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Research

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

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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 and creatine levels were measured in the caudate nucleus, anterior cingulate cortex and hippocampus in 15 patients with mitochondrial disorders compared with 15 healthy controls matched for age and sex.

Results: *N*-acetyl-aspartate levels were significantly lower in the caudate nucleus among patients with mitochondrial disorders (mean 7.04 ± 1.19 standard deviation [SD] institutional units) compared with healthy controls (mean 8.19 ± 1.18 SD institutional units; p = 0.02). Creatine levels were lower in the caudate nucleus among patients compared with controls (patients: mean 6.84 ± 1.42 SD institutional units; controls: mean 7.52 ± 0.76 SD institutional units; p = 0.03), but the results were no longer significant after correction for multiple comparisons. There were no significant differences in metabolite measurements between patients and controls in the anterior cingulate cortex and the hippocampus.

Interpretation: Metabolic abnormalities were identified exclusively in the caudate nucleus, with significantly lower *N*-acetyl-aspartate levels among patients compared with controls. These results suggest that the corpus striatum may be highly susceptible to mitochondrial oxidative phosphorylation defects and resultant cell loss. Given the role of the caudate nucleus in cognitive and executive functions, our findings raise the possibility that metabolic abnormalities in the caudate nucleus may contribute to cognitive impairment and neuropsychiatric symptoms in patients with mitochondrial disorders, which could be investigated in future studies.

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 through oxidative phosphorylation.[1] Mitochondria contain their own genome that is maternally inherited and encodes for 13 subunits of the respiratory chain, 2 ribosomal ribonucleic acids and 22 transfer ribonucleic acids. In addition, more than 1000 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] Although there is growing recognition that many other disorders are associated with mitochondrial dysfunction, the term "mitochondrial disorder" is currently used only in reference to those disorders associated with the mitochondrial respiratory chain. Even with this narrow definition, the prevalence of mitochondrial disorder is estimated to be about 1 in 5000.[3,4]

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Common features include fatigue, muscle weakness, migraines, seizures, stroke-like episodes, ataxia, ptosis, ophthalmoplegia, hearing loss and cardiomyopathy.[2] In addition, our group has shown that a range of psychiatric symptoms can be the most prominent and most often presenting features of mitochondrial disorders.[5,6] This suggests that regional metabolic abnormalities associated with mitochondrial dysfunction in the brain may give rise to psychiatric symptoms in these patients. To our knowledge, there has not been an investigation of metabolic abnormalities in regions of the brain implicated in neuropsychiatric illness in patients with mitochondrial disorders.

Proton magnetic resonance spectroscopy (MRS) is a particularly useful tool for studying patients with mitochondrial disorders, because 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 been reported only in individual cases or small series, and most studies were carried out in areas of the brain that appeared abnormal on magnetic resonance imaging (MRI) as part of the clinical investigation of the patient.[7–15] The most consistent findings have been higher lactate levels and lower *N*-acetyl-aspartate levels, although lower creatine and glutamate levels, and altered levels of choline have also been reported.[7–15]

In this study, we measured metabolites in vivo in patients with mitochondrial disorders compared with age- and sex-matched healthy controls using standardized voxel placement in 3 brain regions commonly implicated in neuropsychiatric disorders: the caudate nucleus, anterior cingulate cortex and hippocampus.[16–19] Our primary hypothesis was that patients with mitochondrial disorders would have reduced levels of markers of mitochondrial dysfunction (*N*-acetyl-aspartate and creatine) compared with healthy controls in the 3 candidate brain regions. A secondary hypothesis was that other metabolic indices, including glycerophosphocholine, glycerophosphocholine and phosphocholine, myo-inositol, and glutamine and glutamate would also be altered in patients with mitochondrial disorders in the candidate brain regions.

Methods

Study participants

Participants were recruited between Nov. 1, 2006 and Mar. 31, 2011. A total of 30 participants were included: 15 patients with a mitochondrial disorder and 15 healthy controls matched for age (± 5 yr) and sex without neurologic, psychiatric or systemic disease. Patients and controls with implanted metallic foreign bodies or inability to undergo MRI were excluded. The study was approved by the research ethics board of St. Joseph's Healthcare Hamilton and McMaster University Medical Centre. Each participant provided informed consent before inclusion. A psychiatric research nurse assessed capacity to consent, and all participants were found to be capable of consenting to participation in the study.

