Impaired ABCA1-dependent Lipid Efflux and Hypoalphalipoproteinemia in Human Niemann-Pick type C Disease*

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The cholesterol trafficking defect in Niemann-Pick type C (NPC) disease leads to impaired regulation of cholesterol esterification, cholesterol synthesis, and low density lipoprotein receptor activity. The ATP-binding cassette transporter A1 (ABCA1), which mediates the rate-limiting step in high density lipoprotein (HDL) particle formation, is also regulated by cell cholesterol content. To determine whether the Niemann-Pick C1 protein alters the expression and activity of ABCA1, we determined the ability of apolipoprotein A-I (apoA-I) to deplete pools of cellular cholesterol and phospholipids in human fibroblasts derived from NPC1−/−, NPC1+/−, and NPC1−/− subjects. Efflux of low density lipoprotein-derived, non-lipoprotein, plasma membrane, and newly synthesized pools of cell cholesterol by apoA-I was diminished in NPC1−/− cells, as was efflux of phosphatidylcholine and sphingomyelin. NPC1+/− cells showed intermediate levels of lipid efflux compared with NPC1−/− and NPC1+/− cells. Binding of apoA-I to cholesterol-loaded and non-cholesterol-loaded cells was highest for NPC1−/− cells, with NPC1+/− and NPC1−/− cells showing similar levels of binding. ABCA1 mRNA and protein levels increased in response to cholesterol loading in NPC1−/− and NPC1+/− cells but showed low levels at base line and in response to cholesterol loading in NPC1−/− cells. Consistent with impaired ABCA1-dependent lipid mobilization to apoA-I for HDL particle formation, we demonstrate for the first time decreased plasma HDL-cholesterol levels in 17 of 21 (81%) NPC1−/− subjects studied. These results indicate that the cholesterol trafficking defect in NPC disease results in reduced activity of ABCA1, which we suggest is responsible for the low HDL-cholesterol in the majority of NPC subjects and partially responsible for the overaccumulation of cellular lipids in this disorder.

* This work was supported in part by the Heart and Stroke Foundation of Alberta, Northwest Territories, and Nunavut (to G. A. F.), Canadian Institutes of Health Research Grant MOP-12660 (to G. A. F.) and MOP-132321 (to J. E. V.), National Institutes of Health Grant DK56732 (to R. A. H.), and the Ara Parseghian Medical Research Foundation (to W. S. G. and J. E. V.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a Postdoctoral Research award from the Heart and Stroke Foundation of Canada.

§ Supported by a Doctoral Research Award from the Heart and Stroke Foundation of Canada.

¶ Scholar of the Alberta Heritage Foundation for Medical Research.

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agonists (16). In addition, HDL-cholesterol levels in NPC-deficient mice have been reported to be normal (17, 18).

In the current studies we characterized apoA-1-mediated efflux of phospholipids and cholesterol from distinct cellular pools, binding of apoA-I, and regulation of ABCA1 expression in normal (NPC), NPC1<sup>+/−</sup>, and NPC1<sup>−/−</sup> human fibroblasts, and we correlated our findings with the plasma lipid profiles of NPC patients. The results support the hypothesis that the regulation and activity of ABCA1, resulting in decreased apoA-I obtained using DEAE-cellulose chromatography as described previously (22). The whole protein fraction of HDL was obtained by delipidating HDL and purified by thin layer chromatography, and assayed for radioactivity as described previously (26). Cell proteins were determined using BSA as standard (30).

**Cellular Binding of ApoA-1**—The binding of apoA-1 to cells was determined as described previously (31). Non-cholesterol-loaded cells or cells cholesterol loaded in 25 μl buffer (DMEM) were incubated for 2 h at 0 °C in DMEM/BSA containing 25 mg/ml HEPES and increasing concentrations of [125I]apo-A-I. Cells were rinsed 5 times with iced PBS/BSA and twice with iced PBS. Cells were washed at 20 °C until lipid extraction. Efflux media were collected and centrifuged (3,000 rpm for 10 min) to remove cell debris. Radioactivity in the medium was then either measured directly (for cells labeled with [125I]cholesterol) or the medium was extracted for determination of radioactivity. Cellular lipids were then rinsed by thin layer chromatography, and assayed for radioactivity as described previously (26). Cell proteins were determined using BSA as standard (30).

