Abstract

Background: Mitochondrial disorders are clinical syndromes associated with mutations in the mitochondrial or nuclear genome that result in impaired oxidative phosphorylation and deficient energy production. Metabolic abnormalities in brain areas associated with cognitive functions could give rise to neuropsychiatric symptomatology. The aim of this study was to use single voxel proton magnetic resonance spectroscopy to identify metabolic abnormalities in regions implicated in neuropsychiatric symptoms in patients with mitochondrial disorders. Methods: N-acetyl-aspartate (NAA), creatine (Cr), glycerophosphocholine (GPC), GPC+phosphocholine (PCh), myoinositol (mI), and glutamine + glutamate (Glx) were measured in the caudate, anterior cingulate cortex and hippocampus in 15 patients with mitochondrial disorders compared to 15 age and sex-matched healthy controls. Results: Patients with mitochondrial disorders had metabolic abnormalities exclusively in the caudate, with significantly lower NAA in compared to controls. Cr, GPC+PCh and Glx were also lower in the caudate in patients compared to controls. Interpretation: These results suggest that NAA is a useful marker of mitochondrial dysfunction and that the striatum may be highly susceptible to mitochondrial oxidative phosphorylation defects and resultant cell loss. Given the caudate's role in cognitive and executive functions, our findings also suggest that metabolic abnormalities in the caudate may contribute to cognitive impairment and neuropsychiatric symptoms in patients with mitochondrial disorders.

Introduction

Mitochondria are intracellular organelles that are involved in a number of important cellular functions including apoptosis, calcium homeostasis and the generation of energy in the form of adenosine triphosphate (ATP) through oxidative phosphorylation (1). Mitochondria contain their own genome that is maternally inherited and encodes for 13 subunits of the respiratory chain, two ribosomal ribonucleic acids (RNAs) and 22 transfer RNAs. In addition over 14,500 proteins are encoded by nuclear DNA and transported into the mitochondria. Therefore, abnormalities in either genome can give rise to mitochondrial dysfunction.

Mitochondrial disorders, by definition, are clinical syndromes produced by mutations in either the mitochondrial genome or nuclear genome that result in impaired oxidative phosphorylation and deficient aerobic energy production (2). While there is growing recognition that many other disorders are associated with mitochondrial dysfunction, the term 'mitochondrial disorder' is at this point in time, used only in reference to those disorders associated with the mitochondrial respiratory chain. Even narrowly defined, the prevalence of mitochondrial disorder is estimated to be approximately 1 in 5000 (3, 4). Common features include fatigue, muscle weakness, migraines, seizures, stroke-like episodes, ataxia, ptosis, ophthalmoplegia, hearing loss, and cardiomyopathy (2). In addition, we have demonstrated that a range of psychiatric symptoms can be the most prominent and presenting features of mitochondrial disorders. This suggests that regional metabolic abnormalities associated with mitochondrial dysfunction in the brain may give rise to psychiatric symptoms in these patients. To date there has never been an investigation of metabolic abnormalities in regions of the brain

implicated in neuropsychiatric illness in patients with mitochondrial disorders.

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a particularly useful tool for studying patients with mitochondrial disorder, as it allows for the measurement of metabolic abnormalities in the brain *in vivo*. To our knowledge, MRS findings in the brain of patients with mitochondrial disorders have only been reported in individual cases or small series and in the majority, the study was carried out in areas of the brain appearing abnormal on MRI as part of the clinical investigation of the patient (5-13). The most consistent findings have been elevated lactate and decreased N-Acetyl aspartate (NAA), although decreased creatine (Cr), decreased glutamate (Glu) and altered levels of choline (Cho) have also been reported (5-13). To date, there has not been a systematic investigation of metabolic abnormalities associated with mitochondrial disorders that has included a matched control group and that has measured metabolites in specific regions of interest using standardized voxel placement.

