ELSEVIER

Journal of the Neurological Sciences 214 (2003) 17–20

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Late onset of stroke-like episode associated with a 3256C→T point mutation of mitochondrial DNA

Tina Dysgaard Jeppesen[a,b,*], Marianne Schwartz[c], Klaus Hansen[b], Else R. Danielsen[d], Flemming Wibrand[e], John Vissing[a,b]

[a] *The Copenhagen Muscle Research Center, National University Hospital, Rigshospitalet, Copenhagen, Denmark*
[b] *Department of Neurology, National University Hospital, Rigshospitalet, Copenhagen, Denmark*
[c] *Department of Clinical Genetics, National University Hospital, Rigshospitalet, Copenhagen, Denmark*
[d] *Department of Radiology, National University Hospital, Rigshospitalet, Copenhagen, Denmark*
[e] *John F. Kennedy Institute, Glostrup, Denmark*

Received 4 February 2003; received in revised form 2 April 2003; accepted 19 May 2003

Abstract

Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) are usually associated with the common 3243A → G mutation of mtDNA. Onset of stroke-like episodes usually occurs before age 30. We report a patient with late onset MELAS harboring a rare 3256C → T mutation in the $tRNA^{Leu(UUR)}$ gene of mtDNA. The patient presented with a stroke-like episode at age 36. MRI showed a stroke-like lesion in the right parietooccipital brain region. Proton MR spectroscopy showed elevated lactate concentrations in the lesion (8.4 mmol/l), and in the mid-occipital region (2.3–3.2 mmol/l) that appeared normal on MRI. Further tests revealed evidence of a severe oxidative defect of muscle metabolism as well.

Keywords: mtDNA mutation; Stroke-like episodes; ^{31}P-Magnetic resonance spectroscopy; Lactate; Mitochondrial enzyme activity

1. Introduction

The clinical picture of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is usually associated with the most common mitochondrial DNA (mtDNA) mutation; the 3243A → G mutation in the $tRNA^{Leu}$ gene. The clinical description of the syndrome was described first by Pavlakis et al. [1] in 1984, and in 1990, Goto et al. [2] were the first to ascribe the common 3243A → G mutation as the cause of MELAS. Since this finding, additional 14 mutations in mtDNA have been found to cause MELAS syndrome [3].

Although diagnostic criteria dictate that stroke-like episodes have to occur before age 40 in MELAS [4], it very rarely occurs after age 30 [5]. Only a total of 11 patients in eight studies have been reported to have onset of MELAS after age 34. Most of these have had the common 3243A → G mutation [5,6], and two have had private point mutations of mtDNA [7,8]. In the present study, we report a patient with the rare 3256C → T point mutation of mtDNA who presented with an acute stroke-like episode at the age of 36.

2. Case report

2.1. Historical background

The patient was a 36-year-old woman, who was admitted to the hospital because of acute behavioral changes. On examination, the patient was confused and had a homonym left-sided upper quadrant anopsia. The patient presented with generalized seizures on admission. EEG showed focal, paroxysmal activity in the right pre-temporal region. She was treated with levetiracetam after loading with phenytoin, and no further attacks occurred. Over the course of a month, the patient recovered slowly, but had transient episodes of psychotic behavior. After 2 months, the patient was back to normal. The patient had a short stature (154 cm) and low body weight (45 kg).

* Corresponding author. Department of Neurology 2082, National University Hospital, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. Tel.: +45-35-45-25-62; fax: +45-35-45-26-26.
E-mail address: dysgaard@rh.dk (T.D. Jeppesen).

0022-510X/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0022-510X(03)00168-0

The patient was normal at birth and had normal motor milestones, but since her early teenage years, mild exercise induced premature fatigue. She could never keep up with her peers when cycling and she had difficulties walking up stairs. In the year before the stroke-like episode, she had difficulties fulfilling her job as a schoolteacher because she had memory problems and felt stressed. A hearing test had revealed a slight hearing impairment a year earlier.

The patient's mother had epilepsy and at the age of 62 she presented with sudden onset of dementia that was diagnosed as Pick's disease, although her MRI was normal. She died at age 64, 10 years before our patient was diagnosed. The patient's only sibling, a sister, had short stature and was physically very weak. She died 37 years before our patient was diagnosed, at the age of eight, with a presumed diagnosis of encephalitis, although spinal fluid examinations could not corroborate the clinical suspicion. It is likely that the fatal incident represented a stroke-like episode. No tissues from the mother or sister were available for analysis. The only other maternal relative, a 79-year-old maternal aunt, did not have neurological symptoms and declined further investigations.

2.2. Histochemistry, biochemistry and genetic investigations

A needle biopsy was performed in the lateral vastus muscle, and stained for trichrome, cytochrome oxidase and succinate dehydrogenase to evaluate the presence of ragged red fibers and COX-negative fibers. Mitochondrial enzyme activities of complexes I, II and IV were measured in postnuclear supernatants of frozen muscle as described previously [9].

Total genomic DNA from blood and muscle was isolated, amplified and analyzed for mutation according to previously described procedures [9].

2.3. Brain imaging

A CT scanning of the brain with and without contrast injection performed at admission was normal. T2- and diffusion-weighted MRI, using a 1.5-T Siemens vision scanner, was performed 2 days, 8 days, 1 month and three months after admission, and proton magnetic resonance spectroscopy (^{1}H-MRS) was performed in conjunction with the three last MRIs. Single photon emission computerized tomography (SPECT) was performed 3 days after admission, using a brain-dedicated gamma camera—Ceraspect (DSI, Waltham, MA, USA).

2.4. Cycle ergometry

Six months after discharge, the patient was studied on a cycle ergometer (MedGraphics CPE 2000) operated via a MedGraphics CPX/D cardiopulmonary exercise test system that measured gas exchanges, workload and heart rate. VO_{2max} and maximal workload was determined by an incremental exercise test to exhaustion with increments of 10 W every other minute. Blood samples were collected from the median cubital vein at rest and at exhaustion and analyzed for plasma lactate levels.

3. Results

3.1. Morphological, biochemical and molecular genetic investigations

The patient's skeletal muscle had 10% ragged red and no COX-negative fibers. Sequencing of the mitochondrial genome revealed the presence of a 3256C → T point mutation in the $tRNA^{Leu(UUR)}$ gene. The mutation was heteroplasmic with a mutant mtDNA/total mtDNA ratio of 85% in skeletal muscle and 35% in blood.

Muscle biochemistry showed the following citrate synthase corrected activities: complex I 18%, complex II 97%, and complex IV 70%, of the normal mean found in 13 age-matched controls. Both complex II and IV activities were within the normal reference range.

3.2. Brain imaging

MRI revealed a high signal area in the upper right pretemporal and parietooccipital regions (Fig. 1) and a small high signal area in the left side of the cerebellum. Hyperemia was detected in the same regions with SPECT scanning of the brain. Eight days after admission, ^{1}H-MRS revealed elevated lactate concentrations in the parietooccipital lesion (8.4 mmol/l), but lactate levels were also elevated in grey matter of the mid-occipital region (2.3 mmol/l) that appeared normal on MRI (Fig. 1). One and three months after admission, MRI showed partial and complete regression, respectively, of the lesion areas in the brain. MRS out side the lesion areas showed consistently higher lactate concentrations in grey vs. white matter regions. Thus, in the recovery phase, MRS revealed consistently elevated lactate concentrations in grey matter of the former stroke-like lesion (2.8 and 2.2 mmol/l) and in grey matter that appeared normal on MRI (mid-occipital region, 2.3 and 3.2 mmol/l; frontal, 2.5 mmol/l). In contrast, lactate concentrations in central white matter, and white matter of the parietooccipital region were either undetectable (<1 mmol/l) or mildly elevated (2.0 mmol/l). The resting venous plasma lactate was elevated (3.1 mmol/l) 2 months after the stroke-like episode.

3.3. Oxidative capacity assessed by cycle ergometry

During the cycle test, the patient reached a peak workload of 67 W at a heart rate of 167. VO_{2max} was low (900 ml/min) in the patient compared to that in seven age-matched, sedentary women (2155 ± 94 ml/min). Peak plas-

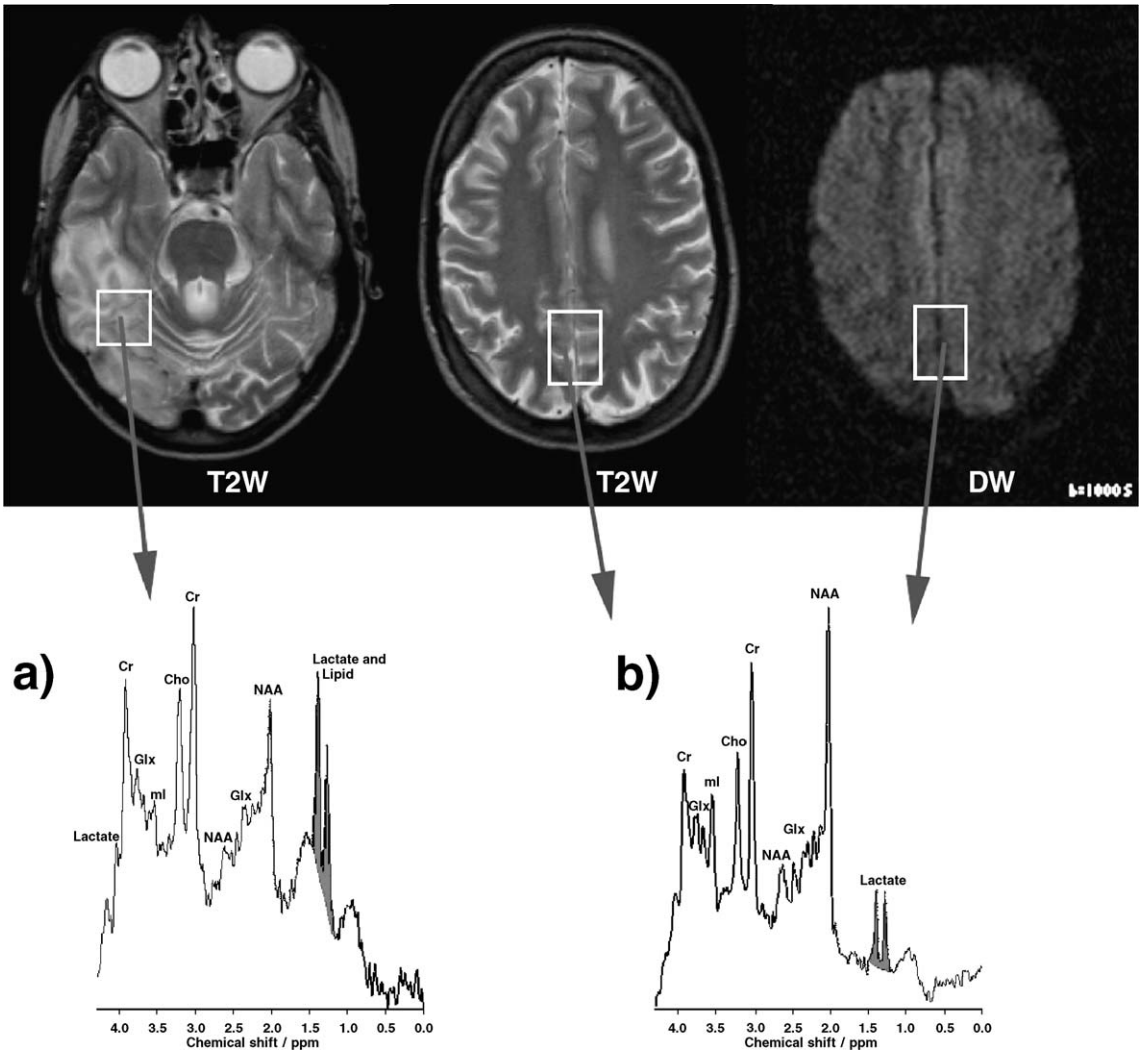

Fig. 1. Left brain slice: A T2-weighted MRI of the brain from a patient with MELAS syndrome showing a high signal area in the upper right pre-temporal and parietooccipital regions. Middle brain slice: A more coronal T2-weighted MRI of the brain showing no lesions. A diffusion-weighted (DW) MRI (right brain slice) from the same slice also appeared normal. ^{1}H-MR spectra were sampled from volumes of interest (inserted boxes on images), using stimulated echo acquisition mode (echo time = 20 ms, repetition time = 1500 ms). ^{1}H-MRS showed normal metabolite ratios of *N*-acethylaspartate/creatine (NAA/Cr), choline/creatine (Cho/Cr), and myoinositol/creatine (mI/Cr) in the stroke-like lesion and in normally appearing brain tissue on MRI. Brain lactate levels, as indicated by the red peaks on the spectra, were elevated in both the stroke-like lesion (a) and in normally appearing tissue (b).

ma lactate concentration was 10.1 mmol/l in the patient, and 6.8 ± 0.6 in controls, although controls reached a significantly higher peak workload (160 ± 11 W).

4. Discussion

Stroke-like episodes in patients with MELAS syndrome usually occur before the age of 20 [5,6]. In this study, we report a patient with a late onset of stroke-like episode associated with the 3256C → T mutation of mtDNA. This mutation has only been reported in three families [10–12]. In two families, the mutation was associated with diabetes [10] or a multisystem disorder [11]. In the third family, a patient presented with recurrent focal seizures, hemiplegia and hemianopia at the age of 10. Besides the uncommon mutation and age of presentation, our patient had classical features of a mitochondrial disease, with exercise intolerance since adulthood, low oxidative capacity and exaggerated lactate responses to cycle exercise, hearing impairment, elevated resting plasma lactate levels, ragged red fibers on muscle biopsy, markedly reduced complex I activity in muscle and a heteroplasmic occurrence of the mutation in muscle and blood.

Patients with mutations of the mtDNA $tRNA^{Leu}$ gene very often lack COX-negative fibers on muscle biopsy [11]. Biochemically, tRNA gene mutations are often associated with a decrease in complex I activity, whereas a decrease in complex IV, in line with histological findings, is less consistent [13]. Our patient had a clear reduction in complex I activity, whereas complex IV activity was on the lower threshold of the normal reference range. In line with the

variable reduction of complexes I and IV in 3243A → G patients [13], a more profound reduction of complex IV activity has also been reported for the 3256C → T [11].

The pathogenesis of stroke-like episodes is unknown. An angiopathy caused by mitochondrial dysfunction of cells in the vessel walls has been suggested to be causative. A number of reports suggest that the mutation load in brain tissue may influence the occurrence of stroke-like episodes, but only one autopsy study of three patients have provided direct evidence for this [6]. The mutation type likely plays a smaller role than the mutation load in brain tissue for the development of MELAS, since many different mtDNA mutation types can give MELAS, and since mtDNA mutations that may produce MELAS often result in other phenotypes. A similar correlation between mutation load and symptoms in the same tissue has recently been described for skeletal muscle [14].

Epileptic activity in the brain is directly correlated with a high metabolic rate in the involved neuronal tissue. Patients with mutations in the mitochondrial $tRNA^{Leu}$ gene have a high prevalence of epilepsy [15]. MELAS could be elicited by regional epileptic activity that requires a metabolic rate that exceeds the oxidative capacity of the brain region. In accordance with this, our patient had focal paroxysmal activity in the stroke-like lesion, but not elsewhere in the brain. If focal paroxystic activity is involved in eliciting stroke-like episodes in patients with mitochondrial disease, pharmacological antiepileptic treatment should be prescribed routinely to these patients. However, further studies are needed to elucidate the pathogenesis of MELAS syndrome in mitochondrial disease.

In this study, elevated lactate concentrations were predominantly detected in grey matter of the brain. Eight studies have evaluated brain metabolism of MELAS patients by ^{1}H-MRS in the acute phase of a stroke-like episode. Three of these studies have found differences in lactate accumulation between white and grey matter similar to that found in the present study [16]. This finding is in accordance with the neuropathological evidence of multifocal encephalomalacia preferentially occurring in the grey matter of MELAS patients.

The present study emphasizes the importance of considering mitochondrial disease as a differential diagnosis to stroke in younger adults.

References

[1] Pavlakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes: a distinctive clinical syndrome. Ann Neurol 1984;16:481–8.

[2] Goto Y, Nonaka I, Horai S. A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 1990;348:651–3.

[3] Hanna MG, Nelson IP, Morgan-Hughes JA, Wood NW. MELAS: a new disease associated mitochondrial DNA mutation and evidence for further genetic heterogeneity. J Neurol Neurosurg Psychiatry 1998;65:512–7.

[4] DiMauro S, Bonilla E, Davidson M, et al. Mitochondria in neuromuscular disorders. Biochim Biophys Acta 1998;1366:199–210.

[5] Ciafaloni E, Ricci E, Shanske S, et al. MELAS: clinical features, biochemistry, and molecular genetics. Ann Neurol 1992;31:391–8.

[6] Macmillan C, Lach B, Shoubridge EA. Variable distribution of mutant mitochondrial DNAs (tRNA(Leu[3243])) in tissues of symptomatic relatives with MELAS: the role of mitotic segregation. Neurology 1993;43:1586–90.

[7] Bataillard M, Chatzoglou E, Rumbach L, et al. Atypical MELAS syndrome associated with a new mitochondrial tRNA glutamine point mutation. Neurology 2001;56:405–7.

[8] Santorelli FM, Tanji K, Kulikova R, et al. Biochem Biophys Res Commun 1997;238:326–8.

[9] Wibrand F, Ravn K, Schwartz M, Rosenberg T, Horn N, Vissing J. Multisystem disorder associated with a missense mutation in the mitochondrial cytochrome *b* gene. Ann Neurol 2001;50:540–3.

[10] Hirai M, Suzuki S, Onoda M, et al. Mitochondrial deoxyribonucleic acid 3256C–T mutation in a Japanese family with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1998;83:992–4.

[11] Moraes CT, Ciacci F, Bonilla E, Jansen C, Hirano M, Rao N, et al. Two novel pathogenic mitochondrial DNA mutations affecting organelle number and protein synthesis. Is the tRNA(Leu(UUR)) gene an etiologic hot spot? J Clin Invest 1993;92:2906–15.

[12] Sato W, Hayasaka K, Shoji Y, et al. A mitochondrial tRNA(Leu)(UUR) mutation at 3256 associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). Biochem Mol Biol Int 1994;33:1055–61.

[13] Mariotti C, Savarese N, Suomalainen A, et al. Genotype to phenotype corelations in mitochondrial encephalomyopathies associated with the A3243G mutation of mitochondrial DNA. J Neurol 1995;242:304–12.

[14] Jeppesen TD, Schwartz M, Olsen DB, Vissing J. Oxidative capacity correlates with muscle mutation load in mitochondrial myopathy. Ann Neurol 2003 [in press].

[15] Chinnery PF, Howell N, Lightowlers RN, Turnbull DM. Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes. Brain 1997;120:1713–21.

[16] Willichowski E, Pouwels PJ, Frahm J, Hanefeld F. Quantitative proton magnetic resonance spectroscopy of cerebral metabolic disturbances in patients with MELAS. Neuropediatrics 1999;30:256–63.