**Reverse Transcription-PCR Analysis of ABCA1 mRNA**—Total RNA was isolated from cells by guanidine isothiocyanate/phenol/chloroform extraction (32). The concentration of RNA was measured spectrophotometrically at a wavelength of 260 nm, and 2 μg of RNA was treated with DNase I (Invitrogen) according to the manufacturer's guidelines. First strand cDNA synthesis was performed using 500 ng of oligo(dT) primer and superscript<sup>TM</sup> II RNase H (Invitrogen). Each reaction mixture contained 100 units of Superscript<sup>TM</sup> enzyme, 1× first strand buffer (50 mM Tris-HCl, pH 8.0), 0.5 μM dNTP mix, 0.01 μM diethiothreitol, 0.05 μg/ml BSA, and 2 units of RNase inhibitor (Invitrogen). The mixtures were incubated at 45 °C for 90 min followed by incubation at 95 °C for 3 min (Whatman Biometra T-gradient thermocycler) and then put promptly on ice. Amplification of ABCA1 and cyclophilin mRNAs was performed in tandem to ensure equal amounts of starting cDNA for each sample. Diethyl pyrocarbonate-treated water, 1× PCR buffer (20 mM Tris-HCl, pH 8.4, and 50 mM KCl), 1.5 mM MgCl<sub>2</sub>, 0.1 μM dNTPs, and cDNA were added to 200 μl of thin walled PCR tubes and mixed, and one-half volume was transferred to another PCR tube. Then 1 unit of Taq DNA polymerase (Invitrogen) and 0.3 μM forward (NPC1<sup>−/−</sup>, CRL-2076) and 0.3 μM reverse (ABCA1 or cyclophilin) were added to complete the reaction mixture. ABCA1 amplification was performed by initially denaturing DNA at 95 °C for 3 min. Thereafter, denaturing was at 95 °C for 75 s, annealing at 54.6 °C for 75 s, and extension at 72 °C for 55 s for a total of 31 cycles with a final extension period of 5 min. Human cyclophilin amplification was performed using similar conditions except the annealing temperature was 48 °C with a total of 33 cycles. PCR products were electrophoresed on a 1.2% agarose gel, stained with ethidium bromide, and visualized under UV light. The primers used are as follows: human ABCA1, 5′-GAC ATC CTG AAG CCA ATC CTG (forward), 5′-TCT GGT AGC GTC ATG AGC G (reverse); human cyclophilin, 5′-ACC CAA GAA CAG CAG GAC G (forward), 5′-CCG CGT CTT TGA GCT GTT TGC AG (reverse).

**Northern Blot Analysis of ABCA1**—Total RNA was isolated from cells as described (32). Seven micrograms of RNA was electrophoresed on a 1% agarose gel containing 5% formaldehyde, and transferred on a nylon membrane (Amersham Biosciences) by capillary transfer. The probe for ABCA1 was obtained by purifying PCR products using a gel extraction kit (Qiagen) and then radiolabeled by the random priming method with [α-<sup>32</sup>P]dCTP (Invitrogen). After cross-linking with UV light (Stratalinker model 1800, Stratagene), the membranes were hybridized with <sup>32</sup>P-labeled probes. The hybridization signal was detected by autoradiography.