In this study, we measured metabolites *in vivo* in patients with mitochondrial disorders compared to age and sex-matched healthy controls in three brain regions commonly implicated in neuropsychiatric disorders: the caudate, anterior cingulate cortex and hippocampus. Our primary hypothesis was that patients with mitochondrial disorders would have reduced markers of mitochondrial dysfunction (NAA and Cr) compared to healthy controls in the three candidate brain regions. A secondary hypothesis was that other metabolic indices, including glycerophosphocholine (GPC), GPC+phosphocholine (PCh), myoinositol (mI), and glutamine + glutamate (Glx) might also be altered in patients with mitochondrial disorders in the candidate brain regions.

Methods

Study Subjects

The study was approved by the Research Ethics Board of St.Joseph's Healthcare Hamilton and McMaster University Medical Centre, and informed consent was obtained from each participant prior to inclusion. Capacity to consent was assessed by a psychiatric research nurse and all participants were found to be capable of consenting to participation in the study. A total of 30 participants were investigated: 15 patients with a mitochondrial disorder, and 15 age (± 5 years) and sex-matched healthy controls without neurological, psychiatric or systemic disease. Patients and controls with implanted metallic foreign bodies or inability to undergo MRI imaging were excluded. Patients with a mitochondrial disorder were recruited from the Neuropsychiatry and Neurometabolic Clinics at McMaster University in Hamilton, Ontario. The diagnosis of a mitochondrial disorder was made using the Thorburn criteria (14), and only those with definite or probable mitochondrial disease were eligible for the study. Of the participants, 11 had a definite mitochondrial disorder with identification of the pathogenic mutation or deletion and 4 had a probable mitochondrial disorder with supportive clinical features, histology and enzymology.

Magnetic resonance spectroscopy imaging

^{1}H-MRS 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 (IR) prepared 3D-fSPGR (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 (^{1}H-MRS) 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}.

Single voxel ^{1}H-MRS 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)(15). 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 (16) 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 (17). 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.(18)*. 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.

Statistical Analysis

Statistical analyses were performed with PASW Statistics 18.0 (SPSS Inc, Chicago, IL). Two-tailed significance was assessed at the $p < 0.05$ threshold. Differences between mitochondrial patients and healthy controls for each metabolite were

tested separately. Our primary *a priori* hypothesis was that patients and controls would differ with respect to NAA and Cr based on previous reports of decreased NAA and the importance of Cr as an energy substrate. Therefore, these analyses were performed first, and the remainder were performed as secondary analyses. A Holm Bonferroni correction for multiple comparisons was used for each region of interest (ROI) for our primary analyses, and subsequently for our secondary analyses. Group differences in metabolite concentration, and full width at half maximum (FWHM) in each ROI were assessed using independent samples t-tests. When there were significant differences in FWHM between groups in an ROI, FWHM was used as a covariate in an analysis of covariance (ANCOVA) for the group differences for each metabolite in that ROI.

Results

Demographics

Patients with mitochondrial disorders (MD) and healthy controls (HC) were pair-matched by age (MD 51.7 ± 8.7, p=0.65, HC 50.2 ± 8.6) and sex (11F/4M in each group). Clinical and demographic information for the 15 patients with mitochondrial disorders is presented in Table 1. Three patients had mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) T3271C mutations, two had MELAS A3243G mutations, two had myoclonic epilepsy with raged red fibers (MERRF) A8363G mutations, three had single, large scale mtDNA deletions of ~4.7kb, three had complex I deficiency (2 patients >3 standard deviations (SD) below the age-matched mean, 1 patient 2 SD below the age-matched mean), one had a C9035T mutation and one had mitochondrial cytopathy based on Thorburn criteria but the mutation has not been

identified. The average age at diagnosis was 45. The most common clinical features included muscle weakness or atrophy, fatigue, depression, anxiety, hearing loss, stroke-like episodes, migraines or headaches, cognitive impairment and Type 2 diabetes. Nine patients had white matter lesions on MRI. Thirteen patients were treated with mitochondrial supplements including creatine, alpha-lipoic acid, riboflavin, vitamin C, vitamin E and coenzyme Q10. None of the healthy controls was receiving any mitochondrial supplements.

