and the cumulative reduction in mass of the 4N-Q mutant (Fig. 2B). We tested the ability of the single N-glycosylation site mutants and the quadruple mutant to correct the NPC cholesterol-trafficking defect and the subcellular localization of the proteins in CT60 cells. Each of the single N-glycosylation site mutant proteins were localized within a lgp95-positive compartment as was wild-type NPC1, indicating a late endosome/lysosomal vesicle (7, 15, 18), and each of the single N-glycosylation site mutants corrected the NPC cholesterol-trafficking defect to the same extent as the wild-type protein (Table I). However, the quadruple mutant displayed about 50% of the capacity to correct the cholesterol-trafficking abnormality compared with wild-type protein, possibly as a result of the lower steady state levels of the quadruple mutant protein in the transfected cells. The reduced expression of the quadruple mutant was not the result of lower transfection efficiency because experiments in which pCH110, a plasmid expressing CoA reductase, to serine residues. We also mutated Tyr-634 to a cysteine residue, a mutation that makes SCAP resistant to inhibition by hydroxysterols (10, 11). Each of the mutant proteins could not correct the lysosomal free cholesterol accumulation (Table I), even though they were targeted to the lysosomal membranes surrounding the cholesterol-laden cores. Fig. 5B shows the distribution of the D76A/L80F mutant, which was identical to distribution found with the two other leucine zipper motif mutants (L73P/L80F and L80F/L94P) (data not shown).

**Mutations in the Sterol-sensing Domain Inactivate NPC1**—To elucidate the functional significance of the putative sterol-sensing domain, we mutated Tyr-634 and Pro-691, both of which are conserved in NPC1, SCAP, Patched, and HMG-CoA reductase, to serine residues. We also mutated Tyr-634 to a cysteine residue, a mutation that makes SCAP resistant to inhibition by hydroxysterols (10, 11). Each of the mutant proteins was detectable in the transfected CT60 cells by Western blot analysis (Fig. 6). In contrast to the wild-type NPC1 protein that yielded proteins of 170 and 190 kDa, the sterol-sensing domain mutant expression constructs each yielded a major immunoreactive protein of 170 kDa (Fig. 4). The mutant proteins could not correct the lysosomal free cholesterol accumulation (Table I), even though they were targeted to the lysosomal membranes surrounding the cholesterol-laden cores. Fig. 5B shows the distribution of the D76A/L80F mutant, which was identical to distribution found with the two other leucine zipper motif mutants (L73P/L80F and L80F/L94P) (data not shown).

**Correction of the NPC cholesterol trafficking defect in CT60 cells by expression of NPC1**

CT60 cells were transfected with the indicated plasmid and the pEGFP expression plasmid. Cells were incubated with LDL and then fixed for filipin staining as described in the text. One hundred EGFP-stained cells were analyzed for filipin staining in each treatment. The values presented are the percentage of EGFP-expressing cells showing a marked reduction in perinuclear filipin-positive granules. Values are means ± S. E. from three separate experiments.

**TABLE I**

| Transfected plasmid | Correction | % |
|---------------------|------------|---|
| Empty vector       | 6.2 ± 0.3a | 0.05 |
| Wild-type NPC1     | 61.1 ± 0.5a| 0.05 |
| N70Q                | 54.3 ± 1.7a| 0.05 |
| N122Q               | 57.7 ± 2.4a| 0.05 |
| N185Q               | 56.1 ± 2.1a| 0.05 |
| N222Q               | 57.7 ± 2.0a| 0.05 |
| N70Q/N122Q/N185Q/N222Q | 33.6 ± 2.4a | 0.05 |
| L73P/L80F           | 7.3 ± 1.7a | 0.05 |
| D76A/L80F           | 7.0 ± 1.2a | 0.05 |
| L80F/L94P           | 7.2 ± 1.7a | 0.05 |
| Y634C               | 4.6 ± 1.3a | 0.05 |
| Y634S               | 5.7 ± 2.6a | 0.05 |
| P691S               | 5.9 ± 1.5a | 0.05 |

* Significantly different from b and c (p < 0.05).
* Significantly different from a and c.
* Significantly different from a and b.

**DISCUSSION**

Here we present strong evidence indicating that NPC1 is N-glycosylated. Wild-type human NPC1 expressed in CT60 cells appears as 170- and 190-kDa immunoreactive proteins that are larger than the 142-kDa size predicted from the deduced amino acid sequence (4). However, treatment of the transfected CT60 cells with tunicamycin, an inhibitor of protein N-glycosylation, resulted in the expression of a 140-kDa protein. At least four N-glycosylation sites appear to be in the NPC1 N terminus surrounding or within the NPC1 domain, as
evidenced by the reduction in molecular mass of the expressed single N-glycosylation site mutants and the quadruple mutant as well as by the impact of N-glycosidase F treatment of a trypsin-digest product detected by our anti-NPC1 N terminus antibody. N-Glycosylation of the NPC1 N terminus appears to account for a 20-kDa increase in mass. Therefore, domains lying beyond the N terminus containing an additional 10 potential N-glycosylation sites must also be glycosylated to account for the size difference between the mass predicted from the deduced amino acid sequence of 142 kDa and the largest form of NPC1 detected in our Western blot analysis of 190 kDa. Further studies are needed to identify these other glycosylation sites.

