Published in final edited form as:

Muscle Nerve. 2011 September ; 44(3): 448–451. doi:10.1002/mus.22149.

MERRF and Kearns-Sayre overlap syndrome due to the mtDNA m.3291T>C mutation

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Abstract

A 48-year-old man presented with a complex phenotype of myoclonus epilepsy with ragged-red fibers (MERRF) syndrome and Kearns-Sayre syndrome (KSS): progressive myoclonus epilepsy, cerebellar ataxia, hearing loss, myopathic weakness, ophthalmoparesis, pigmentary retinopathy, bifascicular heart block, and ragged-red fibers. The m.3291T>C mutation in the tRNA^{Leu(UUR)} gene was found with 92% heteroplasmy in muscle. This mutation has been reported with MELAS, myopathy, and deafness with cognitive impairment. This is the first description with a MERRF/KSS syndrome.

Keywords

Mitochondrial DNA (mtDNA); Point mutation; tRNA^{Leu(UUR)}; Myoclonus epilepsy; ragged-red fibers; Kearns-Sayre syndrome

1. Introduction

Since the identification of the first pathogenic mutations in human mitochondrial DNA (mtDNA) 23 years ago,^{1,2} hundreds of large-scale mtDNA rearrangements and over 200 mtDNA point mutations have been described.³ Point mutations of mtDNA cause a wide spectrum of syndromes, and most of them are in transfer RNA (tRNA) genes. Although some mtDNA mutations tend to be associated with specific clinical syndromes, genotype-phenotype correlations are imprecise. Many individuals who harbor a pathogenic mtDNA mutation manifest overlapping features of typical mitochondrial syndromes.

Myoclonus epilepsy with ragged-red fibers (MERRF) is a multisystem disorder characterized by myoclonic seizures, cerebellar ataxia, mitochondrial myopathy, and ragged-red fibers (RRF) on muscle biopsy, but other common signs include dementia, hearing loss, optic atrophy, peripheral neuropathy, and spasticity. About 80% of MERRF cases are caused by the m.8344A>G mutation in the tRNA^{Lys} gene.⁴

In contrast, Kearns-Sayre syndrome (KSS) is a mitochondrial disorder characterized by the onset before age 20 of progressive external ophthalmoplegia or pigmentary retinopathy,

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together with at least one of a triad of cerebellar ataxia, heart block and elevated cerebrospinal fluid protein. Typically, KSS is due to single large-scale deletions of mtDNA and is almost always sporadic.⁵ Here, we report the second case of an overlap syndrome with features of MERRF and KSS in which there is a point mutation in the tRNA^{Leu(UUR)} gene (m.3291T>C).

2. Case report

A 48-year-old man with a history of premature graying of hair beginning at age 20, hearing loss at age 32, and depression presented with chief complaints of progressive loss of balance and gait instability with leg weakness for five years. At age 43, he noted intermittent gait imbalance and felt unstable while sitting or standing. Neurological examination revealed mild clumsiness of rapid alternating movement and fine finger movements on the left, and a Romberg sign. Brain magnetic resonance imaging showed cerebral and cerebellar atrophy (Fig. 1A–C). At age 45, he developed myoclonus epilepsy. An EEG showed atypical irregular 2.5–3.5 Hz spike and wave complexes. The patient was successfully treated initially with lamotrigine, then with levetiracetam.

There was no history of consanguinity. The mother developed myoclonus epilepsy in her 70s. No other family members presented with similar features.

At age 48, neurological examination showed normal cognition, mild cerebellar dysarthria, atypical pigmentary retinopathy with normal optic discs (Fig. 1D), and abnormal eye movements with right eye amblyopia and exotropia, left exophoria, mild bilateral limitation of eye adduction, and saccadic pursuits. In addition, he had mild proximal leg weakness, left greater than right, dysmetric fine finger movements and heel-to-shin maneuvers, and impaired tandem gait. Sensation and tendon reflexes were normal.

Laboratory tests revealed a mildly elevated creatine kinase of 398 U/L (normal <200) and resting lactate level of 24.1 mg/dL (normal < 22), with normal glucose, thyroid function tests, parathyroid hormone, and liver function panel.

An electrocardiogram (ECG) showed left posterior fascicular and incomplete right bundle branch blocks with right atrial enlargement. Other cardiac function tests were normal, including 30 day ECG loop recording, transthoracic echocardiogram, cardiac MRI and tilt table test. Nerve conduction studies and electromyography did not detect any sign of myopathy or neuropathy.

