Clonal Expansion of Secondary Mitochondrial DNA Deletions Associated With Spinocerebellar Ataxia Type 28

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Case Report/Case Series

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Progressive external ophthalmoplegia (PEO) is a common feature in adults with mitochondrial (mt) DNA maintenance disorders associated with somatic mtDNA deletions in muscle, yet the causal genetic defect in many patients remains undetermined.

Whole-exome sequencing identified a novel, heterozygous p.(Gly671Trp) mutation in the AFG3L2 gene encoding an mt protease—previously associated with dominant spinocerebellar ataxia type 28 disease—in a patient with indolent ataxia and PEO. Targeted analysis of a larger, genetically undetermined cohort of patients with PEO with suspected mtDNA maintenance abnormalities identified a second unrelated patient with a similar phenotype and a novel, heterozygous p.(Tyr689His) AFG3L2 mutation. Analysis of patient fibroblasts revealed mt fragmentation and decreased AFG3L2 transcript expression. Western blotting of patient fibroblast and muscle showed decreased AFG3L2 protein levels.

Our observations suggest that AFG3L2 mutations are another important cause, albeit rare, of a late-onset ataxic PEO phenotype due to a disturbance of mtDNA maintenance.

T he most common presenting neurological feature of adults with mitochondrial (mt) DNA maintenance disorders is progressive external ophthalmoplegia (PEO) and ptosis. Of the known maintenance genes, 10 (POLG, POLG2, SLC25A4, C10orf2, RRM2B, TK2, MFN2, OPA1, MGME1, and DNA2) have been associated with PEO and not infrequently with extraocular manifestations including ataxia. Despite this, the genetic basis of mtDNA maintenance disorders remains unknown in approximately 50% of patients.

Studies have shown that autosomal recessive and autosomal dominant mutations in the SPG7 gene encoding paraplegin cause a complex clinical syndrome, including PEO and spastic ataxia, leading to the accumulation of multiple mtDNA deletions in muscle. Here, we present 2 unrelated patients with novel heterozygous mutations in AFG3L2, typically associated with spinocerebellar ataxia type 28 (SCA28), with late-onset neurological presentations and muscle-restricted mtDNA deletions, extending the clinical phenotype of adult-onset disorders of mtDNA maintenance.

Report of Cases

Patient 1
A 63-year-old woman presented with a 10-year history of slowly progressive ptosis and ophthalmoparesis, recurrent falls, and slurred speech. She had developed indolent gait and limb ataxia since her teenage years. Her sister in her 50s was similarly affected with ataxia but no ptosis and declined clinical assessment. Her father had a progressive ataxia-dementia syndrome but died prior to genetic testing.

Currently, she has striking bilateral ptosis and marked limitation of horizontal and vertical gaze, with broken saccades consistent with PEO (Figure 1A). Findings from fundal examination were normal. She had lower limb proximal muscle weakness (Medical Research Council grade 4/5), brisk tendon reflexes, and flexor plantar reflexes. She had mild dysarthria; finger-nose-finger and heel-knee-shin dysmetria; and an ataxic, broad-based gait.

Serum creatine kinase (50 U/L; normal <200 U/L; to convert to microkatal/s per liter, multiply by 0.0167) and serum lactate (1.6 mmol/L; normal <2.1 mmol/L) levels were normal. Nerve conduction studies and single-fiber electromyography findings were normal. A muscle biopsy at age 63 years revealed a conspicuous mt histochemical defect (20% cytochrome c oxidase-deficient fibers, 9% ragged red fibers; Figure 1B), while clonally expanded multiple mtDNA deletions were detected in muscle DNA (Figure 1C and D).

Patient 2
A woman in her 70s presented with a 15-year history of slowly progressive ataxia, slurred speech, and lower limb
spasticity. Subsequently, she developed progressive ptosis and ophthalmoparesis over the ensuing 10 years. There was no relevant family history. She had bilateral ptosis, PEO, and normal fundal examination findings. She had lower limb proximal muscle weakness (Medical Research Council grade 4/5), brisk tendon reflexes, and flexor plantar reflexes. She
had mild finger-nose-finger and heel-knee-shin dysmetria and an ataxic gait. Serum creatine kinase (97 U/L; normal <200 U/L) and serum lactate (1.8 mmol/L; normal <2.1 mmol/L) were normal. Findings from nerve conduction studies and electromyography were normal. Brain magnetic resonance imaging at the age of 60 years showed marked cerebellar atrophy. Muscle biopsy revealed occasional cytochrome c oxidase-deficient fibers and multiple mtDNA deletions.

Following exclusion of major candidate genes, we undertook whole-exome sequencing (eTables 1 and 2 in the Supplement), identifying a novel heterozygous AFG3L2 mutation (c.2011G>T predicting p.[Gly671Trp]), which was confirmed by Sanger sequencing (Patient 1; Figure 2). Subsequent analysis of a cohort of 68 adult patients with genetically undetermined PEO and multiple mtDNA deletions identified 1 further case with a novel heterozygous (c.2065T>C; p.[Tyr689His]) AFG3L2 mutation (Patient 2; Figure 2).

