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A novel mitochondrial tRNA\(^{\text{Val}}\) T1658C mutation identified in a CPEO family

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Purpose: To analyze mitochondrial DNA (mt DNA) gene mutations in a 19-year-old female patient, who presented with chronic progressive external ophthalmoplegia (CPEO), together with her mother and younger sister.

Methods: The diagnosis of mitochondrial myopathy was made based on clinical and biologic analysis. Histochemical methods were used to detect ragged-red fibers (RRFs) and ragged-blue fibers (RBFs) on a muscle biopsy of the patient. All mitochondrial gene DNA fragments of the patient, her mother, and younger sister were amplified by polymerase chain reaction. The products were sequenced and compared with reference databases.

Results: A novel T1658C mutation and a known A10006G mutation were identified in the mitochondrial tRNA\(^{\text{Val}}\) gene and the tRNA\(^{\text{Gly}}\) gene, respectively, in the patient, her mother, and younger sister. The T1658C mutation changes the T loop structure of mitochondrial tRNA\(^{\text{Val}}\) and the A10006G mutation disturbs the D loop of mitochondrial tRNA\(^{\text{Gly}}\).

Conclusions: The T1658C and A10006G mutations of mtDNA may be responsible for the pathogenesis of the patient with CPEO.
<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Annealing temperature (°C)</th>
<th>Size of product</th>
</tr>
</thead>
</table>
| 1      | Forward: CTCCTCAAAGCAATACACTG  
          Reverse: TGCTAAATCCACCTTCGAC  | 56  | 839  |
| 2      | Forward: CGATCAACCTCACCACCTCT  
          Reverse: TGGACACCCACCTTACGCA  | 57  | 801  |
| 3      | Forward: GGACTAAACCCTATACCTTTGC  
          Reverse: GCCAGGTCATATTCAGCCTG  | 58  | 859  |
| 4      | Forward: AAATCTTACCCGGCCTGTIT  
          Reverse: AGGAATGCCATTGCGATTAG  | 57  | 886  |
| 5      | Forward: TACTTCACAAAGCGCCTTCC  
          Reverse: ATGAAAGATAGGGCGAAGG  | 57  | 831  |
| 6      | Forward: CTGCTCCTTTAACCTCCTCA  
          Reverse: AAGGAATATGGGAGCGGGT  | 56  | 903  |
| 7      | Forward: ACTAATTTCCCTTGCTGCTG  
          Reverse: AATCCGGTGGGTTTCTATG  | 57  | 978  |
| 8      | Forward: CTAACCGCTTTTTTGCC  
          Reverse: ACCTAGAAGGGTGCCCTGCTG  | 56  | 813  |
| 9      | Forward: GAGGCCTAACCCTGTCTTT  
          Reverse: ATTCGAAGCGCTGTTAGGAT  | 59  | 827  |
| 10     | Forward: CTCTTCGCTCTGATCCGCTCT  
          Reverse: AGCGAAGGGCTCTCAATGAA  | 58  | 885  |
| 11     | Forward: AGCCCAAATCCACCTTACCT  
          Reverse: CGGGATATGGCATCCTTTTTT  | 55  | 986  |
| 12     | Forward: ACGAGTACACCGACTACGCC  
          Reverse: TGCGAGGGTGGCCGGTAAATG  | 58  | 909  |
| 13     | Forward: TTTCCCCCTCTATGGCTCCC  
          Reverse: GTGGCCTGGTATTGGTATCC  | 57  | 815  |
| 14     | Forward: CCCACCAATCATGCCTAT  
          Reverse: TGTACCGCTTGGATTGCTT  | 57  | 939  |
| 15     | Forward: CTCTCCATCTATGGAGGCTCT  
          Reverse: AATTAGGCTGGGCTGTTG  | 58  | 892  |
| 16     | Forward: GCCATACTAGTCTTGTGCCG  
          Reverse: TTGAGATGGGTGATCGCGG  | 60  | 859  |
| 17     | Forward: TCAACTCTACCTGCAAGAAGA  
          Reverse: GGAATCTGGGGAATGAGGT  | 56  | 801  |
| 18     | Forward: TATCTACTCTCTACTACAG  
          Reverse: AGAAGGATATAATCCTACG  | 55  | 865  |
| 19     | Forward: AAAACCCACGCTCCCCTCAA  
          Reverse: CGATGATGGTGTCTTGGGA  | 56  | 976  |
| 20     | Forward: ACATCTTGATCCCAGCCCTTC  
          Reverse: AGAGGGTGCTAGGATTTGG  | 58  | 969  |
| 21     | Forward: GCATAATACATTTTACTCT  
          Reverse: AGAATATTGAGGGCGCCATTG  | 55  | 937  |
| 22     | Forward: TGAACCTTGGGCTACCTCCT  
          Reverse: AGCTGGTGGTCTATGTTG  | 57  | 1161 |
| 23     | Forward: TCATTGGAGAAGTAGCATCC  
          Reverse: GAGTTGGAATAGGGTATAG  | 55  | 809  |
| 24     | Forward: CACCATCCTCCGTGAATCA  
          Reverse: AGGCTAAGCGCTTTTGGAGGCT  | 55  | 963  |

