Mitochondrial bioenergetics, acylcarnitine profile and phospholipidomic analysis of fibroblasts derived from trifunctional protein-deficient patients

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Background:
Mitochondrial trifunctional protein (TFP) catalyzes the three last steps of long-chain fatty acid oxidation (1). Mutations in either α (HADHA) or β (HADHB) subunits usually result in combined TFP deficiency, while the HADHA p.E510Q mutation leads to isolated 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (Figure 1).

A fourth enzymatic activity located on the α subunit, monolysocardiolipin acyltransferase (MLCLAT-1), participates in cardiolipin remodeling (CL) (2). Cardiolipin remodeling deregulation may explain many manifestations of TFP/LCHAD deficiency shared with primary disorders of electron transport chain (ETC), including peripheral neuropathy, retinal degeneration, and cardiomyopathy. This study explored the effect of mutations in the TFP subunits on CL and other phospholipid content and composition and the effect of these changes on mitochondrial bioenergetics in TFP/LCHAD-deficient fibroblasts.

Material and Methods:
**Mitochondrial bioenergetics:** Fibroblast oxygen consumption (OCR) was measured with a Seahorse XFe96 Extracellular Flux Analyzer (Agilent Technologies, Santa Clara, CA) (3); 
**Identification and quantification of mitochondrial phospholipids (including CL) and oxidized phospholipids by LC-MS/MS:** measured as described (4). Briefly, lipids were extracted using the Folch method, total phosphate content was quantified, and samples were then analyzed using a LC-MS/MS system. The identification and quantification of the lipid species were achieved with an optimized workflow using SIEVE 2.2 software, and an in-house database; 
**ESI-MS/MS-based acylcarnitine profiling in fibroblasts using unlabeled palmitic acid:** the acylcarnitine profiles were determined in media collected from cells seeded in 6-well plates and incubated for 72 h in medium containing palmitic acid, and L-carnitine; acylcarnitines were analyzed by the precursor-ion scan of m/z 85 mode and quantitated by comparison to isotope-labelled internal standards.
Results:
The spectra of CLs, monolyso-CLs, and oxidized CLs were different among mitochondria isolated from controls and TFP/LCHAD-deficient fibroblasts (Figure 2). The concentration of CLs was higher in control than in TFP/LCHAD deficient fibroblasts, and the reverse was found for monolyso-CLs (Figure 3). An isolated LCHAD-deficient cell line (822), and a HADHB (861) complete TFP-deficient cell line had increased levels of oxidized CLs. These two cell lines plus an HADHA complete TFP-deficient cell line (847) had the highest levels of monolyso-CLs.

Figure 2. Heat map showing the distribution of CLs, monolyso-CLs, and oxidized CLs species in mitochondria isolated from TFP/LCHAD-deficient and control fibroblasts. Z-score of 0 is marked by white, +3 by dark red, and −3 by dark blue cells and they indicate that the sample value is identical to mean value, or 3 SD above or below the mean, respectively.

549, 554, and 902 – control cells from 3-y-o, 8-y-o, and 22-y-o males.

Figure 3. LC-MS/MS quantification of cardiolipins and monolyso-cardiolipins in mitochondria isolated from TFP/LCHAD-deficient fibroblasts and controls.

Other major phospholipid classes, lysophospholipids (LPL), and phosphatidylglycerols (PG), were also significantly varied among these cell lines (Figure 4). PG levels were increased in TFP/LCHAD-deficient cell lines compared to controls.

The orthogonal partial least squares discriminant analysis (OPLS-DA) score plot showed a clear separation between controls and TFP/LCHAD-deficient (HADHA and HADHB) fibroblasts in the lipidomics analysis. Nevertheless, there was a split in HADHA and HADHB groups indicating an important variation among the mutated cell lines analyzed (Figure 5).

Figure 5. Score plot of orthogonal partial least squares discriminant analysis (OPLS-DA) showing the differences in the phospholipidome of control, HADHA, and HADHB fibroblasts.
Variable Importance for Prediction (VIP) revealed that CL, LPL, monolyso-CL and PG were the most significantly discriminatory phospholipid classes among these cell lines. The top 10 VIP score phospholipids were CL(70:6), CL(72:6), CL(72:9), LysoPI(18:2), LysoPI(16:0), LysoPI(16:1), LysoPG(18:0), LysoPE(22:1), PA(40:5)+2O, PEp(34:0) – Figure 6.

A clear reduction of maximal respiration and spare respiratory capacity was observed in all TFP/LCHAD-deficient fibroblasts compared to control. In contrast, most patients’ fibroblasts had values for basal respiration and ATP production equal to or higher than control, with the exception of 822 (LCHAD-deficient), and 861 (HADHB) – Figure 7.

The marker of TFP/LCHAD deficiency, C16-OH, was present in the acylcarnitine profile of all deficient cells, although in different concentrations. C16 was low and medium-chain acylcarnitines (C8 and C10) were high in controls and two deficient cell lines – 854 (HADHB) and 864 (HADHA). In contrast, 822 (LCHAD-deficient) and 847 (HADHA) had very high C16 levels and low levels of medium-chain acylcarnitines (Figure 8).

Discussion:
The findings of increased monolyso-CLs and decreased CLs concentrations in mitochondria isolated from TFP/LCHAD-deficient fibroblasts suggest a disruption in CL reacylation in this disease, similar to Barth syndrome (5). Although the increase in monolyso-CLs in Barth syndrome is more dramatic, this discovery in TFP/LCHAD deficiency points to a possible function of MLCLAT-1 as a CL remodeling enzyme. This finding offers new potential targets for development of drug therapy.

References: