ON-LINE APPENDIX

Proton MR Spectroscopy Data Analysis

For NAA, Cr, Cho, and mIns, absolute concentrations were calculated. The T2 times of NAA, Cr, and Cho were calculated by fitting the monoexponential decay of their signal in the water-suppressed PROBE spectra at different TEs and calculated as follows[1]:

$$S_{met}(TE) = S_{met}(0) \times e^{-TE/T_2},$$

where $S_{met}(TE)$ indicates the metabolite signal at a given TE, and $S_{met}(0)$, the extrapolated metabolite signal at TE = 0.

Water T2 relaxation in vivo is multiexponential,[2] and it is possible to deduce the T2 distribution of different water compartments within brain tissues and separate their contributions by fitting a multiexponential curve to the water signal decay. Spectra acquired at very short TEs can be used to provide quantitative estimates of the relative contribution of the myelin water compartment, but because the TEs we used for acquiring unsuppressed spectra are relatively long, the total water signal amplitude of the unsuppressed water spectra was fitted using a biexponential equation, which describes a 2-water compartment model,[3] namely the intracellular and extracellular, without distinguishing a specific compartment ascribable to the myelin water component within the intracellular component, according to the following equation:

$$W(TE) = S_{BW}(0) \times e^{-TE/T2_{BW}} + S_{CSF}(0) \times e^{-TE/T2_{CSF}},$$

where $W(TE)$ indicates the signal of total water at given TE; $S_{BW}(0)$ and $S_{CSF}(0)$, the signal of brain and cerebrospinal water, reflecting, respectively, the intra- and extracellular compartment, extrapolated at TE = 0. The unsuppressed water signal was used as an internal standard,[1,3] and metabolite concentrations were assessed as follows:

$$C_{met} = \frac{S_{met}(0)}{S_{BW}(0)} \times \frac{2}{N_{met}} \times (R \times M),$$

where C_{met} indicates the millimolar metabolite concentration; $S_{met}(0)$, the metabolite signal extrapolated at TE = 0 ms; $S_{BW}(0)$, the brain-water signal extrapolated at TE = 0; N_{met} the number of metabolite protons; R, the assumed fraction of water per gram of wet weight of brain tissue (0.65 for white matter)[1]; and M, the total water concentration (55,500 mmol/L).

With this method, the T2 of each of the 3 metabolites of interest (NAA, Cho, and Cr) was calculated for both healthy controls and 3 patients; in addition, the T2 of the water signals (T_{2BW} and T_{2CSF}) was calculated for all subjects. Metabolite concentrations were assessed for both control subjects and the patient group using the controls' mean T2 values for NAA, Cho, and Cr. The metabolite absolute concentrations of NAA, Cho, and Cr of patients were quantified extrapolating $S_{met}(0)$ using the S_{met} at TE = 35 ms.

Immunohistochemical Analyses

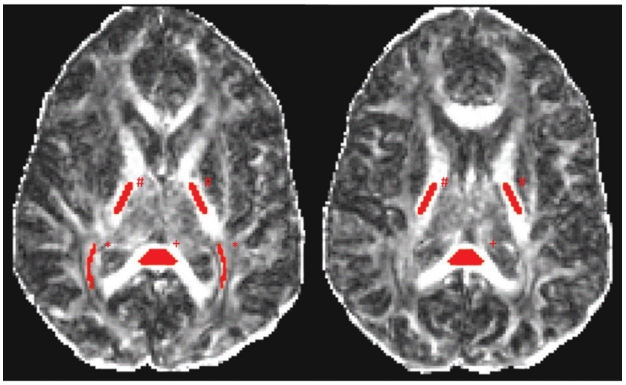
Antibodies against NeuN (Millipore; http://www.emdmillipore.com/US/en/product/NeuN-Antibodies,MM_NF-C87804), glial fibrillary acidic protein (Dako; www.dako.com), smooth-muscle actin (Dako), collagen IV (Dako), CD31 (Dako), CD68 (Dako), HLA-DR β-chain (clone CR3/43; Dako), Neurofilament heavy (Abcam; http://www.abcam.com/), β-Amyloid, 17–24 (clone 4G8; Signet), Phospho-PHF-tau pSer202+Thr205 (clone AT8; Innogenetics, Ghent, Belgium), Amyloid Precursor Protein (clone 348; Chemicon), α-synuclein (clone LB509, Life Technologies; https://www.thermofisher.com/us/en/home/brands/life-technologies.html), Mitochondria, 113–1 (Abcam), MTCOI (Mitosciences; http://mitosciences.com/), and COXIV (Mitosciences) were used for immunohistochemical staining.

