AJNR

MR imaging and proton MR spectroscopy in A-to-G substitution at nucleotide position 3243 of leucine transfer RNA.

J Bowen, T Richards and K Maravilla

AJNR Am J Neuroradiol 1998, 19 (2) 231-234
http://www.ajnr.org/content/19/2/231

AJNR Am J Neuroradiol 19:231–234, February 1998

MR Imaging and Proton MR Spectroscopy in A-to-G Substitution at Nucleotide Position 3243 of Leucine Transfer RNA

James Bowen, Todd Richards, and Kenneth Maravilla

Summary: MR imaging of the brain in a 38-year-old woman with maternally inherited diabetes and deafness (MIDD) showed extensive subcortical and basal ganglia high signal intensity on T2-weighted studies though she was neurologically asymptomatic. High-signal-intensity changes were also seen in the basal ganglia on T1-weighted studies. Proton MR spectroscopy showed increased lactate, an increased choline/creatine ratio, and a decreased *N*-acetylaspartate/creatine ratio. Our observations suggest that patients with MIDD may have subclinical neurologic dysfunction identifiable by proton MR spectroscopy.

Mutation of mitochondrial DNA has been increasingly recognized as a cause of maternally inherited disease (1). The genotypes of many mitochondrial diseases are now understood, and the phenotypes of mutations are being described. We present a case of maternally inherited diabetes and deafness (MIDD) with magnetic resonance (MR) imaging findings consistent with mitochondrial encephalopathy. Proton MR spectroscopic findings are also presented.

Case Report

Our patient had insulin-dependent diabetes mellitus diagnosed at age 22. She had mild renal insufficiency but no retinopathy or peripheral neuropathy. Her only neurologic symptom was slowly progressive sensorineural hearing loss, which was first noted in her mid-teens. She required hearing aids at age 33. Her medical history was notable for mild hypertension and hypercholesterolemia. Both parents had mild hearing impairment but were not formally assessed. Her brother has a similar hearing impairment and diabetes mellitus. At age 38, she was found to be mildly hypothyroid with decreased thyrotropin-stimulating hormone. Results of a thyrotropin-releasing-hormone stimulation test were equivocal. She also suffered a severe episode of diabetic ketoacidosis complicated by hypotension, a small subendocardial myocardial infarction, oliguric renal failure, pulmonary edema, and pneumonia. Cardiac catheterization was normal. On examination, she was 152 cm tall and had normal neurologic findings. Normal laboratory tests included visual evoked potentials, cerebrospinal fluid examination for IgG index and oligoclonal bands, skin biopsy with electron microscopy, arylsulfatase A, and very long chain fatty acids. At age 43, she had a mitochondrial DNA analysis demonstrating an A-to-G mutation at nucleotide position 3243 in an estimated 15% of genomes. She remains neurologically normal at age 44.

MR imaging revealed a normal pituitary and hypothalamic structures but abnormal brain findings. T2-weighted images showed extensive high-signal areas in the periventricular and subcortical white matter, basal ganglia, and pons. On T1-weighted images, there were subtle high-signal-intensity changes in the basal ganglia, including the caudate heads, posterior thalami, and putamina bilaterally. Atrophy of the cerebrum and cerebellum was present (Fig 1A).

Proton MR spectroscopy was performed using a point-resolved spectroscopic pulse sequence (2000/136 [repetition time/echo time]) and water suppression (2). Localized spectra were acquired from an 8-cm^3 voxel located in the periventricular white matter at the level of the corona radiata (Fig 1B–D). This voxel was selected because the mitochondrial disease was initially unrecognized and the extensive white matter changes suggested leukodystrophy. Also, data from healthy subjects using the same MR spectroscopic parameters were available from this region. Resonance areas for choline, creatine, and *N*-acetylaspartate (NAA) were determined by using lorentzian/gaussian curve-fitting and baseline-correction software developed in our laboratory. The software performed the following functions: 1) apodization (3-Hz line broadening) and Fourier transform of the raw data; 2) automatic phasing with a user option of making a small adjustment to the phase; 3) display of a small region of the spectrum around the choline and creatine resonances and a user-defined baseline on each side of the resonances; 4) calculation of a straight line between the user-defined baseline points; 5) fitting of the choline and creatine peaks to a lorentzian (20%)/gaussian (80%) mixed equation after baseline subtraction and adjustment of resonance height, width, and frequency to give minimum least squares; and 6) performance of similar procedures for the NAA peak except that it was fit as a single resonance. In cases with baseline distortions (due to adjacent lipid resonances), the baseline was chosen on both sides of the peak at an oblique angle. Data from 12 healthy volunteers were used to determine normal ratios of choline/creatine and NAA/creatine in this brain region.

