Orbicularis oculi muscle biopsies for mitochondrial DNA analysis in suspected mitochondrial myopathy

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ABSTRACT

Aims We wished to demonstrate the feasibility of performing diagnostic mitochondrial DNA (mtDNA) analysis on biopsies of the orbicularis oculi muscle in patients with a chronic progressive external ophthalmoplegia (CPEO) phenotype and suspicion of an underlying mitochondrial disorder.

Method Case series of three patients who underwent ptosis surgery and had simultaneous biopsy of the orbicularis oculi muscle because of a suspicion of a mitochondrial disorder. Orbicularis muscle samples were divided into two pieces at the time of biopsy. The first was snap-frozen in liquid nitrogen, DNA was extracted and mtDNA deletion analysis was performed by two complementary methods (long PCR and Southern blot analysis). The second piece of muscle was assessed using routine histopathology, electron microscopy and immuno-histochemical analysis.

Results Three patients with clinical features of CPEO, without any positive family history, underwent orbicularis muscle biopsies at time of eyelid ptosis surgery. All biopsies were adequate to conduct histopathological and immuno-histochemical analysis, which showed evidence of abnormal muscle structure and function. mtDNA was successfully extracted from all biopsies, and long PCR and Southern blot analysis confirmed diagnostic large single mtDNA deletions in all three cases.

Conclusions Orbicularis oculi muscle biopsies are useful in patients with CPEO to perform mtDNA analysis, thus avoiding a separate biopsy of skeletal muscle elsewhere.

INTRODUCTION

The most common mitochondrial myopathy involving the eyes is chronic progressive external ophthalmoplegia (CPEO).1 CPEO characteristically presents with progressive upper eyelid ptosis and reduction in ocular movements, and many patients eventually require eyelid surgery to allow them to function. Making the diagnosis usually involves a limb muscle biopsy on which histopathology, immuno-histochemistry, electron microscopy and mitochondrial DNA (mtDNA) analysis are performed. CPEO patients may show cytochrome oxidase negative (COX−) fibres, ragged red fibres (RRF) and/or ultrastructurally abnormal mitochondria in skeletal muscle biopsy.2

While these findings are suggestive of mitochondrial myopathy, the most accurate and definitive diagnostic tool is mtDNA analysis. Most cases of CPEO are sporadic, and these are typically caused by a single large-scale deletion in mtDNA which is not inherited.3,4 Some cases of CPEO are due to an mtDNA point mutation, inherited in matrilineal succession,3 while others are secondary to a nuclear gene defect showing autosomal inheritance.5

Because the levator palpebrae muscle is primarily involved in CPEO patients, biopsies of this muscle have sometimes been used for histopathology to support a diagnosis of a mitochondrial disorder. However, these biopsies often give inadequate results due to the small volume of tissue available for diagnostic testing6 and the technical difficulties in sampling it, as the belly of the muscle is hidden deep in the orbit.

Extraocular muscle has also been successfully used for mtDNA analysis and could be sampled during strabismus repairs, but is not accessible during eyelid ptosis correction.7

The use of orbicularis oculi muscle to assist in the diagnosis of CPEO was first described in 1980 by Eshaghian et al8 who concluded that RRF are more abundant in CPEO patients, but that some RRF are also seen in controls. In 2009, Almousa et al9 developed a protocol for orbicularis oculi muscle biopsies that involved suturing samples of muscle on wooden cotton buds to keep them at isometric length to minimise the contraction artefact. They concluded that orbicularis oculi muscle biopsies are a good source of skeletal muscle for investigating muscle disorders and that their use could avoid the need for limb muscle biopsies. Unlike levator palpebrae muscle biopsies, biopsies of the orbicularis oculi muscle can easily be harvested during ptosis surgery as this circular muscle lies directly below the eyelid skin and permits generous biopsy with no functional effect on lid closure.

However, there have been no reports on the feasibility of using orbicularis muscle for mtDNA studies, the most accurate test for diagnosis and genetic counselling for mitochondrial myopathies.

In this case series, we demonstrate the feasibility of performing such a diagnostic mtDNA analysis on biopsies of the orbicularis oculi muscle.

MATERIALS AND METHODS

Approval of the institutional review board was obtained, and the study adhered to the tenets of the Declaration of Helsinki.

We performed a retrospective chart review of three patients with a sporadic CPEO phenotype on whom testing for large mtDNA deletions was performed using biopsies of the orbicularis oculi muscles.

At the time of ptosis repair (usually using a frontalis sling because of poor levator function), a lid crease incision was created, and a strip of underlying orbicularis muscle was obtained using forceps and Westcott scissors without crushing the
tissue or using cautery. The muscle biopsy was immediately divided into two portions.

The first piece had a minimum weight of 25 mg (approximate dimensions 10×1×1 mm³ or 5×2×1 mm³) and was placed in a plastic vial and snap-frozen in liquid nitrogen. DNA was extracted and mtDNA deletion analysis was performed by two complementary methods. Long PCR analysis was used as a highly sensitive first-line test to identify the presence of deletions. Southern Blot analysis, which is less sensitive but more quantitative, was used for independent confirmation of deletions and to quantify the degree of heteroplasmy, the proportion of mtDNA molecules containing the deletion.

