Acta Neurol. Belg., 2009, **109**, 305-309

Brain white matter 1 H MRS in Leber optic neuropathy mutation carriers

Jelena Ostojic[1], Jasna Jancic[2], Dusko Kozic[1], Robert Semnic[1], Katarina Koprivsek[1], Mladen Prvulovic[1] and Vladimir Kostic[3]

[1]Oncology Institute of Vojvodina, Diagnostic Imaging Center, Sremska Kamenica, Sremska Kamenica, Serbia;
[2]Clinic of Neurology and Psychiatry for Children and Youth, School of Medicine, University of Belgrade, Belgrade, Serbia;
[3]Institute of Neurology, Department of Movement Disorders, Clinical Center of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia

Abstract

Objective: This study was conducted in order to test the hypothesis that proton MR spectroscopic (1H MRS) profile of Leber's hereditary optic neuropathy (LHON) mutation carriers group (including both symptomatic and asymptomatic) differs from group of healthy individuals and to determine metabolite or ratio that contributes most to differentiation.

Patients and methods: We performed single voxel 1H MRS in normal appearing white matter of eighteen LHON mtDNA mutation carriers bearing one of three LHON mtDNA point mutations and in fifty control subjects.

Results: ANOVA showed significant difference for absolute concentration of creatine (Cr) ($p < 0.01$) and N-acetylaspartate to creatine ratio (NAA/Cr) ($p < 0.01$). Discriminant analysis revealed that decreased absolute Cr followed by decreased absolute NAA concentration have the most significant contribution in discriminating LHON mutation carriers from healthy controls.

Conclusion: Abnormal metabolic profile in normal appearing white matter on MR imaging seems to be significantly present in LHON mutation carriers.

Key words: MR spectroscopy; mitochondrial diseases; LHON; creatine; N-acetylaspartate

Introduction

Disorders of oxidative phosphorylation are related to diverse genetic and biochemical defects and heterogeneous clinical symptoms ranging from isolated organ dysfunction to multi-system disorder (Smeitnik *et al.*, 2001). Leber's hereditary optic neuropathy (LHON) is a maternally inherited mitochondrial disease, characterized by bilateral acute or subacute loss of the central vision. The pathogenesis of LHON is understood to be related to the mtDNA point mutations 1178, 3460, and 14484. These mutations may either decrease the net ATP synthesis for a given cell, or induce a chronic overproduction of reactive oxygen species in mitochondria. Depletion of ATP leads to increased dephosphorylation of PCr in patients with mitochondrial diseases. Defective brain and muscle energy metabolism in LHON have been already investigated using phosphorous (31P) MRS (Barbiroli *et al.*, 1999; Barbiroli *et al.*, 1995). To our knowledge, reports on use of 1H MRS in LHON are confined to few publications. Some authors have shown normal 1H MRS spectra in patients with LHON (Salvan *et al.*, 1998) while Jansen *et al.* has shown 1H MRS findings compatible with a chronic multiple sclerosis lesion (Jansen *et al.*, 1996).

In prior 1H MRS studies potential abnormalities in metabolic profile were expressed as ratios of Cho and NAA to Cr peak. Our study focused on absolute quantitative data of these metabolites in LHON mutation carriers. Determination of metabolite ratios only, when absolute concentration of all metabolites is abnormal, could be misleading.

Methods and materials

The examined group consisted of 18 subjects, 6 affected with LHON and 12 twelve unaffected carriers (six bearing the homoplasmic 11778, nine homoplasmic 3460 mutations and 3 heteroplasmic14484 mutation).The ethics committee of our institution approved the study, and all the examinees signed an informed consent form.

Combined MRI and MRS studies were performed on a 1.5T MR imaging unit, using the standard quadrature transmit/receive birdcage head coil. Image guided positioning of the volume of interest (VOI) for 1H MRS was based on coronal and sagittal T1W gradient echo sequence TR/TE/FA = 266/6/80 and axial T2W fast spin echo sequence TR/TE =

5730/98, 5 mm slice thickness, 230 mm FOV and 192 × 256 matrix.

