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Nerve excitability changes related to muscle weakness in chronic progressive external ophthalmoplegia

Antoine Gueguen [a], Claude Jardel [f,b], Marc Polivka [c], S. Veronica Tan [d], Françoise Gray [c], Catherine Vignal [e], Anne Lombès [f,b], Olivier Gout [a], Hugh Bostock [d,*]

[a] Department of Neurology, Fondation Ophtalmologique A. de Rothschild, Paris, France
[b] Department of Metabolic Biochemistry, AP/HP, Hôpital Pitié-Salpêtrière, Paris F-75651, France
[c] Department of Anatomical Pathology, AP/HP, Hôpital Lariboisière, Paris, France
[d] Institute of Neurology, University College London, London, United Kingdom
[e] Department of Neuro-Ophthalmology, Fondation Ophtalmologique A. de Rothschild, Paris, France
[f] INSERM U1016, Institut Cochin, Paris F-75014, France

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HIGHLIGHTS

- Patients with chronic progressive external ophthalmoplegia due to large size deletions of mtDNA were studied.
- Nerve excitability abnormalities were found that correlated with skeletal muscle weakness.
- Abnormalities suggested a spread of mitochondrial dysfunction to Schwann cells.

ABSTRACT

Objective: To explore potential spreading to peripheral nerves of the mitochondrial dysfunction in chronic progressive external ophthalmoplegia (CPEO) by assessing axonal excitability.
Methods: CPEO patients ($n = 13$) with large size deletion of mitochondrial DNA and matching healthy controls ($n = 22$) were included in a case-control study. Muscle strength was quantified using MRC sum-score and used to define two groups of patients: CPEO-weak and CPEO-normal (normal strength). Nerve excitability properties of median motor axons were assessed with the TROND protocol and changes interpreted with the aid of a model.
Results: Alterations of nerve excitability strongly correlated with scores of muscle strength. CPEO-weak displayed abnormal nerve excitability compared to CPEO-normal and healthy controls, with increased superexcitability and responses to hyperpolarizing current. Modeling indicated that the CPEO-weak recordings were best explained by an increase in the 'Barrett-Barrett' conductance across the myelin sheath.
Conclusion: CPEO patients with skeletal weakness presented sub-clinical nerve excitability changes, which were not consistent with axonal membrane depolarization, but suggested Schwann cell involvement.
Significance: This study provides new insights into the spreading of large size deletion of mitochondrial DNA to Schwann cells in CPEO patients.

1. Introduction

Chronic progressive external ophthalmoplegia (CPEO) is one of the most frequent forms of mitochondrial disease. It may be due to diverse genetic alterations, including mitochondrial DNA (mtDNA) large-size deletion, mtDNA point mutation and alteration of a nuclear gene involved in the mtDNA replication (Dey et al., 2000; Hirano and DiMauro, 2001; Holt et al., 1988; Kaukonen et al., 2000; Seneca et al., 2001; Spelbrink et al., 2001; Van Goethem et al., 2001; Zeviani et al., 1989).

In the case of large-size deletions, deleted mtDNA molecules coexist with normal ones (heteroplasmy) and linear correlation has been observed between cellular energy supply and percentage

* Corresponding author at: Institute of Neurology, University College London, Queen Square, London WC1N 3BG, United Kingdom.
E-mail address: h.bostock@ucl.ac.uk (H. Bostock).

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of mutated mtDNA, without a clear threshold above which symptoms appear (Gellerich et al., 2002). The proportion of large-size deletions can differ greatly between different tissues (Shanske et al., 1990), directly influencing the phenotype (Berenberg et al., 1977; Drachman, 1968; Shy et al., 1967). The spreading of large-size deletions to the different tissues is not totally understood. It might be related to the time point at which the genetic alteration occurred during embryogenesis: nervous system as well as muscle and other mesoderm tissues would be involved if the alteration occurred before mesoderm and ectoderm differentiation. Mitotic segregation of deleted mtDNA molecules during oocyte maturation could also explain restricted tissue distribution (Chinnery et al., 2004; Wai et al., 2008).

Peripheral neuropathy is common in mitochondrial diseases (Filosto et al., 2003; Hirano and DiMauro, 2001; Peyronnard et al., 1980; Seneca et al., 2001; Yiannikas et al., 1986). In CPEO it has preferentially been associated with nuclear gene mutations and less classically with large-size deletions. However peripheral nerve functional abnormalities may be overlooked by standard electroneuromyography (ENMG).

The development of computerized threshold-tracking techniques has facilitated non-invasive assessment of the excitability properties of peripheral nerve fibers (Bostock et al., 1998). These techniques have previously been used to explore the pathophysiology of a wide variety of diseases affecting nerve excitability (Lin et al., 2001; Kiernan and Lin, 2012), including the mitochondrial disease MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (Farrar et al., 2010). The aim of this study was to use the high sensitivity of nerve excitability measurements to detect spread of mitochondrial dysfunction to peripheral nerve in CPEO.

