



## Short communication

Mutation in the mitochondrial tRNA<sup>lle</sup> gene causes progressive myoclonus epilepsy

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## ABSTRACT

**Purpose:** The group of the rare progressive myoclonic epilepsies (PME) include a wide spectrum of mitochondrial and metabolic diseases. In juvenile and adult ages, MERRF (myoclonic epilepsy with ragged red fibres) is the most common form. The underlying genetic defect in most patients with the syndrome of MERRF is a mutation in the tRNALys gene, but mutations were also detected in the tRNAPhe gene.

**Method:** Here, we describe a 40 year old patient with prominent myoclonic seizures since 39 years of age without a mutation in the known genes who underwent intensive clinical, genetic and functional workup.

**Results:** The patient had a slight mental retardation and a severe progressive hearing loss based on a defect of the inner ear on both sides. Ictal electroencephalography (EEG) showed bilateral occipital and generalized spikes and polyspikes induced and aggravated by photostimulation. A cranial magnetic resonance imaging (cMRI) detected a global cortical atrophy of the brain and mild periventricular white matter lesions. The electromyography (EMG) was normal but the muscle biopsy showed abundant ragged red fibres. Sequencing of the mitochondrial DNA from the skeletal muscle biopsy revealed a novel heteroplasmic mutation (m.4279A>G) in the tRNA<sup>lle</sup> gene which was functionally relevant as tested in single skeletal muscle fibre investigations.

**Conclusion:** Mutations in tRNA<sup>lle</sup> were described in patients with chronic progressive external ophthalmoplegia (CPEO), prominent deafness or cardiomyopathy but, up to now, not in patients with myoclonic epilepsy. The degree of heteroplasmy of this novel mitochondrial DNA mutation was 70% in skeletal muscle but only 15% in blood, pointing to the diagnostic importance of a skeletal muscle biopsy also in patients with myoclonic epilepsy.

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## 1. Introduction

Progressive myoclonus epilepsies (PME) are a group of rare syndromes including a broad spectrum of neurodegenerative diseases.<sup>1</sup> Main characterizing features are myoclonic seizures

which are defined as sudden, brief involuntary movements without loss of consciousness. Myoclonus can be induced by voluntary movements, sensory or tactile triggers. In adulthood, MERRF (myoclonic epilepsy with ragged red fibres) and MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes) might be the most common forms. Mutations were found in the mitochondrial DNA such as tRNA<sup>Lys</sup> in case of MERRF or in tRNA<sup>Leu</sup> in MELAS. In both syndromes, mutations were also found in tRNA<sup>Phe</sup>.<sup>2</sup> Patients with MERRF present with a combination of epilepsy including myoclonic seizures inducible by photic stimulation, myopathy with ragged red fibres, progressive deafness, dementia and optic atrophy. Rare symptoms are cardiomyopathy, retinitis pigmentosa and pyramidal signs. In up to 80% of patients, one specific mutation (m.8344A>G) in tRNA<sup>Lys</sup> was described.<sup>3</sup> Prominent heteroplasmy, the variable abundance of the mutation in analyzed tissues, seems to be the

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pathophysiological background of the broad variability in the clinical syndrome.<sup>4</sup> Electroencephalography (EEG) can show slow background activity and bursts of generalized or focal epileptic discharges. In brain magnetic resonance imaging (cMRI), global atrophy and white matter lesions as well as calcifications of the basal ganglia were detected.<sup>5</sup> Here, we describe a patient who presents with a classical MERRF phenotype with prominent myoclonic seizures, but does not carry the typical m.8344A>G mutation.