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 by Bernier and colleagues,[20] and only those with definite or probable mitochondrial disease were eligible for inclusion in 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. A history of cognitive impairment was determined by chart review and specific questioning about a history of cognitive impairment during the psychiatric interview.

Controls were healthy individuals recruited by advertisement in hospitals affiliated with McMaster University (McMaster University Medical Centre and St. Joseph's Healthcare Hamilton). They did not undergo genetic testing for a mitochondrial disorder but were required to be free of any medical or psychiatric conditions, which made it highly unlikely that they had an undetected mitochondrial disorder.

Magnetic resonance spectroscopy

Proton MRS was performed using the Signa HD 3.0T MR system by GE Healthcare (General Electric Company) and an 8-channel receive-only phased-array head coil. Following a localizer scan, axial and sagittal scans were performed for use in MRS localization. In addition, an axial T_2 fluid attenuation inversion recovery scan was acquired to aid in ruling out gross pathology. Gross pathology was not identified in any of the participants in the regions of interest.

Proton MRS was done on 3 single voxels placed in the caudate nucleus, hippocampus and anterior cingulate cortex (Figure 1). Voxels were placed using anatomic landmarks to allow for consistency between participants. The caudate nucleus voxel was placed using the sagittal T_1-weighted images and centred on the left caudate nucleus. The axial 3-dimensional scan was used to adjust the voxel to include maximal caudate nucleus volume. The anterior 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 centred on the head of the left hippocampus. Using the axial anatomic series, the voxel was adjusted to be lateral to the medial aspect of the lateral fissure. Voxel size was 20 × 20 × 20 mm^3.

Single-voxel proton MRS was conducted using a point-resolved spectroscopy sequence with a short echo time (echo time = 35 ms, repetition time = 2000 ms, 256 acquisitions). Second-order shimming was performed for each acquisition voxel. The entire MRS protocol took about 60 minutes. Segmentation for every participant and each MRS scan was accomplished using a combination of in-house software and the software Analysis of Functional NeuroImages.[21] Sample spectra from a patient with a mitochondrial disorder and healthy control are included in Figure 2. A Cramér-Rao lower bound of less than 20% was used as a cut-off for inclusion in the analysis. Appendix 1 available at www.cmajopen.ca/content/1/1/E48/suppl/DC1 contains a detailed description of the MRS imaging techniques used in this study.

Statistical analysis

Two-tailed significance was assessed at the $p < 0.05$ threshold. Differences between patients with mitochondrial disorders and healthy controls for each metabolite were tested separately. Our primary a priori hypothesis was that patients and controls would differ with respect to *N*-acetyl-aspartate and creatine levels based on previous reports of decreased *N*-acetyl-aspartate levels and the importance of creatine as an energy substrate. Therefore, these analyses were performed first, and the remaining metabolites were tested as secondary analyses. A Holm–Bonferroni correction for multiple comparisons was used for each region of interest for our primary analyses, and subsequently for our secondary analyses. Group differences in metabolite concentration, and full width at half maximum in each region of interest were assessed using independent-samples *t* tests. Full width at half maximum is the measure of the difference between the upper and lower values (the width) of the frequency on a spectral curve half way up its maximum intensity value. It is an important measure of the quality of an image, and a low value is optimal. When there were significant differences in full width at half maximum between groups in a region of interest, full width at half maximum was used as a covariate in an analysis of covariance for the group differences for each metabolite in that region of interest.

Results

Demographics

Patients with mitochondrial disorders and healthy controls were pair-matched by age (patients: mean 51.7 ± 8.7 standard deviation [SD] yr; $p = 0.6$; controls: mean 50.2 ± 8.6 SD yr) and sex (11 female and 4 male in each group). Clinical and demographic information for the 15 patients with mitochondrial disorders is presented in Table 1 and Appendix 2 available at www.cmajopen.ca/content/1/1/E48/suppl/DC1. Three patients had mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) T3271C mutations, 2 patients had MELAS A3243G mutations, 2 patients had myoclonic epilepsy with ragged red fibres (MERRF) A8363G mutations, 3 patients had single large-scale mitochondrial DNA deletions of about 4.7 kb, 3 patients had complex I deficiency (2 patients > 3 SDs below the age-matched mean, 1 patient 2 SDs below the age-matched mean), 1 patient had a C9035T mutation and 1 patient had mitochondrial cytopathy based on the Thorburn Criteria,[20] but the mutation has not been identified. The average age at diagnosis was 45 years. The most common clinical features included muscle weakness or atrophy, fatigue, depression, anxiety, hearing loss, stroke-like episodes, migraines or headaches, cognitive impairment and typets, including creatine, α-lipoic acid, riboflavin, vitamin C, vitamin E and coenzyme Q_{10}. None of the healthy controls were receiving mitochondrial supplements.

