Chronic progressive external ophthalmoplegia with inflammatory myopathy

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Abstract: Chronic progressive external ophthalmoplegia is one of mitochondrial disorders, characterized by ptosis, limitation of eye movement, variably severe bulbar muscle weakness and proximal limb weakness. Chronic progressive external ophthalmoplegia complicated with acquired disease is extremely rare. We report a 44 years old male patient with more than 20 years of chronic progressive bilateral ptosis and limitation of eye movements manifested dysarthria, dysphagia and neck muscle weakness for 3 years. The first muscle biopsy showed red-ragged fibers and cytochrome c oxidase negative fibers as well as inflammatory cells infiltration. Electron microscopy revealed paracrystalline inclusions. Mitochondrial genetic analysis demonstrated a large-scale mtDNA deletion of m.8470_13446del4977. The patient was treated with prednisone. In a three-year follow-up study, the second biopsy was performed. Before the treatment, except bilateral ptosis and external ophthalmoplegia, this patient presented bulbar muscle weakness and neck muscle weakness. After treated with prednisone, the symptoms of dysphagia, dysarthria and neck muscle weakness were significantly improved, and the second biopsy showed only mitochondrial myopathy pathology but the inflammations disappeared. Here, we report a patient with chronic progressive external ophthalmoplegia complicated with inflammatory myopathy and after treated with prednisone as myositis, he had a significant therapeutic effect.

Keywords: Chronic progressive external ophthalmoplegia, mitochondrial DNA deletions, inflammatory myopathy

Introduction

Chronic progressive external ophthalmoplegia (CPEO) is the most common disease of mitochondrial myopathies, which is clinically characterized by bilateral ptosis, limitation of eye movements, and sometimes with limb and bulbar muscle involvement [1]. The muscle biopsies of CPEO show red ragged fibers (RRF) and cytochrome c oxidase (COX)-deficient fibers, which are key features of mitochondrial disease. This disease can be caused by point mutations, single large scale mitochondrial DNA (mtDNA) deletions, duplications or multiple mtDNA deletions secondary to a nuclear mutation such as ANT1, POLG1, POLG2, OPA1, C10orf2 and SLC25A4 genes [2-5].

Patients with CPEO have variable manifestations ranging from pure CPEO to CPEO+ syndrome with other accompanying multisystem features, such as cataracts, hearing loss, sensory axonal neuropathy, ataxia, depression, hypogonadism and parkinsonism. But mitochondrial disease associated with inflammatory myopathy is very rare. Here we describe a CPEO patient suffered from inflammatory myopathy, who had a 3 years of clinical and treatment follow-up study, and was performed the genetic tests and twice of muscle biopsies for enzymohistochemical staining, immunohistochemical staining and electron microscopic study.

Patient and methods

Patients

On December 8th, 2010, a 44 years old Chinese man first came to our hospital complaining of
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bilateral ptosis and diplopia for twenty years. He developed slowly progressive limitation of eye movements. There was no obviously limb weakness but he had a little exercise intolerance. He had no cataracts, retinitis pigmentosa. At 41 years old, the disease was suddenly getting worse. The patient manifested severe bulbar paralysis. Dysarthria, dysphagia and choking when drinking water were present. Neck muscle weakness was first observed, especially when he lay on the bed, he couldn’t raise his head. There were no skin rashes or myalgia. He had no family history. Neurological examination showed a myopathy face, bilateral ptosis, complete ophthalmoplegia with eyeball fixation (Figure 1A). Double vision presented in horizontal gaze. Facial muscle weakness and decreased facial expressions were noticed. Necker flexor muscle was MRC grade 2. Proximal limb muscles were mild involved with MRC grade 5-. Deep tendon reflex was normal. The laboratory examination revealed normal serum CK (146.9 U/L, normal: 20-200 U/L), slightly elevated lactate (2.82 mmol/L; normal: 0.7-2.1 mmol/L) and pyruvic acid (110.4 umol/L; normal: 10-100 umol/L). Thyroid function and autoantibodies in serum were normal. Needle EMG showed myopathic changes. Nerve conduction and repetitive nerve stimulation tests were normal. Electrocardiogram (ECG) was also normal.