## 2. Methods

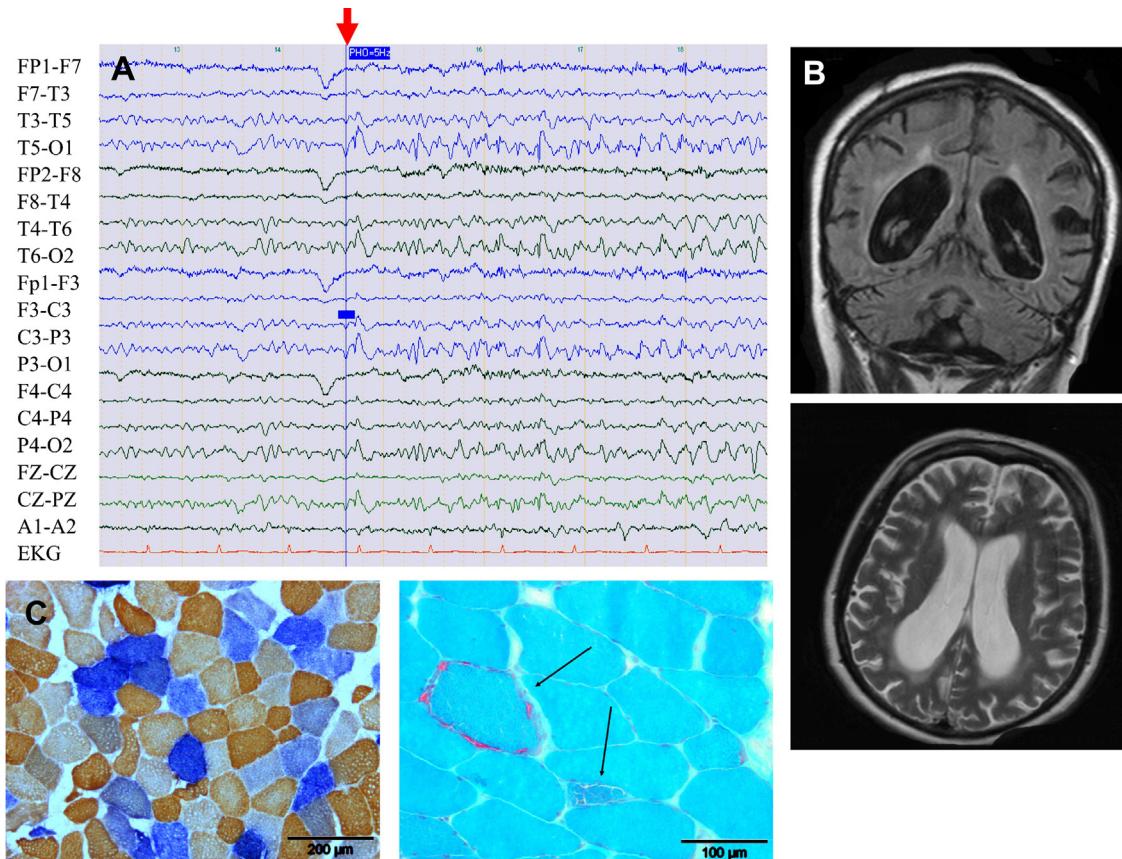
**Standard protocol approvals and patient consent.** The study was conducted according to the guidelines of the Ethical Committees of the University Ulm, Bonn and Tübingen, Germany. Written informed consent was obtained from all individuals.

**Muscle histology.** Skeletal muscle biopsy was performed according to standard procedures. For histological and histochemical analysis, 10 µm frozen sections were stained with haematoxylin and eosine, modified Gomori trichrome, cytochrome c oxidase (COX) and succinate dehydrogenase (SDH).

**Biochemical investigations.** Cytrate synthase activities were determined by standard methods. The activity of NADH:CoQ oxidoreductase (complex I) was measured using a dual-wavelength spectrophotometer (Aminco DW 2000, SLM Instruments, Rochester, NY, USA) at 340/380 nm ( $\epsilon_{\text{red-ox}} = 5.5 \text{ mM}^{-1} \text{ cm}^{-1}$ ). Cytochrome c oxidase (complex IV) activity was measured by monitoring the oxidation of ferrocytochrome c in its  $\beta$ -band at the wavelength pair 510/535 nm ( $\epsilon_{\text{red-ox}} = 5.9 \text{ mM}^{-1} \text{ cm}^{-1}$ ).<sup>6</sup>