The functional consequences of p.(Gly671Trp) AFG3L2 mutation were investigated using confocal microscopy to study the organization of the dynamic mt network in patient fibroblasts. Previous studies of cells harboring SPG7 mutations have shown changes in mt distribution indicative of a disturbance of mtDNA maintenance. Confocal microscopy revealed that the mean (SD) percentage of fragmented mt networks (<2 μm in length; Figure 3A; patient: 53% [9%]; control individuals: 43% [7%]; P < .001), network length (patient: 3.22 μm [0.49]; control individuals: 3.39 μm [7]; P < .05), and volume distribution of fragmented mt networks (<0.2 μm³; patient: 24.6 [9]; control individuals: 12.9 [2.5]; P < .001). The distribution of mitochondrial network lengths (A, stratified by micrometer) and volumes (B, stratified by cubic micrometer) are shown for patient 1 compared with results from 4 control cell lines showing increased fragmentation of mitochondrial networks. C, AFG3L2 transcript levels. All data shown are normalized to GAPDH transcript level. *AFG3L2 transcript levels are modestly decreased in patient fibroblasts compared with 2 controls (P = .03).
control individuals: 34.6 [9]; P < .001) were significantly different in patient fibroblasts compared with control individuals (Figure 3B), while the mean (SD) number of networks per cell (patient: 82.32 [27.69]; control individuals: 88.75 [29.16]) was similar. Expression studies showed a modest decrease in patient transcript levels for \textit{AFG3L2} (P = .03) but no difference in \textit{SPG7}, \textit{OPA1}, \textit{MFN2}, \textit{POLG}, and \textit{SDHA} compared with control individuals (P = .03) (Figure 3C).

Western blot analysis of fibroblasts demonstrated decreased levels of immunoreactive \textit{AFG3L2}, while \textit{OPA1}, \textit{POLG}, and \textit{SDHA} expression overlapped with control individuals (Figure 4A and B). Similarly, in patient muscle tissue, \textit{AFG3L2} levels were decreased, while expression of both \textit{SPG7} and \textit{HSP60} were increased compared with control individuals (Figure 4C), confirmed by densitometric analysis (P < .001) (Figure 4D).

Discussion

We demonstrated in patients with PEO, ataxia, and multiple mtDNA deletions that mutation of the \textit{AFG3L2} gene is an important, albeit rare, diagnostic consideration. The mutations described in this report are novel and likely pathogenic. Both mutations affect amino acids in exon 16 of \textit{AFG3L2}, a hot-spot for disease-causing mutations, while the p.(Gly671Trp) mutation occurs at the same amino acid position as reported mutations causing SCA28.\textsuperscript{6} Both novel mutations are located in an evolutionarily conserved functional domain of the protein\textsuperscript{5} (Figure 2) and, critically, neither variant was represented within the 1000 Genomes Project or National, Heart, Lung, and Blood Institute Exome Sequencing Project databases nor more than 400 in-house exomes. Furthermore, the
family history of patient 1 was suggestive of autosomal dominant inheritance, while Western blot analysis revealed decreased levels of AFG3L2 protein in patient tissues.

AFG3L2 encodes a protein of the mitochondrial ATPase complex linked to a variety of cellular activities and is a recognized partner of paraplegin, the product of the SPG7 gene, responsible for a form of autosomal recessive hereditary spastic paraplegia. Both AFG3L2 and paraplegin are selectively abundant in cerebellar Purkinje cells, while AFG3L2 expression is lower in the human motor system relative to paraplegin. Loss of AFG3L2 expression in patients with SC2A8 appears to affect the cerebellum, which is relatively spared in SPG7/paraplegin-related disease and this may, in part, explain the phenotypic differences between SPG7 and SC2A8-related neurodegenerative disorders.

The presence of only mild spasticity (compared with the SPG7-disease phenotype) in our patients may reflect the lower expression of AFG3L2 in the motor system compared with SPG7.

Haploinsufficiency has been proposed as a possible pathologic mechanism leading to clinical disease expression of SC2A8. Our data support such an hypothesis, with the finding of a modest decrease in AFG3L2 transcript levels accompanied by markedly decreased AFG3L2 protein levels evident in both fibroblasts and, more significantly, in muscle tissue. This suggests that accelerated degradation of mutant AFG3L2 protein results in haploinsufficiency. We also noted elevated SPG7 protein levels compared with controls and, given the morphology, colocalization and similar functions of SPG7 and AFG3L2, postulate that this may be either an attempted compensatory response or opportunistic overexpression. Fibroblast studies have shown that AFG3L2 mutations cause mtDNA fragmentation, while the presence of cytochrome c oxidase-deficient fibers and multiple mtDNA deletions in skeletal muscle indicate a role for AFG3L2 in mtDNA maintenance. Based on these observations, we propose that mutations in AFG3L2 lead to mtDNA fragmentation and impaired mtDNA maintenance with a consequent acceleration of the age-associated accumulation of somatic mtDNA mutation. This may, in part, explain why the extracocular features are a late-onset clinical manifestation. Although PEO, ptosis, abnormal respiratory chain complex activities, and dramatic mitochondrial morphology defects have all been described in patients with AFG3L2 mutations, to our knowledge, these are the first reports of multiple mtDNA deletions in skeletal muscle, confirming AFG3L2 mutations as a novel cause of disordered mtDNA maintenance.

Conclusions

We believe that the multisystem nature, mtDNA dysfunction, and late clinical features evident in our patients are, in part, mediated through disordered mtDNA maintenance. And they suggest that AFG3L2-related disease should be considered in PEO-plus syndromes, in which progressive ataxia and/or spasticity are conspicuous.