The choline/creatine ratio for our patient was elevated at 1.40 (normal, 1.06 ± 0.17). The NAA/creatine ratio was 1.67 (normal, 2.01 ± 0.24). The lactate resonance was differentiated from lipid by its inversion at 1.3 ppm, although it was difficult to differentiate from background noise because of its small size. Elevated lactate was seen at 1.33 and 1.28 ppm along with other abnormal resonances at 0.9 to 1.2 ppm.

Received November 4, 1996; accepted after revision February 17, 1997.

From the Departments of Neurology (J.B.) and Radiology (T.R., K.M.), University of Washington, Seattle.

Address reprint requests to James Bowen, MD, Department of Neurology, Box 356427, University of Washington Medical Center, 1959 NE Pacific, Seattle, WA 98195.

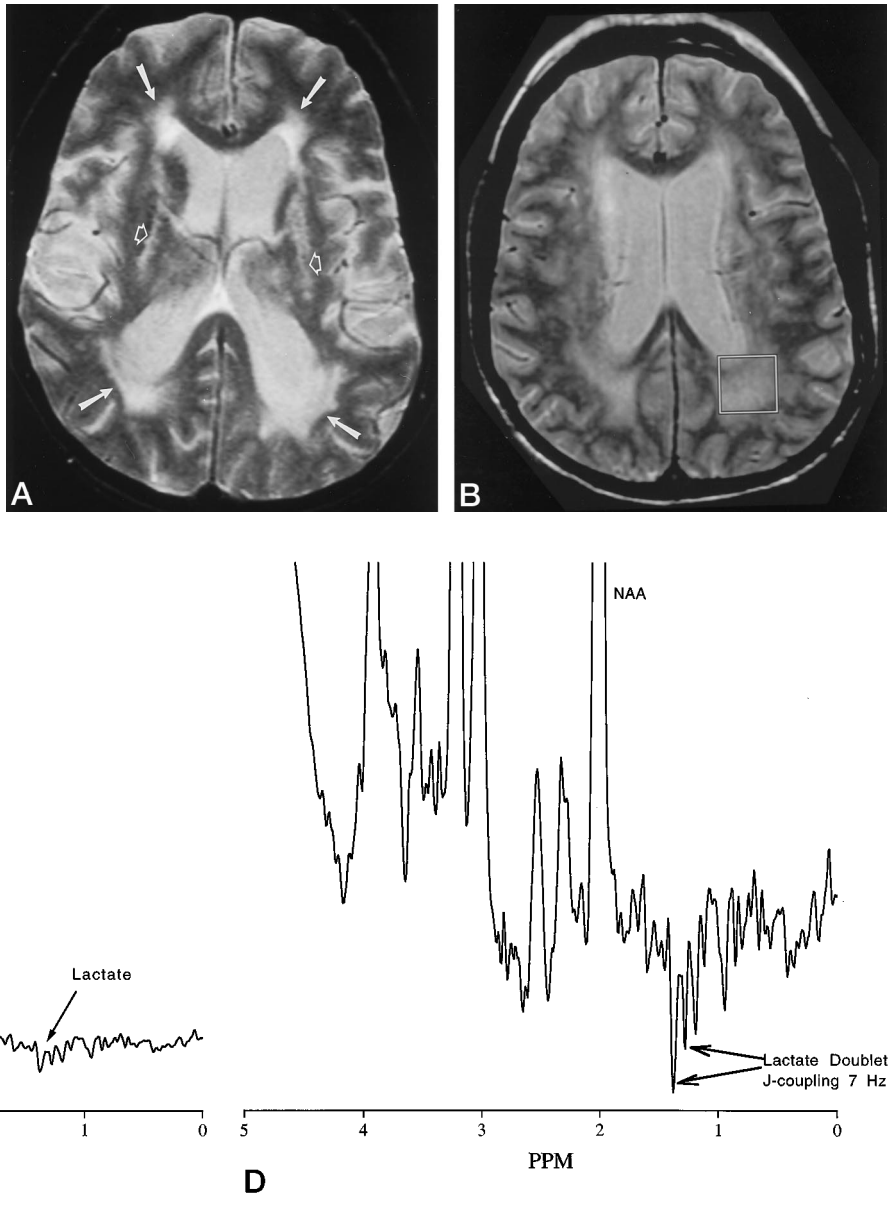

FIG 1. Woman with MIDD and A-to-G mutation at nucleotide position 3243 of $tRNA^{Leu(UUR)}$.

