Article details: 2012-0020	
Title	Metabolic abnormalities in the caudate in patients with mitochondrial disorders measured using proton magnetic resonance spectroscopy
Authors	Rebecca E. Anglin MD PhD, Patricia I. Rosebush MD MSc N, Michael D. Noseworthy PhD P Eng, Mark Tarnopolsky MD PhD, Alexander M. Weber, Noam Soreni MD, Michael F. Mazurek MD
Reviewer 1	Nishant Mishra
Institution	Department of Medicine, University of Glasgow, Scotland, UK
General comments	The authors compared the levels of metabolic markers in fifteen patients suffering from mitochondrial dysfunction (definite and probable; per Thorburn criteria) with an age- and sex- matched cohort of fifteen patients without mitochondrial disorders. The main analysis involved comparison of the main markers, N Acetlyl aspartate and Creatine, between the groups; however, the authors also measured glycerophosphocholine, glycerophosphocholine and phosphocholine, myoinositol, glutamate and glutamine between the disease and normal control group. The regions of interest comprised caudate, cingulate cortex, and hippocampus. The disease group comprised of patients who suffered from MELAS, MERRF, C9035T mutation and mitochondrial cytopathy. Thirteen patients were getting mitochondrial supplements; none of the healthy controls were getting these supplements. Authors report a significantly lower levels of NAA and choline levels in the caudate nucleus of the patients compared to the controls. A few minor comments: 1: ABSTRACT: Please report some data (demography; p for comparisons, etc) in the RESULTS section. Authors contend that "NAA is a a useful marker for mitochondrial dysfunction". Do the authors have sensitivity/specificity/positive predictive value data to support this? If so, this should be reported. I am unsure that the results support the following conclusion can be drawn from the data:"Given caudate's role in cognitive....disorders (last line of the abstract)". 2: INTRODUCTION: Introduction can be shortened. For example, first paragraph is probably not required. In second paragraph, provide reference to the statement :"In addition, we have demonstrated...disorders ". 3: RESULTS: t and F values are usually not reported, unless the journal style specifically requires. 4: INTERPRETATION: authors should use appropriate terms/description for the patients whom they call I "mitochondrial patients", "caudate patients", etc. in the paper. 5: COGNITIVE IMPAIRMENTS: Authors compare NAA levels in pateints with cognifive impairment. Was systematic neuropsychological testing done on all patients? If so, what cognitive impairments did these patients show on your testing? What was the degree of deficit?
Reviewer 2	Erwin Lemche BSc BA MSc PhD
Institution	Institute of Psychiatry, London, UK
General comments	This paper reports the results of a case-control study in mitochondrial patients (N=15) comparing them to age-matched paired healthy controls (N=15), measuring N-acetyl-aspartate, creatine, glycerophosphocholine, phosphocholine, myoinositol, and glutamate in three regions of interest (ROIs) by means of MR spectroscopy. After correction for multiple parallel testing the only significant result surviving is the significant NAA difference in the caudate nucleus. <u>Pros</u> Very mature paper from a highly specialized unit in MR spectroscopy Paper reports important discovery for neurology with implications for treatment Paper will serve as starting point for future larger replication study No errors, mistakes, typos could be detected in the ms <u>Cons</u> The authors think of their paper of a report of negative results The authors feel their study was underpowered These self-attributions are probably not justified, as only future larger studies can provide more accurate results <u>Minor issues</u> Authors should provide a ref or rationale for choosing the three ROIs.
Reviewer 3	Daniel Mandell MD
Institution	Division of Neuroradiology, Department of Medical Imaging , University Health Network and the University of Toronto, Toronto, Ont.
General comments	

