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Case report

Progressive external ophthalmoplegia due to a recurrent *de novo* m.15990C>T *MT-TP* (mt-tRNA^{Pro}) gene variant

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Abstract

Progressive external ophthalmoplegia is typically associated with single or multiple mtDNA deletions but occasionally mtDNA single nucleotide variants within mitochondrial transfer RNAs (mt-tRNAs) are identified. We report a 34-year-old female sporadic patient with progressive external ophthalmoplegia accompanied by exercise intolerance but neither fixed weakness nor multisystemic involvement. Histopathologically, abundant COX-deficient fibres were present in muscle with immunofluorescence analysis confirming the loss of mitochondrial complex I and IV proteins. Molecular genetic analysis identified a rare heteroplasmic m.15990C>T mt-tRNA^{Pro} variant reported previously in a single patient with childhood-onset myopathy. The variant in our patient was restricted to muscle. Single muscle fibre analysis identified higher heteroplasmy load in COX-deficient fibres than COX-normal fibres, confirming segregation of high heteroplasmic load with a biochemical defect. Our case highlights the phenotypic variability typically observed with pathogenic mt-tRNA mutations, whilst the identification of a second case with the m.15990C>T mutation not only confirms pathogenicity but shows that *de novo* mt-tRNA point mutations can arise in multiple, unrelated patients.

Keywords: Mitochondria; Progressive external ophthalmoplegia; tRNA Pro; Pathogenicity; *de novo* mutation.

1. Introduction

Mitochondrial diseases are a group of inherited, metabolic disorders with heterogeneous, systemic or organ-specific symptoms, often showing poor phenotype–genotype correlation. As a direct consequence, the presence of heterogeneous clinical symptoms associated with a unique genetic variant is a classical trait of mitochondrial disorders [1]. Progressive external ophthalmoplegia (PEO) is a common mitochondrial clinical feature that presents with gradually worsening bilateral ptosis and ophthalmoparesis. PEO may be present as the sole clinical feature or can be associated with multisystemic disease presentations. Single, large-scale mtDNA deletions or nuclear gene defects associated with disordered mtDNA maintenance resulting in multiple mtDNA deletions are frequently associated with PEO [2,3]. In addition, mtDNA point mutations are also detected in a small proportion of PEO patients; the m.3243A>G mutation is the most frequently identified mitochondrial point mutation in patients with PEO [3], although more than 30 different

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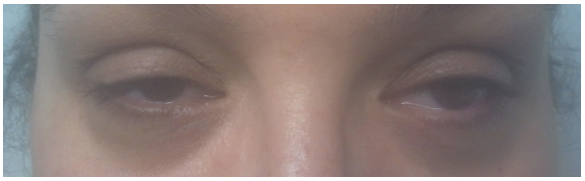

Fig. 1. Clinical photography of the eyes of the patient (aged 35 years) showing bilateral ptosis, affecting the right eye more than the left.

point mutations are reported (https://www.mitomap.org/). These can be inherited or somatic and are almost exclusively localized in mitochondrial (mt-) tRNA genes [4,5].

Here we present a case of adult-onset progressive external ophthalmoplegia (PEO) due to a m.15990C>T *MT-TP* variant that was previously identified in a child with isolated myopathy, demonstrating firm evidence of pathogenicity.

2. Case report

A 34-year-old woman originally from Kosovo presented with an 8-month history of ptosis of the right eye. She complained of ocular pain and watery eyes with increased glare sensitivity without double vision. Dysphagia and dysarthria were denied. The patient later developed myalgia and exercise intolerance involving mainly her proximal limbs. Family history for neuromuscular disorders, especially ptosis or muscle symptoms, was negative. The patient's father died of a stroke; her mother, nine siblings and two children aged 7 and 9 years are all clinically asymptomatic. At age 35 years, she had a miscarriage after 3 months of pregnancy. Neurological examination showed a right greater than left ptosis and a mild ophthalmoparesis in all directions with particularly limited amplitude of upgaze (Fig. 1). Further neurological examination was normal. In particular, there was no evidence for bulbofacial/ limb muscle weakness or neuropathy. Encephalopathic symptoms such as cognitive impairment, epileptic seizures and cerebellar signs were not present. Laboratory testing revealed normal values for blood lactate but level of creatine kinase (CK) was increased 3 to 5 times the upper limit of normal. Electromyography (EMG) was normal. Cardiological examinations, including echocardiogram (ECG, long-term ECG) and echocardiography revealed no abnormalities. An ophthalmological examination did not show signs of a retinopathy. Furthermore, MRI imaging of the brain, EEG and audiometry results were normal.