For each patient, the spectroscopic volume of interest (VOI) measuring 15 × 15 × 15 mm (3, 4 cm^3), was positioned in the centrum semiovale of the supratentorial white matter. The VOIs were placed in areas that appeared normal on MR images, avoiding CSF spaces within the VOI. Localization of the signal was performed using the single voxel Point Resolved Spectroscopy PRES technique with a repetition time of 1500 ms, an echo time of 135 ms and 256 acquisitions. The local magnetic field within the VOI was optimized by manual shimming. The shim currents were changed to achieve the full width of peak at half maximum (FWHM) as small as possible. Usually, FWHM of 5-10 Hz was achieved. Spectroscopic data were accumulated after the optimal water signal had been suppressed by the chemical shift-selective technique. Spectra were sampled to 1024 time points.

Data were zero filled to 2048 data points, Lorentz-Gaussian (half width 256 ms) and Fourier transformed, and then phase and baseline corrected. The spectral peaks from NAA, Cr and Cho were integrated and expressed as ratios to the total creatine resonance at approximately 3.0 ppm, and as absolute values ([NAA], [Cr], [Cho]).

Absolute quantification of the signal intensities of NAA, Cho and Cr was obtained using the external referencing. The signal from acetic acid was measured in separate acquisitions from spectroscopy phantom. Metabolite peak areas were referenced to the resonance area of the acetic acid, with known concentration of 100 mM. Corrections to coil loading using the reciprocity principle were taken into account, as well as corrections to longitudinal and transversal relaxation, temperature and numbers of nuclei contributing to the signal of the respective molecule (Tofts and Waldman, 2003; Kreis, 1997). Published values of longitudinal and transversal relaxation times in normal brain were used (Kreis *et al.*, 1993). Concentration values were expressed as mmol/L (mM) and were not corrected for contributions by CSF.

The control group consisted of 50 healthy age matched volunteers, investigated by the same methodology. Metabolite control values were collected from voxels positioned in the Centrum Semiovale of the supratentorial white matter.

We compared the mutation carriers group, consisted of affected and non affected carriers, to control group. Due to small sample size, it was not possible to separate affected from non affected carriers.

Statistical Analysis included: Computing Descriptive Statistics (Mean, Standard Deviation (SD), Minimum and Maximum, Coefficient of Variance (CV) and Confidence Interval), measure of the asymmetry skewness (sk), measure of the peakedness of the probability distribution Kurtosis (ku), Kolmogorov-Smirnov test, Multivariate Analysis of Variance (MANOVA), T-test and Linear Discriminant Analysis (DA).

Results

MR imaging revealed normal appearing white matter in 16 examinees, while in two of them with 11778 homoplasmic DNA mutation, non-specific white matter lesions were detected.

The sampling distribution is approximately normal for all metabolites and ratios in LHON and control group. The overall difference between group of LHON mutation carriers and control group was significant (MANOVA, $p < 0.001$). T-test showed significant difference for [Cr] ($p<0.01$) and NAA/Cr ($p<0.05$). According to discriminant coefficient the [Cr] contributes most to discrimination between groups, followed by [NAA] (Fig. 1 and 2, Table 1).

The values that contribute most to discrimination between LHON (Ellipse 1) and control group (Ellipse 2) are plotted in two-dimensional representation space (Fig. 3).

Discussion

These data suggest that decreased absolute concentration of creatine acts as a major discriminant value in differentiation between healthy individuals and LHON mutation carriers. Cr and its phosphorylated form (PCr) are essential for energy storage and transfer. Mitochondria are membranous organelles that are responsible for providing and storing most of the energy required by the cell in the form of high-energy bond of ATP.

The CNS is frequently involved in mitochondrial diseases because of its strong dependence on oxidative metabolism. A decreased brain energy reserve, shown by reduced PCr content is reported in patients with mitochondrial disorders (Barbiroli *et al.*, 1995; Lodi R *et al.*, 2002; Valentino *et al.*, 2006). Eleff *et al.* reported a decrease in PCr/ATP in the frontal lobe both in patients with MELAS, MERRF and Leigh's syndrome, KSS and CPEO (Eleff *et al.*, 1990). Barbiroli *et al.* performed 31P MRS on 19 patients with mitochondrial cytopathies (10 had familial LHON). The brains of all patients showed a 31P MRS pattern with low PCr and high Pi, indicating defective mitochondrial respiration (Barbiroli *et al.*, 1999).