A, Axial T2-weighted image shows hyperintense signal within the globi pallidi bilaterally (*open arrows*) and in the periventricular white matter (*solid arrows*). Additional areas of hyperintense T2 signal were seen in the centrum semiovale bilaterally.

B, Axial T2-weighted image shows position of voxel.

C, Proton MR spectrum of an 8-cm^3 volume of brain in the posterior periventricular region as indicated in *B*. This spectrum has elevated choline/creatine, elevated lactate, and low NAA/creatine as compared with control subjects.

D, Magnified view clearly shows inverted lactate doublet.

Discussion

A number of syndromes caused by mutations in mitochondrial DNA are now recognized (1). These include conditions that affect the nervous system, such as Kearns-Sayre syndrome (KSS), Leigh syndrome, mitochondrial encephalopathy with lactic acidosis and strokelike episodes (MELAS), and myoclonus, epilepsy, and ragged red fibers (MERRF) (1). The retina and optic nerves are affected in Leber hereditary optic neuritis (1). Extraocular muscles are affected in KSS, progressive external ophthalmoplegia, and MELAS (1). Myopathy is found in a number of these syndromes, including MELAS, MERRF, and KSS (1). Sensorineural hearing loss may be found in MELAS, MERRF, KSS, and in neuropathy, ataxia, and retinitis pigmentosa (1). Maternally inherited diabetes and deafness (MIDD) is a relatively recent addition to the list of known mitochondrial diseases (3). Clinical findings in MIDD include sensorineural hearing loss and islet cell dysfunction.

Although many patients present with symptoms of recognized syndromes, some patients with mitochondrial diseases have overlapping symptoms from more than one syndrome. This may be due to differences in the proportion of mutant mitochondrial DNA in various tissues or to the presence of other concomitant mutations (4, 5). For example, some patients with KSS may exhibit some features of MELAS or MERRF (1). This is particularly true of patients with the A-to-G substitution at nucleotide position 3243 of leucine transfer RNA ($tRNA^{Leu[UUR]}$) (4, 5). This mutation is associated with MELAS and is also known to be the cause of MIDD (3, 6, 7). Other phenotypes have also been described in this mutation. Folgero et al (8) reported a pedigree with MERRF and the $tRNA^{Leu(UUR)}$ mutation. Sasagasako et al (9) reported a family with MIDD, one member of which also had MELAS. Crimmins et al (10) found cases of MELAS, MERRF, and KSS within a single kindred with the $tRNA^{Leu(UUR)}$ mutation; and deafness, short stature, and non–insulin-dependent diabetes mellitus were associated with many members of the kindred. Gerbitz et al (11) reported that 13% of 199 affected members of 45 families with the $tRNA^{Leu(UUR)}$ mutation had a combination of MIDD and MELAS; and deafness was the only manifestation in some patients. Wolfram syndrome consists of diabetes insipidus, juvenile diabetes mellitus, optic atrophy, and deafness. It differs from MIDD in that it has an autosomal recessive inheritance and the absence of mutation in the $tRNA^{Leu(UUR)}$ gene (12). Our case expands the findings within

the $tRNA^{Leu(UUR)}$ genotype to include clinical features of MIDD combined with the MR imaging abnormalities found in mitochondrial encephalopathies.

