The Role of Brain MRI in Mitochondrial Neurogastrointestinal Encephalomyopathy

MAURO SCARPELLI¹, GIUSEPPE KENNETH RICCIARDI², ALBERTO BELTRAMELLO², ISABELLA ZOCCA³, FRANCESCA CALABRIA¹, ANNA RUSSIGNAN¹, FRANCESCA ZAPPINI¹, MARIA SOFIA COTELLI⁴, ALESSANDRO PADOVANI⁴, GIULIANO TOMELLERI¹, MASSIMILIANO FILOSTO⁴, PAOLA TONIN¹

¹Department of Neurological and Movement Sciences, Section of Neurology, University of Verona; Verona, Italy

²Department of Pathology and Diagnostics, Section of Neuroradiology, University of Verona; Italy

³Department of Medicine, Section of Internal Medicine, University of Verona; Italy

⁴Clinical Neurology, Section for Neuromuscular Diseases and Neuropathies, “Spedali Civili” University Hospital; Brescia, Italy

Key words: mitochondrial neurogastrointestinal encephalomyopathy, MNGIE, MRI, MRS, DWI

SUMMARY – *Leukoencephalopathy is a hallmark of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) a devastating disorder characterized by ptosis, ophthalmoparesis, gastrointestinal dysfunction and polyneuropathy. To characterize MNGIE-associated leukoencephalopathy and to correlate it with clinical, biochemical and molecular data, four MNGIE patients with heterogeneous clinical phenotypes (enteropathic arthritis, exercise intolerance, CIDP-like phenotype and typical presentation) were studied by magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). Diffusion weighted imaging (DWI) with apparent diffusion coefficient (ADC) maps were also obtained. In two patients we also investigated the role of brain MRI in monitoring the evolution of leukoencephalopathy by performing follow-up imaging studies at an interval of one and two years. The extension and distribution of leukoencephalopathy were not clearly linked with age, phenotype or disease severity, and did not seem to be related to TYMP mutations, enzyme activity or pyrimidine levels. In the studied patients MRS revealed reduced N-acetyl-aspartate and increased choline signals. Although DWI appeared normal in all patients but one, ADC maps always showed moderate increased diffusivity. Leukoencephalopathy worsened over a two-year period in two patients, regardless of the clinical course, indicating a lack of correlation between clinical phenotype, size and progression of white matter abnormalities during this period. Brain MRI should be considered a very useful tool to diagnose both classical and atypical MNGIE. Serial MRIs in untreated and treated MNGIE patients will help to establish whether the leukoencephalopathy is a reversible condition or not.*

Introduction

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder of nucleotide metabolism due to TYMP gene mutations that cause loss of thymidine phosphorylase (TP) activity. TP deficiency results in marked elevation of thymidine and deoxyuridine levels, nucleotide pool imbalance, subsequent instability of mitochondrial DNA (mtDNA) and impairment of the mitochondrial respiratory chain^{1,2}.

Patients develop symptoms at a mean age of 18 years with disease onset ranging from five months to more than 50 years of age³⁻⁴. The disease course is relentlessly progressive leading to death at a mean age of 35 years³.

Classically, MNGIE manifests as a multisystemic disorder affecting mainly the gastrointestinal and nervous systems with severe gastrointestinal dysmotility, cachexia, ptosis and ophthalmoparesis, peripheral neuropathy and leukoencephalopathy^{3,5-6}. However, MNGIE patients with incomplete or atypical clinical pres-

entations have been described and frequent misdiagnosis such as anorexia nervosa, inflammatory bowel disease, superior mesenteric artery syndrome, Whipple disease, chronic intestinal pseudo-obstruction, chronic inflammatory demyelinating polyneuropathy (CIDP) and Charcot Marie Tooth (CMT) disease have been reported⁷⁻¹⁰.

Leukoencephalopathy is a hallmark of MNGIE, so far reported in all patients^{3,6}. Brain magnetic resonance imaging (MRI) in MNGIE patients usually shows symmetric and confluent T2-hyperintensity in the white matter of the semioval centres with sparing of subcortical U-fibers, and less frequently in the cerebellar white matter, splenium of the corpus callosum, basal ganglia and thalami^{11,12}. In addition, cerebellar atrophy in a few MNGIE patients^{12,13} and post-contrast MR gadolinium enhancement in one patient were reported¹⁴.

Even though a number of patients showed symptoms and signs of central nervous system involvement such as cognitive impairment, dementia, seizures, headaches or psychiatric symptoms^{3,15}, the clinical correlates of white matter involvement are unclear^{3,5,6}.