Summary of the primer sequences, annealing temperatures, and size of products used for the amplification of the entire mitochondrial genome.
were purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. Next, two mitochondrial regions covering 1658 and 10006 from 148 control DNA samples were amplified by PCR and sequenced.

RESULTS

The patient—the only subject manifesting CPEO in the pedigree—had previously undergone thorough ophthalmic examinations. Her visual acuity was 20/80 in the right eye (O.D.) and 20/100 in the left eye (O.S.). Bilateral and asymmetric ptosis was noticed. The palpebral aperture was 3 mm for the O.D. and 5 mm for the O.S. The abduction and adduction of the left eye were mildly limited, yet the supraduction and deorsumduction appeared normal. The movement of the left eye was normal. Binocular diplopia was noticed in all directions. Both pupils were equal, round, and reactive to light. The anterior segment was unremarkable. Visual evoked potential showed P100 latency (positive peak at around 100 ms) was delayed in both eyes and P100 amplitude was reduced in the O.D.

Other examinations included neurologic examinations, laboratory tests, and a skeletal muscle biopsy. The muscle strength of proximal extremities was normal (5/5), but was slightly weak at the distal end (4/5). The muscular tension of extremities was within normal limits and myotonia was absent. Deep reflexes and sensation were normal. Routine blood and urine tests were unremarkable. A magnetic resonance imaging (MRI) scan of the head showed no abnormality. The antinuclear antibody (ANA) was negative. An electromyogram (EMG) showed the right ulnar nerve has no abnormality in low and high frequency stimulation. Histological examinations showed ragged-red fibers (RRFs) and ragged-blue fibers (RBFs) on the patient’s muscle biopsy (Figure 1), consistent with the pathological features of mitochondrial myopathy.

To determine the role of mitochondrial abnormalities in the pathogenesis of this patient with CPEO, the 24 PCR fragments spanning the entire mitochondrial genome of the affected individual, her mother, and younger sister were analyzed by direct sequencing. The comparison of the resultant sequences with the Revised Cambridge Reference Sequence [5] revealed several nucleotide changes, as shown in Table 2, Figure 2, and Figure 3. The patient’s mitochondrial sequence results are identical to those of her mother and sister, except the T1658C mutation of the patient’s young sister is T/C heterozygous in the mitochondrial tRNAVal gene. There were no T1658C or A10006G mutations in the 148 control subjects.

DISCUSSION

Chronic progressive external ophthalmoplegia, characterized by limited ocular motility in all directions of gaze and ptosis, is the most common manifestation of mitochondrial myopathy and usually occurs in young adulthood. Ptosis is usually the first clinical sign, but ophthalmoplegia may not become apparent for months or even years. The ptosis is usually bilateral and symmetric.

The diagnosis of CPEO relies upon a combination of different approaches, including clinical data, neurologic examinations, laboratory tests, and a skeletal muscle biopsy. Ragged-red fibers on muscle biopsies are observed in a wide variety of mitochondrial diseases. The presence of more than 2% RRFs on skeletal muscle biopsies can be considered one of the criteria required for the diagnosis of mitochondrial disease. Figure 1 shows RRFs and RBFs on the patient’s muscle biopsy. Stained with MGT staining, one can see an accumulation of enlarged mitochondria in the RRFs. Therefore, changes and dysfunction of mitochondria are the pathogenesis of CPEO.