**Western Blot Analysis of ABCA1**—Crude cellular membranes were prepared by homogenizing cells in ice on 50 ml Tris-HCl buffer, pH 7.4, containing protease inhibitors and 2 μg/ml EDTA. The nuclear fraction was removed by centrifugation for 10 min at 700 rpm, and the supernatant was subsequently centrifuged for 20 min at 14,000 rpm. The supernatant was then resuspended in 0.45 μM histone solution (HCl solution: 0.4% acetic acid, 5% sodium acetate, 1 M HEPES and 1% Triton X-100, and 0.05% diethiothreitol and protein concentrations were determined.) Thirty micrograms of membrane proteins were separated by 7.5% SDS-PAGE under reducing conditions and transferred to nitrocellulose membrane. Immunoblotting was performed according to standard protocols using a polyclonal rabbit anti-human ABCA1 antibody (40). Membrane immunofluorescence (a kind gift of Dr. Shunji Yokoyama, Nagoya City University (33)) and a goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:10,000, Sigma). Chemiluminescence was detected by the enhanced chemiluminescence assay (Amersham Biosciences).
Significant differences between experimental groups and in the levels of accumulation of cholesterol in late endosomes/lysosomes and a was approximately 3 times higher in
cholesteryl ester (NPC1) compared with both these other cell

lum in (Fig. 1, A), cell CE (B), and cell UC (C) following subtraction of efflux to medium containing 1 mg/ml BSA alone. Cell [3H]cholesterol immediately prior to addition of apoA-I was 94 ± 5, 115 ± 14, and 346 ± 19 × 10^6 dpm/mg cell protein for NPC1+/+, NPC1−/−, and NPC1−/− cells, respectively. Values are the mean ± S.D. of quadruplicate determinations and are representative of two experiments with similar results. A, values for NPC1−/− cells at ≥4 h and for NPC1+/− cells at 24 h are lower than NPC1+/+ cells. B, values for NPC1−/− cells are lower than NPC1+/− cells at ≥4 h. C, values for NPC1−/− cells are greater than NPC1+/− cells at ≥4 h. For all significant differences, p ≤ 0.05.

**RESULTS**

**ApoA-I-mediated Efflux of LDL-derived Cholesterol Is Impaired in Human NPC1−/− Fibroblasts—**Impaired trafficking of LDL-derived cholesterol in fibroblasts or lymphocytes is a biochemical hallmark of NPC disease (1). To assess the removal of LDL-derived cholesterol by apoA-I in human NPC1-deficient cells, fibroblasts from a normal subject (NPC1+/+) and individuals heterozygous (NPC1+/−) or compound heterozygous (NPC1−/−) for mutations in NPC1 were grown to confluence in lipoprotein-deficient serum. The cells were then labeled with [3H]cholesterol linoleate-labeled LDL for 24 h prior to incubation with apoA-I. Incorporation of LDL-derived [3H]cholesterol was approximately 3 times higher in NPC1−/− than in NPC1+/− or NPC1+/− cells (see Fig. 1, legend), consistent with accumulation of cholesterol in late endosomes/lysosomes and a failure to down-regulate LDL receptor activity in NPC1−/− cells (10, 11). Incubation of cells with 10 μg/ml apoA-I for 48 h resulted in efflux of 13–14% of LDL-derived [3H]cholesterol to the medium from NPC1+/− cells (Fig. 1A). NPC1−/− cells showed a slightly decreased ability to release LDL-derived cholesterol to apoA-I, whereas NPC1−/− cells showed markedly diminished efflux (only 2% above basal levels of efflux to albumin alone) to apoA-I compared with both these other cell lines. Removal of radiolabeled cellular cholesterol to the medium was accompanied by a marked decrease in radiolabeled cellular cholesterol ester (CE) in NPC−/− and NPC+/− cells (Fig. 1B). NPC−/− cells showed a sharper decline in cellular CE levels and a simultaneous accumulation of [3H]cholesterol (Fig. 1, B and C), consistent with normal rates of CE hydrolysis but failure to re-esterify cholesterol in the endoplasmic reticulum in NPC−/− cells (1).
dependent upon apoA-I being first or simultaneously phospholipidated in a process that requires ABCA1 (12). \([\text{H}]\)Choline-labeled NPC1\(^{-/-}\) cells showed a diminished ability to mobilize both PC and SM to apoA-I (Fig. 3). NPC1\(^{+/+}\) cells showed intermediate levels of PC efflux; SM efflux from NPC1\(^{+/+}\) cells was similar to NPC1\(^{+/+}\) cells at early time points (\(\leq 8\) h) but fell to levels similar to those from NPC1\(^{-/-}\) cells at later time points. Impaired efflux of choline-containing phospholipids by NPC1-deficient cells parallels the decreased ability of apoA-I to mobilize cholesterol from all of the cellular cholesterol pools examined (Figs. 1 and 2).