	This is a case-control study to determine whether there are differences in absolute concentration of proton MR spectroscopy metabolites in the caudate nucleus, cingulate gyrus, and hippocampus of patients with mitochondrial disorders compared with healthy controls. There were a total of 30 subjects. Metabolites measured were NAA, creatine, GPC, GPC+PCh, mI, and Glx. The study found that the absolute concentration of NAA in the caudate was lower in subjects with mitochondrial disorders than in controls. The study seems methodologically sound. Some revision of the manuscript is needed to clarify a few points, but the manuscript is generally very well written. Major Recommendations: This study uses absolute quantification of metabolite concentrations rather than relative (eg. NAA/creatine) ratios. How was calibration performed for the absolute quantification? Was endogenous water calibration used or an external reference? If an internal water reference was used, is it possible that the caudate nucleus water content differs between patients with mitochondrial disorders and healthy controls? If this is a potential confounding variable, it should be discussed. Minor Recommendations: 1. The abstract states that the "aim of the study was to use... spectroscopy... to identify metabolic abnormalities in regions implicated in neuropsychiatric symptoms in patients with mitochondrial disorders." The manuscript should provide/cite the evidence that the regions studied are the regions implicated in neuropsychiatric symptoms in patients with mitochondrial disorders. 2. The abstract states that the "results suggest that NAA is a useful marker of mitochondrial dysfunction". I suggest removing the word "useful" as the study does show that NAA is a marker of mitochondrial dysfunction, but does not specifically show that this is a useful marker. I think the latter would require some evidence that NAA measurement alters diagnosis, prognosis, clinical decision-making etc... 3. The abstract states that "metabolic abnormalities in the caudate may contribute to cognitive impairment and neuropsychiatric symptoms in patients with mitochondrial disorders". I suggest removing this from the abstract as the study did not demonstrate a significant effect regarding cognition. 4. pg 3 line 33: word "in" should be removed from "lower NAA in compared" 5. Statement "reduced markers of mitochondrial dysfunction (NAA and Cr)" should be revised for clarity. One option is "reduced concentration of markers of mitochondrial function (NAA and Cr)" 6. How were clinical features evaluated? Was this a retrospective chart review? Given that cognitive impairment is discussed in particular, I suggest including a bit more detail on cognitive evaluation, even if no standardized testing was performed. For example, were all subjects evaluated by the same clinician or several different clinicians? 7. I do not think it is crucial, but it would be a nice addition to briefly describe the clinical MR imaging findings for the subjects. 8. I believe the references need some changes to meet the format of Open CMAJ 9. The part of the figure that is labeled "caudate" show regions-of-interest in the hippocampus on the coronal and axial images, not on the caudate. Thanks for the opportunity to review this interesting manuscript.
Reviewer 4	Vann Chau MD
Institution	Department of Pediatrics, University of British Columbia, Vancouver, BC
General comments	Using single-voxel proton magnetic resonance spectroscopy, Dr. Anglin and colleagues have compared the values of different metabolites in 3 different regions of the brain, that have commonly been reported to be involved in neuropsychiatric symptoms, between 15 patients with mitochondrial disorders and 15 age and sex-matched controls. They found that patient with mitochondrial disorders had significantly lower NAA in the caudate compared to controls. Cr, GPC, PCh and Glx were also lower, but these abnormalities were no longer significant after correction for multiple comparisons. As pertinently mentioned by the authors, given the scarcity of systematic studies on brain metabolism in mitochondrial disorders, this well-written manuscript addresses an important question. The Methods and Results are presented clearly. The Discussion is also well-written and comments nicely each finding with supporting literature. I have only 2 minor points to raise: 1) Due to the variability of metabolite values, most studies compare metabolite ratios (e.g. NAA/choline or NAA/creatine) instead of absolute values. The difference of NAA values between the patients with mitochondrial disorders and controls is interesting, but may not be real unless reported in ratios. The disease process may have decreased all metabolites at different extent. 2) There is a mistake in Figure 1. The authors showed the hippocampus twice (instead of the caudate) in the coronal and axial views. I declare I have no conflict of interest with reviewing this manuscript
Author response	Thank you so much for your thoughtful and detailed review of our manuscript "Metabolic Abnormalities in the Caudate in Patients with Mitochondrial Disorders

Measured Using Proton Magnetic Resonance Spectroscopy." We appreciate your comments, as well as those of the reviewers, and have responded to them below and with changes to the manuscript (identified using track changes).
Reviewer(s)' Comments to Author:

Reviewer: 1
Comments to the Author

The authors compared the levels of metabolic markers in fifteen patients suffering from mitochondrial dysfunction (definite and probable; per Thorburn criteria) with an age- and sex- matched cohort of fifteen patients without mitochondrial disorders. The main analysis involved comparison of the main markers, N Acetlyl aspartate and Creatine, between the groups; however, the authors also measured glycerophosphocholine, glycerophosphocholine and phosphocholine, myoinositol, glutamate and glutamine between the disease and normal control group. The regions of interest comprised caudate, cingulate cortex, and hippocampus. The disease group comprised of patients who suffered from MELAS, MERRF, C9035T mutation and mitochondrial cytopathy. Thirteen patients were getting mitochondrial supplements; none of the healthy controls were getting these supplements. Authors report a significantly lower levels of NAA and choline levels in the caudate nucleus of the patients compared to the controls.