We previously reported that CT60 cells transfected with NPC1 N-terminal cysteine mutants, which did not correct the NPC cholesterol-trafficking defect, yielded a major immunoreactive protein of 170 kDa without the 190-kDa protein present in cells transfected with wild-type NPC1 plasmid (7). The altered structure of the N terminus resulting from the cysteine mutations could have prevented glycosylation of the N terminus. These findings also raised the possibility that glycosylation of the NPC1 N terminus is essential for the biological activity of the protein. However, the present results demonstrate that N-glycosylation of the NPC1 N terminus is not obligatory for either lysosomal targeting or cholesterol-mobilizing activity. Single mutations in the four potential N-glycosylation sites had no significant effect on protein localization or lysosomal cholesterol unloading. However, deletion of all four sites partially impaired function resulting in diminished cor-

FIG. 3. CT60 cells transfected with an empty vector (A and B), wild-type NPC1 (C and D), and a quadruple glycosylation mutant (N70Q/N122Q/N185Q/N222Q) (E and F) and stained for cholesterol with filipin (A, C, and E) and immunostained for NPC1 protein (B, D, and F). The cells transfected with empty vector contain filipin-positive, cholesterol laden lysosomes in a characteristic central location near the nucleus (A) and do not contain NPC1 protein (B). The cell expressing wild-type NPC1 contains NPC1 protein in small cholesterol unloaded lysosomes (D) and does not contain accumulations of intracellular cholesterol (C). The cell expressing the quadruple glycosylation mutant contains NPC1 protein in small lysosomes (F) that contain less accumulation of cholesterol when compared with those of neighboring cells not expressing NPC1 protein. Bar, 10 μm.

FIG. 4. Western blot analysis of leucine zipper motif mutants expressed in CT60 cells. Extracts of CT60 cells transfected with plasmids expressing the indicated proteins were subjected to Western blotting. Each lane contained 40 μg of protein.
The leucine zipper mutant contains NPC1 protein localized as rings (B) at the surface of the cholesterol-laden cores of lysosomes (A). The cell expressing the sterol-sensing mutant contains NPC1 protein localized as rings (D) at the surface of the cores of cholesterol-laden lysosomes (C). Bar, 10 µm. D, inset, the rings of NPC1 protein are shown at higher magnification. Bar, 5 µm.

FIG. 5. CT60 cells transfected with the leucine zipper mutant (D76A/L80F) (A and B) and the sterol-sensing mutant (P691S) (C and D), stained for cholesterol with filipin (A and C), and immunostained for NPC1 protein (B and D). The cell expressing the leucine zipper mutant contains NPC1 protein localized as rings (B) at the surface of the cholesterol-laden cores of lysosomes (A). The cell expressing the sterol-sensing mutant contains NPC1 protein localized as rings (D) at the surface of the cores of cholesterol-laden lysosomes (C). Bar, 10 µm. D, inset, the rings of NPC1 protein are shown at higher magnification. Bar, 5 µm.

FIG. 6. Western blot analysis of sterol-sensing domain mutants expressed in CT60 cells. Extracts of CT60 cells transfected with plasmids expressing the indicated proteins were subjected to Western blotting. Each lane contained 40 µg of protein.
would probably be unable to identify the consequences of an activating mutation in NPC1, if such exists. Thus, we are limited with this end point to the identification of loss of function mutations.

Our studies on the structure-function relationships of NPC1 have identified three patterns of NPC1 intracellular localization at the light microscopic level. Wild-type protein and mutant proteins capable of discharging cholesterol from lysosomes were identified within a subset of small vesicles expressing lysosomal markers (7, 15, 18). An NPC1 mutant lacking the C-terminal lysosome-targeting motif was localized to the endoplasmic reticulum (7). All of the other mutant proteins that were incapable of mobilizing free cholesterol from lysosomes, including those disrupting the NPC1 domain and the sterol-sensing domain, were located in the membrane limiting the cholesterol-loaded lysosome cores (Ref. 7 and the present study). Future studies should explore the localization of the latter mutants within the organelles at an ultrastructural level to determine whether alterations in specific NPC1 domains influences intralysosomal protein localization.

The assay for NPC1 functional activity that we employed is not quantitative, which represents a recognized limitation for studies of NPC1 mutants that partially impair activity. Because the mechanism of NPC1 action remains unknown, there is currently no cell-free assay of NPC1 activity that can be applied to assess the function of the expressed proteins. However, the filipin assay used in our studies is sufficiently robust to permit us to identify proteins that retain function as opposed to those mutants that do not. Indeed, this type of functional assay was originally used to identify the NPC1 protein (4). Our studies on the structure-function relationships of NPC1 indicate that the sterol-sensing domain of NPC1 could harbor the mechanism by which the accumulation of sterol in lysosomal membranes (23) activates the process of cargo unloading by causing a conformational change in NPC1. We also speculate that NPC1 interacts through its leucine zipper motif with other proteins critical for lysosomal cargo release and that the interacting molecules may include the putative NPC2 gene product.