**ABCA1 Expression Is Diminished in NPC1\(^{-/-}\) Human Fibroblasts**—Impaired efflux of phospholipids and various pools of cellular cholesterol to apoA-I from NPC1\(^{-/-}\) fibroblasts suggests ABCA1 regulation and activity is also impaired in these cells. Levels of ABCA1 mRNA and protein were determined under non-cholesterol-loaded and cholesterol-loaded conditions. Semi-quantitative determination of ABCA1 mRNA using...
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Fig. 4. Expression of ABCA1 in human NPC1-deficient fibroblasts. A, cells were grown to confluence in DMEM, 10% FBS, then incubated in the presence or absence of 30 μg/ml non-lioprotein cholesterol for 24 h, and equilibrated in DMEM, 1 mg/ml BSA for 24 h prior to the determination of ABCA1 mRNA and protein levels. Alternatively, cells were grown the last 40% to confluence in lipoprotein-deficient serum and then incubated with 50 μg/ml non-lipoprotein cholesterol and then with 125I-apoA-I. As expected from previous reports (36), binding of apoA-I was markedly higher to cholesterol-loaded (Fig. 5B) than to non-cholesterol-loaded (Fig. 5A) cells of all 3 NPC1 genotypes. With both degrees of cholesterol loading, NPC1+/− cells showed the highest levels of apoA-I binding. Despite marked differences in ABCA1 protein levels in cholesterol-loaded and non-loaded conditions (Fig. 4), NPC1+/− and NPC1−/− cells showed similar levels of apoA-I binding. The results with all three of these cell types suggest that other factors in addition to the amount of ABCA1 determine apoA-I binding to cells.

**HDL Levels Are Low in NPC1−/− Subjects**—Our results using human fibroblasts indicate impaired ABCA1-dependent HDL particle formation by NPC1-deficient cells in culture. Although the lipid profiles of NPC-deficient patients have been reported previously to be normal (1, 42), the only data in the literature are for total plasma cholesterol levels (43). With the help of the Arz Parseghian Medical Research Foundation, we obtained the fasting lipid profiles of 21 NPC1+/− patients (Table 1). The majority of NPC patients are compound heterozygotes for NPC1 mutations (44). Consistent with the finding of impaired ABCA1 expression in human NPC1−/− fibroblasts, we found that 9 of 10 male and 8 of 11 female subjects had HDL-cholesterol levels below the currently identified lower limit of normal for adults and children, 40 mg/dl or 1.03 mmol/liter (Fig. 5B) (45, 46). The very high prevalence of low HDL levels in NPC1−/− subjects is even more striking given that children normally have higher HDL levels than adults. HDL-cholesterol levels fall by an average of 14% in males and 5% in females following puberty (47). The Bogalusa Heart Study of 4074 children reported average HDL levels in pre-pubertal