Metabolites

Concentrations of *N*-acetyl-aspartate, creatine, glycerophosphocholine, glycerophosphocholine and phosphocholine, myo-inositol, and glutamine and glutamate for each region of interest are presented in Table 2. Because the *t* tests showed

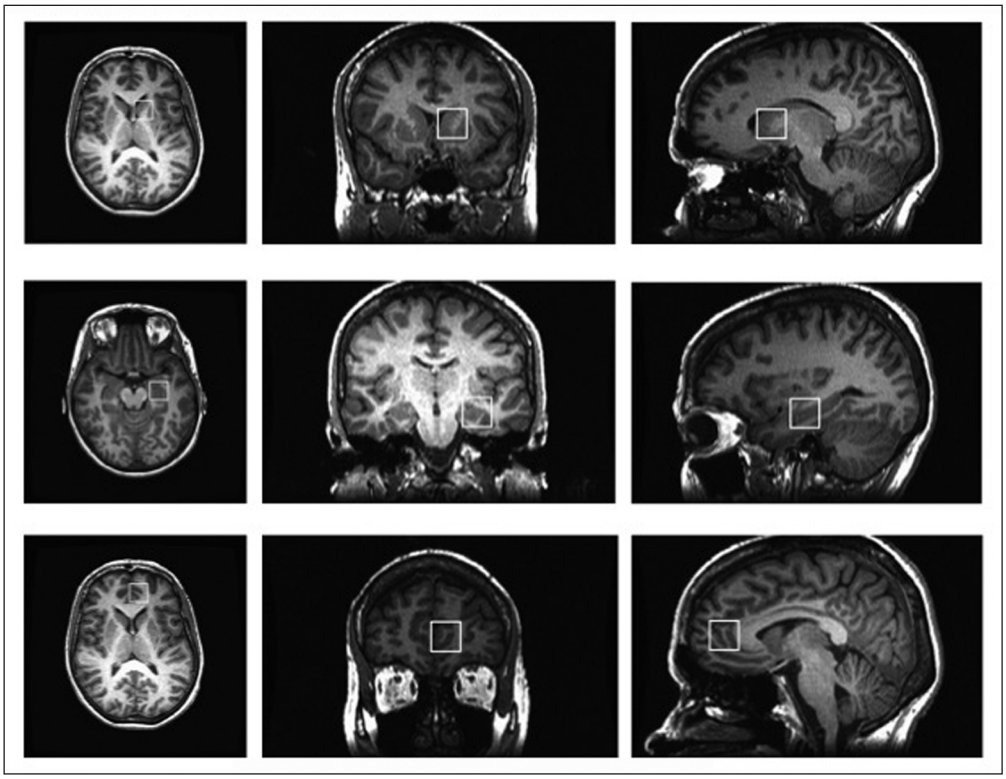

Figure 1: Placement of voxels in the axial, coronal and sagittal planes in the caudate nucleus (top), hippocampus (middle) and anterior cingulate cortex (bottom).