In mitochondrial disorders a correlation between brain imaging and genotype and/or biochemical findings can be difficult to demonstrate^{16,17} and clinical presentation may be disproportionate to the extent of white matter involvement^{18,19}. In childhood, a 'leukodystrophic' (white matter abnormalities) MRI pattern may be the only or the most significant

imaging feature of an underlying respiratory chain disorder, whereas a 'leukodystrophic' pattern is very rarely observed in adult onset mitochondrial disorders, with the exception of MNGIE²⁰. MR spectroscopy (MRS) represents a useful investigational tool in the study of mitochondrial diseases, as it supplies additional information compared to conventional imaging²¹. N-acetyl-aspartate (NAA) decrease is currently interpreted as the most sensitive sign of neuronal rarefaction or dysfunction in many metabolic and neurodegenerative brain diseases²²⁻²⁴. Choline (Cho) decrease is supposed to be the spectroscopic correlate of an impairment of normal membrane maintenance processes due to reduced energy production²¹. High Cho levels in the white matter likely reflect the release of phosphocholine and glycerophosphocholine due to demyelination and membrane remodeling^{25,26}. Elevation of lactate, the end product of nonoxidative metabolism, can also be observed in the absence of plasma lactic acidemia²¹ and usually represents a marker of respiratory chain dysfunction in brain²⁴.

This study used MRI to evaluate four MNGIE patients with both classical and clinically heterogeneous phenotypes. In two of these patients we performed serial imaging studies at an interval of one and two years after the first examination in order to assess the evolution of leukoencephalopathy. MRS and diffusion-weighted imaging (DWI) with calculation of apparent diffusion coefficient (ADC) maps were also obtained in three of them.

Table 1 Clinical, biochemical and molecular findings in the four patients.

Pt	Sex	Onset of symptoms	Age at diagnosis	Presentation & clinical features	TYMP mutations	TP activity	U-Thd (mmol/mol creatinine)	U-dUrd (mmol/mol creatinine)
1	f	childhood	33	ATYPICAL enteropathic arthritis	HOMOZYGOUS c.1160-1 G>A	Undetectable	38.57	75.86
2	m	childhood	38	ATYPICAL exercise intolerance	HOMOZYGOUS c.1160-1 G>A	Undetectable	61.51	172.5
3	m	adulthood	26	ATYPICAL severe neuropathy	HETEROZYGOUS c.866A>C; c.1231_1243 del	Undetectable	13.89	33.28
4	f	childhood	22	TYPICAL multisystem disease	HOMOZYGOUS c.1160-1 G>A	Undetectable	23.01	79.70

TP: thymidine phosphorylase (normal values: 80–1000 nmol/h/mg protein); U-Thd: urinary thymidine (normal values: 0.04–0.05 mmol/mol creatinine); U-dUrd: urinary deoxyuridine (normal values: 0.02–0.04 mmol/mol creatinine).

Patients and Methods

Four biochemically and genetically proven MNGIE patients were studied. Clinical findings of these patients have already been reported^{10,27}; clinical, biochemical and molecular data are summarized in Table 1.

Briefly, Patient 1, a 33-year-old woman, complained of episodes of abdominal pain with alternating diarrhoea and constipation over a ten-year period. At age 25, she had slight persistent fever and recurrent episodes of migrant arthritis. At age 31, she began complaining of paraesthesias in her fingers and toes. A neurological examination revealed partial bilateral blepharoptosis, diffuse muscle atrophy, reduced joint position and vibration sense in the lower limbs, absent ankle tendon reflexes, and reduced knee jerk. An electroneurographic-electromyographic (ENG-EMG) study showed a demyelinating sensory motor polyneuropathy. Further tests showed increased urinary levels of thymidine (26.49 mmol/mol creatinine; n.v. 0.04–0.05) and deoxyuridine (67.77 mmol/mol creatinine; n.v. 0.02–0.04) and virtual absence of blood TP activity in buffy coats. Direct sequencing of TYMP revealed the homozygous c.1160-1 G>A splice acceptor site mutation.

Patient 2, the 38-year-old brother of patient 1, reported lifelong exercise intolerance and occasional muscle cramps and, during the last two years, an alternating bowel function without abdominal pain. Urinary thymidine and deoxyuridine levels were increased (61.51 mmol/mol

creatinine and 172.50 mmol/mol creatinine, respectively) and blood TP activity was undetectable. Direct sequencing of TYMP gene showed the homozygous c.1160-1G>A mutation. At age 35, neurological examination showed only eyelid ptosis and reduced upward gaze. Three years later, the clinical picture remained unchanged.