2.1 Material and methods

A right quadriceps muscle biopsy was analyzed with standard histological and histochemical stains. Biochemical analysis of mitochondrial enzymes in muscle extracts was performed using described techniques.⁶

DNA was extracted from muscle by standard procedures. Screening for mtDNA large-scale rearrangements was performed on muscle DNA by Southern blot as described.⁷ DNA from other tissues was not available. All 22 mitochondrial tRNA genes were amplified using oligonucleotide primers. The PCR fragments were then analyzed using BigDye[®] Termination v3.1 Cycle Sequencing kit with an Applied Biosystem 3130xl Genetic Analyzer.

To quantitate the mutation level, a restriction fragment length polymorphism (RFLP) study was performed on DNA extracted from muscle. A 205 base-pair (bp) fragment was obtained with the following PCR conditions: one cycle of 94 °C for 5 min, and 35 cycles of 94 °C for

1 min, 55 °C for 1 min and 72 °C for 1 min, and a final extension step of 72 °C for 7 min. α -³²P-dCTP (3000 Ci/mmol) was added prior to the last PCR cycle. Wild-type mtDNA was cut into three fragments (96, 77 and 32 bp), while mutant mtDNA was digested into two fragments (173 and 32 bp) by the enzyme *Tsp509I*. The digestion products were separated in a 15% non-denaturing acrylamide gel and analyzed in phospho-imager (Molecular Analyst, BioRad, Hercules, CA) using Image-Quant software (Molecular Dynamics, Sunnyvale, CA) to assess the mutation load.

2.2 Results

Muscle biopsy demonstrated markedly increased RRF on modified Gomori trichrome staining and numerous ragged-blue fibers on succinate dehydrogenase reaction (SDH). Biochemical analysis of respiratory chain enzymes was normal (Table 1).

Genetic tests were negative for the common MERRF mtDNA mutations (m.3243A>G, m.8344A>G, m.8356T>C, m.8363G>A, m.8296A>G), and no deletions in mitochondrial DNA were detected by Southern blot analysis.

Direct sequencing of the 22 mtDNA tRNA genes revealed a T-to-C transition at nucleotide position 3291 in the tRNA^{Leu(UUR)} gene. PCR-RFLP showed 92% mutant genome in muscle.

3. Discussion

This patient's clinical manifestations together with the presence of elevated resting lactate blood level, cerebral and cerebellar atrophy on brain MRI, partial bifascicular cardiac conduction block, RRF on muscle biopsy and myoclonus epilepsy in the mother, suggested a mitochondrial disease. Specifically, the patient appeared to have an overlap syndrome with features of both MERRF and KSS. Myoclonus epilepsy (usually manifesting earlier than in our patient), sensorineural hearing loss, premature graying and ragged-red fibers on muscle biopsy are features of the MERRF syndrome. Brain MRI usually shows brain atrophy with or without basal ganglia calcification.⁸ In contrast, ophthalmoparesis, pigmentary retinopathy, partial heart block, and diffuse white matter MRI alterations are features of the KSS syndrome.^{9,10} Cerebellar ataxia is common in both conditions.

Direct sequencing of the patient's muscle DNA revealed a heteroplasmic mtDNA T-to-C transition at nt.3291 in the tRNA^{Leu(UUR)} gene. The m.3291T>C mutation affects an evolutionarily highly conserved nucleotide in the T- ψ -C loop of the tRNA^{Leu(UUR)} cloverleaf.¹¹ In 2004, Hao and colleagues demonstrated that the m.3291T>C mutation impaired mitochondrial tRNA^{Leu(UUR)} aminoacylation due to instability of the mutated tRNA structure, which might also inhibit aminoacylation of the wild-type tRNA.¹² Furthermore, the mutant tRNA showed partial deficiency of the normal taurine modification at the wobble position, which is required for precise and efficient codon recognition.¹³

Point mutations in the tRNA^{Leu(UUR)} gene have been associated with diverse phenotypes, ranging from mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), to isolated myopathy, myopathy and cardiomyopathy, chronic progressive external ophthalmoplegia, and diabetes mellitus with or without deafness.³ In particular, the m.3291T>C mutation was proven to be pathogenic in previous reports.^{11,14,15} The first patient described by Goto *et al.* in 1994 was a 7-year-old Japanese boy affected by MELAS.¹¹ His skeletal muscle biopsy showed numerous RRF and strongly SDH reactive vessels. Biochemical assays revealed no definite deficiencies of respiratory chain enzymes in muscle. The mutation load in this patient was 86% in muscle, which is similar to our patient. Six years later, Uziel *et al.* found the same mutation in a 7-year-old Italian girl who