Clinical examination suggested PEO and hence, an open muscle biopsy of the deltoid muscle was performed and processed according to standard procedures. Written consent was obtained from the patient for further histological, biochemical and molecular investigations. Standard histology [hematoxylin and eosin (H&E)] and oxidative enzyme histochemistry [cytochrome *c* oxidase (COX), succinate dehydrogenase (SDH) and sequential COX/SDH activities] were performed on 10 μm transversely-orientated frozen skeletal muscle sections as described previously [6]. These histological analyses revealed well-preserved activity of succinate dehydrogenase (SDH) throughout the biopsy. However, both the individual COX and sequential COX/SDH histochemical reactions showed extensive COX-deficiency (75% COX-deficient fibres) (Fig. 2A). Based on these findings, a quantitative, quadruple immunofluorescence analysis was performed to interrogate NDUFB8 (complex I) and COXI (complex IV) immunoreactivities [7] that confirmed a mitochondrial defect involving loss of both complex I and complex IV proteins (Fig. 2B).

For molecular genetic analysis, total DNA of the patient was extracted from available tissues (muscle, blood, urothelial cells, buccal cells and hair shafts) following standard procedures. Total DNA of her mother was also extracted from blood, urothelial cells, buccal cells and hair shafts. Genetic testing of two clinically-asymptomatic children was not performed due to ethical reasons. Asymptomatic siblings were not available for clinical and genetic investigations. Long range PCR of muscle DNA [8,9] of the patient did not identify large scale deletions. A panel of 11 nuclear genes responsible for Mendelian PEO (*SPG7, TWNK, TK2, MGME1, RNASEH1, CHRNE, C12orf65, DGUOK, DNM2, TYMP* and *POLG*) was tested but no pathogenic variants were identified. As a consequence, the entire mitochondrial genome of the patient was sequenced using DNA extracted from muscle using previously described protocols [9]. This identified a heteroplasmic mt-tRNA^{Pro} variant, m.15990C>T, present at high levels. Quantitative pyrosquencing was performed as previously described [10,11], revealing a heteroplasmy level of 78% in muscle. However, the m.15990C>T variant could not be detected in DNA extracted from blood, urothelial cells, buccal cells and hair shafts obtained from either the patient or her mother. Furthermore, analysis of individual COX-deficient and COX-positive fibres using pyrosequencing following laser-capture microdissection detected a statistically-significantly higher mutation load in COX-deficient fibres [89.75 ± 6.84 ($n=20$)] than in COX-positive fibres [14.00 ± 6.84 ($n=17$)] ($p<0.001$) (Fig. 2C), thus confirming causality.

3. Discussion

We present a case of mitochondrial PEO, where the identification of a rare mt-tRNA^{Pro} gene variant - m.15990C>T - supported the clinical and histopathological findings in our patient's muscle biopsy. This rare mtDNA variant was reported previously on one occasion, more than 25 years ago, in single case of a 9-year old US-American Caucasian girl with a generalized myopathy and lactic acidosis since age 7-years without PEO [12]. Family history for neuromuscular disorders was negative. Similar to our patient, the mutation was also restricted to skeletal muscle with a heteroplasmy level of 85%. The variant is listed as possibly pathogenic with a pathogenicity score of only 51.7% (https://www.mitomap.org/). The pathogenicity score of this variant was calculated using mitochondrial tRNA informatics predictor (MitoTIP) scoring system [13].