MR imaging abnormalities have been found in a number of mitochondrial disorders (13–15). Mitochondrial encephalopathies, including MELAS, MERRF, KSS, and Leigh disease, are characterized by T2 prolongation in the deep cerebral nuclei (13–15). The peripheral and retrotrigonal white matter may also show T2 prolongation (14). Patients with a combination of MIDD and MELAS have been reported to have "focal brain abnormalities and cortical atrophy" (16). One patient with MIDD, parkinsonism, myopathy, and supranuclear ophthalmoplegia showed "cerebral atrophy and mild atrophy of the cerebellar vermis as well as mild periventricular hyperintensities [on] T2-weighted images" (17). The T2-weighted abnormalities seen in our patient were similar to those described for other mitochondrial encephalopathies (14, 15). In addition, increased T1 signal intensity was also seen in the basal ganglia, probably as a result of basal ganglia calcification, which has been described with other mitochondrial diseases, although its pathogenesis remains uncertain in our patient (14).

The $tRNA^{Leu(UUR)}$ gene codes for a transfer RNA needed to transport leucine into the mitochondria for synthesis into mitochondrial proteins. Interference with this function leads to severe defects in protein synthesis and respiration. Bresolin et al (18) reported an increase in the ratio of phosphate to phosphocreatine and a decrease in the ratio of phosphocreatine to ATP in the occipital lobe of a patient with the $tRNA^{Leu(UUR)}$ mutation and "migrainous strokes." An increase in lactate is found in the peripheral blood (1) and brain (13, 14) of many patients with mitochondrial encephalopathies. Barkovich et al (14) reported an increase in lactate seen at MR spectroscopy in affected brain areas of patients with mitochondrial diseases. In our case, the lactate resonance was difficult to differentiate from background noise. Nevertheless, we were able to confidently identify the lactate resonance by its inversion at 1.3 ppm, a feature not found with lipid. Although the amount of lactate was low relative to the NAA or creatine peaks, it is an abnormal amount and consistent with mitochondrial disease. While other reports of mitochondrial disease may have described higher levels of lactate, these were generally obtained from spectra sampled in the basal ganglia and not the cerebral white matter (14). Also, our patient's relatively mild symptoms may reflect a relatively mild degree of mitochondrial metabolic disruption with only a mild increase in brain lactate. Our patient also had an increased choline/creatine ratio.

The *N*-methyl groups of choline resonate at 3.2 ppm in the proton spectra in vivo and contain contributions mainly from phosphorylcholine and glycero-3-phosphorylcholine (GPC) (19). Phosphorylcholine is a precursor molecule that is incorporated into phosphatidylcholine and sphingomyelin (20, 21), which are then incorporated into cell and myelin membranes in the brain (21). GPC is a catabolite of phosphatidylcholine (20, 22). The mild elevation of the choline peak seen in our patient may be related to alterations in membrane metabolism. The methyl groups of NAA and other *N*-acetyl compounds have a sharp resonance at 2.0 ppm in the proton spectra. The peak at 3.9 ppm is due to the CH2 protons next to the methyl group in the creatine molecule. The source of the peak at 3.7 ppm is unknown. NAA is a marker for neurons and may have a role in neuronal metabolism (23). A decrease in NAA has been attributed to axonal loss (24), active degradation of NAA in injured neurons (22), and gliosis (25). The mild decrease in the NAA/creatine ratio in our patient suggests that neuronal damage was mild, in agreement with the lack of neurologic symptoms.

Our case extends the phenotype of the $tRNA^{Leu(UUR)}$ mutation. Diabetes and deafness may be accompanied by changes on T2-weighted images without neurologic symptoms. The high-signal-intensity lesions on T1-weighted images have not been previously reported in mitochondrial disorders. Elevated brain lactate was demonstrated, consistent with a mitochondrial disorder. Subclinical dysfunction of axons and myelin was suggested by the MR spectroscopic findings of elevated choline and decreased NAA.

Acknowledgments

We thank Vern Terry and Denise Echlard for assistance in obtaining the MR images.

