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Novel mutation in *C10orf2* associated with multiple mtDNA deletions, chronic progressive external ophthalmoplegia and premature aging

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ABSTRACT

Chronic progressive external ophthalmoplegia (CPEO) is caused by defects in both mitochondrial and nuclear genes, however, the causal genetic factors in large number of patients remains undetermined. Therefore, our aim was to screen 12 unrelated patients with CPEO for mutation/multiple deletions in mtDNA and mutations in the coding regions of *C10orf2*, which is essential for mtDNA replication. Histopathological study of muscle biopsy revealed cytochrome c oxidase-deficient fibers and ragged blue fibers in all the patients. Long-range PCR of DNA from skeletal muscle revealed multiple mtDNA deletions in all the 12 patients. Further, sequencing coding regions of *C10orf2* revealed three variants in three different patients, of which two were novel (c.1964G > A/p.G655D; c.204G > A/p.G68G) variants and one was reported (c.1052A > G/p. N351S). Sequencing of other nuclear genes that are associated with CPEO and multiple mtDNA deletions, such as; *POLG1*, *POLG2*, *TK2*, *ANT1*, *DGUOK*, *MPV17* and *RRM2B* did not reveal any pathogenic mutation in patients with *C10orf2* mutation. Since *in silico* analyses revealed p.G655D could be a potentially pathogenic and it was absent in 200 healthy controls, p.G655D could be the causative factor for CPEO. Therefore, we suggest that *C10orf2* gene should be screened in CPEO individuals with multiple mtDNA deletions, which might help in prognosis of this disease and appropriate genetic counseling.

1. Introduction

Mitochondrial defects have been reported to be a major cause of neurological disease usually due to mutations in nuclear or mtDNA (McFarland et al., 2010; Schon et al., 2012). Accumulation of multiple mtDNA deletions in skeletal muscle and brain produces different phenotypes ranging from severe encephalopathy in infancy and childhood to late-onset CPEO, ataxia and myopathy due to mutations in the nuclear genes that are involved in the maintenance of mtDNA (Fratter et al., 2010).

CPEO and multiple mtDNA deletions are genetically heterogeneous, which may be due to mutations in mtDNA polymerase gamma (*POLG1* and *POLG2*) (Longley et al., 2006; Van Goethem et al., 2001), chromosome 10 open reading frame 2 (*C10orf2*) (Spelbrink et al., 2001), thymidine kinase 2 (*TK2*) (Tyynismaa et al., 2012), adenine nucleotide translocator 1 (*ANT1*) (Kaukonen et al., 2000), p53-induced ribonucleotide reductase B subunit (*RRM2B*) (Tyynismaa et al., 2009), deoxyguanosine kinase (*DGUOK*) (Ronchi et al., 2012) and mitochondrial inner membrane protein (MPV17) (Garone et al., 2012).

Twinkle is encoded by *C10orf2*, which is essential for mtDNA replication and serves as the helicase for polymerase gamma. Mutations in the *C10orf2* gene affect the function of Twinkle protein and cause adult-onset CPEO with multiple mtDNA deletions. Recent study reported 23 mutations in the *C10orf2* gene shown to be associated with CPEO (Goffart et al., 2009). Currently, mutations in the *C10orf2* gene have been documented in patients with clinical phenotypes other than CPEO, such as parkinsonism (Baloh et al., 2007), infantile-onset spinocerebellar ataxia (Hartley et al., 2012), severe early onset encephalopathy (Hakonen et al., 2007), severe epileptic encephalopathy (Lonnqvist et al., 2009) and mtDNA depletion syndrome (Sarzi et al., 2007). Defects in *POLG* and *C10orf2* have recently been identified to enhance age-dependent accumulation of mutations in the control region of mtDNA and multiple mtDNA deletions due to replication stalling (Wanrooij et al., 2004).

Here we report the results of screening in a series of 12 patients with CPEO harboring multiple mtDNA deletions and a total of three different variants in *C10orf2* gene.

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2. Materials and methods

2.1. Clinical and histological study

We identified and studied 12 unrelated patients with CPEO (Table 1A). Patient 1 is a male patient aged 35 years, born to consanguineous parents. He first presented with symptoms of ptosis at the age of 31 years. Subsequently, he developed CPEO, exercise intolerance and proximal muscle weakness of all limbs. He also had premature onset of aging-related phenotypes such as alopecia (hair loss), reduced body size and weight loss (Fig. 1). The EMG revealed both myogenic and chronic neurogenic signs at the limb muscles. The ECG and echocardiogram were normal. He had elevated blood lactate. His father had CPEO but was deceased and this mutation not found in unaffected family members. Patients 2 and 3 born to non-consanguineous parents had ptosis and CPEO. There was no family history of this disease. Histological examination was done using fresh frozen sections of biopsy according to standard procedures. Mitochondrial abnormalities were assessed by histochemical staining for modified Gomori trichrome (MGT), cytochrome c oxidase (COX) succinate dehydrogenase (SDH) and combined COX–SDH. An informed written consent was obtained from the proband and his family members. The study was approved by the Institutional Ethical Committee (IEC) of Centre for Cellular and Molecular Biology, Hyderabad, India; Nizam's Institute of Medical Sciences (NIMS), Hyderabad, India.