Molecular Analysis on Postmortem Brain Samples

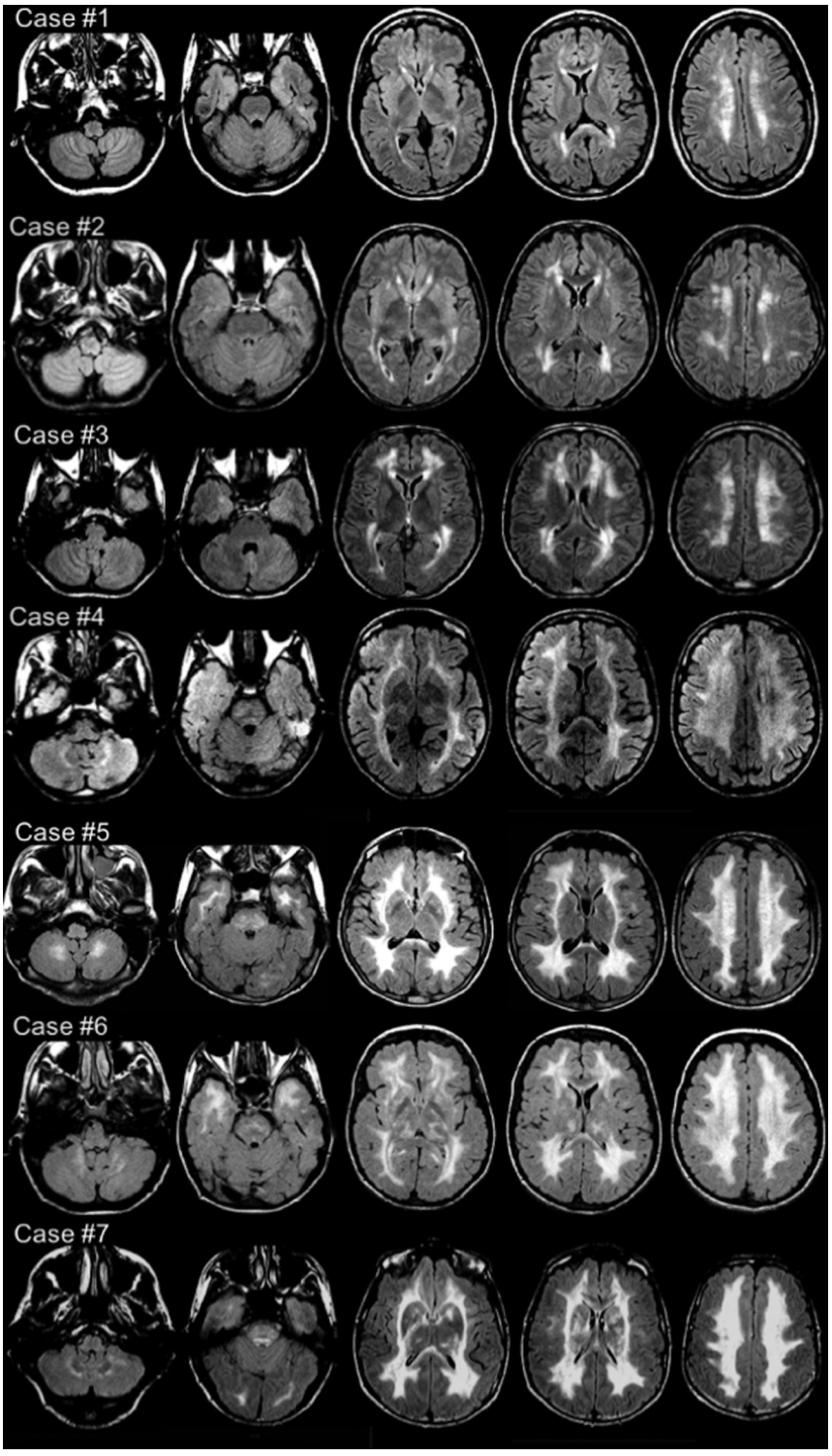
Serial 5-μm-thick cut sections were mounted on a polyethylene foil slide and stained with H&E. Sections were observed under light microscope with a ×40 objective. Between 50 and 100 smooth-muscle and endothelial cells from penetrating arteries and arterioles were microdissected and collected on an adhesive cap of nanotubes for nucleic acid extraction. Samples were digested with Proteinase K (20 mg/100 mL). The mtDNA copy number/cell was measured by a quantitative real-time polymerase chain reaction assay as previously published.[4]