2. Materials and methods

Thirteen CPEO patients with large-size deletions and 22 healthy controls, matching in sex and age, were enrolled in a prospective case-control study. Inclusion criteria were progressive ptosis and ophthalmoplegia, orbital muscle atrophy on MRI or Scan and muscle mitochondrial histologic abnormalities with presence of large-size deletion of mtDNA. The patients were part of the cohort of 69 CPEO patients followed in the neurological department of "Fondation Ophtalmologique A. de Rothschild". The study was approved by the ethical committee of Ile de France VI and conducted in accordance with the provisions of the Declaration of Helsinki. Patients and controls were fully informed of the study modalities and gave their written consent. Medical history was collected in the patients' chart.

Large-size deletion was shown in total DNA extracted from muscle biopsy, using Southern blot and long-range PCR to determine the presence, size and proportion of the deletion. When muscle sample was unavailable for molecular studies, large-size deletion was also looked for in urinary sediment sample using long-range PCR.

MRC sum-score evaluation was performed on eleven groups of muscles (shoulder abductors, elbow flexors and extensors, wrist flexors and extensors, first dorsal interosseous, hip flexors, knee flexors and extensors, foot flexors and extensors) (Kleyweg et al., 1991; Merkies et al., 2003). The MRC sum-score was used to define two groups of CPEO patients: with normal strength (CPEO-normal) or with weakness (CPEO-weak). The patients of the latter group presented with skeletal muscle weakness in the four limbs and MRC sum-score <108/110. Slight deltoid weakness was frequent and did not suffice to include in the CPEO-weak patients in the absence of additional muscle weakness.

The multiple measures of nerve excitability were performed on the median motor nerve, once an ENMG procedure had been achieved. The cutaneous temperature was monitored before and after excitability recordings at the stimulation site, to ensure a temperature around 32 °C. The stimulating current was applied over the median nerve at the wrist. The anode was placed 10 cm proximally to the cathode over muscle on the lateral part of the forearm. Stimuli were delivered using bipolar constant current stimulator (DS5 stimulator; Digitimer, U.K.). The current intensity was adjusted to reach a CMAP of target amplitude by means of the Qtrac program (written by H. Bostock, copyright Institute of Neurology, UCL, London, UK). CMAP was recorded from the abductor pollicis brevis using a belly-tendon configuration. The signal had 50 Hz noise eliminated with a Humbug (Quest Scientific, Canada) before digitization with a data acquisition card (PCI-6221 National Instruments, U.S.A.) and recording in a computer. Assessment of nerve excitability was performed using the TRONDNF Qtrac recording protocol.

The stimulus-response relationship was established by manually increasing the intensity of stimulation until a CMAP of maximum amplitude was obtained. The program then generated a descending stimulus-response relationship and fitted a curve, which was afterwards used to optimally track a target response set to 40% of the maximal CMAP. Threshold tracking was used to determine the following excitability properties: (i) strength-duration relationship (evaluated as the 'threshold current' to reach the target response when the duration of the stimulation decreased from 1 ms to 0.2 ms in 0.2 ms steps); (ii) threshold electrotonus (assessed as the threshold changes during and 100 ms after 100 ms polarizing currents set to ±20% and ±40% of the control threshold); (iii) current/threshold relationship (evaluated as the threshold changes at the end of 200 ms polarizing currents varied from 50% to −100% of the control current threshold in 10% steps); and (iv) recovery cycle (determined as the threshold changes following a supra maximal conditioning pulse at inter-pulse intervals that were gradually decreased from 200 ms to 2 ms). Analysis of the excitability recordings was carried out automatically by the Qtrac software, which generated multiple excitability measurements as described previously (e.g. Table 2 in Tomlinson et al. (2010).

2.1. Statistical analysis

Clinical characteristics and excitability parameters were analyzed with Fischer test (discrete variables) and Mann-Whitney U-test (continuous variables). Wilcoxon-test was used to analyze repetitive assessments of excitability parameters. Since

Table 1
CPEO characteristics.

	CPEO-normal $n = 7$	CPEO-weak $n = 6$	P
Age at onset (years)	19.3 [6–20]	11.6 [7–14]	ns
Sex F/M	3/4	4/2	ns
Ptosis	7	6	ns
Ophthalmoplegia	7	6	ns
Bulbar	1 (14)	4 (66)	0.006
Skeletal UL/LL	1/0	6/6	–
MRC sum-score	110 [109–110]	101 [98–104]	–
Heart	2 (28)	1 (16)	ns
Ataxia	0	4 (67)	<0.0001
Retinopathy	0	0	–
Large size deletion mt DNA	7	6	
Southern blot muscle	7	5	
Long range PCR urine	–	1	–
Size of LSD (Kb)	7 [3–8]	6 [4–8]	–
mtDNA with LSD (%)	20 [10–40]	45 [10–50]	–

Data: median [range] or number of patients (percentage); Medical Research Council: MRC; Mitochondrial DNA: mtDNA; skeletal weakness in the upper/lower limbs: skeletal UL/LL; PCR: polymerase chain reaction; LSD: large-size deletion; Percentage of mitochondrial DNA with large size deletion: mtDNA with LSD.