Appendix 1: Detailed methods: magnetic resonance spectroscopy imaging (as supplied by the authors)

Proton magnetic resonance spectroscopy was performed using a GE Healthcare 3.0T Signa HD MRI system (General Electric Healthcare, Milwaukee, WI) and an 8-channel receive-only phased array head coil. Following a 3-plane localizer scan, a T_1 weighted inversion recovery prepared 3D- fast SPoiled GRadient echo axial scan (TR=7.5ms, TE=2.1ms, TI=450ms , flip angle =12^o , FOV=240mm, slice thickness = 2.0mm, NEX=1, matrix=192x320 (zero padded to 512x512), 163 slices) and a sagittal IR prepared 3D-fSPGR (TR=10.1ms, TE=2ms, flip angle=20^o, FOV=220mm, slice thickness=1.2 mm, NEX=2, matrix=192x320 (zero padded to 512x152), 123 images) were performed for use in MRS localization. In addition, an axial T_2 fluid attenuation inversion recovery (FLAIR) scan (TR=9000, TE=139.8ms, FOV=220mm, matrix=192x320 (zero padded to 512x512) 25 slices 5.0mm thick, NEX=2) was acquired to aid in ruling out gross pathology. Gross pathology was not identified in any of the subjects in the regions of interest.

Proton magnetic resonance spectroscopy acquisition was done on three single voxels placed in the caudate, cingulate cortex, and hippocampus using a standardized positioning procedure (Figure 1). The caudate voxel was placed using the sagittal T1-weighted images and centered on the left caudate. The axial 3D scan was used to adjust the voxel to include maximal caudate volume. The cingulate cortex voxel was placed using the sagittal and axial series adjacent to the edge of the corpus callosum on the left side. The hippocampal voxel was first prescribed on the sagittal anatomic scan and centered on the head of the left hippocampus. Using the axial anatomical series, the voxel was adjusted to be lateral to the medial aspect of the lateral fissure. Voxel size was 20 x 20 x 20 mm^3.

Appendix to: Anglin RE, Rosebush PI, Noseworthy MD. Metabolite measurements in the caudate nucleus, anterior cingulate cortex and hippocampus among patients with mitochondrial disorders: a case–control study using proton magnetic resonance spectroscopy. *CMAJ Open* 2013. DOI:10.9788/cmajo.20120020

Single voxel proton magnetic resonance spectroscopy was conducted using a short echo PRESS (Point RESolved Spectroscopy) sequence (TE=35 ms, TR = 2000 ms, 256 acquisitions). Second order shimming was performed for each acquisition voxel. The entire MRS protocol took approximately 60 minutes.

Segmentation for every subject and each MRS scan was accomplished using a combination of in-house software and the freeware software Analysis of Functional NeuroImages (AFNI)[1]. Using high-resolution anatomical images (T1-weighted fSPGR axial), white matter (WM) and cerebrospinal fluid (CSF) masks were created in order to determine each tissue's grey-scale intensity (grey matter (GM) was taken to be the intensities between WM and CSF). MRS voxel masks were then segmented based on these intensity values giving the fraction of GM, WM and CSF within each voxel. Spectra were analyzed using LCModel[2] using the included simulated metabolite basis set for PRESS and TE = 35 ms. LCModel partial volume correction was performed according to the LCModel manual, section 10.2.2.2[2]. Total water concentration for each VOI was adjusted using the following equation:

wconc = $43300f_{gm} + 35880f_{wm} + 55556f_{csf}$,where f_{gm}, f_{wm}, and f_{csf} are the fraction of GM, WM and CSF tissue within each voxel, respectively. Water concentrations in GM, WM and CSF were taken from Ernst *et al.*[3]. Since metabolites are concentrated in only the GM and WM, the final concentration was then divided by: $1 - f_{csf}$.

Sample spectra from a mitochondrial patient and healthy control are included in Figure 2. A Cramér-Rao lower bound less than 20% was used as a cut-off for inclusion in the analysis.

1. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res. 1996; **29**(3): 162-73.
2. Provencher SW. LC Model Manual. 2011 [cited 2011 November 20, 2011]; Available from: http://lcmodel.ca/pages/lcm-manual.shtml

3. Ernst T, Kreis, R., Kreis, R. Absolute quantitation of water and metabolites in the human brain. 1. Compartments and Water. journal of Magnetic resonance, series B. 1993; **102**(1): 1064-866.