Patient 3, a 25-year-old man, had a two-year history of paraesthesias and muscle weakness at lower limbs, bilateral lid ptosis with extraocular muscle paresis and sensorineural deafness. Diabetes had been diagnosed three years before. ENG revealed a demyelinating polyneuropathy. Thymidine and deoxyuridine in urine were increased (13.89 mmol/mol creatinine and 33.28 mmol/mol creatinine) and TP activity in buffy coats was absent. TYMP sequence analysis showed compound heterozygous mutations (c.866A>C and c.1231_1243 del). One year later he reported exercise intolerance and reduced muscle strength in the upper limbs. He started carrier erythrocyte entrapped thymidine phosphorylase (CEETP) therapy and three years after the first examination the neurological picture was characterized by a reduction in numbness and tingling sensations in both hands and improvement in sensory ataxia, strength in upper limbs and fine finger functioning.

Patient 4, a 22-year-old woman, complained of recurrent abdominal pain, frequent episodes of diarrhoea and vomiting since childhood associated with several episodes of intestinal pseudo-obstruction. She was cachectic with a body mass index of 10, had bilateral lid pto-

Table 2 Results of brain MRI study in the four patients.

Pt	WMA	rDWI	rADC	MRS	Follow-up MRI and MRS	Fig.
1	++	–	–	–	–	1A
2	++	Isointensity	↑	NAA =	Increase WMA, ADC, CHO/NAA	1B,3
				CHO =		
				H ₂ O ↑		
3	+	Isointensity	↑	NAA =	Increase WMA, ADC, CHO/NAA	1C,2,4
				CHO =		
				H ₂ O ↑		
4	+++	Isointensity	↑	NAA ↓	–	1D
				CHO ↑		
				H ₂ O ↑		

WMA: white matter alterations; rDWI: relative signal in diffusion weighted images; rADC relative apparent diffusion coefficient; NAA: N-acetyl-aspartate; CHO: choline; H₂O: water. = : normal; ↑ : increased; +: grade of extension of leukoencephalopathy.

Figure 1 FLAIR images of semioval centres in the four patients (Pt) investigated. A) Pt 1, B) Pt 2, C) Pt 3, D) Pt 4. Intensity, morphology and distribution of the signal alteration significantly differ among the four patients, although always maintaining a common pattern. Patient 4, with a typical clinical picture at presentation, had the most widespread white matter T2 hyperintensity involving not only periventricular regions, but also extensively the subcortical areas. Deep pyramidal and subcortical U-fibers appear grossly spared.

Figure 2 FLAIR imaging of semioval centres acquired in Patient 3 at the time of the first study (A), 15 (B) and 28 (C) months later. Hyperintensity progressively spread centrifugally towards frontal subcortical white matter (B) and became more evident in the third study with the appearance of focal bright areas within the deep part of precentral gyri.

sis and ophthalmoplegia, sensorineural deafness, diffuse muscle atrophy with severe feet dorsiflexor weakness, reduced joint position and vibration sense in the lower limbs and no deep tendon reflexes. Nerve conduction study showed a severe demyelinating sensory and motor polyneuropathy. Urine thymidine was 23.01 mmol/mol creatinine and urine deoxyuridine was 79.70 mmol/mol creatinine. Blood TP activity was undetectable. Direct sequencing of TYMP gene showed the homozygous c.1160-1G>A mutation. All four patients underwent MRI; in patients 2, 3 and 4 an MRS study was performed at the time of diagnosis. Patients 2 and 3 also underwent repeated MRI and MRS studies at an interval of one and two years. All the MRI studies were performed on a 3T Allegra MRI scanner (Siemens AG, Erlangen Germany) with a transmitting-receiving head coil. Axial T2-weighted (T2w) (TE/TR=100/4000 ms) and axial and coronal fluid attenuate inversion recovery (FLAIR) (TE/TI/TR=88/2500/9000 ms) images were acquired. To guide the spectroscopic volume-of-interest (VOI) sagittal T1-weighted (T1w) MP-RAGE (TE/TI/TR=2.6/1800/1360 ms) was obtained and reconstructed in axial and coronal slices.

Diffusion-weighted images were acquired in the transverse plane using a spin-echo echo-planar imaging sequence with the following parameters: TE/TR/TI=100/12,000/2,200 ms diffusion gradient encoding in three orthogonal directions; $b = 500$ and 1.000 s/mm^2 ; section thickness 5 mm. After pixel-by-pixel calculation ADC maps were always obtained.