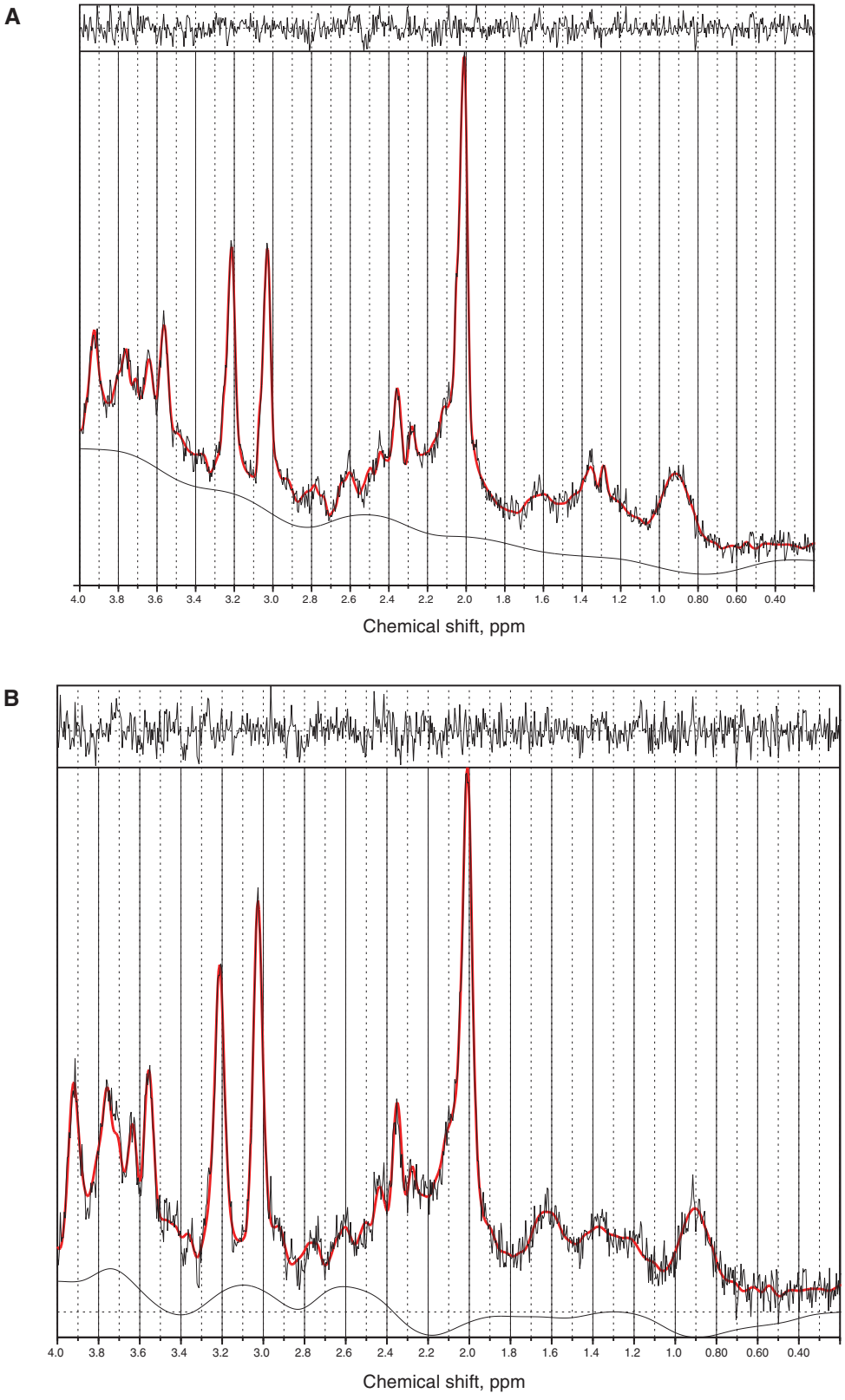

Figure 2: Representative magnetic resonance spectra from the anterior cingulate cortex of a patient with a mitochondrial disorder (A) and a healthy control (B).

significant differences in the full width at half maximum between the groups in the caudate nucleus (p = 0.005), full width at half maximum was used as a covariate in the analyses for this region. For our primary analysis, analyses of covariance examining *N*-acetyl-aspartate and creatine between the 2 groups in each region of interest showed significantly lower *N*-acetyl-aspartate (p = 0.02) and creatine (p = 0.03) levels in the caudate nucleus in patients compared with controls. However, after Holm–Bonferroni correction for multiple comparisons, only lower *N*-acetyl-aspartate levels in the caudate nucleus remained significant. For our secondary analyses, all metabolites were analyzed, and significantly lower levels of glycerophosphocholine and phosphocholine (p = 0.04), and glutamine and glutamate (p = 0.02) in the caudate nucleus were found in patients compared with 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 anterior cingulate cortex or hippocampus.