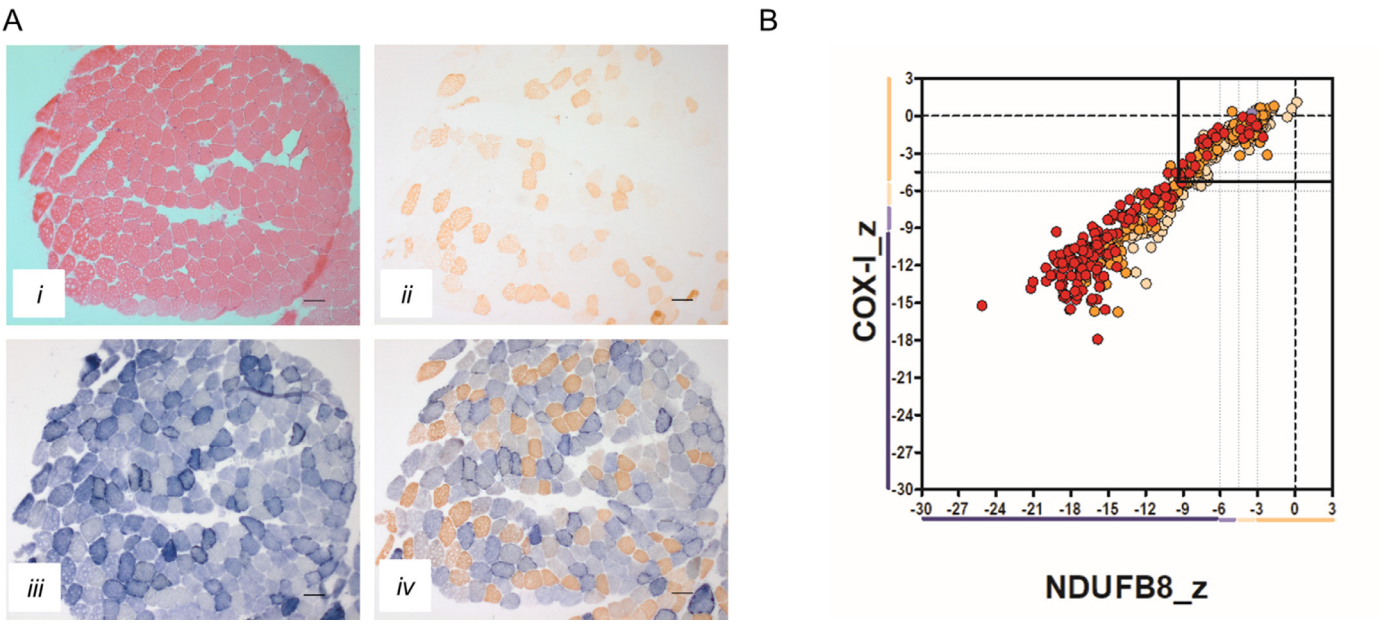

Fig. 2. **Histopathological evaluation of skeletal muscle**. (**A**) Histological and histochemical analyses of the patient's skeletal muscle biopsy showing hematoxylin and eosin (H&E) staining (i), cytochrome c oxidase (COX) histochemistry (ii), succinate dehydrogenase (SDH) histochemistry (iii) and sequential COX/SDH histochemistry (iv), highlighting the marked COX defect; scale bar = 100 μm. (**B**) Quadruple immunofluorescence analysis of NDUFB8 (complex I) and COXI (complex IV) mitochondrial subunits depicting loss of complex I (NDUFB8) and complex IV (COX I) proteins. Each dot represents the measurement from an individual muscle fibre, colour co-ordinated according to its mitochondrial mass (low=blue, normal=beige, high=orange, very high=red). grey dashed lines represent SD limits for classification of the fibres. Lines next to x- and y-axes represent the levels of NDUFB8 and COXI: beige=normal (> −3), light beige=intermediate positive (−3 to −4.5), light purple=intermediate negative (−4.5 to −6), purple=deficient (<−6). Bold dashed lines represent the mean expression level of normal fibres.

