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Novel Mitochondrial DNA tRNA^{Val} Mutation in MELAS: A Second Mutation at Nucleotide 1644 Causing the Same Clinical Syndrome

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Abstract

Background—More than 30 different mutations in mitochondrial DNA(mtDNA) have been associated with mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS), one of the most common mitochondrial encephalopathies.

Objective—To describe a novel mutation in the tRNA^{Val} gene of mtDNA in a patient with clinical features of MELAS.

Design—Case report.

Patient—A 37-year-old woman had encephalopathy with seizures, stroke, and psychiatric symptoms. A muscle biopsy specimen showed pre-ragged red fibers and strongly succinate dehydrogenase-reactive blood vessels.

Results—Biochemical analysis showed normal respiratory chain enzyme activities. Molecular analysis revealed a novel heteroplasmic mutation (G1644A) in the tRNA^{Val} gene of mtDNA. A different mutation at the same location has also been associated with MELAS.

Conclusions—tRNA^{Val} mutations are rare causes of mitochondrial encephalomyopathy. Our data show that G1644A should be added to the long list of mtDNA mutations associated with MELAS.

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 four heteroplasmic point mutations have been associated with encephalomyopathies. The first (G1642A) was identified in two unrelated patients with the MELAS phenotype [3, 4] (Taylor et al 1996; de Coo et al 1998); 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]. A 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.

REPORT OF A CASE

PATIENT

A 37-year-old woman emigrated from China to the United States in May 2006. In early December she had a seizure and was given phenytoin. On December 16, 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, parietal, and occipital lobes, with contrast enhancement of the adjacent meninges [Fig. 1, 2]. She refused lumbar puncture. She was given ceftriaxone 2g IV and acyclovir 500mg IV and then transferred to the Columbia University Medical Center on December 26.

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.

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 (0.7mM/L; normal range: 0.5-1.6). A muscle biopsy was performed to confirm the diagnosis. At the time of discharge (date?), she still had the mild left hemiparesis with hyperreflexia and normal speech.

A month later, she was readmitted for episodic restlessness, anxiety, echolalia, profuse irrelevant speech, possible visual and auditory hallucinations, and brief 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 showed no seizure activity. Her behavior improved with quetiapine and she was discharged home on (date). When last seen on (date) her behavior was normal and there was only a trace of left-sided weakness.

RESULTS

Morphology and Biochemistry

Muscle biopsy showed sparse, mildly atrophic fibers by conventional hematoxylin-eosin. Succinate dehydrogenase (SDH) histochemical analysis showed early signs of segmental mitochondrial proliferation in 1% to 2% of myofibers [Fig. 4A]. In addition, strongly SDH-reactive vessels (SSV) 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).

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]. [Is it necessary to include

these methodological details in the Results section?] 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]. [I presume that the mutation was absent in controls and, if so, this result should be noted here.] Single or multiple deletions of mtDNA were excluded using long-range PCR methodology as described previously [12]. Only a single band of 16kb representing intact mitochondrial DNA was observed on the gel indicating the absence of mtDNA deletion.

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]. Polymerase chain reaction conditions were 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. After 35 cycles, 4 µCi of α³²P-dCTP (3000 Ci/mmol) were 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% nondenaturing acrylamide gel. [Again, it is necessary to include detailed methods in the Results?] The G1644GA transition creates *TSP* 509I restriction site in the mutant PCR product, cutting the amplicon into additional two fragments 93, and 57 base pair in length. The proportion of mutant mtDNA was evaluated in a phosphorimager (Molecular Analyst; BioRad, Hercules, Calif) using Image-Quant software (Molecular Dynamics, Sunnyvale, Calif). The mutation was heteroplasmic, and the patient harbored 91 % mutant mtDNA in her muscle [Fig. 6].

COMMENT

The youth 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 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 mutation 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 a possible 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. To date, five point mutations in the tRNA^{Val} gene have been associated with mitochondrial encephalopathy. Two patients with a G1642A mutation had typical MELAS [3, 4]. Two other unrelated patients with a different mutation (G1606A) 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 important because our patient had a transition mutation at the very same position (G1644A). Although it is puzzling that a different mutation 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 with the Gomori trichrome stain, while occasional fibers showed peripheral enhancement of SDH activity. COX-negative fibers accounted for 11% and 13% of the total in 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 gut vessels [19, 20, 21]. This focal and segmental proliferation of mitochondria in MELAS vasculature is thought to be a compensatory reaction to the respiratory chain dysfunction, which has been well documented by histochemical and immunohistochemical COX deficiency in MELAS autopsy cases [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 [Figure: Dr. Khanji's contribution].

In conclusion, we found a novel heteroplasmic mtDNA mutation 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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Figure 1.

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) map 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.

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Figure 2.
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).

Figure 3.

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), choline (cho), and creatine (cr) are not affected.

Figure 4. The muscle biopsy shows a few 'pre-ragged' red fibers (A. SDH, x200), SSV (B. SDH, x200), and COX-negative fibers (C. cytochrome *c* oxidase, x200). These COX-negative fibers account for roughly 25% of myofibers on the section.

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

M: molecular size marker

P: patient

C: normal control

Figure 6.

RPLP analysis of DNAs of a normal control and the patient. In the patient's DNA, the G1644A mutation creates a restriction site in the mutant PCR, creating additional two fragments, 93 and 57 base pair in length. The proportion of mutant mtDNA in the patient is 91%.