I have a few minor comments:

1: ABSTRACT: Please report some data (demography; p for comparisons, etc) in the RESULTS section. Authors contend that "NAA is a a useful marker for mitochondrial dysfunction". Do the authors have sensitivity/specificity/positive predictive value data to support this? If so, this should be reported. I am unsure that the results support the following conclusion can be drawn from the data:"Given caudate's role in cognitive....disorders (last line of the abstract)".
Thank you for these suggestions. We have added data to the results section and removed the statement that "NAA is a useful marker of mitochondrial dysfunction."

2: INTRODUCTION: Introduction can be shortened. For example, first paragraph is probably not required. In second paragraph, provide reference to the statement :"In addition, we have demonstrated...disorders ".
We have not removed the first paragraph as we feel it is a useful primer for readers. However it can be removed or shortened if requested by the editor. We have added a reference for the statement in the second paragraph as suggested.

3: RESULTS: t and F values are usually not reported, unless the journal style specifically requires.
These have been removed.

4: INTERPRETATION: authors should use appropriate terms/description for the patients whom they call I "mitochondrial patients", "caudate patients", etc in the paper.
We have used the term mitochondrial patients throughout the paper to refer to all patients with mitochondrial disorders in the study (as opposed to controls). The term "caudate patients" was used in error – this has been corrected to read: "in the caudate in patients compared to controls."

5: COGNITIVE IMPAIRMENTS: Authors compare NAA levels in pateints with cognifive impairment. Was systematic neuropsychological testing done on all patients? If so, what cognitive impairments did these patients show on your testing? What was the degree of deficit?
A history of cognitive impairment was ascertained through chart review and a detailed psychiatric interview that included a review of systems and specific questioning about a prior diagnosis of cognitive impairment. Although the patients with a diagnosis of cognitive impairment had undergone formal neuropsychological testing, this was not performed on all patients in the study. This has been clarified (last paragraph, page 9) and acknowledged as a limitation of the study in the Limitations section.

Reviewer: 2
Comments to the Author

This paper reports the results of a case-control study in mitochondrial patients (N=15) comparing them to age-matched paired healthy controls (N=15), measuring N-acetyl-aspartate, creatine, glycerophosphocholine, phosphocholine, myoinositol, and glutamate in three regions of interest (ROIs) by means of MR spectroscopy. After correction for multiple parallel testing, the only significant result surviving is the significant NAA difference in the caudate nucleus.

Pros
Very mature paper from a highly specialized unit in MR spectroscopy
Paper reports important discovery for neurology with implications for treatment
Paper will serve as starting point for future larger replication study
No errors, mistakes, typos could be detected in the ms

We appreciate these kind comments.
Cons

1. The authors think of their paper of a report of negative results. The authors feel their study was underpowered. These self-attributions are probably not justified, as only future larger studies can provide more accurate results
Thank you for this statement. We agree that only future larger studies can provide more accurate results, but have still included our relatively small sample size in the limitation section of the paper.

Minor issues
2. Authors should provide a ref or rationale for choosing the three ROIs
Thank you for this suggestion. References to justify the use of the three ROIs have been added.

Reviewer: 3
Comments to the Author
This is a case-control study to determine whether there are differences in absolute concentration of proton MR spectroscopy metabolites in the caudate nucleus, cingulate gyrus, and hippocampus of patients with mitochondrial disorders compared with healthy controls. There were a total of 30 subjects. Metabolites measured were NAA, creatine, GPC, GPC+PCh, mI, and Glx. The study found that the absolute concentration of NAA in the caudate was lower in subjects with mitochondrial disorders than in controls. The study seems methodologically sound. Some revision of the manuscript is needed to clarify a few points, but the manuscript is generally very well written.

Major Recommendations:

1. This study uses absolute quantification of metabolite concentrations rather than relative (eg. NAA/creatine) ratios. How was calibration performed for the absolute quantification? Was endogenous water calibration used or an external reference? If an internal water reference was used, is it possible that the caudate nucleus water content differs between patients with mitochondrial disorders and healthy controls? If this is a potential confounding variable, it should be discussed.
Please refer to our response above (number 12). Endogenous water calibration was used, as reported in the Methods section under Absolute Metabolite Levels: "This method uses the unsuppressed 'internal' water signal, along with tissue fractions, and water-tissue and metabolite relaxation times." **The reviewer raises the possibility that the caudate nucleus water content differs between patients with mitochondrial disorders and healthy controls. We therefore did an analysis of CSF, WM and GM segmentation volumes within the MRS voxel of the caudate nucleus in each participant and there was no difference between the two groups.**

Minor Recommendations:

1. The abstract states that the "aim of the study was to use... spectroscopy... to identify metabolic abnormalities in regions implicated in neuropsychiatric symptoms in patients with mitochondrial disorders." The manuscript should provide/cite the evidence that the regions studied are the regions implicated in neuropsychiatric symptoms in patients with mitochondrial disorders.
Thank you for this suggestion. These regions were selected as they are implicated in neuropsychiatric symptoms/disorders in general, and appropriate

references have now been added. There are no prior studies looking at regions implicated in neuropsychiatric symptoms in patients with mitochondrial disorders.