**ABCA1 Expression Levels Do Not Predict Binding of ApoA-I to NPC-deficient Fibroblasts**—Lipid efflux to apoA-I has been shown to correlate directly with binding of apoA-I to cells (36) and with levels of ABCA1 expression (reviewed in Ref. 12). Cross-linking studies have suggested a direct protein-protein interaction between apoA-I and ABCA1 (37–39), and apoA-I binding appears to enhance ABCA1 activity by preventing its degradation by a calpain protease (40, 41). To assess binding of apoA-I to NPC1-deficient cells, fibroblasts grown to confluence in 10% FBS were incubated in the presence or absence of non-lipoprotein cholesterol and then with 125I-apoA-I. As expected from previous reports (36), binding of apoA-I was markedly higher to cholesterol-loaded (Fig. 5A) than to non-cholesterol-loaded (Fig. 5B) cells of all 3 NPC1 genotypes. With both degrees of cholesterol loading, NPC1+/− cells showed the highest levels of apoA-I binding. Despite marked differences in ABCA1 protein levels in cholesterol-loaded and non-loaded conditions (Fig. 4), NPC1+/− and NPC1−/− cells showed similar levels of apoA-I binding. The results with all three of these cell types suggest that other factors in addition to the amount of ABCA1 determine apoA-I binding to cells.
Caucasian children ages 5–9 of 1.73 ± 0.57 mmol/liter (mean ± S.D., n = 459) for boys and 1.69 ± 0.56 mmol/liter (n = 450) for girls (47). In contrast, HDL levels for children aged 5–9 in our study were strikingly lower, 0.63 ± 0.21 for boys (mean ± S.D., n = 5) and 0.81 ± 0.24 (n = 5) for girls, p < 0.005 for both boys and girls compared with Bogalusa Heart Study children in this age group. Other than low HDL-cholesterol, no consistent abnormalities were found in the remaining plasma lipid parameters of NPC1−/− subjects (Table I). Although 2 of the 21 subjects had mildly elevated plasma triglyceride levels, the low incidence of this finding suggests the absence of an association between hypertriglyceridemia and the low HDL-cholesterol of human NPC disease.

Fasting lipid profiles were also obtained for 31 parents of NPC subjects in this study. Of these, 4 of 15 male and 2 of 16 female heterozygotes had low HDL-cholesterol (0.93, 0.90, 0.90, 0.93, 0.88, and 0.77 mmol/liter, respectively). Again, no consistent abnormalities were found among the other lipid parameters in the NPC heterozygote profiles, including those with low HDL (data not shown).

**DISCUSSION**

Niemann-Pick type C disease is characterized by the accumulation of LDL-derived cholesterol in late endosomes/lysosomes and an inability to regulate normally the central mechanisms of cholesterol homeostasis: delivery of unesterified cholesterol to the endoplasmic reticulum for esterification by acyl-CoA cholesterol acyltransferase, regulation of cholesterol synthesis by 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and regulation of LDL receptor activity (1, 9–11). In the current studies we demonstrate that regulation of another pivotal mediator of cholesterol homeostasis, ABCA1, is also impaired in human NPC1-deficient fibroblasts. ApoA-I showed a diminished ability to mobilize cholesterol in NPC1−/− cells from LDL-derived and non-lipoprotein-derived cholesterol pools, and to mobilize cellular phosphatidylcholine and sphingomyelin. ABCA1 mRNA and protein levels in NPC1−/− cells were diminished at basal levels of cell cholesterol and following loading of cells with either non-lipoprotein- or LDL-derived cholesterol, when compared with NPC+/+ and NPC+−/− cells. Consistent with impaired regulation of ABCA1 at the cellular level, we found a strikingly high incidence of hypoalphalipoproteinemia (90% of males and 73% of females) in the lipid profiles of 21 NPC1−/− subjects.