Following our chemical-shift imaging (CSI)-based shimming procedure, an 11 cm anterior-posterior $\times$ 8 cm left-right $\times$ 2 cm inferior-superior ^1H -MRSI VOI was centred on the semioval centres and excited using TE/TR=135/1700 ms point resolved spectroscopy (PRESS). The VOI was encoded with 16×16 2D-CSI over a 18×18 cm FOV. Voxel size was $1.3 \times 1.3 \times 2$ cm. At four averages, the ^1H -MRSI study, repeated twice with and without water suppression for subsequent eddy current correction, took approximately 12 minutes.

Finally the initial sagittal T1-weighted MP-RAGE was repeated after iv contrast injection and again reconstructed in axial and coronal slices.

The entire protocol took 40 minutes.

MRS post-processing was performed on an off-line workstation. Voxels were interpolated to $6.5 \times 6.5 \times 20$ mm.; peak areas of water, n-acetyl-aspartate (NAA), choline (Cho), lipid, lactate and creatine/phosphocreatine (Cr) were assessed and metabolite ratios of Cho/NAA, NAA/Cr and Cho/Cr were calculated for each given voxel.

Colour maps summarizing results distribution for each of these parameters were obtained. For each patient the values found in the white matter presenting areas of hyperintensity in T2-weighted images were compared with those obtained from normal appearing white matter (NAWM) and/or subcortical areas of the same patient, and with those collected from a pool of healthy subjects previously studied in our Department.

Figure 3 Images of caudate nuclei and lateral ventricle bodies acquired in Patient 2. A) FLAIR. B) EPI T2-w b0. C) DWI b1000. D) ADC map. Two regions of interest show the technique used to compare T2 hyperintense areas (ROI 1) with NAWM (ROI 2). While the ADC map effectively reveals the increased diffusivity in ROI 1 corresponding to T2 hyperintensity, b1000 DWI shows a comparable signal in the two areas because of a balance between T2 shine through hyperintensity and signal loss due to increased diffusivity as seen in the ventricles (T2 washout phenomenon). This finding, together with increased water signal in MRS studies, could confirm that, at least partially, T2 WM hyperintensity is due to vasogenic interstitial oedema.

Figure 4 MR spectroscopy colour maps, obtained by means of multivoxel studies with 135 ms TE in Patient 3 at the time of the third study (A,C) and in a control subject (B,D). Red areas indicate higher N-acetyl-aspartate (NAA) or N-acetyl-aspartate/choline ratio levels, green areas indicate lower levels. Colour scale legend is shown on the left in Figures 4A,B. The colour map showed lower levels of NAA distribution in T2 hyperintense supraventricular areas in a MNGIE affected patient (A) compared to a normal subject (B). In normal patients NAA tended to be uniformly higher in the semioval centres far from cortical grey matter. The choline/NAA ratio was significantly higher in T2 hyperintense areas (red and yellow) compared to subcortical regions in MNGIE-affected patients (C). This pattern was not found in normal patients where the ratio was stable and comparatively lower (D).

ADC values were calculated on maps obtained from DWI imaging, using 5 mm diameter ROIs positioned on areas of T2 white matter hyperintensity and on areas of T2 NAWM.

Results

MRI findings obtained at first evaluation and in two patients in the follow-up are summarized in Table 2.

Patient 1. T2-weighted and FLAIR brain MRI sequences showed diffuse hyperintensity in the white matter of supratentorial areas (Figure 1A) and subtle T2 hyperintensity within the pons.

Patient 2. A first brain MRI revealed large confluent almost symmetrical hyperintense areas on T2-weighted and FLAIR images in the white matter of the semioval centres (Figure 1B). Serial brain MRI, performed one and two years later, showed the appearance of new lesions on T2-weighted imaging and FLAIR located eccentrically from the initial periventricular lesions and expanding towards subcortical white matter. Signal alterations already present in the first study appeared more evident in the following images.

Patient 3. Brain MRI showed almost symmetric hyperintense areas in the white matter of the semioval centres and periventricular white matter on T2-weighted and FLAIR images. Signal alterations on FLAIR images appeared less diffuse than in patients 1 and 2 (Figure 1C). A second and third follow-up brain MRI showed a progressive increase in supratentorial signal alterations with an evolution comparable to that observed in patient 2 (Figure 2).