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REFERENCES

1. Pentchev, P. G., Kruth, H. S., Comly, M. E., Butler, J. D., Vanier, M. T., Wenger, D. A., and Patel, S. (1986) J. Biol. Chem. 261, 16775–16780
2. Pentchev, P. G., Comly, M. E., Kruth, H. S., Vanier, M. T., Wenger, D. A., Patel, S., and Brady, R. O. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8247–8251
3. Blanchette-Mackie, E. J., Dwyer, N. K., Amend, L. M., Kruth, H. S., Butler, J. D., Sokol, J., Comly, M. E., Vanier, M. T., August, J. T., Brady, R. O., and Pentchev, P. G. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8022–8026
4. Carstea, E. D., Morris, J. A., Coleman, K. G., Loths, S. K., Zhang, D., Cummings, C., Gu, J., Rosenfeld, M. A., Pavan, W. J., Krizman, D. B., Nagle, J., Polymeryopoulos, M. H., Sturley, S. L., Ioannou, Y. A., Higgins, M. E., et al. (1997) Science 277, 228–231
5. Gu, J. Z., Carstea, E. D., Cummings, C., Morris, J. A., Lofts, S. K., Zhang, D., Coleman, K. G., Cooney, A. M., Comly, M. E., Fandino, L., Roff, C., Tagle, D. A., Pavan, W. J., Pentchev, P. G., and Rosenfeld, M. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7378–7383
6. Lofts, S. K., Morris, J. A., Carstea, E. D., Gu, J. Z., Cummings, C., Brown, A., Elson, J., Ohno, K., Rosenfeld, M. A., Tagle, D. A., Pentchev, P. G., and Pavan, W. J. (1997) Science 277, 232–235
7. Watari, H., Blanchette-Mackie, E. J., Dwyer, N. K., Glick, J. M., Patel, S., Neufeld, E. B., Brady, R. O., Pentchev, P. G., and Straus, J. F., III (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 805–810
8. Randale, E. E., Kingsman, S. M., and Kingsman, A. J. (1996) Virology 220, 100–108
9. Tong, Q., Xing, S., and Jhiang, S. M. (1997) J. Biol. Chem. 272, 9043–9047
10. Oleaneder, E. H., and Simoni, R. D. (1992) J. Biol. Chem. 267, 4223–4235
11. Hua, X., Sakai, J., Ho, Y. K., Goldstein, J. L., and Brown, M. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7378–7383
12. Neufeld, E. B., Wastney, M., Patel, S., Suresh, S., Cooney, A. M., Dwyer, N. K., and Chang, T.-Y., eds) pp. 93–107, Kluwer, Norwell, MA
13. Liscum, L., and Underwood, K. W. (1993) J. Biol. Chem. 270, 15443–15446
14. Liscum, L., and Klanecky, J. (1998) Cur. Opin. Lipidol. 9, 131–135
15. Neufeld, E. B., Wastney, M., Patel, S., Suresh, S., Cooney, A. M., Dwyer, N. K., Roff, C. F., Ohno, K., Morris, J. A., Carstea, E. D., Indarciona, J. P., Straus, J. F., III, Vanier, M. T., Patterson, M. C., Brady, R. O., Pentchev, P. G., and Pavan, W. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 277–281
16. Neufeld, E. B. (1998) In Intracellular Cholesterol Transport (Freeman, D. A., and Chang, T.-Y., eds) pp. 93–107, Kluwer, Norwell, MA
17. Spillane, D. M., Beagan, J. W., Kennedy, N. J., Schneider, D. L., and Chang, T.-Y. (1995) Biochim. Biophys. Acta 1254, 283–284
18. Patel, S. C., Suresh, S., Kumar, U., Hu C. Y., Cooney, A., Blanchette-Mackie, E. J., Neufeld, E. B., Patel, R. C., Brady, R. O., Patel, Y. C., Pentchev, P. G., and Ong, W. Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1657–1662
19. Porre, D., Oertel-Buchheit, P., Granger-Schnurr, M., and Schnarr, M. (1995) J. Biol. Chem. 270, 22721–22730
20. Neufeld, E. B., Brown, M. S., and Goldstein, J. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12848–12853
21. Vanier, M. T., Duthie, S., Rodriguez-Latrasse, C., Pentchev, P., and Carstea, E. D. (1996) Am. J. Hum. Genet. 58, 118–125
22. Dahl, N. K., Duane, D. A., and Liscum, L. (1994) J. Lipid Res. 35, 1839–1849
23. Coxe, R. A., Pentchev, P. G., Campbell, G., and Blanchette-Mackie, E. J. (1993) J. Lipid Res. 34, 1165–1176