Impaired activity of ABCA1 in NPC-deficient cells is strongly suggested by the diminished basal and cholesterol-stimulated levels of ABCA1 mRNA and protein, and decreased levels of phospholipid and cholesterol efflux to apoA-I from these cells. The pattern of accumulation of cell cholesterol in NPC disease and localization of the NPC1 protein has led to the conclusion that the major site of action of NPC1 is in late endosomes/lysosomes (5, 35). ABCA1 mobilizes cellular lipids to apoA-I at the plasma membrane (12, 48) and may also facilitate the delivery of intracellular lipids to internalized or cell surface apolipoproteins from late endosomes/lysosomes (16, 49, 50). As such, mutations in NPC1 might adversely affect the function of ABCA1 in facilitating the removal of late endosomal/lysosomal cholesterol. We found the greatest degree of inhibition of apoA-I-mediated cholesterol mobilization from NPC1−/− cells from LDL-derived cholesterol (Fig. 1), which accumulates mainly in late endosomes/lysosomes in these cells (51). We also found a >50% decrease in cholesterol mobilization to apoA-I from non-lipoprotein-derived cholesterol pools, including newly synthesized cholesterol, in NPC1−/− cells (Fig. 2). Although the initial delivery of newly synthesized cholesterol to the plasma membrane is normal in NPC cells (6, 51, 52), subsequent trafficking of this cholesterol back to intracellular compartments and therefore mobilization to apoA-I may be impaired in the presence of NPC1 mutations. Of the several cholesterol labeling methods utilized, efflux to apoA-I of cholesterol from cells pulse-labeled with [3H]cholesterol may represent the pathway least dependent on NPC1, as NPC1 is not currently known to function directly in the plasma membrane. ABCA1, on the other hand, is thought to function, at least in part, at the cell surface to deliver lipids to apoA-I. Impaired efflux of cholesterol from NPC1−/− cells labeled using this method therefore provides further evidence for decreased ABCA1 activity in NPC1−/− cells, and for ABCA1 mobilizing cholesterol from plasma membrane as well as late endosomal/lysosomal pools.

Intermediate levels of esterification of LDL-derived cholesterol have been reported previously (3) in heterozygous NPC1 cells during the first 6 h of incubation with LDL, with normal levels of esterification in these cells incubated over 24 h with LDL. We found similar overall levels of esterification and efflux to apoA-I of LDL-derived [3H]cholesterol in NPC1−/− and NPC1+/− cells following a 24-h incubation with labeled LDL (Fig. 1). Efflux of total cellular, plasma membrane, and newly synthesized [3H]cholesterol from NPC1−/− cells were intermediate between NPC1+/− and NPC1−/− cells (Fig. 2), as was efflux of phosphatidylcholine (Fig. 3). Northern blot analysis indicated a moderate decrease in cholesterol-induced levels of ABCA1 mRNA in NPC1−/− relative to NPC1+/− cells, whereas ABCA1 protein levels in response to cholesterol and LDL load-
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The markedly decreased ABCA1 expression and decreased ABCA1-dependent lipid efflux to apoA-I in the classic NPC1 heterozygote lipid profiles studied. The cell culture results in NPC1−/− cells, although interesting in showing intermediate levels of lipid efflux in our study, are likely to reflect heterogeneity of the NPC1 mutations and may not be useful in predicting low HDL formation or plasma levels of HDL in NPC heterozygotes generally. The differences between our findings of impaired ABCA1-dependent efflux of lipids to apoA-I in non-cholesterol-loaded and cholesterol-loaded cells (Fig. 5). In addition, despite marked differences in ABCA1 expression, levels of apoA-I binding to NPC1−/− and NPC1+/− cells were similar under both conditions. The reasons for this are unclear; however, they strongly suggest factors other than ABCA1, possibly extracellular matrix components (53), are important in facilitating the apoA-I-cell interaction. These results suggest NPC cells may be an excellent model to study other key determinants of apoA-I binding.

Interestingly, NPC1+/− fibroblasts showed the highest levels of 125I-apoA-I binding in both non-cholesterol-loaded and cholesterol-loaded cells (Fig. 5). In addition, despite marked differences in ABCA1 expression, levels of apoA-I binding to NPC1+/− and NPC1−/− cells were similar under both conditions. The reasons for this are unclear; however, they strongly suggest factors other than ABCA1, possibly extracellular matrix components (53), are important in facilitating the apoA-I-cell interaction. These results suggest NPC cells may be an excellent model to study other key determinants of apoA-I binding.