Muscle biopsy

With informed consent of the patient, twice of open muscle biopsies were performed sepa-

Figure 1. This patient shows myopathic face, classic bilateral ptosis and eyeball fixation (A). In the first muscle biopsy, there are a cluster of muscle fibers presenting necrosis and phagocytosis. Around the small blood vessel there are a large number of lymphocytes infiltrating on hematoxylin-eosin (H&E) staining (B, ×200); but in the second biopsy, there is no inflammation (C, ×200). RRFs on modified Gomori-Trichrome (MGT) staining (D, ×400), increased enzyme activity in succinate dehydrogenase (SDH) staining (E, ×400) and cytochrome c oxidase (COX) negative fibers (F, ×400) suggest a mitochondrial disorder. Immunohistochemical staining shows CD4+ cells (G, ×200) and CD68+ cells invasion (H, ×200). The electron microscopy shows many subsarcolemmal paracrystalline inclusions and glycogen granules (I, ×10000).
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| Table 1. Primers used for long-PCR and detection of the 4977 bp deletion |
|-----------------------------|-----------------------------|
| Primers Sequence            |                             |
| "mtF15149"                  | 5'-TGAGGCCAAATATCATATTCTGAGGGGC-3' |
| "mtR14816"                  | 5'-TTTCATCATGCGGAGATTTGGATGG-3' |
| "mtF10"                     | 5'-TCTATACCTATTAACACTACCCGGAGCT-3' |
| "mtR16463"                  | 5'-GATACAGTCTACCTTGAAGCTCCAAGTGTT-3' |
| "F7259"                     | 5'-GCTACACCACATGAAAATC-3' |
| "R14559"                    | 5'-GATTTGACGTGGTGTCG-3' |
| "F8187"                     | 5'-GAGCAACCCAGATTTGATG-3' |
| "R13710"                    | 5'-AACCTCCTGCAAATGCGCTCCGGCT-3' |

*primer pairs used for mtDNA long-PCR amplification. *primer pairs used for detecting the 4977 bp deletion.

Histological changes of muscle

The first muscle biopsy was performed. On H&E staining, it revealed variation in muscle fiber size and the fibers showed frequently hypertrophy, regenerated, splitting and nuclear internalization. Meanwhile, groups of muscle fibers were observed degeneration, necrosis and phagocytosis with obvious perimysium inflammatory cells infiltration. Noticeably, a large number of lymphocytes were found infiltrating surrounding the small blood vessel. There was no perifascicular distribution of atrophy fibers (Figure 1B). MGT staining showed about 15% RRFs, which corresponded to the negative reacted ones in the COX staining. The fibers had increased enzyme activity on SDH staining (Figure 1D-F). Immunohistochemical staining showed massive CD4+ cells invading perivascular regions and the fiber sarcolemma (Figure 1G). Antibodies against CD8, MHC-I and C5b9 membrane attack complex were weakly deposited between the muscle fibers. Strong expression of CD68 was present in both perivascular region and the sarcolemma of fibers (Figure 1H). Ultrastructural study revealed a large number of subsarcolemmal paracrystalline inclusions and glycogen granules (Figure 1I).

Genetic analysis

DNA was extracted from the proband’s skeletal muscle tissue using QIAamp DNA Blood MiNi Kit (Qiagen, Hilden, Germany). The mtDNA was amplified by the long-PCR. Specific primers were previously reported to amplify the entire mtDNA genomes listed in Table 1 [6, 7]. The qualified DNA products were fragmented to 350 bp and were sequenced by paired-end reads for 151 cycles per read on the Miseq Analyzers (Illumina, San Diego, California). We used BWA (Burrows-Wheeler Aligner) to align all reads onto the human mitochondrial genome (NC_012920.1). In order to get accurate breakpoint of deletion, we chose re-aligning soft clipped reads method [8, 9]. Two pairs of specific primers which flank the 4977 bp deletion (the common deletion) were designed using Primer Premier 6.0 listed in Table 1. The PCR products were amplified and if the deletion is present, the PCR products will be 2363 bp and 570 bp respectively. The 570 bp PCR product purified was sequenced by Sanger sequencing and sequencing data were compared with reference sequences to confirm the deletion.

