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Letter to the Editor

A unique Japanese CPEO family with a novel homozygous m.14819 T > G (p. S25A) substitution

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1. Introduction

Chronic progressive external ophthalmoplegia (CPEO), which is an inherited mitochondrial disease associated with the mutation or deletion of mitochondrial DNA (mtDNA), is induced by nuclear DNA mutations in the DNA polymerase gamma (POLG) gene [1], but is rarely induced by mtDNA mutations. Here we report a unique Japanese CPEO family with a novel homozygous m.14819 T > G (p. S25A) substitution accompanied by an mtDNA deletion (m.8483_13,459, del 4977), showing bilateral ptosis and muscle weakness in the proband and the proband's mother, but not in the brother. The heterozygous c. 2890C > T (p. R964C) substitution in the POLG gene was also found in the proband and the proband's brother, but not in the mother.

2. Case report

A 47 year old woman (Fig. 1a) showed bilateral ptosis, followed by muscle weakness in all limbs at the age of 55. At the age of 57, her bilateral ptosis worsened and she experienced double vision in the left gaze, and was admitted for a diagnosis.

The patient was 147.3 cm tall and weighed 47.5 kg. A neurological examination showed bilateral ptosis, external dislocation of the right eye, and upper and lateral gaze limitation of bilateral eyes (Fig. 1b (iii)). She showed muscle weakness and atrophy (proximal > distal, upper > lower), and hyperreflexia in all extremities without any pathological reflex. Examination of the serum showed normal creatine kinase (116 U/L, normal 41–153 U/L) but a slightly high level of myoglobin (102 ng/mL, normal 18–70 ng/mL). A muscle biopsy of her right biceps brachii showed ragged red fibers (RRFs) when stained by both hematoxylin and eosin, and for Gomori trichrome (Fig. 1c (i, ii), arrows). Negative fibers were observed with cytochrome *c* oxidase staining (Fig. 1c (iii), arrows). Consequently, she was diagnosed with CPEO.

The proband's mother (Fig. 1a) began to show bilateral ptosis at the age of 60, and underwent blepharoplasty at the age of 78. However, when she was 83, she showed bilateral ptosis again, upper and lateral gaze limitation of bilateral eyes without double vision (Fig. 1b (i)), and proximal dominant muscle weakness in all limbs. The proband's older brother (Fig. 1a) showed bilateral upper and lateral gaze limitation without ptosis, double vision, or muscle weakness of limbs at the age of 61 (Fig. 1b (ii)). The proband's father died at the age of 79 without ptosis, double vision, or muscle weakness of limbs before the proband's admission to our hospital.

A genetic study was carried out with written informed consent from all three family members. Nuclear DNA analysis of the POLG gene revealed a heterozygous c. 2890C > T (p. R964C) substitution in the brother and the proband, but not in the mother (Fig. 1d (i)). This finding was confirmed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) using *Hae*II (Fig. 1d (ii)). MtDNA analysis of DNA from the proband's skeletal muscle of biceps brachii revealed DNA deletion (m.8483_13,459, del 4977) accompanied with 13-bp direct repeats (ACCTCCCTCACCA), which was confirmed by PCR (Fig. 1e). Sanger sequence analysis revealed the novel homozygous m.14819 T > G (p. S25A) substitution in the mother, brother and proband, which was confirmed by PCR-RFLP using *Bse*YI (Fig. 1f).

3. Discussion

Here we report a unique Japanese family with CPEO (Fig. 1a). The proband, as well as her mother and older brother, showed upper and lateral gaze limitation of bilateral eyes (Fig. 1b). The proband and her mother also showed bilateral ptosis and muscle weakness, which are often observed in CPEO (Fig. 1g) [2]. However, the brother did not show such blepharoptosis and skeletal muscle weakness (Fig. 1g), suggesting a wide clinical spectrum in this family. Following a muscle biopsy, the proband's muscle biopsy showed RRFs and negative cytochrome *c* oxidase staining (Fig. 1c). The proband and her brother, but not the mother, showed the heterozygous c. 2890C > T (p. R964C) substitution in the POLG gene (Fig. 1d). Analysis of mtDNA indicated that the proband had a deletion (m.8483_13,459, del 4977) in muscle and all three family members showed a novel homozygous m.14819 T > G (p. S25A) substitution in peripheral white blood cells (Fig. 1e, f).