Over the past two decades, numerous genetic causes of CPEO have been described [6-30]. Most present at mt tRNA genes, affecting mitochondrial tRNA^Leu(UUR), tRNA^Ile, tRNA^Ala, tRNA^Asp, tRNA^Lys, tRNA^Leu(CUN), and tRNA^Gly genes, including A3243G, T3250C, and C3254T in the tRNA^Leu(UUR) gene; T4274C, T4285C, G4298A, and G4309A in the tRNA^Ile gene; T5628C in the tRNA^Ala gene; T5692C and G5703A in the tRNA^Asp gene; G12294A, A12308G, T12311C, and 12315A in the tRNA^Leu(CUN) gene; and an A10006G mutation in the tRNA^Gly genes [6-30]. Most of these
point mutations in mt tRNA genes have been proved only once and appeared to be limited to one patient or one family; some point mutations have been reported in different pedigrees [22]. Therefore, mt tRNA mutations play a pivotal role in the pathogenesis of CPEO.

There are 44 nucleotide changes in this CPEO subject’s mitochondrial genome that belong to haplogroup D4f. Most nucleotide changes are polymorphisms and are not associated with human diseases. However, two mutations in this subject, T1658C in the tRNA<sup>Val</sup> gene and A10006G in the tRNA<sup>Gly</sup> gene, may be responsible for this disorder.

In fact, Lauber et al. [22] first reported a CPEO patient with a tRNA<sup>Gly</sup> A10006G mutation. Sternberg et al. [16] also found this mutation in a patient with oculomotor myopathy.

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**Table 2. mtDNA nucleotide changes in the affected subject.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Previously reported*</th>
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<td></td>
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<tr>
<td></td>
<td>263</td>
<td>A→G</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>T→CTC</td>
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<td>+</td>
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<td></td>
<td>489</td>
<td>T→C</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>514</td>
<td>C→DEL</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>515</td>
<td>A→DEL</td>
<td></td>
<td>+</td>
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<tr>
<td></td>
<td>568</td>
<td>C→CCCC</td>
<td></td>
<td>+</td>
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<tr>
<td></td>
<td>16223</td>
<td>C→T</td>
<td></td>
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</tr>
<tr>
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<td>16362</td>
<td>T→C</td>
<td></td>
<td>+</td>
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<tr>
<td></td>
<td>16519</td>
<td>T→C</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>12SrNA</td>
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<td>A→G</td>
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<td>+</td>
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<tr>
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<td>1382</td>
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<td>A→G</td>
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<td>-</td>
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<td></td>
<td>5178</td>
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<td>15326</td>
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<td>T→A</td>
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</table>

*See [http://www.mitomap.org](http://www.mitomap.org). The boldfaced are pathogenic mutations.
Münscher et al. [14] identified tRNA\textsuperscript{Gly}\textsubscript{A10006G}, a mutation known to be associated with aging, in patients with chronic intestinal pseudo-obstruction (CIPO) and CPEO. Our results, as exhibited in Figure 2D,E and Figure 3B, show the A10006G mutation is located at position 16 on the D loop of tRNA\textsuperscript{Gly}. Most importantly, this mutation changes the structure of the D loop of mt tRNA\textsuperscript{Gly}. T1658C is a novel mutation, located at position 57 of tRNA\textsuperscript{Val}. Figure 2A-C show that wild type T at position 57 matches with A at position 50 in tRNA\textsuperscript{Val}. When position 57 T changes to C, the mutant 57 C does not match with position 50 A. Therefore, one base pair on the T arm of tRNA\textsuperscript{Val} is split and the T loop is extended.

Mitochondrion has its own tRNAs to carry amino acids to synthesize mitochondrial proteins essential for ATP production. Each tRNA is charged with the proper amino acid via a covalent ester bond at their 3' end by the specific aminocacyl-tRNA synthetase. In our study, the structural changes of mt tRNA\textsuperscript{Gly} and tRNA\textsuperscript{Val}, like the A3243G mutation in mt tRNA\textsuperscript{Leu(UUR)} [30,31], may influence their structural stability, modifications, 3' end processing, and aminocacylations and decrease mitochondrial protein synthesis and ATP production. Valine is one of these essential amino acids. The defective mt tRNA\textsuperscript{Val} will reduce the transportation efficiency for valine, leading to insufficient valine in the mitochondrial translation. Therefore, the T1658C mutation leads to the T loop structural change of mt tRNA\textsuperscript{Val}, indicating a role in the development of CPEO in this individual. Furthermore, the mitochondrial sequence of the patient’s younger sister is 1658 T/C heterozygous; however, her sister has not manifested CPEO.

Muscle, especially extraocular muscle, consumes a lot of energy. Mitochondrial tRNA mutations may cause the
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Dr. Ronghua Li and Dr. Xuyang Liu contributed equally to the science of the project and can be considered as co-corresponding authors. The authors wish to thank Dr. Yun Yuan from Beijing University First Hospital for histological examination.

REFERENCES