Patient 4. Brain MRI revealed diffuse and symmetric T2-weighted and FLAIR hyperintense areas which were more obvious in the white matter of the semioval centres (Figure 1D). Signal alterations appeared more widespread than in the other patients, also involving the temporal lobes.

Cerebellum and internal capsules were spared in all patients, while the brainstem was involved only in patient 1. In all four patients no enhancement was noted after gadolinium administration. ADC MAPS showed increased diffusivity corresponding to signal alterations in FLAIR sequences, while b1000 DWI images did not show significant signal alterations with isointensity of T2 altered regions and NAWM (Figure 3).

Brain MRS in patient 4, who had the most widespread lesions, showed a reduction of NAA and increased Cho signal in the white matter T2 hyperintense areas. In patients 2 and 3 the Cho/NAA signal ratio was just below the upper limit of normal range in the hyperintense white matter areas, as compared to NAWM and to white matter in healthy volunteers, at the MRS first study. However, the reduction of NAA and the increase in Cho have become progressively more evident in the follow-up MRSs in parallel with the extension and progression of the white matter T2 signal hyperintensities.

By MRS metabolite distribution colour

MAPS, the NAA reduction was most marked (Figure 4) in T2 hyperintense periventricular areas as compared with subcortical white matter from the patients and with periventricular white matter from control subjects. The water signal in non-suppressed MRS sequences was significantly higher than normal in T2 hyperintense areas of the studied patients and showed a gradual centrifugal decrease in NAWM. This higher signal further increased in follow-up studies in patients 2 and 3. No lactate or lipid peaks were observed.

Discussion

MNGIE is a devastating progressive disease that leads inevitably to death if not treated. Nowadays, promising new therapeutic options, such as allogeneic haematopoietic stem cell transplantation (HSCT)²⁸ and CEETP therapy are available²⁹. This implies that the diagnosis should be made as early as possible to prevent organ damage and to maximize the benefits of treatments²⁸⁻³⁰.

White matter alterations in supratentorial areas can be considered a hallmark of MNGIE because they are present in patients with both classical phenotype and atypical presentation^{3,6,10-12}. Therefore, if a clinical suspicion emerges, MRI often addresses the correct diagnosis.

By contrast, patients with MNGIE-like phenotypes and mutations in genes other than TYMP such as ribonucleoside-diphosphate reductase subunit M2 B (RRM2B)³¹, polymerase gamma (POLG)^{32,33} and mitochondrial DNA (mtDNA)³⁴, usually present with hyperintense, often symmetric non-enhancing lesions in the basal ganglia or patchy signals throughout the white matter. An MNGIE-like patient having gastrointestinal dysmotility, cachexia, peripheral neuropathy and ophthalmoplegia diagnosed by us with a POLG mutation did not show any brain MRI abnormality (data not published).

Our study confirms the usefulness of MRI in diagnosing MNGIE also when patients showed non-classical phenotypes. The three patients with atypical presentation, patient 1 with enteropathic arthritis, patient 2 with exercise intolerance and patient 3 having a CIDP-like phenotype, had MRI findings consistent with the diagnosis of MNGIE. Although a recent single voxel brain MRI/MRS study on three MNGIE siblings suggested a direct correlation between clinical features and the extent of leukoencephalopathy³⁵, in our patients, age, clinical phenotype and

severity of the disease did not significantly correlate with the extension and distribution of the white matter abnormalities. Patients 1 and 2 had almost the same brain MRI picture despite the different presentation, severity and course of the disease; patient 2, the eldest, had the mildest clinical phenotype, but brain involvement on MRI was more widespread than that observed in patient 3 who had a more severe neurological condition. Patient 4, the youngest and the only one with typical presentation, was severely affected with the most widespread involvement of brain white matter. It is noteworthy that our patients did not manifest clinical signs of CNS involvement and were apparently cognitively unaffected, thus suggesting that the signal alterations seen on brain MRI do not reflect any extensive structural damage of the white matter. Nor could we find any clear correlation between the extension and distribution of white matter abnormalities and biochemical and molecular findings.

TP activity in buffy coats was virtually absent in all of our patients while urine nucleoside levels varied among them. In particular, patient 2 had the highest urine levels, the mildest phenotype and a brain MRI picture very similar to that of patient 1, who had lower nucleoside urinary levels. Moreover, three patients with different MRI patterns (patients 1 and 2 with very similar white matter alterations in supratentorial areas, and patient 4 with the most widespread involvement of white matter) harboured the same molecular defect, suggesting again a lack of correlation data. These results differ from a previous study reporting that brain MRI findings might vary with TYMP mutations and residual TP activity¹¹.