Metabolites

Concentrations of NAA, Cr, CPG, PCh, mI, and Glx for each ROI are presented in Table 2. Since the T-tests revealed significant differences in the FWHM between the groups in the caudate ($t=3.24$, $p=0.005$), FWHM was used as a co-variate in the analyses for this region. For our primary analysis, ANCOVAs examining NAA and Cr between the two groups in each ROI revealed significantly lower NAA ($F=5.86$, $p=0.023$), and Cr ($F= 5.60$, $p=0.026$) in the caudate in patients compared to controls. However, after Holm Bonferroni correction for multiple comparisons, only lower NAA in the caudate remained significant. For our secondary analyses, all metabolites were analyzed and significantly lower GPC+PCh ($F = 4.79$, $p=0.038$) and Glx ($F= 5.97$, $p=0.022$) in the caudate were found in patients compared to controls. Neither of these findings remained significant after Holm Bonferroni correction for multiple comparisons. There were no significant differences for metabolites between the groups in the cingulate cortex or hippocampus.

Given patients with mitochondrial disorders had significantly lower NAA in the caudate, we hypothesized that this finding might contribute to the cognitive impairment

observed in patients with mitochondrial disorders. We therefore performed an additional *post-hoc* analysis comparing NAA levels in the caudate in mitochondrial patients with and without a history of cognitive impairment. Although patients with cognitive impairment had lower mean NAA levels (6.61 ± 1.34 in those with cognitive impairment versus 7.25 ± 1.12 in those without) the difference was not significant (p=0.34).

Interpretation

To our knowledge, this is the first systematic study of a range of metabolites measured using ^{1}H-MRS in patients with mitochondrial disorders compared to matched controls. It is also the first study in mitochondrial patients to use standardized voxel placement and partial volume correction. Our results indicate that patients with mitochondrial disorders have significantly lower NAA in the caudate compared to healthy controls. We also found reduced Cr, GPC+PCh and Glx in the caudate patients compared to controls, although these results were no longer significant after correcting for multiple comparisons. Metabolic abnormalities were exclusively identified in the caudate, with no differences identified between patients and controls in the other regions of interest.

Although reduced NAA has previously been reported in cortical gray matter and the cerebellum in individual patients with mitochondrial disorders, our study is the first to examine multiple metabolites in several ROIs using standardized voxel placement and partial volume correction and find reduced NAA in the caudate (5-7, 19). Despite its abundance in the brain and decades of research, the function of NAA remains unclear. Proposed functions have included providing a source of acetate for myelin lipid

synthesis, and acting as an organic osmolyte (20, 21). NAA is synthesized from L-aspartic acid and acetyl-coA in neuronal mitochondria, and it has been suggested that NAA may facilitate mitochondrial energy metabolism (21). NAA synthesis in the brain is decreased when oxidative phosphorylation is impaired by inhibitors of complexes III, IV and V of the respiratory chain (22). This coupling of NAA synthesis with mitochondrial energy production has been further supported by the demonstration of lower NAA levels in experimental models of mitochondrial dysfunction including striatal neurodegeneration induced by the mitochondrial toxin 3-nitropropionic acid, and other models of Huntington's disease (23-25). Our results lend additional support to the notion that NAA may be an important marker of mitochondrial dysfunction.

We identified metabolic abnormalities specifically in the caudate and not other ROIs, which adds to a growing body of literature suggesting that the striatum (composed of the caudate and putamen) is highly susceptible to impaired mitochondrial oxidative phosphorylation (26-28). The particular vulnerability of the striatum to oxidative phosphorylation defects may result from intrinsically higher levels of oxidative phosphorylation in this region and a higher basal mitochondrial membrane potential, leading to increased cytosolic calcium and apoptosis under conditions of impaired mitochondrial function (26).