2. The abstract states that the "results suggest that NAA is a useful marker of mitochondrial dysfunction". I suggest removing the word "useful" as the study does show that NAA is a marker of mitochondrial dysfunction, but does not specifically show that this is a useful marker. I think the latter would require some evidence that NAA measurement alters diagnosis, prognosis, clinical decision-making etc...
This statement has been removed as suggested.

3. The abstract states that "metabolic abnormalities in the caudate may contribute to cognitive impairment and neuropsychiatric symptoms in patients with mitochondrial disorders". I suggest removing this from the abstract as the study did not demonstrate a significant effect regarding cognition.

We appreciate this suggestion. However, we feel that our finding of metabolic alterations exclusively in the caudate in these patients does suggest that caudate dysfunction may be playing a central role in their neuropsychiatric symptomatology. This is a novel finding of our study, and our study was not designed to assess cognition (as described our post hoc analysis was only using the available data on a history of cognitive impairment, not formal neuropsychological testing). We have amended the statement in the abstract as follows, but can also remove it if required:
"Given the caudate's role in cognitive and executive functions, our findings raise the intriguing possibility that metabolic abnormalities in the caudate may contribute to cognitive impairment and neuropsychiatric symptoms in patients with mitochondrial disorders, which can be investigated in future studies."

4. pg 3 line 33: word "in" should be removed from "lower NAA in compared"
Thank you for this observation. It has been corrected.

5. Statement "reduced markers of mitochondrial dysfunction (NAA and Cr)" should be revised for clarity. One option is "reduced concentration of markers of mitochondrial function (NAA and Cr)"
Thank you for this suggestion. The statement has been changed to "reduced levels of markers of mitochondrial function."

6. How were clinical features evaluated? Was this a retrospective chart review? Given that cognitive impairment is discussed in particular, I suggest including a bit more detail on cognitive evaluation, even if no standardized testing was performed. For example, were all subjects evaluated by the same clinician or several different clinicians?
Clinical features were evaluated by an experienced psychiatric nurse in a comprehensive medical and psychiatric evaluation as well as with chart review. Further details on the cognitive evaluation have been added (see response to the editor above, number 12).

7. I do not think it is crucial, but it would be a nice addition to briefly describe the clinical MR imaging findings for the subjects.
We appreciate this suggestion. We feel this may be beyond the scope of this paper, but can add a brief description if requested by the editor.

8. I believe the references need some changes to meet the format of Open CMAJ
Thank you for this observation. The reference format has been corrected.

9. The part of the figure that is labeled "caudate" show regions-of-interest in the hippocampus on the coronal and axial images, not on the caudate.
We apologize for this error. This has been corrected.

Thanks for the opportunity to review this interesting manuscript,

Reviewer: 4
Comments to the Author
Using single-voxel proton magnetic resonance spectroscopy, Dr. Anglin and colleagues have compared the values of different metabolites in 3 different regions of the brain,

	that have commonly been reported to be involved in neuropsychiatric symptoms, between 15 patients with mitochondrial disorders and 15 age and sex-matched controls. They found that patient with mitochondrial disorders had significantly lower NAA in the caudate compared to controls. Cr, GPC, PCh and Glx were also lower, but these abnormalities were no longer significant after correction for multiple comparisons. As pertinently mentioned by the authors, given the scarcity of systematic studies on brain metabolism in mitochondrial disorders, this well-written manuscript addresses an important question. The Methods and Results are presented clearly. The Discussion is also well-written and comments nicely each finding with supporting literature. I have only 2 minor points to raise: 1) Due to the variability of metabolite values, most studies compare metabolite ratios (e.g. NAA/choline or NAA/creatine) instead of absolute values. The difference of NAA values between the patients with mitochondrial disorders and controls is interesting, but may not be real unless reported in ratios. The disease process may have decreased all metabolites at different extent. Please refer to our response to the editor above, number 13. **We feel that the use of absolute values is more accurate and reliable than using ratios, particularly given creatine and choline are expected to be altered in patients with mitochondrial disorders and therefore would be problematic to use as constant, reference metabolites.** 2) There is a mistake in Figure 1. The authors showed the hippocampus twice (instead of the caudate) in the coronal and axial views. **We apologize for this error. This has been corrected.** I declare I have no conflict of interest with reviewing this manuscript.