Cr signal has been assumed to be constant in different cell types and to be invariant over differing

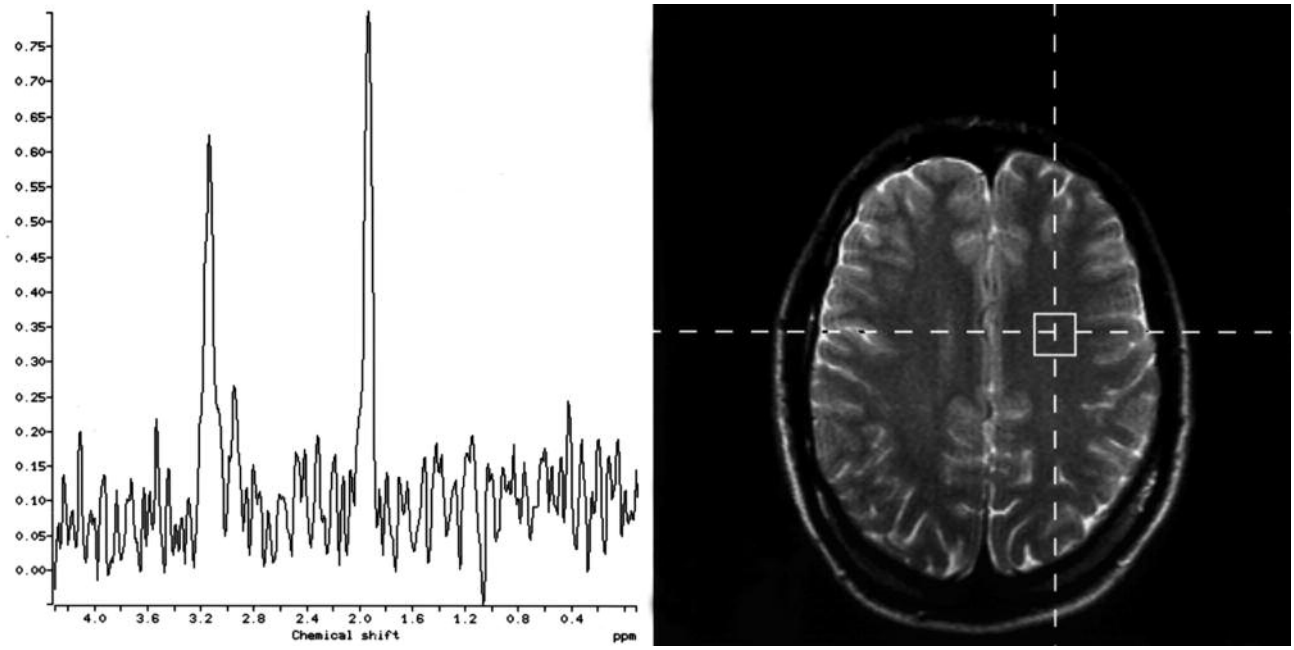

FIG. 1. — Low absolute creatine concentration in a patient with 3460 mutation

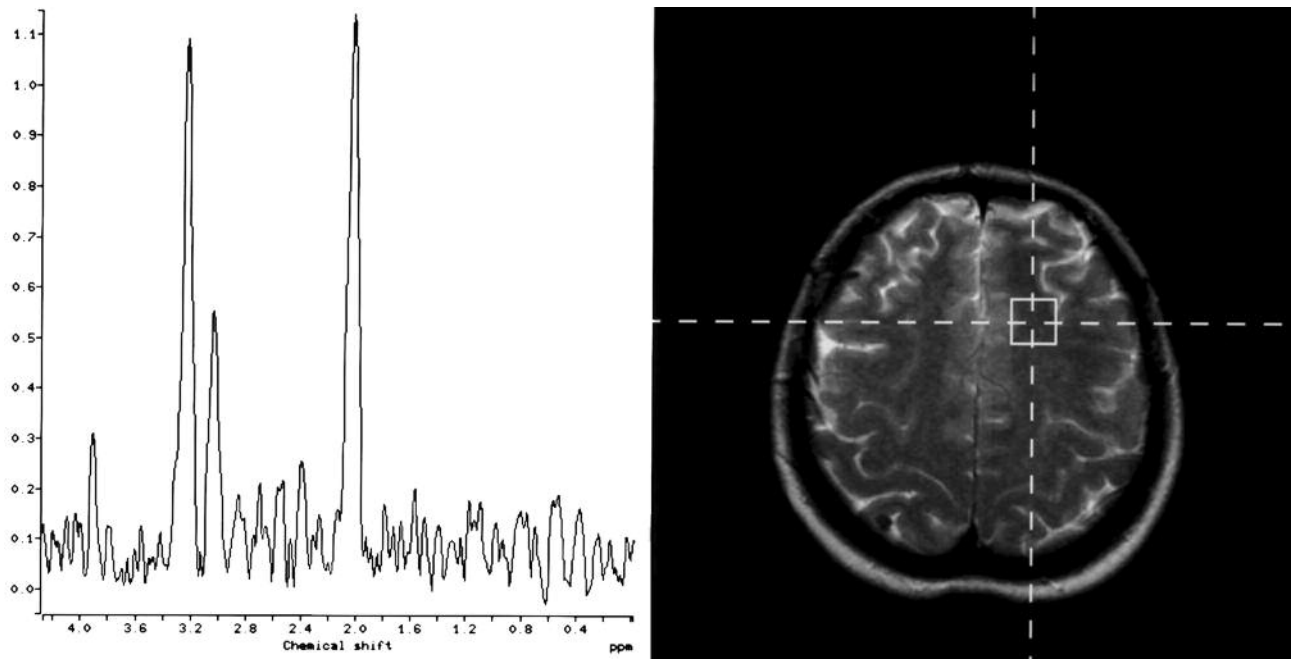

FIG. 2. — Low absolute creatine concentration in an asymptomatic carrier with 11778 mutation

conditions of oxygenation and perfusion. It is therefore often used as an internal calibration standard.

In most contexts NAA is considered a surrogate marker of neural tissue integrity. Decreased NAA is associated with neuronal loss in a wide range of diseases. The study on isolated rat brain mitochondria has shown that when mitochondrial ATP synthesis and or/ oxygen consumption are inhibited, NAA production will also be inhibited (Bates *et al.