An interesting issue is that follow-up brain MRIs, performed at an interval of one and two years in patients 2 and 3, revealed a progression of white matter signal alterations, regardless of the clinical course. Patient 2 was clinically stable and still without therapy, while the clinical conditions of patient 3 initially worsened, but subsequently showed a mild improvement after CEETP therapy was begun. It is unknown whether white matter MRI alterations progress with the patient's age and/or clinical disease and if so, at what rate¹¹. In particular, no data about white matter alterations in treated MNGIE patients have been reported so far. We observed a worsening of white matter alterations in the course of treatment in patient 3 and we do not know if the progression of the leukoencephalopathy may slow down or be halted by therapy, and at what pace. Further

studies are necessary to clarify these issues.

MR spectroscopy study, performed in three of our patients, showed a reduction of NAA in periventricular areas and an increase in Cho signals in the T2-hyperintense areas of the semioval centres, without lactate peaks. In patients 2 and 3 MRS alterations became evident in the follow-up studies, when T2 hyperintensity increased.

The few MRS studies performed in MNGIE patients show inconsistent results. In one report, all three studied patients had increased water content, reduced NAA, Cho and Cr and no lactate peaks in the areas of white matter T2-hyperintensity³⁵, whereas in another study no abnormalities of the main metabolites were detected³⁶.

Divergent results were also obtained in pathological studies of brain autopsies in which loss of myelin and reduction of the number of myelinated fibres without gliosis were found in one patient³⁷, while in two other studies no demyelination, gliosis, or spongy degeneration were detected^{38,39}. On the other hand, a MNGIE mouse model showed increased T2 signal in the cerebral white matter on MRI and, at pathological evaluation, multiple vacuoles in subcortical, periventricular, internal capsule and cerebellar white matter without signs of focal demyelination⁴⁰. In spite of the paucity of signs of central nervous system involvement, our results (reduction in NAA and increase in Cho peak) seem to confirm not only "functional" damage of the white matter but rather a neuronal and myelin dysfunction in the MNGIE brain.

The DWI and ADC MRI studies performed in our patients merit some comment. The isointensity on b1000 DW images between hyperintense and normally intense regions in T2-w images associated with increased ADC values in T2 hyperintense areas observed in our patients (Figure 3) may be the result of a balance of DW images between the increased T2 relaxation (hyperintensity in DWI because of T2 prolongation in Epi T2-w images, known as T2 shine through) and the increased diffusivity (hypointensity in DWI, like cerebrospinal fluid in the ventricles). This may explain why, although DWI images show diffuse nearly isointense white matter, ADC maps reveal increased diffusivity in regions of signal alterations in T2. The phenomenon is known as T2 washout^{41,42} and is determined by annulment of DWI hypointensity because of T2 hyperintensity, as seen in posterior reversible encephalopathy syndrome⁴³. This finding could indicate vasogenic interstitial oedema and correlate with the results of pathological studies³⁹.

Conclusions

Brain MRI is a very useful tool in diagnosing MNGIE patients and distinguishing MNGIE from MNGIE-like patients. Therefore, it should be performed even when symptoms and signs of central nervous system involvement are absent. The extension and distribution of leukoencephalopathy do not clearly correlate with age and clinical picture, hence they should not be considered reliable markers of disease severity in MNGIE. In our patients, leukoencephalopathy slowly worsened over a two-year period regardless of the clinical course, suggesting the tendency of white matter alterations to become diffuse and confluent. In addition, this is the first study reporting a specific analysis of MRS multi-voxel colour maps and serial quantitative ADC evaluation in MNGIE, indicating

that the coexistence of vasogenic oedema and glial dysfunction may play a synergistic role in causing the T2 white matter hyperintensity observed in MNGIE patients. Considering the new therapeutic options, the systematic study of brain MRI in untreated and treated MNGIE patients will probably help clarify the pathophysiology of white matter abnormalities and establish whether MNGIE-associated leukoencephalopathy is a reversible condition or not.

Acknowledgment

We kindly thank Dr Alice Donati from the Unit of Hereditary Metabolic and Neuromuscular Diseases, Meyer Children Hospital, Florence, Italy, for thymidine and deoxyuridine assay in urine.