**DNA mutation analysis.** Total DNA was extracted from 10 ml aliquots of EDTA blood using a salting-out method, and from muscle biopsy specimens using the QiaAmp DNA Mini Kit (Qiagen, Germany). For direct sequence analysis of the entire mitochondrial genome, 28 overlapping PCR fragments were amplified from skeletal muscle DNA and sequenced by a commercial sequencing service (Eurofins MWG Operon, Germany). For quantification of m.4279A>G mutation loads by restriction fragment length analysis (RFLP), we used primers 3922F25 (5'-GAAGTAGTCT-CAGGCTAACATCG-3') and 4388R20 (5'-TGATAGGTGGCACGGGAAT-3') to amplify a product that contained an Eam1104I restriction site in the presence of the mutant allele. Primer 4388R20 in combination with primer 4252PsiF25 (5'-CTCAAACCTAAGAAATATGTCTTAT-3') was used to introduce a mismatched nucleotide (underlined), thus, creating a PsiI restriction site in the presence of the wild-type allele. Conditions for PCR amplification were as follows: 95 °C/10 min; 33 cycles of 95 °C/15 s, 55 °C/30 s, 72 °C/1 min; and finally 72 °C/7 min. After restriction endonuclease digestion of the PCR products, DNA fragments were separated on a 10% polyacrylamide gel and visualized by SYBR Green I staining (Sigma-Aldrich, Steinheim, Germany). Optical densities of bands were measured using the ImageJ program. Heteroplasmy was calculated as the ratio of the sum of mutation-specific band amplitudes to the total sum of band amplitudes.

**Single-fibre analysis.** Genotyping of individual muscle fibres were performed as described before.<sup>7</sup> Briefly, randomly selected fibres in a 10 µm section of unstained frozen skeletal muscle were cut and catapulted to tube cap using the P.A.L.M. MicroBeam laser microdissection system. After incubation at 55 °C/30 min with

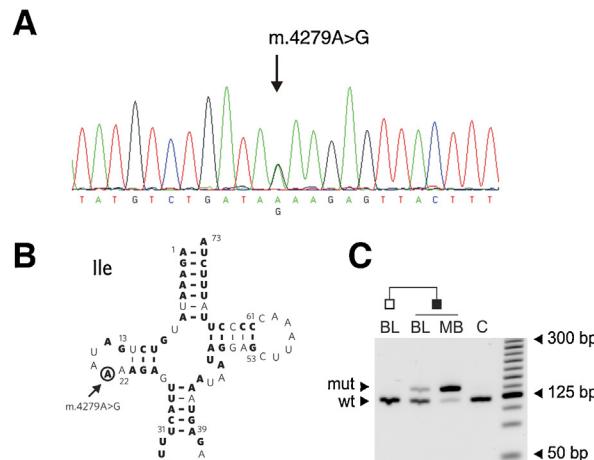


**Fig. 1.** Results of the EEG, the cMRI and the muscle biopsy in the index case. (A) The EEG shows bilateral occipital spikes here induced by photic stimulation and combined with myoclonic jerks of the upper extremities. (B) The cMRI detected a global atrophy of the brain and mild periventricular white matter lesions. (C) The electromyography found no pathological changes leading to a myopathy, but the muscle biopsy demonstrated a COX-deficiency in a high quantity of muscle fibres (blue fibres, left) in a cytochrome c oxidase/succinate dehydrogenase (COX/SDH) staining (brown fibres = regular COX expression). The modified Gomori trichrome staining showed abundant ragged red fibres (see arrows, right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

proteinase K and inactivation at 95 °C/10 min, aliquots were used for PCR-RFLP genotyping. COX and SDH activities of individual fibres were determined in consecutive sections by histochemical staining and grey-level determination.<sup>7</sup>