Our results showing impaired phospholipid efflux and cholesterol efflux from non-LDL-derived cholesterol pools are in contrast to results reported previously (16) for macrophages from a murine model of NPC disease. Chen et al. (16) reported normal levels of [3H]choline-labeled phospholipid efflux to apoA-I from these cells and concluded that ABCA1 function was intact. Similar levels of induction of AbcA1 mRNA and protein were reported for Npc1−/− and wild type mouse macrophages in response to treatment with LXR/retinoid X-receptor agonists; however, basal levels of AbcA1 expression were not indicated (16). Up-regulation of AbcA1 in Npc1−/− cells by these agonists is consistent with the known ability of exogenously added oxysterols to correct the defects in cholesterol esterification, cholesterol synthesis, LDL receptor activity, and lysosomal cholesterol accumulation in NPC cells (11, 54), and provides support for our conclusion that ABCA1 regulation is also impaired in this disorder. The decreased ability of cell cholesterol content to regulate cholesterol homeostasis in human NPC1−/− and mouse Npc1−/− cells suggests either a defect in oxysterol synthesis, sensing, or trafficking in these cells. A recent paper by Ory and colleagues (55) suggests synthesis of 25- and 27-hydroxycholesterol is impaired in human NPC1-deficient cells, leading to the failure to suppress sterol regulatory element-binding protein-dependent gene expression and to promote LXR-mediated responses. Our finding of impaired ABCA1 regulation in NPC1−/− cells is consistent with this finding.

The differences between our findings of impaired ABCA1-dependent efflux of lipids to apoA-I from human NPC1−/− cells and those using Npc1-deficient mouse cells may have been due to differences in the expression of this gene defect in the particular human and BALB/c mouse cell lines used in these studies. To determine whether impaired ABCA1 function in cultured human NPC1−/− fibroblasts is indicative of impaired HDL particle formation in vivo, we obtained the lipid profiles of NPC1−/− subjects. The results shown in Table I and Fig. 6 show low HDL-cholesterol levels in the vast majority (81%) of NPC lipid profiles obtained. This very high incidence of hypoalphalipoproteinemia suggests these results cannot be explained by chance. Although the incidence of heterozygous ABCA1 mutations in the general population is unknown, they are unlikely to represent a frequent cause of low HDL-cholesterol (56), and our results also cannot be explained on this basis. ABCA1-mediated lipidation of apoA-I is now widely accepted to be the rate-limiting step in HDL particle formation and a key predictor of circulating HDL levels (12, 57). Impaired passive efflux of cholesterol from NPC1−/− cells would not explain our findings, as apoA-I does not act as an effective acceptor of passively desorbed cholesterol (58, 59). Impaired regulation of ABCA1 activity, as indicated by the lipid efflux results and ABCA1 expression levels in human NPC1−/− cells, is the most likely explanation for such a high incidence of low HDL-cholesterol values in NPC disease. The absence of low HDL in all the NPC patient lipid profiles obtained is likely an additional demonstration of the known heterogeneity of biochemical and clinical presentations in this disorder (60, 61), which would include variable regulation of ABCA1 expression. The very high inci-