This method uses commonly accepted criteria for pathogenic mtDNA variants such as available databases of benign and pathogenic variants, alignment between diverse tRNAs, structural information and comparative genomics to predict the impact of all possible single-base variants and deletions. Assigning clear pathogenicity to mt-tRNA variants is very important as their causative role in mitochondrial diseases have been clearly established [14]. In the present report, we were able to assign pathogenicity to this m.15990C>T base substitution based on a validated pathogenicity scoring system that uses more standard tools such as biochemical and molecular genetic findings to assign pathogenicity [15]. With a total score of 15 out of 20 points, this substitution clearly exceeded the suggested threshold of 11 points to judge a variant as definitively pathogenic. In addition to the functional criteria used in this scoring system, the pathogenicity of this mutation is strongly established due to its location within the highly evolutionary-conserved and critical anticodon domain of the mt-tRNA^{Pro} (Fig. 3A & B). So far, 7 pathogenic variants (MitoTIP possibly, likely or confirmed) in mt-tRNA anticodon loops are known (5 of those variants resulting in anticodon swaps) amongst a total of 157 pathogenic (possibly, likely, or confirmed) mt-tRNA variants (https://www.mitomap.org/; website accessed on 10.02.2020). The reported pathogenic variants in mt-tRNA anticodon loops are listed in supplemental Table S1.

A calculation based on a ratio of possibly, likely or confirmed pathogenic variants to mt-DNA nucleotides reveals 4.6% in the mt-anticodon loops (7 variants in 154 nucleotides) and 7.6% resulting in anticodon swaps (5 variants in 66 nucleotides) compared to 11% besides anticodon loops (150 variants in 1355 nucleotides). This suggests that the anticodon loop is not a particular hot-spot for pathogenic mt-tRNA variants. A possible explanation could be that most anticodon changes would act in a dominant fashion, resulting in lethal mutations, even when present at low levels [12]. Moreover, mt-anticodon swap variants are believed to contribute to high fidelity of codon recognition, the structural formation and stabilization of functional mt-tRNAs [16].

Absence of detectable levels of the pathogenic variant in other tissues apart from muscle and asymptomatic status of the mother and 9 siblings strengthen *de novo* occurrence of the mutation in our patient. This is strongly supported by the fact that the reported variant was not detected in different tissues of the mother. *De novo* point mutations are common in mitochondrial disease and in spite of the strict maternal inheritance of many mtDNA point mutations, *de novo* point mutations have a low risk of recurrence [17]. However, identification of the m.15990C>T variant in a second independent sporadic case shows that recurrence of de-novo pathogenic mtDNA variants can occur in independent families. This was previously observed in patients with the m.10191T>C variant located in a protein coding gene (complex I subunit) [18] but not in patients with mt-tRNA gene mutations.