### 3. Case report

**Clinical investigations.** The 40 year old patient presented with prominent myoclonic seizures since one year which were aggravated upon movement but also appeared spontaneously. Other triggers were not observed. In the absence of medication the patient had myocloni several times daily. He never had complex-partial nor generalized tonic–clonic seizures. In the family history, a cousin might be also affected, but she did not accept a participation in the study as well as the parents of the patient. In the neurological examination, a severe hearing loss on both sides was evident. Further neurological pathologies were not found interictally, but slight deficits were detected in a neuropsychological testing: reduction in speed of working memory as well as a verbal and episodic memory deficit. Tendon reflexes were symmetric and normal and the patient never complained about muscle cramps, myalgias nor about exercise intolerance. We excluded the affection of the heart by transthoracal echocardiogram and long-term electrocardiogram. A retinitis pigmentosa was not found in the patient; the eye movements were not disturbed. The patient suffered from a severe progressive hearing loss based on a defect of the inner ear probably starting in childhood. Without knowing the genetic basis of the disease, he was initially treated by valproic acid which reduced the myoclonic jerks only marginally. Therefore, the medication was later changed to levetiracetam yielding seizure freedom at a dose of 2000 mg/d. A video-EEG monitoring showed ictal and interictal bilateral occipital and generalized spikes and polyspikes. The seizures and epileptic discharges could be elicited by photic stimulation at frequencies of 20 Hz (Fig. 1A). Background EEG was normal. A cMRI detected a global atrophy of the brain and periventricular white matter lesions (Fig. 1B). The electromyography (EMG) was normal, but in the muscle biopsy abundant ragged red fibres were found (Fig. 1C). Nerve conduction studies were normal.

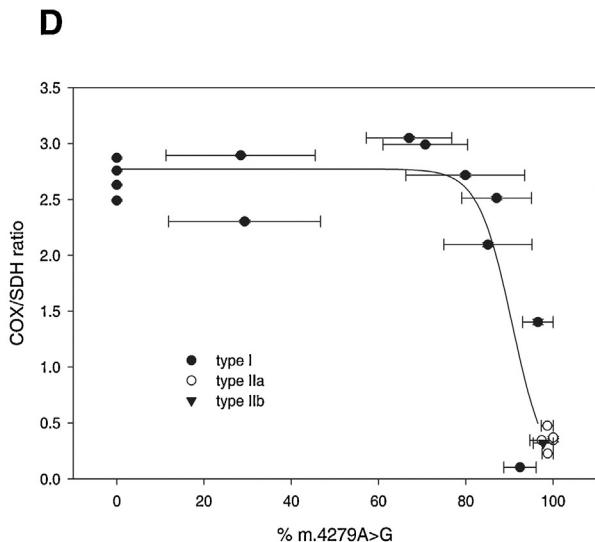


**Fig. 2.** Detection of the mitochondrial tRNA<sup>Ile</sup> mutation m.4279A>G. (A) Partial sequence of the mitochondrial tRNA<sup>Ile</sup> gene from skeletal muscle DNA of the index patient. The arrow indicates the position of the heteroplasmic mutation m.4279A>G. (B) Secondary structure of the mitochondrial tRNA<sup>Ile</sup>. The mutated position observed in the patient is encircled. (C) Quantification of m.4279A>G mutation loads by RFLP shows lower mutation load in blood compared to muscle. BL, blood samples from the patient and his healthy brother; MB, patient's muscle biopsy; C, control blood; wt, wild-type; mut, mutant. (D) Correlation of COX/SDH activity ratios with m.4279A>G mutation loads in single skeletal muscle fibres. Fibres that harboured nearly exclusively mutant mtDNA show a relevant dysfunction of cytochrome c oxidase. Horizontal error bars indicate standard errors of the mean as determined by two different RFLP methods. Fibre types were distinguished based on myosin ATPase staining at pH 4.6 and 9.4, and are indicated as shown in the inset. Note the very high threshold that is required for respiratory chain dysfunction.

