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A novel tRNA^{Val} mitochondrial DNA mutation causing MELAS

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Abstract

Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is the most common mitochondrial disease due to mitochondrial DNA (mtDNA) mutations. At least 15 distinct mtDNA mutations have been associated with MELAS, and about 80% of the cases are caused by the A3243G tRNA$^{Leu(UUR)}$ gene mutation. We report here a novel tRNA^{Val} mutation in a 37-year-old woman with manifestations of MELAS, and compare her clinicopathological phenotype with other rare cases associated tRNA^{Val} mutations.

Keywords: tRNAVal; Mitochondrial DNA; Stroke; MELAS

1. Introduction

Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) is, after Leber hereditary optic neuropathy (LHON), the second most common mitochondrial disease due to mitochondrial DNA (mtDNA) mutations [1]. Although at least 15 distinct mtDNA mutations have been associated with MELAS, about 80% of patients harbor the A3243G tRNA$^{Leu(UUR)}$ gene mutation [2]. Mitochondrial disorders due to mutations in the tRNA^{Val} of mtDNA are rare, but five heteroplasmic point mutations have been associated with encephalomyopathies. The first (G1642A) was identified in two unrelated patients with the MELAS phenotype [3,4]; the second (G1644T) in two siblings with adult-onset Leigh syndrome (LS) [5]; the third (G1606A) in two unrelated patients with a similar "complex" phenotype that included hearing loss, cataracts, ataxia, myoclonus, and dementia [6,7]; and the fourth (T1659C) in a patient with psychomotor developmental delay and mental retardation, right hemidystonia, and mild hypertrophic cardiomyopathy [8]. The fifth mutation (C1624T) was homoplasmic and caused infantile and fatal LS [9]. Here, we report a patient with MELAS, who had a previously undescribed and apparently *de novo* G-to-A mutation at nucleotide position 1644 in the tRNA^{Val} gene.

2. Case report

2.1. Patient

A 37-year-old woman emigrated from China to the United States in 2006. In 2007, she had a seizure and was given phenytoin. Subsequently, she developed right temporal headache, visual hallucinations involving insects, and had 5 more generalized tonic–clonic seizures. At another hospital, she showed aphasia, left hemiplegia and left hemineglect. Brain MRI showed restricted diffusion and T2 FLAIR hyperintensity in the right thalamus and temporal,

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parietal and occipital lobes, with contrast enhancement of the adjacent meninges (Figs. 1 and 2). She refused a lumbar puncture. She was given ceftriaxone 2 g IV and acyclovir 500 mg IV and as transferred to Columbia University Medical Center. Her medical history included two spontaneous abortions and one successful pregnancy. She had had no headaches, visual changes, hearing loss, vertigo, diabetes, gastrointestinal problems, fever, chills, or weight loss. There was no family history of neurological disease. Her mother is alive and well, and one brother and five sisters do not have any neurological signs or symptoms.

2.2. Examination

She was awake and oriented with a mild left hemiparesis and hyperreflexia. CSF contents were normal. Continuous EEG monitoring showed frequent runs of right posterior hemisphere sharp waves. Levetiracetam was added with good response. An MR spectroscopy of the right temporal lobe showed a small lactate peak, decreased NAA, and slightly increased choline (Fig. 3). Arterial lactate was normal (0.7 mM/L; normal range:0. 5–1.6). A muscle biopsy was performed to confirm the diagnosis. At the time of discharge after a month-hospital stay, she still had the mild left hemiparesis with hyperreflexia and normal speech.

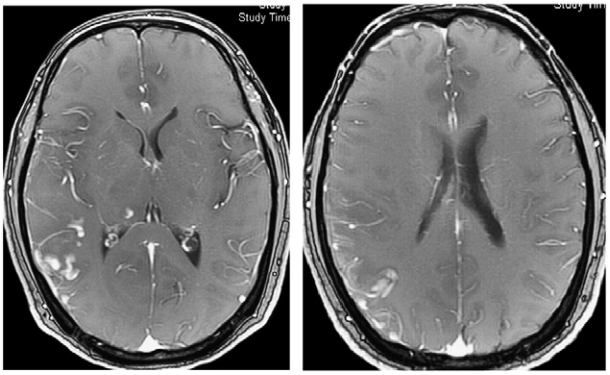

Fig. 2. Brain MRI. T1 weighted images after the administration of contrast show scattered areas of irregular abnormal cortical enhancement in the affected regions on the right side (arrows).

Approximately a month later, she was readmitted for episodic restlessness, anxiety, echolalia, profuse irrelevant speech, possible visual and auditory hallucinations, and brief

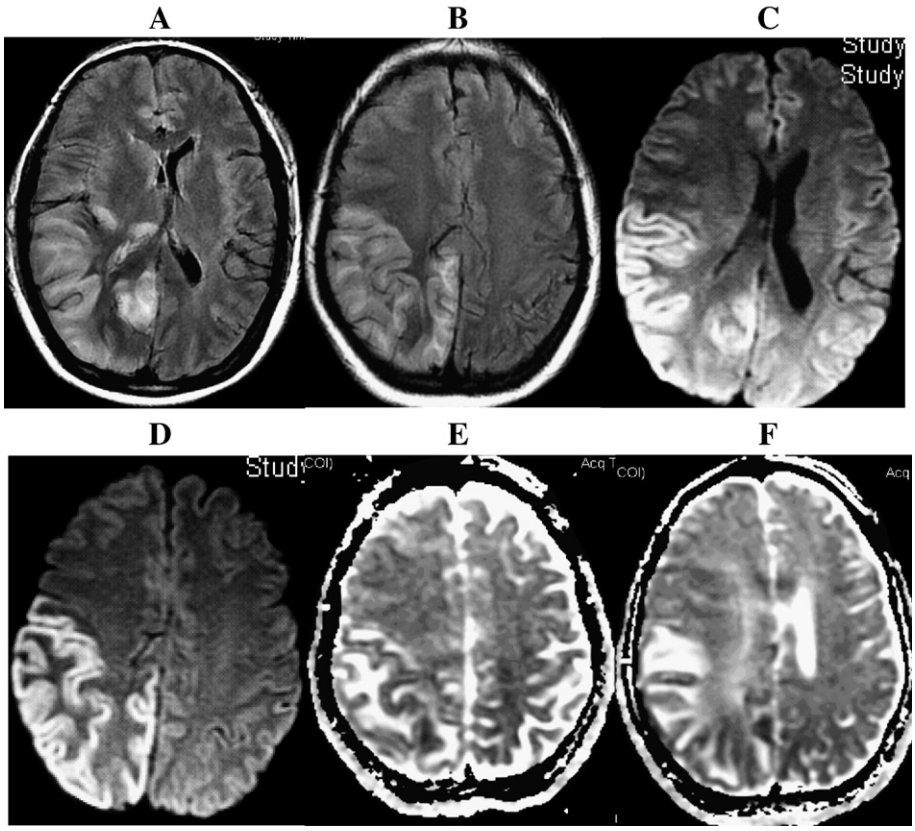

Fig. 1. Brain MRI. A and B: Axial fluid attenuated inversion recovery (FLAIR) images show increased signal intensity and sulcal effacement involving a large portion of the right medial occipital and parietal temporal regions. C and D: The diffusion-weighted image obtained in this patient shows increased cortical signal (restricted diffusion) but decreased white matter signal (vasogenic edema). E and F: The corresponding apparent diffusion coefficient (ADC) maps confirm slightly decreased diffusion in the cortex (ischemia and cytotoxic edema) as compared to the contralateral side. The subcortical white matter has increased diffusion (non-restricted) consistent with vasogenic edema.

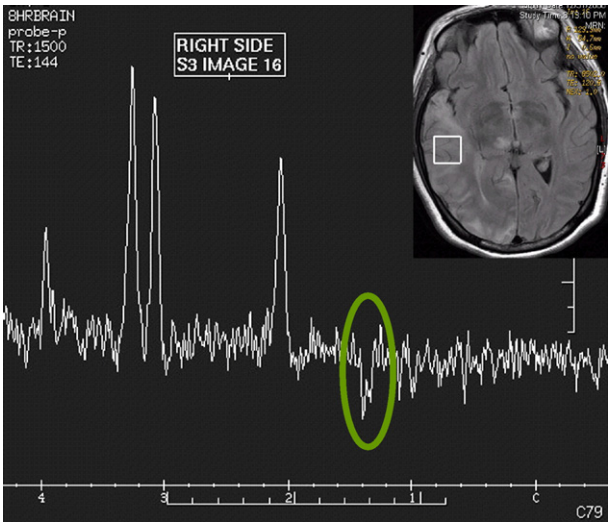

Fig. 3. MR spectroscopy. Magnetic resonance spectroscopy obtained at a TE value of 144 shows a small but definite lactate peak (circle) in the sampled region of the right temporal lobe. The other metabolites specifically *N*-acetylaspartate (NAA) is slightly decreased, and choline (cho) is slightly elevated.

episodes of unresponsiveness. Brain MRI showed no new lesions, but a decrease in the previously noted FLAIR hyperintensities. Continuous EEG monitoring during episodes of confusion and agitation was negative for seizure activity. Her behavior improved with quetiapine and she was discharged home. When last seen on a follow-up visit, her behavior was normal and there was only a trace of left-sided weakness.

3. Results

3.1. Morphology and biochemistry

Muscle biopsy showed sparse, mildly atrophic fibers by conventional hematoxylin–eosin. Histochemical analysis showed early signs of segmental mitochondrial proliferation in 1% to 2% of myofibers, which was well documented by succinate dehydrogenase (SDH) (Fig. 4A). In addition, strongly SDH-reactive vessels (SSVs) were also observed (Fig. 4B). The cytochrome *c* oxidase (COX) histochemical stain revealed varying degrees of COX deficiency in approximately 25% of myofibers (Fig. 4C). Biochemical analysis showed normal activities of respiratory chain complexes (data not shown).

3.2. DNA sequencing

Total genomic DNA was extracted from skeletal muscle using standard protocols. After sequencing $tRNA^{Leu(UUR)}$, the gene most commonly involved in MELAS, the other 21 mitochondrial *tRNA* genes were amplified by PCR using oligonucleotide primers designed with the Primer 3 program (Whitehead Institute, MIT, Cambridge, MA). PCR fragments were sequenced using the BigDye Terminator v3.1 Cycle sequencing reaction kit and an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) and sequences were compared to the revised Cambridge reference sequence [10]. This sequence analysis revealed a G- to -A transition at nucleotide position 1644 in the tRNA gene for valine (Fig. 5). This site is highly conserved across different species [11]. The mutation was absent in more than 500 control mtDNAs. Single or multiple deletions of mtDNA were excluded using long-range PCR methodology as described previously [12]. Only a single band of 16 kb representing intact mitochondrial DNA was observed on the gel indicating the absence of mtDNA deletion.

3.3. PCR/restriction fragment length polymorphism (RFLP) analysis

The level of G1644A heteroplasmy in the $tRNA^{val}$ was determined by last hot cycle technique as described previously [13]. A 184 bp-fragment encompassing the site of mutation was amplified. Polymerase chain reaction conditions were 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. After 35 cycles, 4 μCi of α^{32}P-dCTP (3000 Ci/mmol) was added and a last cycle was performed. Aliquots (10 μl) of PCR products were digested with *TSP* 509I restriction enzyme overnight and electrophoresed on 15% non-

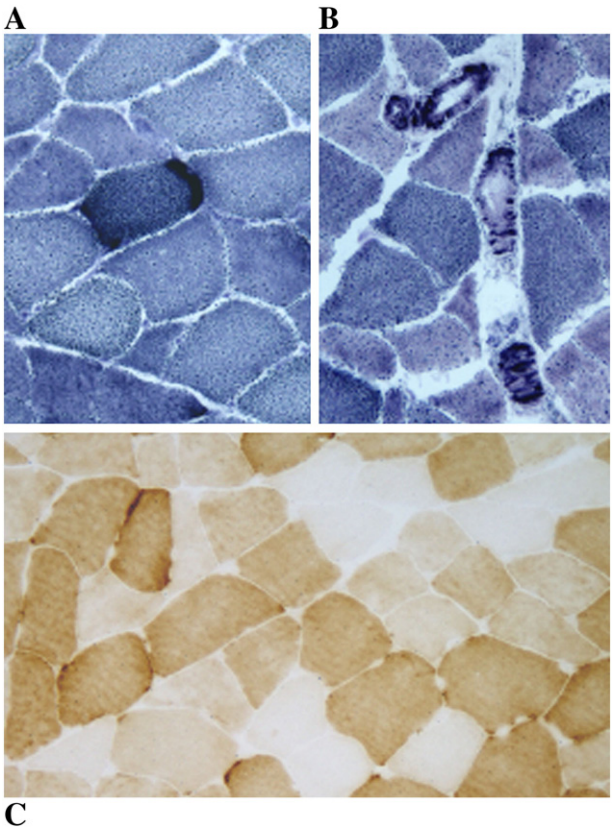

Fig. 4. Muscle biopsy. The muscle biopsy shows a few 'pre-ragged' red fibers (A. SDH, ×200), SSVs (B. SDH, ×200), and COX-negative fibers (C. cytochrome *c* oxidase, ×200). These COX-negative fibers account for roughly 25% of myofibers in the section.

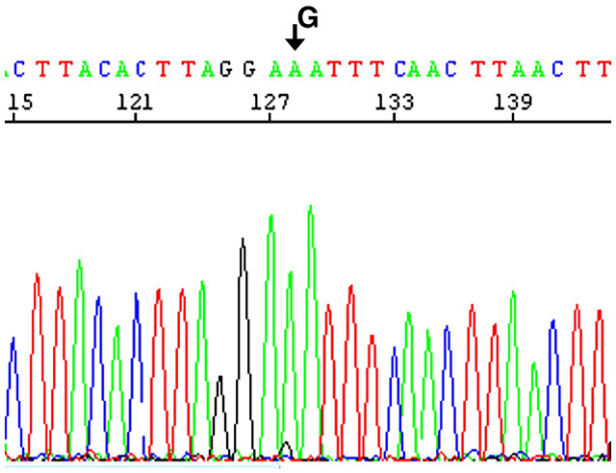

Fig. 5. DNA sequence analysis. The DNA sequence analysis discloses a G- to -A transition at nucleotide position 1644 in the $tRNA^{Val}$ gene (arrow).

denaturing acrylamide gel. In the wild type, the enzyme cuts the 184 bp-PCR product into two fragments, 164 bp and 20 bp. The G1644GA transition creates a new *TSP* 509I restriction site in the mutant PCR product, cutting the 164 bp-fragment into additional two fragments, 112 and 52 bp in length. The proportion of mutant mtDNA was evaluated in a phosphoimager (Molecular Analyst; BioRad, Hercules, Calif) using image-Quant software (Molecular Dynamics, Sunnyvale, Calif). The mutation was heteroplasmic, and the patient harbored 85% mutant mtDNA in her muscle (Fig. 6). To confirm pathogenicity, we intended to perform single fiber PCR, but we ran out of muscle and could not convince the patient to undergo a second biopsy, not even a needle biopsy.

4. Comment

The young age of this patient at the time of stroke and seizures together with radiographic findings raised the possibility of MELAS [14,15], despite the lack of maternal inheritance, normal arterial lactate level, and the small brain MRS lactate peak. Muscle biopsy confirmed the clinical impression of mitochondrial disease because it showed segmental proliferation of mitochondria in a few fibers detected by hyperintense SDH stain ("pre-ragged red" fibers), SSVs, and COX-negative fibers. Muscle biopsies from most MELAS cases, especially those associated with the most common mutation, A3243G in the $tRNA^{Leu(UUR)}$ gene, have COX-positive RRFs and SSV [16]. In contrast, the pre-RRFs and SSVs in our patient were COX-negative, which made us suspect that the putative mtDNA mutation might not be the common A3243G. In fact, we did find a novel heteroplasmic mutation in the $tRNA^{Val}$ gene (G1644A). This mutation may lead to a conformational change of the $tRNA^{Val}$ and result in a faulty translation of the mitochondrial genome either directly or by altering the splicing of 12S and 16S ribosomal RNAs, which "bracket" the $tRNA^{Val}$ [5]. Our patient had no known maternal family history of neurological diseases, which may indicate that the new mutation occurred *de novo*. Unfortunately, due to adamant lack of cooperation on the part of this patient, we could not verify the presence of the mutation in easily accessible non-muscle tissues, such as leukocytes or urinary sediment. To date, five point mutations in the $tRNA^{Val}$ gene have been associated with mitochondrial encephalopathy. Two patients with the G1642A mutation had typical MELAS [3,4]. Two other unrelated patients with the G1606A mutation had a complex but similar phenotype characterized by severe and early hearing loss, cataracts, myoclonus, ataxia, and dementia [6,7]. A third mutation (T1659C) was found in a young woman, who had psychomotor delay in childhood and later developed hemiplegia, dystonia, and mild hypertrophic cardiomyopathy [8].

A fourth mutation (G1644T) caused adult-onset LS in two siblings with a history of maternal inheritance [5]. This transversion mutation is of particular interest, because our patient had a transition mutation at the very same position (G1644A). Although it is puzzling that two mutations at the very same mtDNA site should result in different clinical phenotypes, the muscle biopsy findings in our patient and in two siblings with LS showed similar changes [5]. Both lacked typical ragged-red fibers by Gomori trichrome, while occasional fibers showed peripheral enhancement of SDH activity. COX-negative fibers were 11% and 13% of all fibers in the LS cases, and 25% in our patient. Notably, however, SSVs were not described in the patients with LS. SSVs are characteristically found in mitochondrial encephalomyopathies [17,18], including MELAS, and can affect not only skeletal muscle but also brain and intestinal vessels [19–21]. This focal and segmental proliferation of vascular mitochondria in MELAS is thought to be a compensatory reaction to the

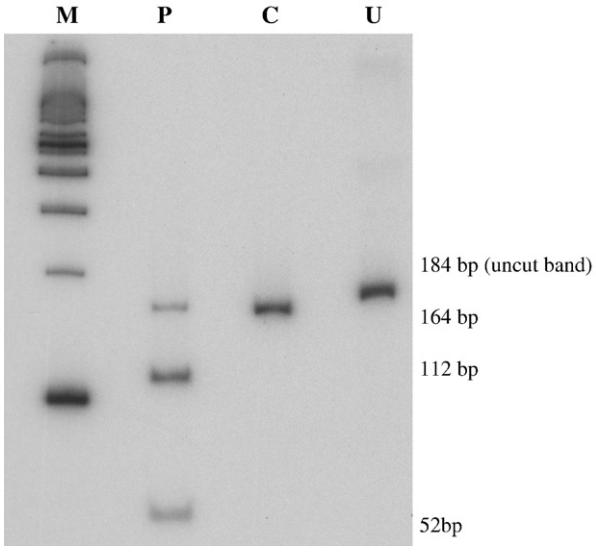

Fig. 6. RFLP analysis. RPLP analysis of DNAs from a normal control and the patient. In the patient's DNA, the G1644A mutation creates an additional Tsp509I restriction site, resulting in two additional fragments, 112 and 52 base pair in length. The proportion of mutant mtDNA in the patient is 85%.

respiratory chain dysfunction, which has been well documented at autopsy by histochemical and immunohistochemical COX deficiency [22,23]. Correspondingly, radiologic studies have indicated breakdown of blood–brain-barrier (BBB) [24,25], a finding also supported by our patient's diffusion-weighted MRI abnormalities (Figs. 1 and 2).

In conclusion, we found a novel heteroplasmic mtDNA mutation, associated with MELAS. Transfer RNA^{Val} mutations are rare, but should be considered when the typical changes of MELAS muscle pathology, such as COX-positive RRFs, are not evident and the more common mtDNA mutations associated with MELAS are not found.

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