DNA polymerase gamma encoded by the POLG gene oversees mtDNA replication [1]. Mutations in the POLG gene, which are a common cause of inherited mitochondrial diseases, including CPEO, induce multiple mtDNA deletions [3,4]. However, the c. 2890C > T substitution of the POLG gene has not been reported in CPEO patients except for previous cases with ataxia and neuropathy [3,5,6]. Only the brother and the proband, but not her mother, showed the above POLG gene mutation, which would come from the father without symptoms, so the mutation in this nuclear gene might not be the principal cause of

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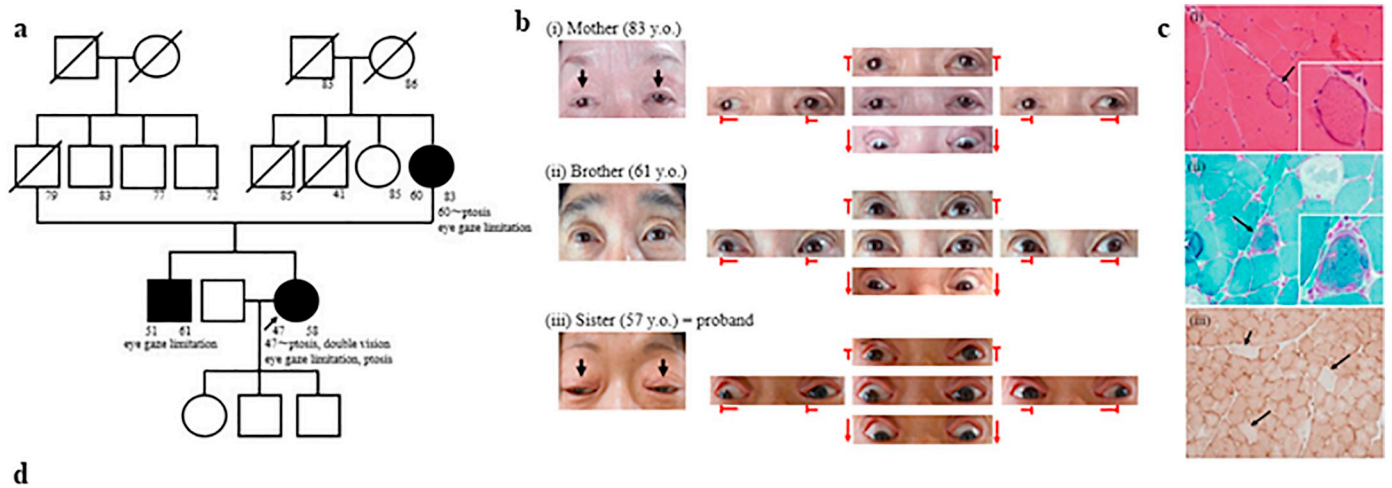

