SHORT REPORT

Frequency of dystrophic muscle abnormalities in chronic progressive external ophthalmoplegia: analysis of 86 patients

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J Neurol Neurosurg Psychiatry 2006;**77**:541–543. doi: 10.1136/jnnp.2005.079954

Background: There are few reports describing the coexistence of dystrophic features with those typical of mitochondrial myopathies in muscle biopsy. A recent study suggested that dystrophic features are frequent in patients with chronic progressive external ophthalmoplegia (CPEO) with a high mutation load, but the actual frequency of these abnormalities in CPEO remains undetermined.

Objective: To review the occurrence of dystrophic abnormalities in a large series of patients with CPEO to assess the frequency of such abnormalities and to verify whether they are correlated with specific mitochondrial DNA (mtDNA) mutations.

Methods: Retrospective survey of case series (86 patients with CPEO).

Results: Only three cases with dystrophic abnormalities were found: two with a large scale mtDNA deletion and one with the A3251G mutation. All three patients showed predominantly proximal muscular weakness resembling limb girdle muscular dystrophy.

Conclusions: Dystrophic abnormalities are rare in CPEO and are not correlated with a specific molecular defect.

Mitochondrial diseases comprise a heterogeneous group of disorders characterised by defects in mitochondrial function.¹ Chronic progressive external ophthalmoplegia (CPEO), one of the most common manifestations of mitochondrial myopathies, is clinically characterised by weakness of extraocular muscles, ptosis, and in many cases limb weakness. These patients commonly have subsarcolemmal accumulations of mitochondria in skeletal muscle (the so called ragged-red fibres) detected by modified Gomori trichrome or succinate dehydrogenase stains. Histochemistry may also show cytochrome c oxidase (COX) deficient fibres, which indicate mitochondrial dysfunction.² A small number of cases of mitochondrial myopathy with prominent morphological features of muscular dystrophy have been reported to date.^{3–5} Although dystrophic changes—such as increased connective tissue and necrosis in muscle—are considered rare abnormalities in mitochondrial myopathies,² a study of 16 patients with various presentations of this condition suggested that dystrophic involvement of the skeletal muscle was a frequent feature in the CPEO subgroup with a high mutation load.⁶

Here, we review the concurrence of dystrophic abnormalities in a series of 86 patients affected by CPEO to assess their frequency and to correlate them with specific mitochondrial genetic abnormalities.

METHODS

Patients

We reviewed the records of 3802 patients investigated by muscle biopsy at the Neuromuscular Diseases Group, Federal University of São Paulo, Brazil between 1989 and 2001. Eighty six patients were diagnosed as having CPEO on the basis of clinical, histopathological, and molecular findings. All 86 patients had ocular symptoms which included ptosis and ophthalmoplegia, and all showed ragged-red fibres, COX deficient fibres, or both in their muscle biopsies. Clinical and molecular findings of some of these patients have been published elsewhere.⁷ Of these patients, three had morphological evidence of muscular dystrophy which coexisted with mitochondrial proliferation. Dystrophic features were characterised by the presence of necrosis, marked increase in fibre size variation with abnormal enlargement or fibre atrophy, loss of fibres and replacement with connective tissue, and fat infiltration.

The patients, aged 28, 62, and 34 years, had very similar clinical features. Bilateral ptosis and ophthalmoplegia were the first symptoms, followed by predominantly proximal shoulder and pelvic girdle muscle weakness (MRC grade 4). None had limitation on physical activity. Age at onset was 18, 60, and 12 years, respectively. None had retinopathy, ataxia, heart block, endocrinopathies, or encephalopathy. Patients 1 and 3 had a high serum creatine kinase (CK) level (555 and 4762 IU/l, respectively; normal <165). CK was not done in patient 2. Electromyographic studies showed myopathic changes in all three patients.

The other 83 patients without dystrophic changes presented with variable degrees of proximal muscle weakness: normal strength to muscle power, grade 3. CK was normal in 50 of the 60 patients examined and was less than three times the upper limit of normal in 10.