The second piece of muscle was divided into a portion measuring 1×1×1 mm³ fixed in 2.5% glutaraldehyde for electron microscopy and another measuring 3×1×1 mm³ snap-frozen in an isopentane bath in liquid nitrogen for histochemical analysis. If there was any muscle left over, this was placed in formalin for routine paraffin histopathology, though this last step is not necessary to diagnose mitochondrial disorders. None of the biopsies were sutured onto a wooden stick as recommended by Almousa et al as this was considered unnecessary.

RESULTS

The three patients each had a clinical phenotype consistent with CPEO, including profound ptosis with poor levator function and markedly reduced ocular excursions. All were sporadic cases with no family history of CPEO, suggesting a large mtDNA deletion to be the most likely aetiology.

Clinical features and family history

Patient 1 was a 46-year-old woman with a progressive, bilateral ptosis completely occluding her visual axis, a right hypotropia with bilateral limitation in upgaze and bilateral weakness of the orbicularis muscle. The family history showed no abnormalities. A previous biopsy of her quadriceps muscle had identified a single large deletion in her mtDNA.

Patient 2 was a 65-year-old woman who presented with recurrent, progressive bilateral ptosis with very poor levator function, marked ophthalmoplegia and subtle weakness of her masseter muscles. A previous bilateral levator resection performed elsewhere had only provided short-term benefit. The family history showed no abnormalities.

Figure 1  Histopathology, histochemistry and electron microscopy on orbicularis oculi muscle for patient 1. (A) H&E-stained frozen section showing muscle fibres in cross-section with variability in diameter in a fibrous background. (B) Trichrome stain showing ragged-red fibres. (C) Nicotinamide adenine dinucleotide dehydrogenase reaction illustrating fibres with irregular and focally intense staining; these are sometimes referred to as ‘ragged-blue fibres’. (D) Similar but more pronounced changes are seen on the succinic dehydrogenase reaction. (E) Increased sarcoplasmic and subsarcolemmal staining is evident in the cytochrome oxidase reaction; a negative fibre is barely evident in relief (asterisk). (F) Electron micrograph showing giant bizarre mitochondria with haphazardly arranged cristae; some mitochondrial granules (arrow) are much larger than normal. Scale bars: white=20 μm, black=50 μm, white with black border=0.5 μm.
Patient 3 was a 64-year-old woman with a recurrent right upper lid ptosis following two previous levator advancements performed elsewhere with short-term benefit. She had markedly reduced ocular movements, particularly in vertical gaze, and reduced levator function bilaterally. There was no family history of ptosis or ophthalmoplegia.

None of these three patients had generalised weakness, pigmentary retinopathy or any cardiac conduction abnormalities. All three patients improved after performing frontalis sling procedures on each eye sequentially.

In the three cases presented here, we have shown that it is possible to perform histopathology and electron microscopy on orbicularis oculi muscle biopsies without stretching the sample on a wooden stick, and that it is feasible to perform mtDNA analysis yielding diagnostic results. While the morphological changes in limb muscle biopsies that are associated with mitochondrial disorders have been shown to be present in orbicularis oculi muscle in normal controls and thus may not be specific, the demonstration of an mtDNA abnormality is diagnostic.

In our patient series, none of the three patients had any family history suggestive of CPEO, and all had a clearly identifiable single mtDNA deletion present in a substantial proportion of mtDNA molecules. In each case, this finding provided a definitive diagnosis of a mitochondrial disorder, and also offered valuable information for counselling of the patients and their family members, as such single deletions are usually not transmitted to the next generation.

**DISCUSSION**

Limb muscle biopsies have generally been considered the gold standard to diagnose patients with a suspicion of a mitochondrial disorder, although limb muscle is usually less affected than are ocular muscles in patients with CPEO. Extraocular muscles have also been shown to allow mtDNA analysis and might allow biopsy during strabismus repair.

Because these patients often present with ptosis, ophthalmologists need to be familiar with diagnosing mitochondrial disorders. Levator palpebrae muscle biopsies have sometimes been used diagnostically but often give inadequate results due to the small volume of tissue available for diagnostic testing and the technical difficulty in reaching the belly of the muscle deep in the superior orbit. There has been very little written about the use of orbicularis oculi muscle biopsies in patients with a suspicion of a mitochondrial disorder and no previous reported attempts to perform mtDNA analysis on orbicularis specimens.
Although this is a small series, our results confirm that mtDNA deletion analysis has a high diagnostic yield in patients with sporadic CPEO and demonstrate that orbicularis oculi muscle biopsies yield DNA of sufficient quantity and quality for this analysis to be performed reliably. We have shown that these biopsies can easily be harvested during ptosis surgery and can provide large, good-quality muscle samples without functionally affecting the patient. While removal of preseptal orbicularis muscle might theoretically increase the risk of lagophthalmos following ptosis surgery in patients with mitochondrial myopathy, we did not find that the biopsied side had an asymmetrically worse eyelid closure in any of our three cases.

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