Table 1: Characteristics of 15 patients with mitochondrial disorders

Characteristic	No. of patients*
Age, yr, mean	52
Age at diagnosis, yr, mean	45
Clinical features	
Muscle weakness or atrophy	14
Fatigue	10
Depression	10
Anxiety	9
White matter lesions on MRI	9
Hearing loss	8
Stroke or 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
Use of mitochondrial supplements	
Creatine	13
α-Lipoic acid	13
Riboflavin	13
Vitamin C	13
Vitamin E	13
Coenzyme Q_{10}	13

Note: MRI = magnetic resonance imaging.
*Unless stated otherwise.

Given that patients with mitochondrial disorders had significantly lower *N*-acetyl-aspartate levels in the caudate nucleus, we hypothesized that this finding might contribute to the cognitive impairment observed in patients with mitochondrial disorders. We therefore performed a post hoc analysis comparing *N*-acetyl-aspartate levels in the caudate nucleus in patients with mitochondrial disorders with and without a history of cognitive impairment. Although patients with cognitive impairment had lower mean *N*-acetyl-aspartate levels (6.61 ± 1.34 SD institutional units in those with cognitive impairment v. 7.25 ± 1.12 SD institutional units in those without), the difference was not significant (p = 0.3).

Interpretation

In this study, we systematically measured a range of metabolites using proton MRS in patients with mitochondrial disorders compared with matched controls, using standardized voxel placement and partial volume correction. Our results indicate that the patients with mitochondrial disorders had significantly lower *N*-acetyl-aspartate levels in the caudate nucleus compared with healthy controls. We also found reduced creatine, glycerophosphocholine and phosphocholine, and glutamine and glutamate levels in the caudate nucleus in patients compared with controls, although these results were no longer significant after correction for multiple comparisons. Metabolic abnormalities were exclusively identified in the caudate nucleus, with no differences identified between patients and controls in the other regions of interest.

Comparison with other studies

Reduced *N*-acetyl-aspartate levels have previously been reported in cortical grey matter and the cerebellum in individual patients with mitochondrial disorders. However, we examined multiple metabolites in several regions of interest using standardized voxel placement and partial volume correction, and found reduced *N*-acetyl-aspartate levels in the caudate nucleus.[7–9,22] Despite its abundance in the brain and decades of research, the function of *N*-acetyl-aspartate remains unclear. Proposed functions have included providing a source of acetate for myelin lipid synthesis, and acting as an organic osmolyte.[23,24] *N*-acetyl-aspartate is synthesized from L-aspartic acid and acetyl coenzyme A in neuronal mitochondria, and it has been suggested that *N*-acetyl-aspartate may facilitate

mitochondrial energy metabolism.[24] *N*-acetyl-aspartate synthesis in the brain is decreased when oxidative phosphorylation is impaired by inhibitors of complexes III, IV and V of the respiratory chain.[25] This coupling of *N*-acetyl-aspartate synthesis with mitochondrial energy production has been further supported by the demonstration of lower *N*-acetyl-aspartate levels in experimental models of mitochondrial dysfunction, including striatal neurodegeneration induced by the mitochondrial toxin 3-nitropropionic acid, and other models of Huntington disease.[26–28] Our results lend additional support to the notion that *N*-acetyl-aspartate may be an important marker of mitochondrial dysfunction.

We identified metabolic abnormalities specifically in the caudate nucleus and not in other regions of interest, which adds to a growing body of literature that suggests the corpus striatum (composed of the caudate nucleus and putamen) is highly susceptible to impaired mitochondrial oxidative phosphorylation.[29–31] The particular vulnerability of the corpus 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.[29]

Although the basal ganglia and corpus striatum have traditionally been associated with motor coordination, it is now recognized that the caudate nucleus plays a central role in cognitive processes, particularly executive functions.[16,32] Specifically, the caudate nucleus is responsible for planning goal-directed behaviour and weighing different contingencies based on possible outcomes.[16] 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 nucleus 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 shown in patients with caudate nucleus pathology, including those with Parkinson disease, Huntington disease and schizophrenia.[16,33,34] Importantly, mitochondrial dysfunction and mitochondrial abnormalities have been observed in the corpus striatum in all of these conditions, and may underlie some of the observed abnormalities.[31,35,36] Cognitive impairment was a common clinical feature in the 15 patients exam-

Table 2: Absolute metabolite concentrations for patients with mitochondrial disorders compared with healthy controls