Muscle histology

Muscle biopsy specimens were obtained from the left deltoid muscle. Serial 10 µm frozen sections were stained with haematoxylin and eosin, Gomori trichrome, and a battery of histochemical methods, including ATP at pH 9.4, 4.6, and 4.3, NADH-tetrazolium reductase, succinate dehydrogenase, COX, periodic acid Schiff, acid and alkaline phosphatase, non-specific esterase, and oil red O. We also screened muscle biopsies with mouse monoclonal antibodies against dystrophin and α -, β -, γ -, and δ -sarcoglycans (Novocastra, UK) using an indirect immunofluorescence method.

Histological and histochemical examination revealed similar dystrophic findings in all three patients. There was increased variation in fibre size, with diameters ranging from 10 to 120 µm, connective tissue proliferation, fat infiltration,

Abbreviations: COX, cytochrome c oxidase; CPEO, chronic progressive external ophthalmoplegia; mtDNA, mitochondrial DNA

Figure 1 Morphological analysis of deltoid muscle. Modified Gomori trichrome (A) shows scattered ragged-red fibres (RRF) and dystrophic changes. A necrotic fibre is arrowed. Serial succinate dehydrogenase staining (B) and cytochrome c oxidase (COX) staining (C) show three different types of fibre: (1) COX positive/RRF, (2) COX negative/non-RRF, and (3) COX negative with positive subsarcolemmal enhancement/RRF. Bar = 50 μ m.

large fibres with multiple internal nuclei, and fibre splitting. Necrosis was found in all three patients, but was more conspicuous in patient 3 (fig 1A). Gomori trichrome and succinate dehydrogenase stains showed typical ragged-red fibres. No lobulated fibres on NADH-tetrazolium reductase stain were seen. The proportions of ragged-red fibres were

20%, 24%, and 40%, respectively. COX negative fibres were present in 12%, 14%, and 7%, respectively. There were scattered ragged-red fibres which stained intensely for COX. Some ragged-red fibres showed reduced enzymatic activity in the sarcoplasm with subsarcolemmal positive staining on COX staining (fig 1, panels B and C). By immunohistochemistry, dystrophin and α -, β -, γ -, and δ -sarcoglycans were normally expressed on muscle fibres from the three patients.

The proportion of ragged-red fibres in patients without dystrophic changes observed by the succinate dehydrogenase stain ranged from 0 to 30%. COX negative fibres ranged from 0.5% to 50% of fibres examined.

mtDNA analysis

Total DNA was extracted from muscle biopsy specimens and screened for large scale rearrangements of mtDNA by Southern blotting. The ratio of deleted mtDNA to total mtDNA was measured by densitometric analysis of non-saturated autoradiographs. We found a single 3.7 kilobase (kb) deletion in the first patient and a 3.8 kb deletion in the second. The amounts of mutant mtDNA were 65% and 4%, respectively. The most common point mutations of the mtDNA—A3243G, A8344G, and T8993G/C—were excluded by PCR/RFLP and sequencing of polymerase chain reaction (PCR) products spanning the nucleotides 3116–3353, 8273–8372, and 8273–9950 with an ABI Prism 377 automated sequencer. The T4409C and G5650A mtDNA point mutations, previously reported^{4,5} to be associated with dystrophic muscle, were excluded by sequencing nucleotides 4241 to 4893 and 5472 to 5971.

Sequencing analysis of the 3116–3353 segment showed a heteroplasmic A→G substitution at position 3251 in the third patient. The 3251 mutation was confirmed by PCR/RFLP (restriction fragment length polymorphism) analysis, as described previously,⁸ with 55% mutant mtDNA, estimated by densitometry from the photography of the ethidium bromide stained agarose gel.

Of the 83 patients without dystrophic changes, 39 had single deletions, two had point mutations, and 19 had multiple deletions. In the remaining 23, we did not find large rearrangements or the most common point mutations.

DISCUSSION

In this study we found that dystrophic changes in skeletal muscle, similar to the muscular dystrophies, were not frequent in CPEO patients. Dystrophic features in mitochondrial myopathies are rare and have only been reported in skeletal muscle from one patient with Kearns-Sayre syndrome and multiple mtDNA deletions,³ one with the T4409C mutation presenting exercise intolerance and growth retardation,⁴ and in one patient clinically resembling myotonic dystrophy harbouring the G5650A mutation.⁵ However, Olsen *et al*⁶ reported dystrophic features in muscle of six of eight CPEO patients with a single mtDNA deletion and concluded that it was a common phenomenon in the CPEO patients with a high mutation load (over 45% in their group).