*, 1996). Other studies found NAA to be sensitive to oxygen consumption and neuronal ATP synthesis, suggesting that NAA synthesis is closely related to mitochondrial energy metabolism (Patel and Clark, 1979; Clark, 1998). Although decreased NAA in our group

Table 1

The table shows results of Discriminant analysis (DA) with confidence intervals for LHON mutation carriers group and control group

	LHON mutation carriers	Control	Discriminant coefficient
[Cr]	4.12-5.13	5.04-5.53	31.780
[NAA]	7.36-8.97	8.23-8.92	30.676
NAA/Cr	2.12-2.59	2.01-2.42	24.299
Cho/Cr	1.11-1.77	1.14-1.23	8.370
[Cho]	1.45-2.04	1.64-1.82	4.875

[Cr], [Cho], [NAA]-Absolute metabolite concentrations in mmol/L (mM).

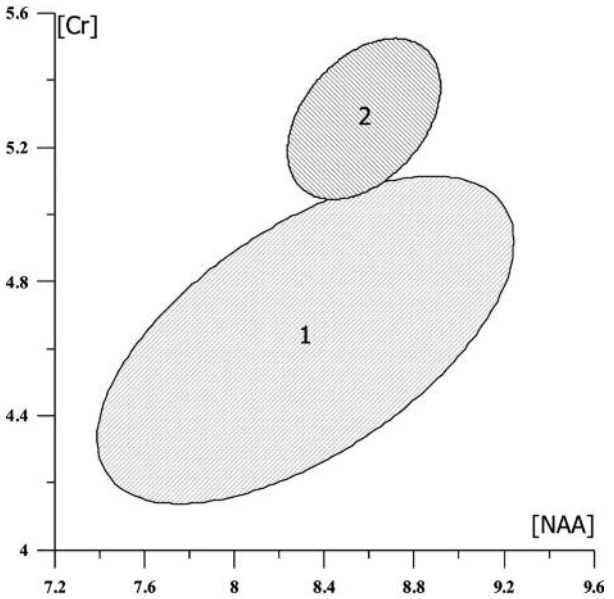

FIG. 3. — Confidence ellipses for LHON (1) and control group (2), [Cr] is markedly lower in LHON comparing to control group.