Although the basal ganglia and striatum have traditionally been associated with motor coordination, it is now recognized that the caudate plays a central role in cognitive processes, particularly executive functions (29, 30). Specifically, the caudate is responsible for planning goal-directed behaviour and weighing different contingencies based on possible outcomes (29). This can range from simple binary decisions based on

known results, to complex decision-making incorporating emotional, rational and social values. Whereas basal ganglia circuits involving the putamen carry out motor programs that coordinate sensorimotor processes, the caudate appears to operate cognitive programs that allow for rapid decision making based on selecting the most appropriate course of action as determined by underlying motivations and goals. Deficits in these cognitive programs and complex cognitive and executive functioning have been demonstrated in patients with caudate pathology, including those with Parkinson's disease, Huntington's disease and schizophrenia (29, 31, 32). Importantly, mitochondrial dysfunction and mitochondrial abnormalities have been observed in the striatum in all of these conditions, and may underlie some of the observed abnormalities (28, 33, 34). Cognitive impairment, was a common clinical feature in the 15 patients examined in this study (Table 1). This is consistent with our previous reports and review of the literature, which identified significant cognitive dysfunction and neuropsychiatric symptoms in patients with mitochondrial disorders (35, 36). Our present findings suggest that metabolic abnormalities in the caudate may contribute to these problems in affected patients. Unfortunately we were not able to identify significant differences in NAA levels between patients with mitochondrial disorders and cognitive impairment and those without. This may be attributable to the relatively small number of patients in each group. In addition, a history of cognitive impairment, was used as opposed to a standardized measurement of current symptomatology. Performing formal neuropsychological evaluations in patients with mitochondrial disorders, and correlating those results with MRS findings in the caudate would be of interest and should be pursued in future studies.

Although this is the largest study to date using ^{1}H-MRS in adult patients with

mitochondrial disorders, it is possible that we were underpowered to detect some differences in metabolites between patients and controls. Given that patients had significant reductions of Cr, GPC+PCh and Glx in the caudate before correction for multiple comparisons, futures studies with larger sample sizes should measure these metabolites. In particular, our finding of increased creatine in these patients despite creatine supplementation is intriguing and highly suggestive that creatine is lower in the caudate in patients with mitochondrial disorders. Our study also focused on the caudate, cingulate cortex and hippocampus; it is possible that patients with mitochondrial disorders might have metabolic abnormalities in other regions. Finally, using a relatively short TE we were able to measure a large number of metabolites at the expense of reliably detecting lactate. At shorter TE values lactate is confounded by broad lipid resonances, which disappear at longer TEs dues to their shorter T2 relaxation values. As elevated lactate has been described in a range of regions in patients with mitochondrial disorders, we chose to focus on metabolites that have not been consistently measured, or for which there have been conflicting results.

Conclusions

Patients with mitochondrial disorders had reduced NAA in the caudate, a brain region known to play a key role in cognitive and executive processes. These findings indicate that (1) reduced NAA may be a useful marker of mitochondrial dysfunction; and (2) the striatum is highly susceptible to mitochondrial oxidative phosphorylation defects. Metabolic abnormalities in the caudate may contribute to the cognitive impairment and neuropsychiatric symptoms exhibited by patients with mitochondrial disorders.

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References

1. Wallace DC. Mitochondrial diseases in man and mouse. Science. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.
Review]. 1999 Mar 5;283(5407):1482-8.

2. van Adel BA, Tarnopolsky MA. Metabolic myopathies: update 2009. J Clin Neuromuscul Dis. [Review]. 2009 Mar;10(3):97-121.

3. Chinnery PF, Johnson MA, Wardell TM, Singh-Kler R, Hayes C, Brown DT, et al. The epidemiology of pathogenic mitochondrial DNA mutations. Ann Neurol. [Research Support, Non-U.S. Gov't]. 2000 Aug;48(2):188-93.

4. Elliott HR, Samuels DC, Eden JA, Relton CL, Chinnery PF. Pathogenic mitochondrial DNA mutations are common in the general population. Am J Hum Genet. [Research Support, Non-U.S. Gov't]. 2008 Aug;83(2):254-60.