In order to investigate the frequency of dystrophic features associated with typical mitochondrial proliferation, we examined muscle biopsies from 86 patients affected by CPEO. Specific mtDNA abnormalities were detected in 62. Among these patients, we found this specific association in three—a much lower frequency (4.8%) than reported by Olsen *et al*.⁶ Our patients showed marked dystrophic changes in skeletal muscle and predominantly proximal muscular weakness resembling limb girdle muscular dystrophy (LGMD). The LGMDs are a heterogeneous group of diseases characterised by progressive pelvic and shoulder girdle musculature weakness and dystrophic changes on muscle biopsy.⁹ The clinical course is variable, ranging from mild

forms with late onset to severe forms with rapid progression and earlier onset; however, the extraocular muscles are consistently spared in LGMD.¹⁰ Moreover, mitochondrial abnormalities and the onset of weakness in extraocular muscles exclude the diagnosis of LGMD,⁹ making such a diagnosis in our patients unlikely.

Direct sequencing of the tRNA^{Leu} gene revealed a A→G transition at position 3251 in our patient 3. This mutation was originally reported by Sweeney *et al*⁸ in a family with CPEO, psychiatric symptoms, and sudden death in early adult life following respiratory failure, probably resulting from muscle weakness. Another young patient was described with rapidly progressive muscle weakness and development of cardiorespiratory failure and death, similar to patients previously reported but without extraocular muscle involvement.¹¹ Our patient—the third described in reports of the A3251G mutation—showed the CPEO phenotype but did not have psychiatric symptoms or severe muscle weakness. Although we cannot exclude the possibility that our patient will develop catastrophic respiratory failure and psychiatric features in the future, the disease duration of 22 years indicates that her disease remains moderate and confined to skeletal muscle. In addition to dystrophic features this patient had COX negative fibres and a very high CK level (4762 IU/l), not previously found in A3251G mutation cases. The patient described here expands the phenotypic range associated with the A3251G mutation to include the dystrophic features, COX negative fibres in skeletal muscle, and a high CK level. The other two patients had large scale mtDNA deletions. The proportion of deleted mtDNA in patient 2 seemed extremely low (4%), but in skeletal muscle there is no well defined mtDNA deletion threshold.¹² Laforêt *et al*¹³ studied 43 patients affected by CPEO and found that the lowest proportion of deleted mutant in their patients was 5%, reinforcing the view that such low levels of mutant mtDNA can cause clinical manifestations. In addition, the high percentage of ragged-red fibres (24%) and COX negative fibres (14%) in skeletal muscle, together with the presence of important clinical signs and symptoms in our patient, allowed us to exclude the deleted mtDNA as an age related phenomenon. It is possible that other factors such as nuclear gene modifiers may aggravate our patient's disease condition, and may even be related to the development of the muscular dystrophy process.

On examination, all three patients with dystrophic features had predominantly proximal shoulder and pelvic girdle muscles weakness (MRC grade 4). The other 83 patients showed variable degrees of weakness (normal to MRC grade 3), so we do not believe that the histological features correlate with the degree of muscle weakness.

The small number of reports (only 12 patients, including the three presented here) suggests that dystrophic features on muscle biopsy are rare in patients with mitochondrial myopathies, and illustrates the histological heterogeneity of these disorders. Moreover, our patients had numerous ragged-red fibres with preserved or high COX activity. Ragged-red fibres with increased COX staining have only been seen in non-CPEO mitochondrial diseases, especially MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes).¹⁴ It is reasonable to

hypothesise that they share a common pathogenic mechanism because of the similarity of their pathological features. The mechanism behind this histological alteration in our CPEO patients remains to be elucidated.

ACKNOWLEDGEMENTS

This study was supported by a research grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil) to BHK and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, Brazil) to CHT.

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Competing interests: none declared

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Received 20 September 2005

In revised form 10 November 2005

Accepted 16 November 2005

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