REFERENCES

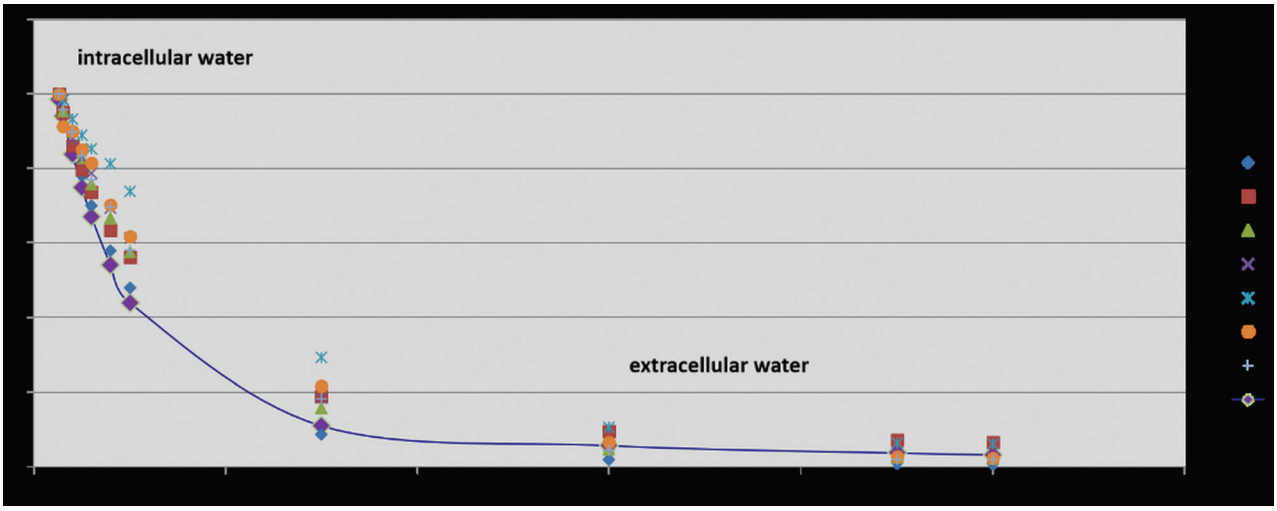
1. Kreis R, Ernst T, Ross BD. **Development of the human brain: in vivo quantification of metabolite and water content with proton magnetic resonance spectroscopy.** *Magn Reson Med* 1993;30:424–37 CrossRef Medline
2. Brief EE, Whittall KP, Li DK, et al. **Proton T2 relaxation of cerebral metabolites of normal human brain over large TE range.** *NMR Biomed* 2005;18:14–18 CrossRef Medline
3. Keevil SF, Barbiroli B, Brooks JC, et al. **Absolute metabolite quantification by in vivo NMR spectroscopy, II: a multicentre trial of protocols for in vivo localised proton studies of human brain.** *Magn Reson Imaging* 1998;16:1093–106 CrossRef Medline
4. Giordano C, d'Amati G. **Evaluation of gastrointestinal mtDNA depletion in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE).** *Methods Mol Biol* 2011;755:223–32 CrossRef Medline



ON-LINE FIG 1. Axial fractional anisotropy maps of patient 2 with the localization of the ROI in brain areas with more orthogonalized fibers such as the posterior limb of the internal capsule (*hashtag*), splenium of the corpus callosum (*plus sign*), and optic radiation (a*sterisk*).



ON-LINE FIG 2. MR imaging. Axial FLAIR T2 images from the 7 patients with MNGIE at supra- and infratentorial levels. Patients 6, 1, and 2 do not show any infratentorial involvement, while a variable extension of cerebral signal intensity increase can be seen in all patients with MNGIE.



ON-LINE FIG 3. Unsuppressed water signal in the white matter of the 7 patients with MNGIE and 9 controls (*solid line* represents mean values). The decay of the unsuppressed water signal was calculated by acquiring multiple TEs from 25 to 1000 ms in the parieto-occipital white matter. The signal acquired at short TEs (from 25 to 300 ms) is considered related to the intracellular water compartment at a longer TE than the extracellular one.

On-line Table. Brain distribution of increased signal intensity in FLAIR T2 and FSE T2 images in patients with MNGIE[a]

Patient No.	Distribution of Cerebral White Matter Abnormalities	CWM	Basal Ganglia	Brain Stem	Cerebellum	Optic Radiation	Corpus Callosum	PLIC
1	Mild and patchy involvement of periventricular white matter in frontal, parietal, and occipital lobes	+	−	−	−	+	+	−
2	Mild and patchy involvement of periventricular white matter in frontal, parietal, and occipital lobes	+	+	−	−	+	−	+
3	Patchy involvement of periventricular white matter in frontal, parietal, and occipital lobes	++	−	−	−	++	−	−
4	Diffuse involvement of white matter of frontal, parietal, occipital, and temporal lobes	++±	++	++	++	+++	+	+
5	Diffuse involvement of white matter of frontal, parietal, occipital, and temporal lobes	+++	++	+++	+++	+++	+	++
6	Diffuse involvement of white matter of frontal, parietal, occipital, and temporal lobes	+++	+++	++	+	+++	++	++
7	Diffuse involvement of white matter of frontal, parietal, occipital, and temporal lobes	+++	+++	+++	++	+++	++	+++

Note:—CWM indicates cerebral white matter.

[a] All brain MRI scans have been evaluated by a senior neuroradiologist (R.L.). The distribution of the cerebral white matter involvement was described and scored according to the severity of the involvement (− = absent; + = mild; ++ = moderate; +++ = severe), for the cerebral white matter, basal ganglia, brain stem, cerebellum, optic radiation, corpus callosum, and PLIC.