References

- Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science*. 1999; 283: 689-692.
- Lara MC, Valentino ML, Torres-Torronteras J, et al. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): biochemical features and therapeutic approaches. *Biosci Rep*. 2007; 27: 151-163.
- Garone C, Tadesse S, Hirano M. Clinical and genetic spectrum of mitochondrial neurogastrointestinal encephalomyopathy. *Brain*. 2011; 134: 3326-3332.
- Martí R, Verschuuren JJ, Buchman A, et al. Late-onset MNGIE due to partial loss of thymidine phosphorylase activity. *Ann Neurol*. 2005; 58: 649-652.
- Hirano M, Silvestri G, Blake DM, et al. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. *Neurology*. 1994; 44: 721-727.
- Hirano M, Nishigaki Y, Martí R. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): a disease of two genomes. *Neurologist*. 2004; 10: 8-17.
- Martín MA, Blázquez A, Martí R, et al. Lack of gastrointestinal symptoms in a 60-year-old patient with MNGIE. *Neurology*. 2004; 63: 1536-1537.
- Massa R, Tessa A, Margollicci M, et al. Late-onset MNGIE without peripheral neuropathy due to incomplete loss of thymidine phosphorylase activity. *Neuromuscul Disord*. 2009; 19: 837-840.
- Needham M, Duley J, Hammond S, et al. Mitochondrial disease mimicking Charcot-Marie Tooth disease. *J Neurol Neurosurg Psychiatry*. 2007; 78: 99-100.
- Filosto M, Scarpelli M, Tonin P, et al. Pitfalls in diagnosing mitochondrial neurogastrointestinal encephalomyopathy. *J Inherit Metab Dis*. 2011; 34: 1199-1203.
- Millar WS, Lignelli A, Hirano M. MRI of five patients with mitochondrial neurogastrointestinal encephalomyopathy. *Am J Roentgenol*. 2004; 182: 1537-1541.
- Barragán-Campos HM, Vallée J, Ló D, et al. Brain magnetic resonance imaging findings in patients with mitochondrial cytopathies. *Arch Neurol*. 2005; 62: 737-742.
- Scaglia F, Wong L-JC, Vladutiu GD, et al. Predominant cerebellar volume loss as a neuroradiologic feature of pediatric respiratory chain defects. *Am J Neuroradiol*. 2005; 26: 1675-1680.
- Petcharunpaisan S, Castillo M. Multiple cranial nerve enhancement in mitochondrial neurogastrointestinal encephalomyopathy. *J Comput Assist Tomogr*. 2010; 34: 47-48.
- Carod-Artal FJ, Herrero MD, Lara MC, et al. Cognitive dysfunction and hypogonadotrophic hypogonadism in a Brazilian patient with mitochondrial neurogastrointestinal encephalomyopathy and a novel ECGF1 mutation. *Eur J Neurol*. 2007; 14: 581-585.
- Diogo L, Cordeiro M, Garcia P, et al. Value of brain magnetic resonance imaging in mitochondrial respiratory chain disorders. *Pediatr Neurol*. 2010; 42: 196-200.
- Friedman SD, Shaw DW, Ishak G, et al. The use of neuroimaging in the diagnosis of mitochondrial disease. *Dev Disabil Res Rev*. 2010; 16: 129-135.
- Finsterer J, Zarrouk Mahjoub S. Leukoencephalopathies in mitochondrial disorders: clinical and MRI Findings. *J Neuroimaging*. 2012; 22: e1-11.
- Lerman-Sagie T, Leshinsky-Silver E, Waternberg N, et al. White matter involvement in mitochondrial diseases. *Mol Gen Metab*. 2005; 84: 127-136.
- Salsano E, Farina L, Lamperti C, et al. Adult-onset leukodystrophies from respiratory chain disorders: do they exist? *J Neurol*. 2013; 260: 1617-1623.
- Bianchi MC, Tosetti M, Battini R, et al. Proton MR spectroscopy of mitochondrial diseases: analysis of brain metabolic abnormalities and their possible diagnostic relevance. *Am J Neuroradiol*. 2003; 24: 1958-1966.
- Urenjak J, Williams SR, Gadian DG, et al. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neuronal cell types. *J Neurosci*. 1993; 13: 981-989.
- Vion-Dury J, Nicoli P, Salvan AM, et al. Reversal of brain metabolic alterations with zidovudine detected by proton localized magnetic resonance spectroscopy. *Lancet*. 1995; 345: 60-61.
- Finsterer J. Central nervous system imaging in mitochondrial disorders. *Can J Neurol Sci*. 2009; 36: 143-153.