5. Moller HE, Kurlemann G, Putzler M, Wiedermann D, Hilbich T, Fiedler B. Magnetic resonance spectroscopy in patients with MELAS. J Neurol Sci. [Research Support, Non-U.S. Gov't]. 2005 Mar 15;229-230:131-9.

6. Mathews PM, Andermann F, Silver K, Karpati G, Arnold DL. Proton MR spectroscopic characterization of differences in regional brain metabolic abnormalities in mitochondrial encephalomyopathies. Neurology. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1993 Dec;43(12):2484-90.

7. Wilichowski E, Pouwels PJ, Frahm J, Hanefeld F. Quantitative proton magnetic resonance spectroscopy of cerebral metabolic disturbances in patients with MELAS. Neuropediatrics. [Case Reports
Research Support, Non-U.S. Gov't]. 1999 Oct;30(5):256-63.

8. Barkovich AJ, Good WV, Koch TK, Berg BO. Mitochondrial disorders: analysis of their clinical and imaging characteristics. AJNR Am J Neuroradiol. 1993 Sep-Oct;14(5):1119-37.

9. Boddaert N, Romano S, Funalot B, Rio M, Sarzi E, Lebre AS, et al. 1H MRS spectroscopy evidence of cerebellar high lactate in mitochondrial respiratory chain deficiency. Mol Genet Metab. [Evaluation Studies
Research Support, Non-U.S. Gov't]. 2008 Jan;93(1):85-8.

10. Sijens PE, Smit GP, Rodiger LA, van Spronsen FJ, Oudkerk M, Rodenburg RJ, et al. MR spectroscopy of the brain in Leigh syndrome. Brain Dev. 2008 Oct;30(9):579-83.

11. Chuang CS, Lo MC, Lee KW, Liu CS. Magnetic resonance spectroscopy study in basal ganglia of patients with myoclonic epilepsy with ragged-red fibers. Neurol India. [Case Reports]. 2007 Oct-Dec;55(4):385-7.

12. Bowen J, Richards T, Maravilla K. MR imaging and proton MR spectroscopy in A-to-G substitution at nucleotide position 3243 of leucine transfer RNA. AJNR Am J Neuroradiol. [Case Reports]. 1998 Feb;19(2):231-4.

13. Lee SK, Kim J, Kim HD, Lee JS, Lee YM. Initial experiences with proton MR spectroscopy in treatment monitoring of mitochondrial encephalopathy. Yonsei Med J. 2010 Sep;51(5):672-5.

14. Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. Neurology. [Research Support, Non-U.S. Gov't]. 2002 Nov 12;59(9):1406-11.

15. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res. [Research Support, U.S. Gov't, P.H.S.]. 1996 Jun;29(3):162-73.

16. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med. 1993 Dec;30(6):672-9.

17. Provencher SW. LC Model Manual. 2011 [cited 2011 November 20, 2011]; Available from: http://lcmodel.ca/pages/lcm-manual.shtml.

18. 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.

19. Bianchi MC, Tosetti M, Battini R, Manca ML, Mancuso M, Cioni G, et al. Proton MR spectroscopy of mitochondrial diseases: analysis of brain metabolic abnormalities and their possible diagnostic relevance. AJNR Am J Neuroradiol. [Research Support, Non-U.S. Gov't]. 2003 Nov-Dec;24(10):1958-66.

20. Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AM. N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol. [Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't
Review]. 2007 Feb;81(2):89-131.

21. Imamura K. Proton MR spectroscopy of the brain with a focus on chemical issues. Magn Reson Med Sci. [Review]. 2003 Oct 1;2(3):117-32.

22. Bates TE, Strangward M, Keelan J, Davey GP, Munro PM, Clark JB. Inhibition of N-acetylaspartate production: implications for 1H MRS studies in vivo. Neuroreport. [Research Support, Non-U.S. Gov't]. 1996 May 31;7(8):1397-400.