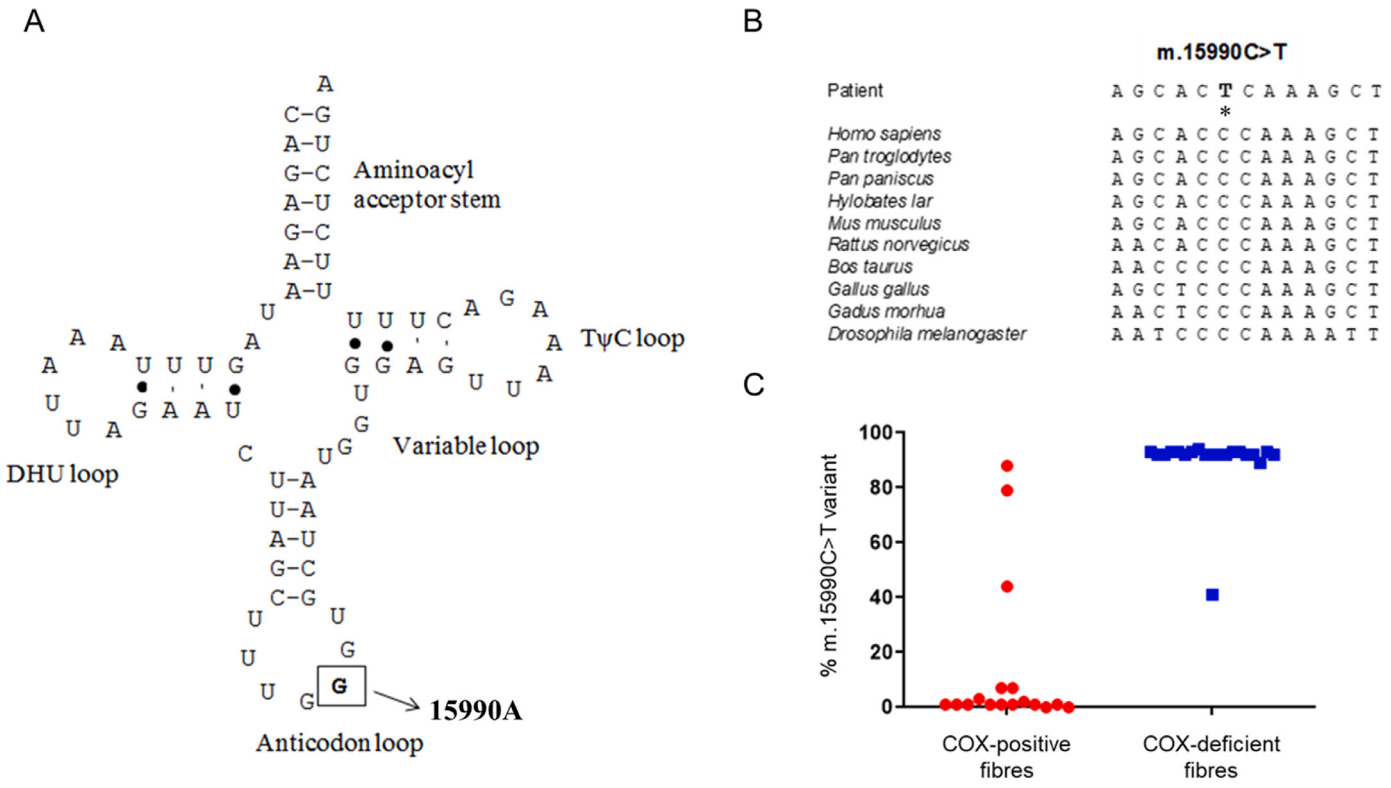

Fig. 3. Mitochondrial DNA studies reveal a pathogenic m.15990C>T variant that affects an evolutionary conserved residue and base pair within the anticodon loop of the *MT-TP* gene. (**A**) Schematic representation of the mt-RNA^{Pro} cloverleaf structure, highlighting the position of the pathogenic m.15990C>T variant. (**B**) Multiple sequence alignment highlighting the evolutionary conservation of the affected nucleotide at this position (denoted by an asterisk). (**C**) Single fibre PCR analysis showing definitive segregation of the m.15990C>T variant with a biochemical defect in individual COX-deficient muscle fibres.

The risk of transmission to offspring of pathogenic mt-tRNA mutations is found to be very high only when the mutation is present, to some extent, in rapidly replicating tissue such as blood [19]. The identified mutation in our patient was restricted only to muscle, as it could not be identified in the DNA of other tissues, including blood, urothelial cells, buccal cells and hair shaft predicting a very low probability of transmission of this mutation to offspring. Hence, the recent miscarriage that our patient suffered with might not be as a consequence of the pathogenic mtDNA variant she harbors. The fact that the patient had no extra-muscular symptoms and clinically asymptomatic status of both her children suggest that this variant might result due to somatic mosaicism, in which other tissues besides muscle are spared. Somatic mtDNA point mutations seem to occur early in life due to mtDNA replication errors and/or spontaneous cytosine deamination and clonally expand throughout adulthood [20]. Our strong feeling however is that the mutation occurs a *de novo* mutational event, present only in the patient's muscle, and that her miscarriage is not related to the m.15990C>T variant.

Identification of the m.15990C>T variant only in muscle strengthens the significance of genetic testing of suspected mitochondrial patients in skeletal muscle despite the high sensitivity of the current NGS-based mtDNA screening techniques [19,21]. To date, 13 different pathogenic point mutations are reported in mt-tRNA^{Pro} gene (https://www.mitomap.org/). The phenotypes associated with these reported variants are heterogeneous, including a pure myopathy [12,21], symptoms related to Parkinson disease [22], multisystem disorders preferentially affecting the proximal muscle and nervous system (mitochondrial cytopathy) [23,24], dilated cardiomyopathy [25] and MERRF-like syndromes [14]. The m.15990C>T variant found in our patient thus extends the clinical spectrum of mt-tRNA^{Pro} gene defects and strengthens the notion that a single, pathogenic mt-tRNA gene variant can result in widely different clinical phenotypes.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2020.02.020.

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