- 25 Barkovich AJ. Magnetic resonance techniques in the assessment of myelin and myelination. *J Inherit Metab Dis.* 2005; 28: 311-343.
- 26 Padma MV, Adineh M, Pugar K, et al. Functional imaging of a large demyelinating lesion. *J Clin Neurosci.* 2005; 12: 176-178.
- 27 Filosto M, Scarpelli M, Tonin P, et al. Course and management of allogeneic stem cell transplantation in patients with mitochondrial neurogastrointestinal encephalomyopathy. *J Neurol.* 2012; 259: 2699-2706.
- 28 Halter J, Schüpbach WM, Casali C, et al. Allogeneic hematopoietic SCT as treatment option for patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): a consensus conference proposal for a standardized approach. *Bone Marrow Transplant.* 2011; 46: 330-337.
- 29 Moran NF, Bain MD, Muqit MM, et al. Carrier erythrocyte entrapped thymidine phosphorylase therapy for MNGIE. *Neurology.* 2008; 71: 686-688.
- 30 Scarpelli M, Russignan A, Zombor M, et al. Poor outcome in a mitochondrial neurogastrointestinal encephalomyopathy patient with a novel TYMP mutation: the need for early diagnosis. *Case Rep Neurol.* 2012; 4: 248-253.
- 31 Shaibani A, Shchelochkov OA, Zhang S, et al. Mitochondrial neurogastrointestinal encephalopathy due to mutations in RRM2B. *Arch Neurol.* 2009; 66: 1028-1032.
- 32 Van Goethem G, Schwartz M, Lofgren A, et al. Novel POLG mutations in progressive external ophthalmoplegia mimicking mitochondrial neurogastrointestinal encephalomyopathy. *Eur J Hum Genet.* 2003; 11: 547-549.
- 33 Tang S, Dimberg EL, Milone M, et al. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)-like phenotype: an expanded clinical spectrum of POLG1 mutations. *J Neurol.* 2012; 259: 862-868.
- 34 Horváth R, Bender A, Abicht A, et al. Heteroplasmic mutation in the anticodon-stem of mitochondrial tRNA(Val) causing MNGIE-like gastrointestinal dysmotility and cachexia. *J Neurol.* 2009; 256: 810-815.
- 35 Schüpbach WMM, Vadday KM, Schaller A, et al. Mitochondrial neurogastrointestinal encephalomyopathy in three siblings: clinical, genetic and neuroradiological features. *J Neurol.* 2007; 254: 146-153.
- 36 Rousset P, Elmaleh-Bergès M, Ogier de Baulny H, et al. Mitochondrial neurogastrointestinal encephalomyopathy. *J Neuroradiol.* 2008; 35: 121-124.
- 37 Simon L, Horoupian D, Dorfman L, et al. Polyneuropathy, ophthalmoplegia, leukoencephalopathy and intestinal pseudo-obstruction: POLIP syndrome. *Ann Neurol.* 1990; 28: 349-360.
- 38 Bardosi A, Creutzfeldt W, DiMauro S, et al. Myo-neurogastrointestinal encephalopathy (MNGIE syndrome) due to partial deficiency of cytochrome c oxidase: a new mitochondrial multisystem disorder. *Acta Neuropathol (Berl).* 1987; 74: 248-258.
- 39 Szigeti K, Sule N, Adesina AM, et al. Increased blood-brain barrier permeability with thymidine phosphorylase deficiency. *Ann Neurol.* 2004; 56: 881-886.
- 40 López LC, Akman HO, García-Cazorla A, et al. Unbalanced deoxynucleotide pools cause mitochondrial DNA instability in thymidine phosphorylase-deficient mice. *Hum Mol Genet.* 2009; 18: 714-722.
- 41 Hiwatashi A, Zhong J. Pitfalls and artifacts of DW imaging. In: Moritani T, Ekholm S, Westesson PL, eds. *Diffusion-weighted MR imaging of the brain.* New York: Springer-Verlag. 2005; p.11-24.
- 42 Casey S. "T2 washout": an explanation for normal diffusion weighted images despite abnormal apparent diffusion coefficient maps. *Am J Neuroradiol.* 2001; 22: 1450-1451.
- 43 Provenzale JM, Petrella JR, Cruz LC Jr, et al. Quantitative assessment of diffusion abnormalities in posterior reversible encephalopathy syndrome. *Am J Neuroradiol.* 2001; 22: 1455-1461.

Mauro Scarpelli, MD
 Section of Neurology
 Department of Neurological and Movement Sciences
 University of Verona
 P.le L.A. Scuro, 10
 37134 Verona, Italy
 Tel.: +39-045-8124286
 Fax: +39-045-8027492
 E-mail: mauro.scarpelli@univr.it