This study was approved by the Institutional Review Board of Chinese PLA General Hospital.

Table 1. Primers used for long-PCR and detection of the 4977 bp deletion

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*primer pairs used for mtDNA long-PCR amplification. *primer pairs used for detecting the 4977 bp deletion.

Results

Histological changes of muscle

The first muscle biopsy was performed. On H&E staining, it revealed variation in muscle fiber size and the fibers showed frequently hypertrophy, regenerated, splitting and nuclear internalization. Meanwhile, groups of muscle fibers were observed degeneration, necrosis and phagocytosis with obvious perimysium inflammatory cells infiltration. Noticeably, a large number of lymphocytes were found infiltrating surrounding the small blood vessel. There was no perifascicular distribution of atrophy fibers (Figure 1B). MGT staining showed about 15% RRFs, which corresponded to the negative reacted ones in the COX staining. The fibers had increased enzyme activity on SDH staining (Figure 1D-F). Immunohistochemical staining showed massive CD4+ cells invading perivascular regions and the fiber sarcolemma (Figure 1G). Antibodies against CD8, MHC-I and C5b9 membrane attack complex were weakly deposited between the muscle fibers. Strong expression of CD68 was present in both perivascular region and the sarcolemma of fibers (Figure 1H). Ultrastructural study revealed a large number of subsarcolemmal paracrystalline inclusions and glycogen granules (Figure 1I).

Genetic alterations

The genetic analysis results showed that the patient fragments were shorter than the control's with long-PCR amplification suggested that the presence of the deletion. The PCR products of 2363 bp and 570 bp respectively revealed the presence of the 4977 bp deletion in the muscle of the patient (Figure 2A). Analysis of the mtDNA revealed that the deleted mtDNA was heteroplasmic in the muscle of the patient, and the proportion of deleted mtDNA was about 84%. The deletion was found to be identical with the 4977-bp deletion (the common deletion) as reported previously (Figure 2B), which has been associated with CPEO. The breakpoints occurred at position
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8470-13446 or 8483-13459, which was flanked by a perfect 13 bp direct repeat at bp 8470-8482 and 13447-13459 in normal mitochondrial genome. As this 13 bp sequence may be derived from either side of the flanking region or contributed in part by both sides, the breakpoints of the deletion could not be determined precisely [10]. To verify the deletion boundaries identified by the high-throughput sequencing, the 570 bp PCR product of the deletion region was sequenced using 3730 DNA analyzer. Direct Sanger sequencing revealed that the sequence agreed with the Cambridge Reference Sequence up to bp 8469, then the 13 bp directly repeated sequence (5'-ACCTCCCTCACCA-3') occurred and the sequence continued at bp 13460 (Figure 2C). The result was exactly the same as obtained using the Miseq sequencing.

Treatment and results

According to the clinical manifestation, muscle pathology and genetic analysis, the patient was diagnosed as pure CPEO complicated inflammatory myopathy. So, he was treated orally with CoQ10, Vitamin E and prednisone. The dose of prednisone was initially 50 mg/d for 2 weeks and was gradually reduced 5mg per week until the dose of 15 mg/d, which maintained for one month. And then this drug was reduced 5mg per week until 5 mg/d for 3 months.

After taking the drugs for more than six months, dysphagia and dysarthria were greatly improved. The choking when drinking water had never appeared and the neck muscle weakness was a little improved. Meanwhile, the second muscle biopsy was performed. It showed no perivascular or perimysium inflammatory cell invasion (Figure 1C) and no positive reactions on the immunohistochemical staining of CD4, CD8, CD68, MHC-I and C5b9. RRFs and COX-negative fibers were still observed. So he stopped taking the prednisone but CoQ10 and Vitamin E were still using. On March 24th, 2013, the patient came to us for re-visiting. He presented bilateral ptosis and eyeball fixation as before, and proximal limb muscle weakness was a little progressive with MRC grade 4. But the bulbar muscle weakness almost disappeared.
Discussion

With a history of bilateral ptosis and limitation of eye movements for more than 20 years, RRFs and COX-negative fibers in muscle biopsy and the mtDNA deletion of m.8470_13-446del4977, the diagnosis of CPEO is definite for this patient.

