Original Article

Mutation in *TWINKLE* in a Large Iranian Family with Progressive External Ophthalmoplegia, Myopathy, Dysphagia and Dysphonia, and Behavior Change

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Abstract

Background: *TWINKLE* (*c10orf2*) gene is responsible for autosomal dominant progressive external ophthalmoplegia (PEO). In rare cases, additional features such as muscle weakness, peripheral neuropathy, ataxia, cardiomyopathy, dysphagia, dysphonia, cataracts, depression, dementia, parkinsonism, and hearing loss have been reported in association with heterozygous mutations of the *TWINKLE* gene.

Methods: We have studied a large Iranian family with myopathy, dysphonia, dysphagia, and behavior change in addition to PEO in affected members.

Results: We identified a missense mutation c.1121G > A in the *c10orf2* gene in all affected members. Early death is a novel feature seen in affected members of this family that has not been reported to date.

Conclusion: The association of PEO, myopathy, dysphonia, dysphagia, behavior change and early death has not been previously reported in the literature or other patients with this mutation.

Keywords: Dysphonia, myopathy, ptosis, progressive external ophthalmoplegia, *TWINKLE*

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Introduction

Mutations in the *TWINKLE* (*C10orf2*) gene are responsible for a wide range of clinical presentations.[1–6] The most common phenotype associated with heterozygote mutations is external progressive ophthalmoplegia (OMIM157640). The presenting symptom is ptosis followed by ophthalmoplegia in adult age. Abnormal muscle biopsy findings include cytochrome c oxidase (COX) deficient fibers and ragged red fibers. Southern blot analysis of muscle mt DNA reveals multiple deletions.[1] Additional features can be muscle weakness (usually proximal), peripheral neuropathy, ataxia, cardiomyopathy, dysphagia, dysphonia, cataracts, depression, dementia, parkinsonism and hearing loss. Reports of patients with these additional features are rare and only few families with each phenotype have been reported to date.[2–9] Autosomal recessive mutations in *TWINKLE* (*C10orf2*) are associated with infantile-onset spinocerebellar ataxia (IOSCA)[10] and hepatocerebral mt DNA deletion disorder.[11,12] Follow-up of patients with spinocerebellar ataxia shows that refractory status epilepticus, migraine-like headaches and severe psychiatric symptoms develop in these patients.[13]

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Here, we describe a large Iranian family with ptosis, ophthalmoplegia, muscle weakness, dysphonia and dysphagia, behavior change and early death with affected individuals spanning five generations. All affected members were found to carry a monoallelic variant in *TWINKLE* causing a pathogenic p.Arg374Gln missense mutation.

Patients and Methods

Family studies

We studied a large Iranian family with 10 living affected members (Figure 1). The age of the affected members ranged from 21 to 54 years. Physical examination was performed on 7 affected members (Figure 1) (III11, III13, III15, IV12, IV18, IV19, IV20) and 4 unaffected members (III10, IV15, IV16, IV22). Blood was drawn from 15 members of this family, 7 affected (III11, III13, III15, IV18, IV19, IV20, IV21) and 8 unaffected members (III12, III14, III16, IV16, IV22, IV14, IV15, IV8). DNA was extracted using standard methods. This study was performed under local IRB approval and after obtaining informed consent from all participating family members. The examination and sampling took place in 2014 at the Iranian Center of Neurological Research, Neurology department, Tehran University of Medical Sciences, Tehran, Iran and Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran. The molecular study was performed at the Institute of Molecular and Cell Biology, A*STAR, Singapore, Singapore.

SNP genotyping and linkage analysis

3.1 DNA was extracted from case III11 from peripheral blood

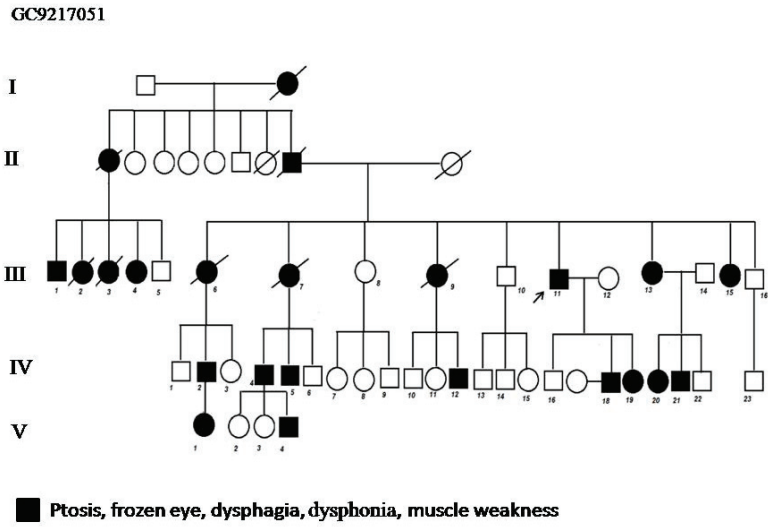

Figure 1. Pedigree of family.