23. Demougeot C, Garnier P, Mossiat C, Bertrand N, Giroud M, Beley A, et al. N-Acetylaspartate, a marker of both cellular dysfunction and neuronal loss: its relevance to studies of acute brain injury. J Neurochem. [Research Support, Non-U.S. Gov't]. 2001 Apr;77(2):408-15.

24. Dautry C, Vaufrey F, Brouillet E, Bizat N, Henry PG, Conde F, et al. Early N-acetylaspartate depletion is a marker of neuronal dysfunction in rats and primates chronically treated with the mitochondrial toxin 3-nitropropionic acid. J Cereb Blood Flow Metab. [Research Support, Non-U.S. Gov't]. 2000 May;20(5):789-99.

25. Jenkins BG, Klivenyi P, Kustermann E, Andreassen OA, Ferrante RJ, Rosen BR, et al. Nonlinear decrease over time in N-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington's disease mice. J Neurochem. [Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.]. 2000 May;74(5):2108-19.

26. Pickrell AM, Fukui H, Wang X, Pinto M, Moraes CT. The striatum is highly susceptible to mitochondrial oxidative phosphorylation dysfunctions. J Neurosci. [In Vitro
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. 2011 Jul 6;31(27):9895-904.

27. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, et al. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell. [Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.]. 2004 Oct 1;119(1):121-35.

28. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, et al. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol. [In Vitro
Research Support, Non-U.S. Gov't]. 1997 May;41(5):646-53.

29. Grahn JA, Parkinson JA, Owen AM. The cognitive functions of the caudate nucleus. Prog Neurobiol. [Review]. 2008 Nov;86(3):141-55.

30. Middleton FA, Strick PL. Basal ganglia output and cognition: evidence from anatomical, behavioral, and clinical studies. Brain Cogn. [Review]. 2000 Mar;42(2):183-200.

31. White NM. Some highlights of research on the effects of caudate nucleus lesions over the past 200 years. Behav Brain Res. [Historical Article
Review]. 2009 Apr 12;199(1):3-23.

32. Simpson EH, Kellendonk C, Kandel E. A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. Neuron. [Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't
Review]. 2010 Mar 11;65(5):585-96.

33. Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol. [Research Support, Non-U.S. Gov't
Review]. 2008 Nov;4(11):600-9.

34. Somerville SM, Lahti AC, Conley RR, Roberts RC. Mitochondria in the striatum of subjects with schizophrenia: relationship to treatment response. Synapse. [Research Support, N.I.H., Extramural]. 2011 Mar;65(3):215-24.

35. Anglin R, Garside, S., Tarnopolsky, M.A., Mazurek M.F., Rosebush, P.I. The Psychiatric Manifestations of Mitochondrial Disorders: A Case and Review of the Literature. Journal of Clinical Psychiatry. In Press.

36. Anglin R, Tarnopolsky, MA, Mazurek MF, Rosebush PI. The Psychiatric Presentation of Mitochondrial Disorders in Adults. Journal of Neuropsychiatry and Clinical Neurosciences. 2012;In Press.