presented with a 1-year history of progressive leg weakness. A muscle biopsy revealed a mild myopathy associated with COX (cytochrome oxidase)-negative fibers and RRF.¹⁴ Biochemical assays on muscle homogenate showed severely reduced activities of complexes I, III, and IV. The patient's muscle contained approximately 87% mutant mtDNA. In 2009, a complete mtDNA analysis carried out in a large cohort of patients with mitochondrial disorders detected the same mutation in 2 additional patients, a 51-year-old with isolated mild myopathy and a 12-year-old with deafness and cognitive impairment.¹⁵ Both muscle biopsies showed RRF/COX-negative fibers, and muscle biochemistry revealed normal respiratory chain activities in the patient with isolated myopathy and decreased complex I activity in the patient with deafness and cognitive impairment. Mutation loads were not reported.

As described, patients who harbor the m.3291T>C mutation have presented with different phenotypes. Age at onset ranged from childhood to adulthood. Clinical phenotype varied from isolated myopathy to more complex neurological disorders that included deafness and cognitive impairment, MELAS, and, as we describe here, MERRF/KSS overlap syndrome. Muscle biopsy always showed mitochondrial abnormalities, but COX-negative fibers were variably present. The biochemical consequences of the mutation were also different. Despite high mutation loads, both our patient and the patient with MELAS had virtually normal respiratory chain activities,^{11,14} whereas the patient with isolated myopathy described by Uziel *et al.* had markedly reduced activities of complexes I, III and IV.¹⁴

From a clinical point of view, our patient is strikingly different from the others, as he is the first example of MERRF/KSS overlap syndrome associated with the m.3291T>C mutation in the tRNA^{Leu(UUR)} gene. Interestingly, one previous report described a patient who also had features of MERRF and KSS but harbored a different m.3251A>G mutation within the DHU-arm of the same gene.¹⁶ However, compared to ours, that patient's phenotype had notable differences, as it included endocrinological problems and migraine headache while lacking cerebellar ataxia, depression, and premature graying. Muscle biochemistry also differed. Although the patient with the m.3251A>G mutation had a lower mutation load (53%) than ours, the activities of complexes I, III and IV were markedly decreased.

In conclusion, the mechanisms by which mutations in tRNA affect respiratory chain activities and how this correlates with the clinical phenotype are still largely unanswered questions. Our data reinforce the well-established concept that in mtDNA related disorders, the same genetic abnormality can be associated with a wide spectrum of phenotypes, and the observation that tRNA^{Leu(UUR)} is a hotspot for mtDNA mutations.

Acknowledgments

This work has been supported by NIH Grant HD32062 and by the Marriott Mitochondrial Disorders Clinical Research Fund (MMDCRF). MH is supported by grants from the NIH (R01HD57543, R01HD056103, and RCNS070232) and by the Muscular Dystrophy Association.

Abbreviations

COX	cytochrome oxidase
ECG	electrocardiogram
EEG	electroencephalogram
KSS	Kearns-Sayre syndrome
MELAS	mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes

MERRF	myoclonus epilepsy with ragged-red fibers
MRI	magnetic resonance imaging
mtDNA	mitochondrial DNA
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RRF	ragged-red fibers
SDH	succinate dehydrogenase
tRNA	transfer RN

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Figure 1.

(A,C) Neuroradiologic findings. A–B: Transverse sections of FLAIR brain MRI study sequences showing cortical atrophy. C: Sagittal section of T1-weighted brain MRI study sequence showing cerebellar atrophy. (D) Left fundus photograph showing pigmentary retinopathy.

Table 1

Mitochondrial respiratory chain enzyme activities in muscle

Enzyme (complex)	Control (mean $\pm$ SD)	Patient (% of mean control values)
Cytochrome c oxidase (IV)	2.80 $\pm$ 0.52	2.61 (93.2%)
Succinate cyt. <i>c</i> reductase (II and III)	0.70 $\pm$ 0.23	0.74 (105.71%)
NADH [*] -cyt. <i>c</i> reductase [†] (I and III)	1.02 $\pm$ 0.38	0.75 (73.53%)
NADH [*] -dehydrogenase (I)	35.48 $\pm$ 7.07	29.90 (84.27%)
Succinate dehydrogenase (II)	1.00 $\pm$ 0.53	1.39 (139%)
Citrate synthase	9.88 $\pm$ 2.55	9.50

Activity expressed as $\mu\text{mol/min/g}$. Controls as means $\pm$ standard deviations, $n = 78$.

* nicotinamide adenine dinucleotide-dehydrogenase.

[†] Cyt *c* reductase, cytochrome *c* reductase.