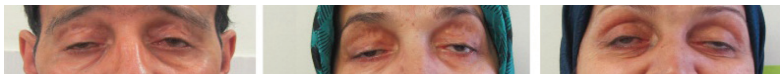

Figure 2. Frontal view of cases III11, III13, III15. Note ptosis and expressionless face. C. Note inability to fully show teeth.

according to the standard protocol. A total of 14 family members (III11, III12, III13, III15, III16, IV8, IV14, IV15, IV16, IV18, IV19, IV20, IV21, IV22) were genotyped using Illumina HumanCore-12v1 BeadChips following manufacturer's instructions. Call rates were above 99%, gender and relationship were verified using Illumina GenomeStudio software. Linkage analysis was performed by searching for shared regions in the 7 affected individuals (III11, III13, III15, IV18, IV19, IV20, IV21) using custom programs written in Mathematica (Wofram Research, Inc.).

Whole exome sequencing

One microgram of high-molecular weight DNA of proband III11 was used for exome capture with Ion TargetSeq™ Exome and Custom Enrichment Kit. This kit targeted 37.3 Mb and permitted capture of >98% VEGA and CCDS coding regions and RefSeq exons. DNA was sheared using Covaris M220 Focused-ultrasonicator (Covaris Inc., Woburn, MA, USA) to target an average fragment size of ~200 bp. Shearing was followed by end repair, ligation of adapters, nick repair, purification, size selection and final amplification prior to exome capture as per TargetSeq protocol. The amplified DNA was cleaned with Ampure XP reagent (Agencourt, Boston, USA) and the DNA was eluted in 30 µL low TE buffer. The libraries were quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). The exome library was used for emulsion PCR on an Ion OneTouch System (Life Technologies, Carlsbad, CA, USA) following the manufacturer's protocol. Each library was sequenced on an Ion Proton instrument (Life Technologies, Carlsbad, CA, USA) using one ION PI chip.

Sequence reads were aligned to the human reference genome [Human GRCh37 (hg19) build] using Torrent Mapping Alignment Program (TMAP) from the Torrent Suite (v4.2.1). PCR duplicates in the BAM file were identified by the Filter Duplicates plugin (v4.2) and removed. The variants were called using the Torrent Variant Caller (TVC) plugin (v4.2.1) and were imported into Ion Reporter (v4.2). Each variant was annotated using the "annotate single sample variants" workflow, including the associated gene, variant location, quality-score, coverage, predicted functional consequences, protein position and amino acid changes, SIFT,[14] PolyPhen2,[15] and Granthan[16] prediction scores, phyloP conservation scores[17] and 5000 genomes Minor Allele Frequencies. Variants were filtered for common SNPs using the NCBI's "common and no known medical impacts" database (URL: http://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh37/) and the Exome Sequencing Project (Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) common SNP database. Variants that were predicted to be synonymous or not having a location on either a coding exon, UTR, splice site junction or flanking intron were filtered out. Variants were next compared to an in-house database of 138 previously sequenced samples. Those variants that were present in more than 1% of the previously sequenced samples were removed. A total of 35,822 variants were identified across protein-coding exons, UTRs, splice sites and flanking introns. After applying all filters and following an autosomal dominant mode of inheritance, a final set of 689 heterozygous variants were identified. Only 7 Htz protein-changing variants were present in the IBD regions including the *C10orf2* gene. Sanger sequencing of the 14 family members confirmed that the missense mutation c.1121G>A in the *c10orf2* gene is only present at the heterozygous state in all the affected individuals of this family. None of the unaffected family members carry the mutation. This missense variant in *C10orf2* leads to the amino-acid alteration p.Arg374Gln, a residue that is phylogenetically invariant. This mutation was previously reported in patients present with progressive external ophthalmoplegia.[8,18,19] The overlapping of the clinical manifestations with previous patients confirms our genetic finding.