2.2. Genetic analyses

DNA was isolated from skeletal muscle/blood using the protocol described previously (Thangaraj et al., 2002) with slight modifications. Complete mtDNA was amplified using 24 sets of overlapping primers (Rieder et al., 1998) to generate overlapping amplicons followed by direct sequencing. Long-range PCR to assess multiple mtDNA deletions (Longley et al., 2006) and real-time PCR for mtDNA depletion (Blakely et al., 2008) were performed as previously described. All exons and intron–exon boundaries of *POLG1*, *POLG2*, *C10orf2*, *TK2*, *ANT1*, *DGUOK*, *MPV17* and *RRM2B* genes were amplified using primer sequences that were published elsewhere (Ronchi et al., 2012; Spinazzola et al., 2006) and bidirectionally sequenced using BigDye terminator cycle sequencing kit and 3730XL Genetic Analyzer. DNA variations were identified after assembling patient's sequence with the reference sequence using Auto-Assembler software (Applied Bio-systems).

2.3. In silico analyses

To evaluate the potential functional impact of the identified mutation, we utilized five widely used algorithms, such as; SIFT, PolyPhen-2, Align-GVGD, Mutation Taster and I-Mutant 2.0.

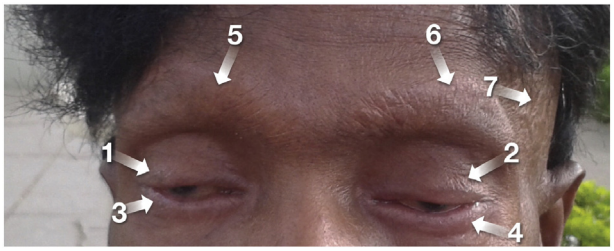

Fig. 1. Patient 1 showed bilateral ptosis, CPEO and premature onset of aging-related phenotypes of CPEO. Arrow 1 and 2 showing bilateral ptosis, arrow 3 and 4 showing loss of eyelashes, arrow 5 and 6 showing poor and thinning eyebrows, arrow 7 showing alopecia (hair loss).

3. Results

3.1. Clinical phenotype and muscle histology

The clinical features and family histories of 12 unrelated patients are summarized in Table 1A. All the patients had both ptosis and CPEO with the exception of two patients (patients 11 and 12) with CPEO and no ptosis. Histochemical staining of skeletal muscle from all the unrelated patients showed ragged red fiber (Fig. 2A), ragged blue fibers (Fig. 2B and C) and COX-negative fibers (Fig. 2D) with the exception of three patients (patients 3, 8 and 10) with COX-negative fibers and no ragged red fiber, which indicate mitochondrial dysfunctions.

3.2. mtDNA analysis

Real time quantitative PCR analysis for the mtDNA copy number did not reveal any significant quantitative difference compared to age and tissue matched controls. However, long-range PCR using DNA isolated from skeletal muscle of all patients revealed multiple deletions of mtDNA (Fig. 2E), further confirming a disorder of mtDNA maintenance. Complete sequencing of mtDNA did not show any pathogenic mutation, but the control region of mtDNA showed higher level mutation in the muscle sample of patient 1 in comparison to age-matched control group.

3.3. Identification of C10orf2 gene mutations

Sequencing of all the exons and exon-intron boundaries of *C10orf2* revealed a total of three heterozygous variants (p.G655D, p.N351S, p.G68G) in three of 12 patients (Table 1B). Among these, two are novel include one missense (p.G655D) and one silent (p.G68G). A novel heterozygous p.G655D mutation in *C10orf2* gene (Fig. 3A),

Table 1A
Clinical features of patients.

Patient	Sex	Age at study (years) deletions	Family history	Clinical features	Muscles histology COX-ve/RRF	Multiple mtDNA
1[a]	M	35	Yes	CPEO, ptosis, exercise intolerance proximal muscle weakness of all limbs premature onset of aging-related phenotypes such as alopecia (hair loss), reduced body size and weight loss	+/+	Yes
2[a]	F	48	No	CPEO, ptosis, weakness of lower limbs	+/+	Yes
3[a]	M	39	No	CPEO, ptosis, diabetes	+/−	Yes
4	F	35	No	CPEO, ptosis, exercise intolerance	+/+	Yes
5	M	59	Yes	CPEO, ptosis, hearing loss	+/+	Yes
6	M	56	yes	CPEO, ptosis	+/+	Yes
7	F	52	No	CPEO, ptosis	+/+	Yes
8	M	22	No	CPEO, ptosis	+/−	Yes
9	M	43	No	CPEO, ptosis	+/+	Yes
10	M	26	Yes	CPEO, ptosis	+/−	Yes
11	F	11	No	CPEO	+/+	Yes
12	M	32	No	CPEO	+/+	Yes

Abbreviations: M = male; F = female; CPEO = chronic progressive external ophtalmoplegia; COX = cytochrome c oxidase deficient fiber; RRF = ragged-red fiber; + = present; − = absent.
[a] Mutations identified in patients.