Metabolite, institutional units	Treatment group; mean ± SD		*p* value
	Patients with mitochondrial disorders	Controls	
Caudate nucleus			
NAA	7.04 ± 1.19	8.19 ± 1.18	0.02
Cr	6.84 ± 1.42	7.52 ± 0.76	0.03
GPC	1.77 ± 0.47	1.88 ± 0.21	0.052
GPC and PCh	1.77 ± 0.47	1.89 ± 0.19	0.04
MI	4.47 ± 1.83	4.13 ± 0.81	0.96
Glx	12.81 ± 2.38	14.87 ± 1.98	0.02
Anterior cingulate cortex			
NAA	8.20 ± 1.53	8.20 ± 1.04	0.99
Cr	7.81 ± 1.32	7.10 ± 0.99	0.1
GPC	2.56 ± 0.51	2.40 ± 0.40	0.4
GPC and PCh	2.56 ± 0.51	2.40 ± 0.37	0.3
MI	6.20 ± 1.55	5.65 ± 1.74	0.4
Glx	17.18 ± 3.17	16.47 ± 2.94	0.54
Hippocampus			
NAA	8.65 ± 1.85	8.88 ± 1.33	0.7
Cr	6.82 ± 1.27	6.94 ± 1.12	0.8
GPC	2.32 ± 0.49	2.48 ± 0.53	0.4
GPC and PCh	2.32 ± 0.49	2.48 ± 0.53	0.4
MI	5.60 ± 1.30	5.91 ± 1.54	0.9
Glx	12.49 ± 3.03	12.55 ± 3.14	0.96

Note: Cr = creatine, Glx = glutamine and glutamate, GPC = glycerophosphocholine, MI = myo-inositol, NAA = *N*-acetyl-aspartate, PCh = phosphocholine, SD = standard deviation.

ined in this study (Table 1). This is consistent with our group's previous reports and review of the literature, which identified marked cognitive dysfunction and neuropsychiatric symptoms in patients with mitochondrial disorders.[5,6] Our present findings suggest that metabolic abnormalities in the caudate nucleus may contribute to these problems in affected patients. However, we were not able to identify significant differences in *N*-acetyl-aspartate levels between patients with mitochondrial disorders and cognitive impairment and those without.

Limitations

Our study had several limitations we would like to acknowledge. First, patients with a variety of mitochondrial disorders were included, which resulted in heterogeneity. However, all patients had disorders that resulted in impaired oxidative phosphorylation, and despite the heterogeneity of the patients, we were able to detect significant differences compared with healthy controls. Second, it is possible that our study was underpowered to detect some differences in metabolites between patients and controls. Given our finding that the patients with mitochondrial disorders had significant reductions of creatine, glycerophosphocholine and phosphocholine, and glutamine and glutamate in the caudate nucleus before correction for multiple comparisons, future studies with larger samples should attempt to replicate our findings. In particular, our finding of decreased creatine levels in these patients, despite creatine supplementation, is intriguing and highly suggestive that creatine levels are lower in the caudate nucleus of patients with mitochondrial disorders. Third, given the limitation on scanning time that is imposed by the need to collect high-quality single-voxel MRS measurements, our study included only 3 regions of interest (caudate nucleus, anterior cingulate cortex and hippocampus). Naturally, it is possible that patients with mitochondrial disorders might have metabolic abnormalities in other regions. Fourth, we chose to use a relatively short echo time to measure a large number of metabolites. However, shorter echo times compromised our ability to reliably measure the lactate signal because it was confounded by broad lipid resonances. Because elevated lactate levels have 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. Finally, a clinical history of cognitive impairment was used instead of a standardized measurement of current symptomatology. Formal neuropsychological evaluations in patients with mitochondrial disorders and the correlation of those results with MRS findings in the caudate nucleus would be of interest and should be pursued in future studies.

Conclusion

Patients with mitochondrial disorders had reduced *N*-acetyl-aspartate levels in the caudate nucleus, a brain region known to play a key role in cognitive and executive processes. These findings indicate that reduced *N*-acetyl-aspartate levels may be a useful marker of mitochondrial dysfunction and the corpus striatum may be highly susceptible to mitochondrial oxidative phosphorylation defects. Metabolic abnormalities in the caudate nucleus may contribute to the cognitive impairment and neuropsychiatric symptoms exhibited by patients with mitochondrial disorders.

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