But it is noteworthy that when the patient was 41 years old, the disease had a subacute and significant progression. Bulbar paralysis was present and the neck extensor muscle was involved, causing significant dysphagia, dysarthria and difficulty in rising head when lying on the bed. This subacute exacerbation is not consistent with the disease course of CPEO. Though some CPEO patients have oropharyngeal weakness, these manifestations often insidiously develop. The symptom of dysphagia in our patient was not chronic progressive but presented subacutely. Axial muscles are rarely involved in CPEO, but this patient has a severe neck weakness. The diagnosis of CPEO can’t explain the subacute progression and the new clinical manifestations, which are more like an acquired myopathy such as myositis. In myositis, the neck extensor muscles may be involved, causing difficulty in holding up the head (head drop), and in advanced cases and rare acute cases of myositis, dysphagia with choking episodes and respiratory muscle weakness occur [11]. To confirm the diagnosis, the muscle biopsy was performed. Interestingly, the biopsy revealed not only RRFs and COX-negative fibers but also the inflammations in the perivascular and perimysium regions. Moreover, immunohistochemical staining showed CD4 and CD68 positive on the specimen. So the pathology demonstrated the patient had both inflammatory myopathy and the mitochondrial dysfunction. Then we treated the patient with prednisone, which had a good therapeutic effect that the bulbar paralysis was obviously improved and neck muscle weakness was also better than before. After the treatment, the second biopsies showed the inflammation and immunohistochemical staining positive region disappeared. So according to all the evidence of clinic, pathology and therapeutic efficacy, it proves that this patient suffered from not only CPEO but inflammatory myopathy. But it isn’t classic dermatomyositis or polymyositis and should be identified as inflammatory myopathy.

Mancuso et al reported an adult male patient of CPEO with 3251A > G mtDNA mutation, who present an episode of sudden respiratory failure. The muscle biopsy showed inflammatory changes [12]. Marotta et al reported a case of mitochondrial encephalomyopathy, lactic acidosis and stroke like episodes (MELAS) with the m.3243A > G mutation, whose histology showed non-specific myositis [13]. And in our case, he is a CPEO patient but also suffered inflammatory myopathy. So this would be taken into consideration that if there are some factors caused by the mitochondrial disorders trigger the inflammatory response? Though in experimental animals, mtDNA changes can increase susceptibility to immune syndromes that arise spontaneously, including collagen induced arthritis, diabetes, nephritis and pancreatitis, or after immunization, such as collagen-induced arthritis and experimental autoimmune encephalomyelitis [14], the mitochondrial disorders complicated with inflammation are really rare. So there is not enough evidence, and these cases are much more like just coincidences, but it still needs future study. But on the other hand, it has been reported that mitochondrial abnormalities are observed in immune and inflammatory myopathies. Reduced COX staining suggests mitochondrial disorders associated with mitochondrial DNA (mtDNA) defects, which can be observed in sporadic inclusion body myopathy, polymyositis and dermatomyositis. But this is considered to be related to oxidative mtDNA damage, alterations of nucleotide pools or increased mtDNA polymerase error rates.

In conclusion, though CPEO with 3251A > G mtDNA mutation complicated with inflammatory myopathy was previously reported, CPEO with the mtDNA deletion of m.8470_13-446del4977 which is the most common deletion in mitochondrial DNA deletion syndrome [15], has never been reported before. From this case we can see repeated muscle biopsies and prompt treatment are very important for the diagnosis and the patient himself, so to some hereditary myopathies with chronic progressive disease course, if there is an acute or subacute progression with some atypical symptoms, we should consider the possibility of complicating with other disease.
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Disclosure of conflict of interest

None.

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