of LHON mutation carriers did not reach the statistical significance (t-test), together with creatine it contributes significantly to differentiation between LHON mutation carriers and group of healthy (DA). In the 31P and 1H MRS study of cerebral energetic effects of creatine supplementation in humans, Cr-induced change affects high energy phosphates and NAA (Pan, 2007). The authors suggested that ADP regulates NAA, i.e. cellular energetic state regulates mitochondrial function.

In the study of 15 patients with mitochondrial diseases, 93% showed NAA/Cr ratio reductions in cerebellum and 87% in normal appearing gray matter. No decrease in the NAA/Cr signal was found in normal appearing white matter (Bianchi *et al.*, 2003). In our results NAA/Cr shows higher values in LHON compared to control group, due to greatly decreased white matter absolute Cr concentration. Thus, an absolute quantification is important for an adequate investigation of metabolic changes.

The elevation of absolute Cho concentration was noted in our LHON group, but it did not have statistical significance. The association of the 11778 mtDNA mutation and demyelinating disease described in literature (Flanigan and Johns, 1993; Lerman-Sagie *et al.*, 2005) could explain increased white matter Cho in some 11778 mtDNA mutation carriers. MRS is very sensitive to detect phospholipid alterations in patients with demyelinating diseases.

Abnormal presence of lactate peak is frequently detected by proton MR spectroscopy in patients with proven mitochondrial disease (Barkovich *et al.*, 1993; Wilichowski *et al.*, 1999; Moroni *et al.*, 2002). In our study, lactate accumulation in the brain was detected neither in the symptomatic nor in the asymptomatic mutation carriers, suggesting that the presence of this metabolite may depend on the type of mitochondrial disorder and regional and temporal variations, which is in accordance with the studies of Heidenreich and Lin (Heidenreich *et al.*, 2006; Lin *et al.*, 2003).

We are well aware that our study has limitations: the number of subjects is limited and the evaluation of metabolic changes was based on single-voxel MR spectroscopy. Also, in order to make valid statistical analysis, merging the symptomatic and asymptomatic mutation carriers was the only solution.

However, our study undoubtedly supports the hypothesis that MR spectroscopy could be useful in the detection of abnormal brain metabolism, especially in patients with normal imaging findings. As a particular feature of our study, we emphasize the importance of quantification of MR spectroscopy data to distinguish LHON mutation carriers and healthy volunteers. Further multicentric MR spectroscopy studies, using multi-voxel approach, are warranted for better understanding of this rare mitochondrial disease.

Acknowledgments

This scientific research was supported by: Ministry of Science and Technological Development, Belgrade, Serbia and Provincial Secretariat for Science and Technological Development, Novi Sad, Serbia.

We thank Dr. Valerio Carelli, MD Dipartimento di Scienze Neurologiche, Università di Bologna, Bologna, Italy for Mitochondrial DNA analysis and Mr Milan Dolga, Smart-Line agency for statistical analysis.

REFERENCES

Smeitnik J, van den Heuvel L, DiMauro S. The genetics and pathology of oxidative phosphorylation. Nat Rev Genet. 2001;2:342-352.

Barbiroli B, Iotti S, Cortelli P. *et al.* Low Brain Intracellular free magnesium in mitochondrial cytopathies. JCBFM J Cereb Blood Flow Metab. 1999;19:528-532.

Barbiroli B, Montagna P, Cortelli P. *et al.* Defective brain and muscle energy metabolism shown by in vivo 31P magnetic resonance spectroscopy in non-affected carriers of 11778 mtDNA mutation. Neurology. 1995;45(7):1364-1369.

Salvan AM, Vion-Dury J, Confort-Gouny S. *et al.* Brain metabolic profiles obtained by proton MRS in two forms of mitochondriopathies:Leber's Hereditary Optic Neuropathy and Chronic Progressive External Ophthalmoplegia. Eur Neurol. 1998;40:46-49.