References

1. DiMauro S, Bonilla E, Lombes A, Shanske S, Minetti C, Moraes C. **Mitochondrial encephalomyopathies.** *Neurol Clin* 1990;8:483–506
2. Bottomley P. **Selective volume method for performing localized NMR spectroscopy.** US patent 4 480 228.1984
3. Kadowaki T, Kadowaki H, Mori Y, et al. **A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA.** *N Engl J Med* 1994;330:962–968
4. Hammans S, Sweeney M, Hanna M, Brockington M, Morgan-Hughes J, Harding A. **The mitochondrial DNA transfer RNALeu(UUR) A→G (3243) mutation: a clinical and genetic study.** *Brain* 1995;118:721–734
5. Moraes C, Ciacci F, Silvestri G, et al. **Atypical clinical presentations associated with the MELAS mutation at position 3243 of human mitochondrial DNA.** *Neuromuscul Disord* 1993;3:43–50
6. Goto Y, Nonaka I, Horai S. **A mutation in the tRNALeu(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies.** *Nature* 1990;348:651–653
7. Kobayashi Y, Momoi M, Tominaga K, et al. **A point mutation in the mitochondrial tRNALeu(UUR) gene in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes).** *Biochem Biophys Res Commun* 1990;173:816–822
8. Folgero T, Torbergsen T, Oian P. **The 3243 MELAS mutation in a pedigree with MERRF.** *Eur Neurol* 1995;35:168–171
9. Sasagasako N, Shida N, Yoshimura T, Kobayashi T, Goto I. **A family with MELAS whose main manifestations are maternally-transmitted deafness and diabetes mellitus.** *Rinsho Shiol* 1993;33:657–659
10. Crimmins D, Morris J, Walker G, et al. **Mitochondrial encephalomyopathy: variable clinical expression within a single kindred.** *J Neurol Neurosurg Psychiatry* 1993;56:900–905
11. Gerbitz K, van den Ouweland J, Maassen J, Jaksch M. **Mitochondrial diabetes mellitus: a review.** *Biocim Biophys Acta* 1995;1271:253–260
12. Barrett T, Bundey S, Macleod A. **Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome.** *Lancet* 1995;346:1458–1463
13. Castillo M, Kwock L, Green C. **MELAS syndrome: imaging and proton MR spectroscopic findings.** *AJNR Am J Neuroradiol* 1995;16:233–239
14. Barkovich A, Good W, Koch T, Berg B. **Mitochondrial disorders: analysis of their clinical and imaging characteristics.** *AJNR Am J Neuroradiol* 1993;14:1119–1137
15. Wray S, Provenzale J, Johns D, Thulborn K. **MR of the brain in mitochondrial myopathy.** *AJNR Am J Neuroradiol* 1995;16:1167–1173
16. Campos Y, Bautista J, Gutierrez-Rivas E, et al. **Clinical heterogeneity in two pedigrees with the 3243 bp tRNALeu(UUR) mutation of mitochondrial DNA.** *Acta Neurol Scand* 1995;91:62–65
17. Hara K, Yamamoto M, Anegawa T, Sakuta R, Nakamura M. **Mitochondrial encephalomyopathy associated with parkinsonism and a point mutation in the mitochondrial tRNA(Leu)(UUR) gene.** *Rinsho Shinkeigaku* 1994;34:361–365
18. Bresolin N, Martinelli P, Barbiroli B, et al. **Muscle mitochondrial DNA deletion and 31P-NMR spectroscopy alteration in a migraine patient.** *J Neurol Sci* 1991;104:182–189
19. Arus C, Chang Y, Barany M. **Proton nuclear magnetic resonance spectra of excised rat brain: assignment of resonances.** *Physiol Chem Phys Med NMR* 1985;17:23–33
20. Pettegrew JW, Kopp JS, et al. **31P nuclear magnetic resonance studies of phosphoglyceride metabolism in developing and degenerating brain: preliminary observations.** *J Neuropathol Exp Neurol* 1987;46:419–430
21. Tunggal B, Hofmann K, Stoffel W. **In vivo 13C nuclear magnetic**

resonance investigations of choline metabolism in rabbit brain. *Magn Reson Med* 1990;13:90–102
22. Bruhn H, Frahm J, Gyngell M, Merboldt K, Hanicke W, Sauter R. **Cerebral metabolism in man after acute stroke: new observations using localized proton NMR spectroscopy.** *Magn Reson Med* 1989; 9:126–131
23. Birken DL, Oldendorf W. **N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain.** *Neurosci Biobehav Rev* 1989;13:23–31
24. Arnold D, Matthews P, Francis G, Antel J. **Proton magnetic resonance spectroscopy of human brain in vivo in the evaluation of multiple sclerosis: assessment of the load of disease.** *Magn Reson Med* 1990;14:154–159
25. vanHecke P, Marchal G, Johannik K, et al. **Human brain proton localized NMR spectroscopy in multiple sclerosis.** *Magn Reson Med* 1991;18:199–206