**Plasma lipid profiles in NPC1−/− patients**

| No. | Sex | Age | TC (mmol/liter) | TG (mmol/liter) | LDL (mmol/liter) | HDL (mmol/liter) |
|-----|-----|-----|----------------|----------------|-----------------|-----------------|
| 1   | M   | 5   | 5.8            | 2.51           | 4.45            | 0.28            |
| 2   | M   | 7   | 4.09           | 2.28           | 2.38            | 0.67            |
| 3   | M   | 7   | 3.39           | 0.94           | 2.12            | 0.83            |
| 4   | M   | 9   | 2.79           | 1.49           | 1.27            | 0.67            |
| 5   | M   | 9   | 3.08           | 1.37           | 1.76            | 0.70            |
| 6   | M   | 11  | 4.09           | 0.98           | 2.38            | 1.27            |
| 7   | M   | 17  | 3.78           | 1.26           | 2.33            | 0.88            |
| 8   | M   | 33  | 4.22           | 1.74           | 2.48            | 0.95            |
| 9   | M   | 40  | 3.44           | 0.98           | 2.35            | 0.83            |
| 10  | M   | 42  | 3.05           | 0.71           | 1.99            | 0.75            |
| 11  | F   | 3   | 3.65           | 1.43           | 2.46            | 0.54            |
| 12  | F   | 5   | 3.59           | 1.87           | 2.15            | 0.59            |
| 13  | F   | 7   | 5.5            | 1.1            | 3.9             | 1.09            |
| 14  | F   | 8   | 2.87           | 1.2            | 1.47            | 0.85            |
| 15  | F   | 8   | 3.72           | 1.13           | 2.22            | 0.98            |
| 16  | F   | 10  | 3.49           | 1.31           | 2.28            | 0.62            |
| 17  | F   | 11  | 3.39           | 3.42           | 1.22            | 0.59            |
| 18  | F   | 17  | 5.59           | 2.28           | 4.45            | 0.91            |
| 19  | F   | 18  | 3.49           | 0.93           | 1.66            | 1.42            |
| 20  | F   | 20  | 4.78           | 2.19           | 2.97            | 0.80            |
| 21  | F   | 32  | 3.59           | 2.21           | 1.19            | 1.19            |

* H. level lower than normal range. TC, total cholesterol; TG, triglycerides.

**Fig. 6. Plasma HDL levels in NPC1-deficient subjects.** HDL-cholesterol levels (mmol/liter) obtained for male and female subjects are taken from Table I. The dashed line represents the lower limit of normal HDL-cholesterol for children and adults (1.03 mmol/liter) (45, 46).
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dence of low HDL in NPC disease patients, however, suggests
this could be used as an additional diagnostic criterion to help
rule in or out Niemann Pick C disease in children, which is
frequently a difficult diagnosis to make.

The reasons for the differences in cell culture results and
HDL levels between human NPC1 disease and the mouse
model of this disease are unknown. Normal HDL levels in
BALB/c Npc1−/− mice (17, 18) are consistent with normal
expression of AbcA1 in these animals. Although the biochemi-

cal and pathologic changes in Npc1−/− mice are similar to
those seen in humans (62, 63), lipoprotein physiology varies
considerably between rodents and humans (64). The findings
presented here suggest striking differences in the impact
of NPC deficiency on HDL metabolism in mice compared with
humans.

Of note, low HDL-cholesterol levels have also recently been
reported in two family members with the acid sphingomyeli-
nase deficiency Niemann-Pick Type B disease (65). In contrast
to the defect in ABCA1-dependent lipid mobilization reported
here in human NPC disease, apoA-I-dependent cholesterol mo-
bilization was normal in fibroblasts of these Niemann-Pick B
patients. It was suggested that the low HDL in these subjects
might be due to impaired lecithin cholesterol acyltransferase
activity (65).

Our data do not allow us to draw conclusions about whether
the severity of clinical disease in NPC patients correlates with
their level of ABCA1 dysfunction and/or HDL-cholesterol level.
High levels of ABCA1 expression in the brain (66, 67), how-
er, raise the intriguing possibility that neurodegeneration in this
disease might be related to impaired regulation of ABCA1
in the central nervous system. Cholesterol trafficking defects in
neurons (68, 69) and glia (70) suggest that ABCA1 expression is
reduced in these cells in the brain, as we have found in
NPC1−/− fibroblasts.

In conclusion, the results presented here demonstrate an
additional defect in regulation of a cholesterol-dependent gene,
ABCA1, in NPC disease. We suggest that this dysregulation is
responsible for the hypoalphalipoproteinemia in the majority of
NPC disease patients studied. Further studies will be aimed at
understanding the role of ABCA1 in the central nervous system
and in the pathogenesis of this disease.

Acknowledgments—We thank Dr. Francis Meany for assistance with
statistical analysis and Glen Shepherd and Ryan Graver for assistance in
obtaining the lipid profiles.

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