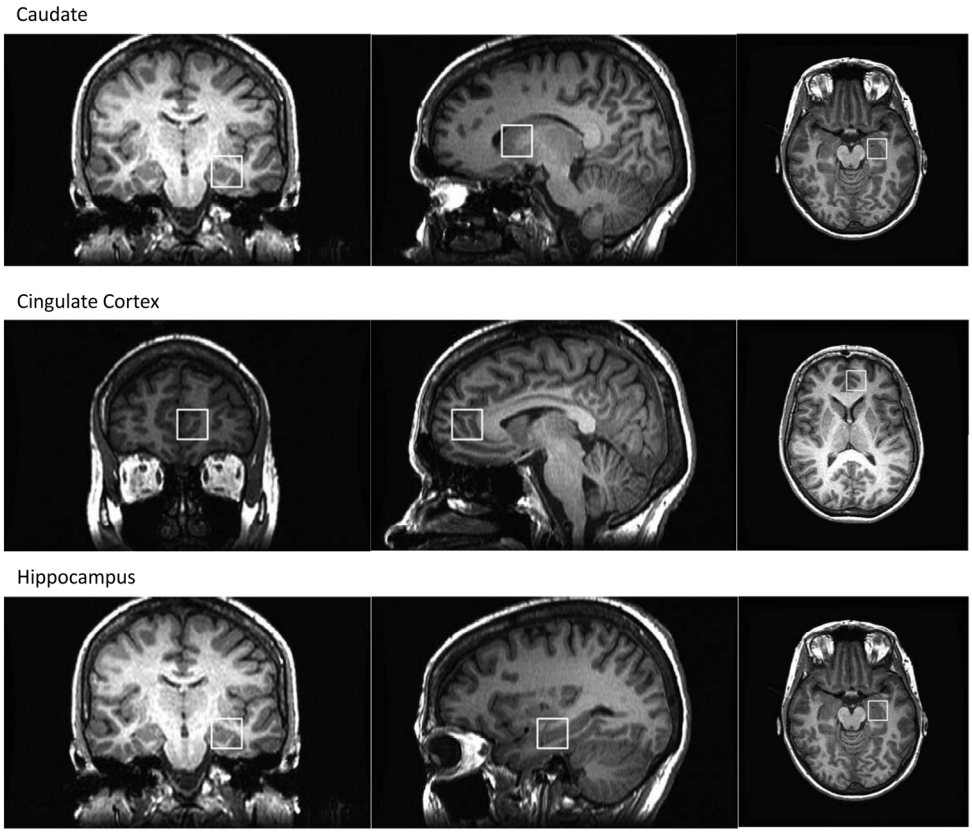
Table 1. Clinical Features and Demographics of 15 Patients with Mitochondrial Disorders

Patient/Sex	1F	2M	3F	4M	5M	6F	7M	8F	9F	10F	11F	12F	13F	14F	15F	
Mitochondrial disorder	MELAS A3243G	mtDNA Deletion	MELAS T3271C	Mitochondrial Cytopathy	mtDNA Deletion	Complex I Deficiency	C9035T Mutation	MELAS 3271	MELAS 3271	mtDNA Deletion	Complex I Deficiency	Complex I Deficiency	MELAS A3243G	MERRF A8363G	MERRF A8363G	AVG TOTA
Age	50	50	50	56	57	35	50	39	60	52	41	65	64	48	58	52
Age at Diagnosis	49	43	47	54	56	29	28	29	46	47	32	64	51	47	50	45
Clinical Features																
Muscle weakness or atrophy	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	14
Fatigue	-	-	+	+	+	+	-	+	+	+	+	-	+	+	-	10
Depression	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	10
Anxiety	+	-	-	+	+	+	-	+	+	+	+	-	-	-	+	9
White Matter Lesions on MRI	+	-	+	+	+	-	-	-	+	+	-	+	+	+	-	9
Hearing loss	+	-	+	+	-	-	-	+	+	+	-	-	+	+	-	8
Stroke/stroke-like episodes	-	+	+	-	-	-	-	+	+	+	+	+	+	-	-	8
Migraines or headaches	-	-	-	-	-	+	-	+	-	+	+	-	+	-	+	6
Cognitive Impairment	+	-	+	+	+	-	-	-	-	-	-	-	-	+	-	5
Type 2 Diabetes	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	5
Learning Disability	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	4
Constipation	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-	4
Dysphagia	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	3
Seizure disorder	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	2
Lipomas	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	2
Cataracts	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	2
Ptosis	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	2
Peripheral Neuropathy	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	2
Short stature	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	2
Bipolar Disorder	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
Personality Disorder	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	1
Dysarthria	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
Cardiomyopathy	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Arrhythmia	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1
Recurrent miscarriages	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Nystagmus	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	1
Ophthalmoplegia	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1
Ataxia	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
Mitochondrial Supplements																
Creatine	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	13
Alpha-lipoic acid	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	13
Riboflavin	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	13
Vitamin C	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	13
Vitamin E	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	13
Coenzyme Q10	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	13