**Biochemistry and Genetics.** Enzymatic measurements in muscle homogenate showed a marked decrease in activities of complex I (1.23 U/g wet weight, in 48 controls 2.48 ± 0.80) and complex IV (5.66 U/g wet weight, in controls 9.63 ± 2.84) of the respiratory chain at almost unchanged citrate synthase activity (16.3 U/g wet weight, in controls 14.1 ± 4.3). Sequencing of the mitochondrial DNA from the skeletal muscle biopsy revealed a novel heteroplasmic mutation (m.4279A>G) in the MT-TI gene coding for the mitochondrial tRNA<sup>Ile</sup> (Fig. 2A). This mutation is changing a highly conserved nucleotide in the DHU-loop of this particular tRNA (Fig. 2B; the position is identical in all species of a consensus panel proposed by Yarham et al.).<sup>8</sup> The nucleotide exchange m.4279A>G was not present in 8466 publicly available complete human mtDNA sequences (NCBI Nucleotide Sequence Database). All other differences from the reference sequence found were known homoplasmic polymorphisms. The degree of heteroplasmy of the novel mitochondrial DNA mutation was 70% in skeletal muscle and 15% in blood (Fig. 2C). The mutation was not detected in the blood of the patient's healthy brother. To obtain evidence on the pathogenicity of the m.4279A>G mutation, we investigated the correlation of mutation loads with the biochemical phenotype in single skeletal muscle fibres. We found that fibres that carried the mutation in less than 90% of their mtDNA molecules did not display an altered function of cytochrome c oxidase, whereas a dysfunctional respiratory chain was observed in fibres harbouring nearly exclusively mutant mtDNA (Fig. 2D). This very high threshold is typical for many pathogenic mitochondrial tRNA mutations.

### 4. Discussion

PMEs are rare entities in adult epilepsy centres. One of the most common forms in adulthood might be MERRF, which is an important differential diagnosis in patients with prominent myoclonic seizures. Mitochondrial tRNA mutations often display lower degrees of heteroplasmy in blood than in postmitotic tissues,<sup>4</sup> as in our case (15% versus 70% in muscle). Therefore, genetic analysis of the muscle biopsy is essential for the diagnosis. Valproic acid can deteriorate the condition of patients with mitochondrial diseases since it influences the mitochondrial



metabolism of fatty acids.<sup>1</sup> Therefore, valproic acid should be avoided in such patients. On the other hand, valproic acid is the first medication chosen in patients with myoclonia underlining the early necessity for a genetic diagnosis. Our case fulfilled the classical characteristics of MERRF, however, we did not find the common mutation, m.8344A>G. Instead, we identified a novel mutation, m.4279A>G, in the mitochondrial tRNA<sup>Ile</sup> gene as the underlying cause of the disease. We provided evidence supporting the functional relevance of the mutation by showing a correlation between genotype and biochemical phenotype in single muscle fibres. This mutation has not been reported in the literature before. According to the revised pathogenicity scoring system for mitochondrial tRNA mutations,<sup>9</sup> our investigations classify the m.4279A>G mutation as definitely pathogenic (evolutionary conservation: 2 points, heteroplasmy: 2 points, histochemical evidence: 2 points, biochemical defect: 2 points, single-fibre analysis: 3 points, total: score 11). The clinical picture in patients with the previously described 14 tRNA<sup>Ile</sup> mutations is dominated by chronic progressive external ophthalmoplegia (CPEO), but also progressive hearing loss and cardiomyopathy have been reported.<sup>10</sup> In two cases, also generalized tonic-clonic seizures were described.<sup>11,12</sup> Myoclonic epilepsy was not reported before in patients with tRNA<sup>Ile</sup> mutations. Our case shows, that prominent biochemical alterations and high levels of mtDNA mutations can be detected in skeletal muscle of a patient with a pure CNS (central nervous system) phenotype. Therefore, histological and genetic analyses of a skeletal muscle biopsy are essential for the diagnosis of myoclonic epilepsy.

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