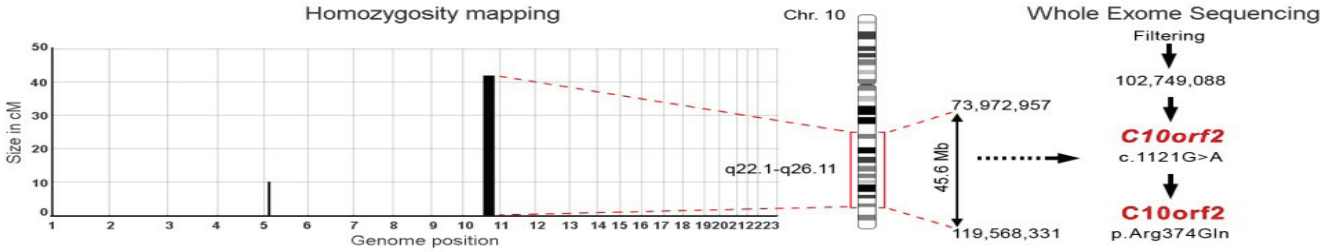

Figure 3. Result of homozygosity mapping and whole exome sequencing.

Results

Representative case histories

The proband (III11) developed ptosis by the age of 14 followed by progressive external ophthalmoplegia four years later. Muscle weakness was observed in his thirties, and dysphonia together with dysphagia in his forties. All symptoms were progressive. He developed dysarthria, fatigue, lethargy, and loss of initiative over the years, starting at the age of 45. Examination at 52 years showed that he had complete ophthalmoparesis with no movement in any direction, ptosis, generalized muscle weakness (distal > proximal) and mild facial muscle weakness (Figure 2). He could blow but could not whistle or completely show his teeth. There was no atrophy of muscles or sensory deficit. Dysphagia first developed at the age of 40, and started with dysphagia to solid foods which had worsened and now included liquids. He had frequent episodes of aspiration during eating. He had a low dysarthric nasal voice.

His sister (III13) also showed ptosis as the first sign of this disease at the age of 24 followed by progressive external ophthalmoplegia. The onset of muscle weakness was in her thirties, upper limbs > lower limbs, distal > proximal, no sensory deficit or atrophy was noted. There was mild facial weakness (Figure 2). The onset of dysphagia was in her late forties, first to solid foods, which progressed to liquids. Dysphonia was present but milder compared to her brother. Hearing loss occurred in the early fifties. They had episodes of respiratory insufficiency, which has been the cause of death in certain cases of affected family members.

Both patients did not initially mention diurnal variation in symptoms, but when asked they said that they feel generally better in the mornings and worse in the evenings and at night.

Early death has been noted in this family. Affected members II1, II8 died at the ages of 55 and 56, respectively while their healthy siblings II2, II3, II4, II5, II6 were still alive at the ages of 83, 81, 79, 75, and 70 years, respectively. In the next generation, the affected members III1, III2, III3, III6, III7, III9 died at the ages of 42, 52, 27, 49, 48, 53 years, respectively while the healthy siblings (III5, III8, III10, III16 were still living at the ages of 46, 55, 53, and 46, respectively. The exact cause of death is not known but was said to be cardiac and respiratory failure in the affected members. The affected members above fifty were said to have behavioral changes and had become lethargic and apathetic with no initiative during their last years of life.

Clinical findings in 7 affected family members are summarized in Table 1.

Electromyography (EMG) and nerve conduction velocity studies (NCV) were performed on III11 and III15 and revealed normal NCV study and myopathic changes in needle EMG and mild active denervation. There was not any after discharge in CMAP (Compound Motor Action Potential) and the Repetitive Nerve Stimulation (RNS) test was within normal limits.

Electrocardiogram and echocardiography were performed on the affected sister of the proband, III 13 showing normal ECG pattern and no cardiomyopathy in echocardiography. During her stay in the neurology department, she felt severe chest pain and after evaluation, myocardial infarction was diagnosed with ST-T changes in ECG and raised cardiac enzymes. There was no history of atherosclerosis or other risk factors such as hypertension, diabetes or smoking in this patient. This was an unexpected event and it was thought that it could be related to her condition and one of the conditions that led to early death in affected members of this family.

Routine laboratory tests performed before her myocardial infarction were as follows: Creatine kinase level was 300U/L (normal range 24–170 U/L), CBC, Sodium, Potassium, Phosphate, Calcium, Urea, ALT, AST, Alkaline Phosphokinase, Creatinine, Thyroid function test, cholesterol, triglyceride, Vitamin D3, LDL, HDL, and urine analysis were all normal.

