In vivo brain 1H-MRS and DWI, and post-mortem study of patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)

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Introduction

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive multisystem disorder caused by mutations in the thymidine phosphorilase (TP) gene. The lack of TP activity leads to the accumulation of thymidine and mitochondrial DNA defects. The neurological manifestations include: ptosis, external opthalmoparesis, hearing loss, peripheral neuropathy, skeletal myopathy. Generally the gastrointestinal symptoms are more severe leading to cachexia and adverse outcome in early adulthood (average age of death 37 years) (1). The presence of diffuse white matter hyperintensities is typical, usually without signs of mental retardation or dementia. The aim of this study was to clarify the pathophysiology of the white matter abnormalities detectable on conventional MR imaging using ^{1}H-MRS and diffusion-weighted MRI with calculation of apparent diffusion coefficient (ADC).

Methods

Three patients with molecular diagnosis of MNGIE (2 males; 28, 28 and 36 years) and 10 sex- and age-matched healthy subjects were studied. All patients underwent neurological and electrophysiological examination, muscle and intestinal biopsy. Informed consent was obtained from all the subjects. Neuropathological examination was carried out in patient 1. ^{1}H-MRS and MRI studies were performed in a 1.5T General Electrics Medical Systems (Milwaukee, Wisconsin) Signa Horizon LX whole-body scanner using a 25cm diameter quadrature birdcage head coil. After structural imaging including SE T1 and FLAIR sequences, one voxel was selected in the left parietal white matter (2x2x2 cm^3). Spectra were acquired using the PRESS localisation sequence (TE=35 ms; TR= 4000 ms, number of acquisitions = 64). Absolute concentrations of N-acetylaspartate (NAA), creatine-phosphocreatine (Cr) and choline (Cho) were calculated by acquiring spectra of unsuppressed water (TE from 25 to 1000ms; TR= 15000ms) (2) and using mean T2 for metabolites values (T2 for NAA = 396ms; for Cho =191ms, for Cr=235ms) calculated in our laboratory in 15 healthy subjects. Peak areas for NAA at 2.02 ppm, for Cr at 3.03 ppm and for Cho at 3.22 were calculated using the time domain fitting program AMARES/MRUI. Statistical significance, determined by Student's unpaired *t* test, was taken as $p<0.05$. Diffusion-weighting EPI images (matrix= 128x128, FOV= 24 cm, slice separation= 6 mm, TE= 98.8 ms, TR= 10 s, b = 0, 300, 600, 900 mm^2/s, in 3 axes) were acquired. For each pixel, the apparent diffusion coefficient (ADC) was calculated. A semi-automated procedure defined CSF, white matter (WM) and grey matter (GM) regions of interest (ROIs). Statistical significance, determined by the non-parametric Mann-Whitney U test, was taken as $p<0.05$.

Results

At the time of the study each patient presented a different clinical and MRI severity ranging from severe to mild. Brain FLAIR sequences showed the typical white matter hyperintensities in all three patients. In the patients WM [NAA], [Cr], and [Cho] were all significantly lower than controls. Consequently the NAA/Cr ratio was not significantly abnormal (Table). In the ADC images abnormal pixels were defined as those having a value greater than 2 standard deviations above the mean control value. The percentage of abnormal pixels was calculated by counting the proportion of such pixels in the WM ROIs. All patients showed a proportion of abnormal WM ADC outside the normal range. Case 1, the most severely affected, showed the greatest reduction in [NAA] and the most abnormal ADC. Conversely, case 3, the least affected, showed the least [NAA] and ADC alterations (Table-Figure). Pathological examination of case 1 did not show significant abnormalities. In particular, demyelination, neuronal loss and gliosis were absent.

Table . Brain ^{1}H-MRS and MRI findings from white matter of MNGIE patients and controls.

	Clinical/ MRI severity	[NAA]	[Cr]	[Cho]	NAA/Cr	ADC % abnormal
Case 1	Severe	8.81	6.99	2.86	1.39	48.5
Case 2	Moderate	9.64	6.27	2.52	1.69	21.0
Case 3	Mild	10.38	7.99	2.83	1.43	10.8
Mean±Sd		9.61±0.79	7.08±0.86	2.74±0.19	1.50±0.16	26.8±26.6
Controls		13.26±1.24	8.79±0.66	3.15±0.22	1.66±0.09	2.9±0.9
***p* value**		*0.003*	*0.013*	*0.027*	*n.s.*	*<0.05*

Metabolite concentrations are expressed as mmol/kg wet weight.

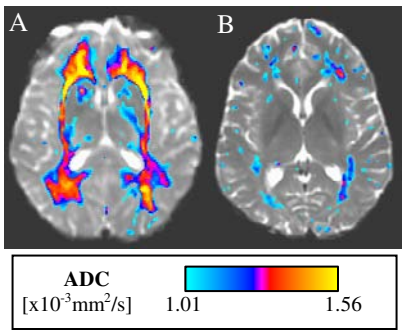

Figure. (A) case1 and (B) case 3 T2-weighted images: greyscale, ADC images color. ADC<1.0x10^{-3} mm^2/s & CSF: transparent.

Discussion

Our study showed that increase ADC was associated with a global reduction in ^{1}H-MRS metabolites in the brain white matter of MINGIE patients. Symmetric increase in white matter ADC is a common findings in a several hereditary leukoencephalopathies including adrenoleukodystrophy, Krabbe disease, and congenital muscular dystrophies (3). In these conditions ADC changes are interpreted as secondary to myelin breakdown as demonstrated by increased Cho and axonal loss as demonstrated by reduced NAA on ^{1}H-MRS (4). In the cases reported here ^{1}H-MRS disclosed reduced levels of Cho in the WM suggesting that myelin pathology does not occur and that both ADC and ^{1}HMRS changes may be secondary to an increase in WM water content in the absence of pathological changes as demonstrated in case 1. This interpretation is supported by a recent post-mortem study of the brain of two MINGIE patients providing evidence of blood brain barrier breakdown in the absence of demyelination, axonal/neuronal loss and gliosis (5).

References

1. Hirano M *et al*, The neurologist 2004;10:8-17.
2. Keevil S.F. *et al*, Magn. Reson. Imaging, 1998;16:1093-1106.
3.Engelbrecht V *et al* Radiology 2002;22:410-418.
4. Oz G. *et al*, Neurology; 64, 434, 2005.
5. Szigeti K *et al*, Ann Neurol 2004;56:881-886.