Jansen PHP, van der Knaap MS, de Coo IFM. Leber's hereditary optic neuropathy with the 11778 mtDNA mutation and white matter disease resembling multiple sclerosis:clinical, MRI and MRS findings. J Neurol Sci. 1996;135:176-80.

Tofts PS, Waldman AD. Spectroscopy: 1H Metabolite Concentrations. In: Tofts P, ed. Quantitative MRI of the Brain:Measuring Changes Caused by Disease. West Sussex, England:John Wiley&Sons Ltd. 2003;299-339.

Kreis R. Quantitative localized 1H MR spectroscopy for clinical use. Prog Nucl Magn Reson Spectrosc. 1997;31:299-339.

Kreis R, Ernst T, Ross BD. Absolute Quantitation of Water and Metabolites in the Human Brain II. Metabolite Concentrations, Journal of Magnetic Resonance, Series B. 1993;102:9-19.

Lodi R, Carelli V, Cortelli P. *et al.* Phosphorus MR spectroscopy shows a tissue specific in vivo distribution of biochemical expression of the G3460A mutation in Leber' s hereditary optic neuropathy. J Neurol Neurosurg Psychiatry. 2002;72:805-807.

Valentino ML, Barboni P, Rengo C. *et al.* J Med Genet. 2006;43:e38.

Eleff SM, Barker PB, Phil D. *et al.* Phosphorus magnetic resonance spectroscopy of patients with mitochondrial cytopathies demonstrates decreased levels of brain phosphocreatine. Ann Neurol. 1990;27:626-630.

Bates TE, Strangward M, Keelan J. *et al.* Inhibition of N-acetylaspartate production:implications for 1H MRS studies *in vivo*. NeuroReport. 1996;7:1397-1400.

Patel TB, Clark JB. Synthesis of N-acetyl-L-aspartate by rat brain mitochondria and its involvement in mitochondrial/cytosolic carbon transport. Biochem J. 1979;184:539-546.

Clark JB. N-Acetyl aspartate: A marker for Neuronal loss or mitochondrial dysfunction. Dev Neurosci. 1998; 20:271-276.

Pan JW. Cerebral energetic effects of creatine supplementation in humans. Am J Physiol Regul Integr Comp Physiol. 2007;292:1745-1750.

Bianchi MC, Tosetti M, Battini R. *et al.* Proton MR spectroscopy of mitochondrial diseases:analysis of brain metabolic abnormalities and their possible diagnostic relevance. AJNR Am J Neuroradiol. 2003;24:1958-1966.

Flanigan KM, Johns DR. Association of the 11778 mitochondria1 DNA mutation and demyelinating disease. Neurology. 1993;43(12):2720-2722.

Lerman-Sagie T, Leshinsky-Silver E, Watemberg N. *et al.* White matter involvement in mitochondrial diseases. Molecular Genetics and Metabolism. 2005;84:127-136.

Barkovich AJ, Good WV, Koch TK. *et al.* Mitochondrial disorders:analysis of their clinical and imaging characteristics. AJNR Am J Neuroradiol. 1993; 14(5):1119-1137.

Wilichowski E, Pouwels PJ, Frahm J, *et al.* Quantitative proton magnetic resonance spectroscopy of cerebral metabolic disturbances in patients with MELAS. Neuropediatrics. 1999;30:256-263.

Moroni I, Bugiani M, Bizzi A. *et al.* Cerebral white matter involvement in children with mitochondrial encephalopathies. Neuropediatrics. 2002;33(2):79-85.

Heidenreich JO, Klopstock T, Schirmer T. *et al.* Chronic Progressive External Ophthalmoplegia:MR Spectroscopy and MR Diffusion Studies in the Brain. AJR Am J Roentgenol. 2006;187:820-824.

Lin DM, Crawford TO, Barker PB. Proton MR Spectroscopy in the Diagnostic Evaluation of Suspected Mitochondrial Disease. AJNR Am. J. Neuroradiol. Jan 2003;24:33-41.

Jelena Ostojic,
Oncology Institute of Vojvodina,
Diagnostic Imaging Center,
Institutski put 4,
21204 Sremska Kamenica (Serbia).
E-mail: sunns@eunet.rs