Anti-AchR antibodies were absent. There was no evidence of fluctuation or respiratory insufficiency and there was no response (positive or even increased weakness) after edrophonium test.

Sequencing of *PABN1* gene responsible for oculopharyngeal dystrophy was performed and no mutation was detected. SNP genotyping followed by linkage analysis was performed to identify the shared regions in the 7 affected individuals (III11, III13, III15, IV18, IV19, IV20, IV21). Allowing 1% error rate, only regions that were >2cM were examined. Candidate regions were further refined by exclusion of common segments with any unaffected family member (III12, III16, IV8, IV14, IV15, IV16, IV22). Two shared loci totaling 53.5 Mb were delineated on chromosomes chr5:10,984,714–18,936,590 and chr10:73,972,957–119,568,331 (hg19) (Figure 3).

Whole exome sequencing of the proband III11 gDNA generated a total of 14.2 Gb with an average read length of 169 bp. An average coverage of 165X was achieved across the exome, with 96% of the targeted sequences covered at ≥20X.

Discussion

We studied a large Iranian family with a novel association of findings, namely, ptosis, progressive external ophthalmoplegia, muscle weakness, dysphagia, dyphonia, behavior change and ear-

ly death over 5 generations. Even though the missense mutation c.1121G>A was previously reported in patients presenting with progressive external ophthalmoplegia, and additional features such as parkinsonism, dysphagia, dysarthria and amyotrophy, and severe respiratory muscle weakness,[8,18,19] the association of clinical findings seen in our family has not been reported previously. Early death (in the fifties) of affected individuals is a new finding associated with this mutation. Early death occurred as early as 27 years and as late as 56 years in the affected members, while the healthy siblings are still living in their seventies or eighties. The cause of death is not specifically known in each case, but is said to be related to cardiac and respiratory failure. Cardiac findings including ventricular enlargement, nonfatal arrhythmias, and nonspecific ECG changes have been previously reported in *TWINKLE* mutation phenotype.[9]

Case III11 showed apathy, mutism, fatigue, and loss of initiative since the age of 45 years, but since all the affected have died in their fifties, there are not many affected members above 45 years to be evaluated for apathy, mutism, fatigue, lethargy and loss of initiative, but the family indicate that all deceased affected members would have behavior change starting in their late forties and fifties, so that they would not speak much, did not work, and spent most of their time sleeping.

Differential diagnoses

The differential diagnoses that can be considered in patients with ophthalmoplegia, ptosis, muscle weakness, dysphagia and dysphonia are Oculopharyngeal Muscular Dystrophy (OPMD) (OMIM 164300), distal oculopharyngeal myopathy, and congenital myasthenic syndromes.

Oculopharyngeal muscular dystrophy (OPMD) is caused by trinucleotide repeat expansion mutations in Poly(A) binding protein 1 (PABPN1). This is a late onset disease with dysphagia, progressive ptosis, and occasional limb-girdle muscle weakness and ophthalmoplegia. Although life expectancy is not shortened, complications secondary to weakness such as aspiration pneumonia, and malnutrition due to difficulty in swallowing, seriously impair the quality of life.[20] Our first impression was that the late onset of the symptoms, dominant inheritance and distribution of the affected muscles with borderline EMG changes strongly suggest dominantly inherited oculopharyngeal muscular dystrophy. Muscle biopsy was not performed in our patient because of the invasive nature of the procedure, but if it had been performed, it could have been helpful in ruling out this condition as they show nuclear aggregates in muscle tissue[21] similar to other polyalanine and polyglutamine diseases,[22–24] however, they are only present in 3%–6% of cases. The presence of a positive family history with involvement of two or generations, presence of ptosis and dysphagia were diagnostic criteria for OFMD.[25] All of these criteria were present in our family, suggesting OPMD.

What did not correlate with this diagnosis in our family was change of behavior, namely apathy, lethargy and lack of initiative seen in the elderly patients and early death. As mentioned above, OFMD significantly diminishes the quality of life, but does not cause early death. Genetic testing was performed to detect GCG expansion in exon 1 of PABPN1 gene in the proband and was negative.