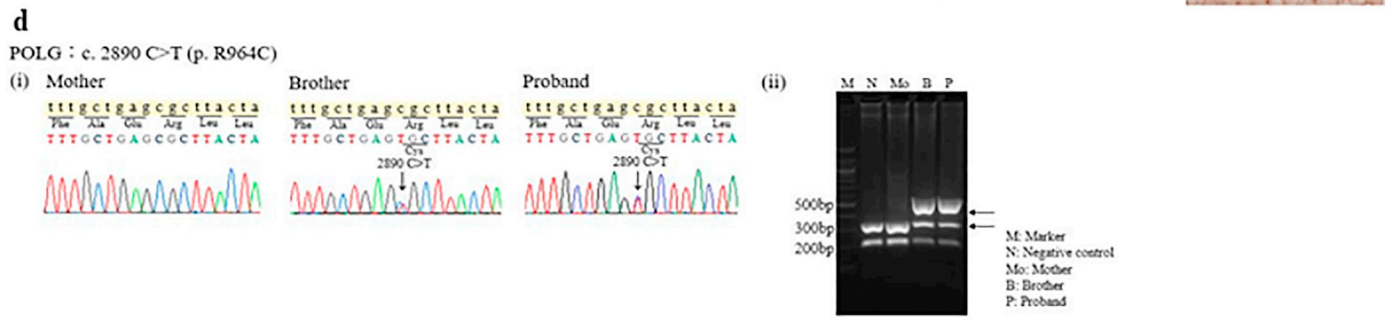

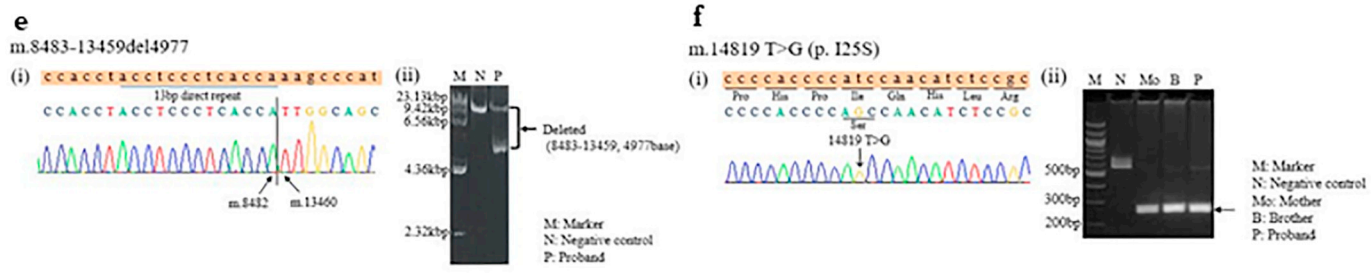

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Symptoms, Signs, and Findings	Gerald Pfeffer, et al (2011) n=40	Present case		
		Mother	Brother	Proband
Onset	39 (1-50)	25	n/a	47
Present age	55 (27-74)	83	61	58
Neurological system				
Ptosis	100%	+ +	−	+ +
Ophthalmoplegia	100%	+ +	+	+ +
Double vision	63%	−	−	+
Muscle weakness	63%	+	−	+
Sensorineural hearing loss	48%	−	−	−
Fatigue	43%	−	−	+
Migraine	40%	−	−	−
Neuropsychiatric disorder	38%	−	−	−
Exertional myalgia	35%	−	−	−
Polyneuropathy	33%	−	−	−
Ataxia	18%	−	−	−

Fig. 1. (a) Family tree of the three patients. Filled symbols are patients. Arrow shows the proband. (b) Photographs of eyes and eye movement of the patients. Mother (i) and proband (iii) showed blepharoptosis, but the brother (ii) did not. All members showed external dislocation of the right eye, and upper and lateral gaze limitation of bilateral eyes. (c) Muscle biopsy of the proband's right biceps brachii. Hematoxylin and eosin staining (i) and Gomori trichrome staining (ii) showed ragged red fibers (arrows). Cytochrome c oxidase staining showed a few negative fibers (iii, arrows). (d) Nuclear DNA analysis on the POLG gene revealed a heterozygous c. 2890C > T (p. R964C) substitution. (i) DNA sequence analysis of POLG showed a c. 2890C > T substitution in the brother and proband, but not in the mother. (ii) PCR-RFLP using *Hae*II confirmed the mutation in the brother and proband (arrows), but not in the mother. (e) MtDNA analysis of DNA from the proband's skeletal muscle of biceps brachii revealed a DNA deletion (i, m.8483.13,459, del 4977) in the DNA sequence; this was confirmed by electrophoresis of long PCR products of the proband's DNA (ii). (f) MtDNA analysis of DNA from peripheral white blood cells of the three patients revealed a novel homozygous m.14819 T > G (p. S25A) substitution (i, proband's mtDNA, arrow) in the DNA sequence; this was confirmed by PCR-RFLP using *Bse*YI in the mother, brother, and proband (ii, arrow). (g) Clinical features of the past and present CPEO cases. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

their symptoms. On the other hand, the novel mtDNA mutation (m.14819 T > G) found in all three members was suspected to be causative in the present three family members. This novel mutation is located in mitochondrial cytochrome *b* (mtCyB), which is one of the central parts of the mitochondrial respiratory chain. The deficiency in the mitochondrial respiratory chain induced by the mtCyB mutation is associated with mitochondrial disease [7]. Mitochondrial respiratory chain dysfunction enhances oxidative stress by producing reactive oxygen species, which causes mtDNA deletions [8,9]. The present cases displayed mtDNA deletions and 13-bp direct repeats (ACCTCCCTCA CCA), which is one of the breakpoints of mtDNA deletions (Fig. 1e (i)) [10]. We suspect that the present mtDNA mutation located in mtCyB might induce mtDNA deletions due to mitochondrial respiratory chain dysfunction, perhaps triggered by the 13-bp direct repeats.

We report a unique CPEO family with a novel mtDNA mutation (m.14819 T > G) accompanied by an mtDNA deletion, showing different clinical symptoms. A further study will be needed to clarify the relationship between their clinical phenotype and mtDNA and nuclear DNA mutations.

Conflicts of interest

The authors disclose no potential conflicts of interest.

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