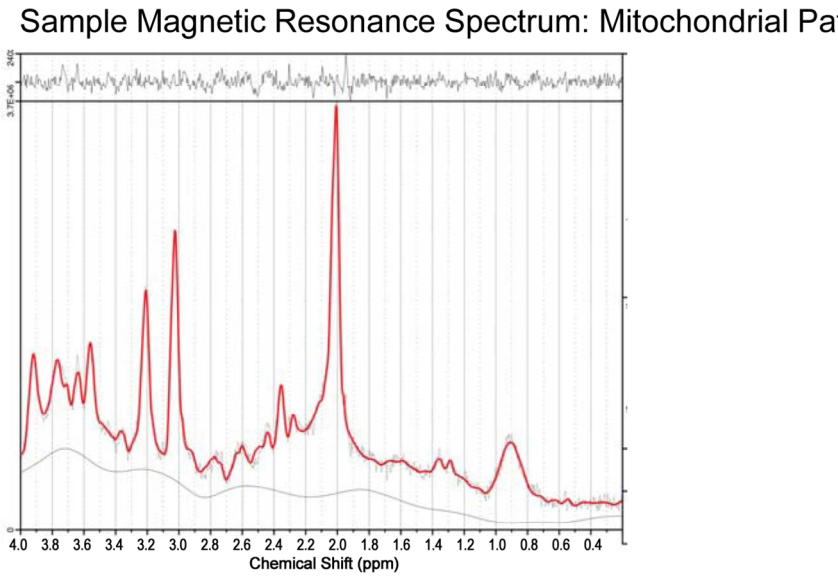
Table 2. Absolute metabolite concentrations for mitochondrial patients compared to healthy controls.

Metabolites	Mito Patients	Controls	T-test or ANCOVA
Caudate			
NAA	7.04 ± 1.19	8.19 ± 1.18	F = 5.86, p = 0.023**
Cr	6.84 ± 1.42	7.52 ± 0.76	F = 5.60, p = 0.026*
GPC	1.77 ± 0.47	1.88 ± 0.21	F = 4.18, p = 0.052
GPC+PCh	1.77 ± 0.47	1.89 ± 0.19	F = 4.79, p = 0.038*
mI	4.47 ± 1.83	4.13 ± 0.81	F = 0.002, p = 0.96
Glx	12.81 ± 2.38	14.87 ± 1.98	F = 5.97, p = 0.022*
Cingulate cortex			
NAA	8.20 ± 1.53	8.20 ± 1.04	T = 0.011, p = 0.99
Cr	7.81 ± 1.32	7.10 ± 0.99	T = 1.64, p = 0.11
GPC	2.56 ± 0.51	2.40 ± 0.40	T = 0.89, p = 0.38
GPC+PCh	2.56 ± 0.51	2.40 ± 0.37	T = 0.96, p = 0.35
mI	6.20 ± 1.55	5.65 ± 1.74	T = 0.89, p = 0.38
Glx	17.18 ± 3.17	16.47 ± 2.94	T = 0.62 p = 0.54
Hippocampus			
NAA	8.65 ± 1.85	8.88 ± 1.33	T = -0.37, p = 0.71
Cr	6.82 ± 1.27	6.94 ± 1.12	T = -0.28, p = 0.78
GPC	2.32 ± 0.49	2.48 ± 0.53	T = -0.86, p = 0.40
GPC+PCh	2.32 ± 0.49	2.48 ± 0.53	T = -0.86 p = 0.40
mI	5.60 ± 1.30	5.91 ± 1.54	T = 0.08, p = 0.94
Glx	12.49 ± 3.03	12.55 ± 3.14	T = -0.56 p = 0.96

* $p<0.05$
** Significant after Holm Bonferroni correction for multiple comparisons



Representative voxel placement in the axial, coronal and sagittal planes
173x147mm (300 x 300 DPI)



Representative MRS spectra from a mitochondrial patient and a healthy control
125x159mm (300 x 300 DPI)