Oculopharyngeal Distal Myopathy (OPDM) (OMIM164310) is another condition that was considered for our family. Both autosomal dominant and recessive pattern of inheritance have been reported. It is an adult onset hereditary muscle disease with involvement of ocular, oropharyngeal and distal muscles.[26–29] Usually the first presenting sign is ptosis, followed variably by dysphagia, oculopharyngeal, distal and proximal muscle weakness. Hearing loss, respiratory difficulties, and facial muscle weakness and muscle atrophy can be additional features.[28–31]

Distal muscle involvement is much more common but proximal involvement has also been reported.[29] Creatine kinase is mildly elevated and EMG shows myopathic pattern, some with myotonic discharges.[29] Muscle biopsy shows rimmed vacuoles with myopathic changes. The genetic cause of this condition is not known yet.

This condition was also a good candidate for our family and almost all features were compatible except change in behavior, early death, and slow rate of progression. In OPMD onset of first clinical findings which are ptosis and ophthalmoplegia, additional clinical symptoms usually follow in the next 5–10-year period[29] while the occurrence of additional features is much slower in our family and each additional new symptom occurs about every ten years; for example ptosis in the twenties, muscle weakness in the thirties, dysphonia and dysphagia in the forties and behavior change in the late forties and early fifties.

There is clinical overlap with congenital myasthenic syndromes (CMS). CMS are a group of heterogeneous diseases caused by genetic defects that affect neuromuscular transmission. The clinical symptoms can present at any time between birth and adult-life. Depending on the site of the defect, they can be classified as presynaptic, synaptic or postsynaptic. The severity and course of the disease are highly variable. Eyelid ptosis, facial, bulbar, and generalized weakness and respiratory insufficiency with sudden apnea and cyanosis can be all part of the clinical picture. Fluctuation of symptoms (mostly seen in ptosis of eyelids and extraocular muscles) is characteristic for this condition.

The diagnosis of CMS is based on clinical findings, a decremental EMG response of the compound muscle action potential (CMAP) on low-frequency (2–3 Hz) stimulation, absence of anti-acetylcholine receptor (AChR) and anti-MuSK antibodies in the serum, and lack of improvement of clinical symptoms with immunosuppressive therapy. Most individuals with CMS benefit from acetylcholine esterase (AChE) inhibitors and/or the potassium channel blocker 3,4-diaminopyridine (3,4-DAP)s. The clinical findings in our family have clinical overlap with CMS which considered in the differential diagnosis. Anti-acetylcholine receptor and anti-MuSK antibodies were negative. On the other hand, there was no evidence of fluctuation or respiratory insufficiency and there was no response (positive or even increased weakness) after edrophonium test and the EMG, repeated three times, did not show decremental EMG response of the compound muscle action potential on low-frequency (2–3 Hz stimulation). So, while the clinical features found in our patients could all be explained by CMS, the laboratory findings, EMB and response to edrophonium test were not consistent with this condition. In Table 2, we have compared the clinical findings of OPMD, OPDM, CMS, PEO caused by mutations in the *TWINKLE* gene with clinical findings found in our proband.

The missense mutation c.1121G > A has been previously reported in three other families. Naimi *et al*. in 2006 reported one patient with this mutation with PEO and severe respiratory muscle weakness, while the additional features found in our family were not reported[18]. Baloh *et al*. in 2007, reported this mutation in a

three generation family with PEO (ptosis and ophthalmoplegia) and Parkinsonism (bradykinesia, tremor, rigidity, cogwheeling).[8] This family did not show dysarthria, dysphagia, muscle weakness, behavior change or early death.

Martin-Negrier *et al*. in 2010, reported this mutation in a French family in which two members had neuropathy and myopathy in addition to PEO.[19] They also had amyotrophy of muscles which was not reported in previous patients. The family with the most similarity with ours was reported by Echaniz-Laguna *et al*. in 2009 in two families with c.1120C > T mutation in the *TWINKLE* gene.[6] The affected members presented with PEO, sensory neuropathy, myopathy, hearing loss, dysphagia, dysphonia and dementia in their 70s and 80s. Only one of our affected patients had hearing loss, and none had sensory neuropathy, while they all have or had (deceased members) behavior change in the fifties. Early death occurred in all of our affected members and none exceeded the age of 56 years which has not yet been reported as a feature of mutation in the *TWINKLE* gene.

In conclusion, in this report, we identified a missense mutation in the c10orf2 gene in a large Iranian family with a novel association of findings, including PEO, myopathy, dysphonia, dysphagia, and behavior change. Early death is a novel feature seen in affected members of this family that has not been reported to date.

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