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Short communication

Beyond dystonia and ataxia: Expanding the phenotype of *SQSTM1* mutations

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ABSTRACT

Background: Homozygous sequestomosome-1 gene mutations have been recently linked to neurodegeneration with dystonia, ataxia and gaze palsy. Seven affected families were identified thus far.
Objective: To describe four new cases with additional phenotypical features.
Results: Four affected patients from two unrelated families were identified. Two compound heterozygous variants of the gene (c.257_259delins35 and c.301+1G > T) were found in one family (cases 1 and 2), and homozygous c.823_824delAG variant was identified in cases 3 and 4. In addition to the previously described syndrome characterized by cerebellar ataxia, dystonia, choreoathetosis, cognitive impairment and gaze palsy, two subjects presented with iridoplegia. Furthermore, we report dysautonomic features such as orthostatic hypotension and sudomotor dysfunction, along with other non-motor symptoms.
Conclusions: We expand the phenotype of dystonia caused by Sequestomosome-1 gene by identifying dysautonomic features along with other non-motor symptoms.

A broad spectrum of diseases can cause dystonia in combination with ataxia [1]. In 2016, Haack et al. described 9 subjects from 4 non-related families (2 from Europe, 2 more from Middle East) featuring dystonia, ataxia and gaze palsy due to mutations of the sequestosome-1 (SQSTM1) gene [2]. More recently, 11 affected individuals from 3 unrelated families were reported by Muto et al. [3]. No other phenotypic descriptions of *SQSTM1*-related dystonia (DYT-SQSTM1) have been published thus far.

Herein, we describe four new cases (two from Mexico and two originally from Jordan). Written informed consent was obtained from all patients for online publication of videos and photographs. Whole exome sequencing was performed in Cases 1 and 4. Genomic DNA was submitted for whole exome sequencing to Centogene (Rostock, Germany). Library enrichment was performed with the SureSelect Human All Exon V6 kit (Agilent). Sequencing was performed with the Illumina platform at a read depth of approximately 100x. Sequencing reads were aligned to the human reference genome (hg19/GRCh37). Filtering of germline variants was performed by consulting HGMD®, ClinVar and CentoMD® databases. *SQSTM1* sequencing was performed in Cases 2 and 3.

CASE 1. A 27-year-old Mexican right-handed male presented with left foot dystonia 20 years earlier. The subject had two siblings, one of them affected by the same disease (CASE 2). Parents were not consanguineous, father and mother had Spanish and French ancestry, respectively. Mydriatic, unresponsive pupils on both eyes were present since he was 8 years old (Fig. 1A). Dystonia presented at the same age, spreading to the face and trunk. Incoordination and gait disturbances were also evident by age 13. Dysphagia and fecal incontinence started when he was 17-year-old; while recurrent falls, writing and speech disturbances presented at age 19. Neurological assessment showed ophthalmoplegia, iridoplegia, and a generalized dystonia combined with a cerebellar syndrome. There was no near-light dissociation, funduscopy was normal and there was no vision loss. A low intellectual quotient (IQ) was also found (Suppl Table 1). Non-motor symptoms were also referred: constipation by age 7, poor concentration

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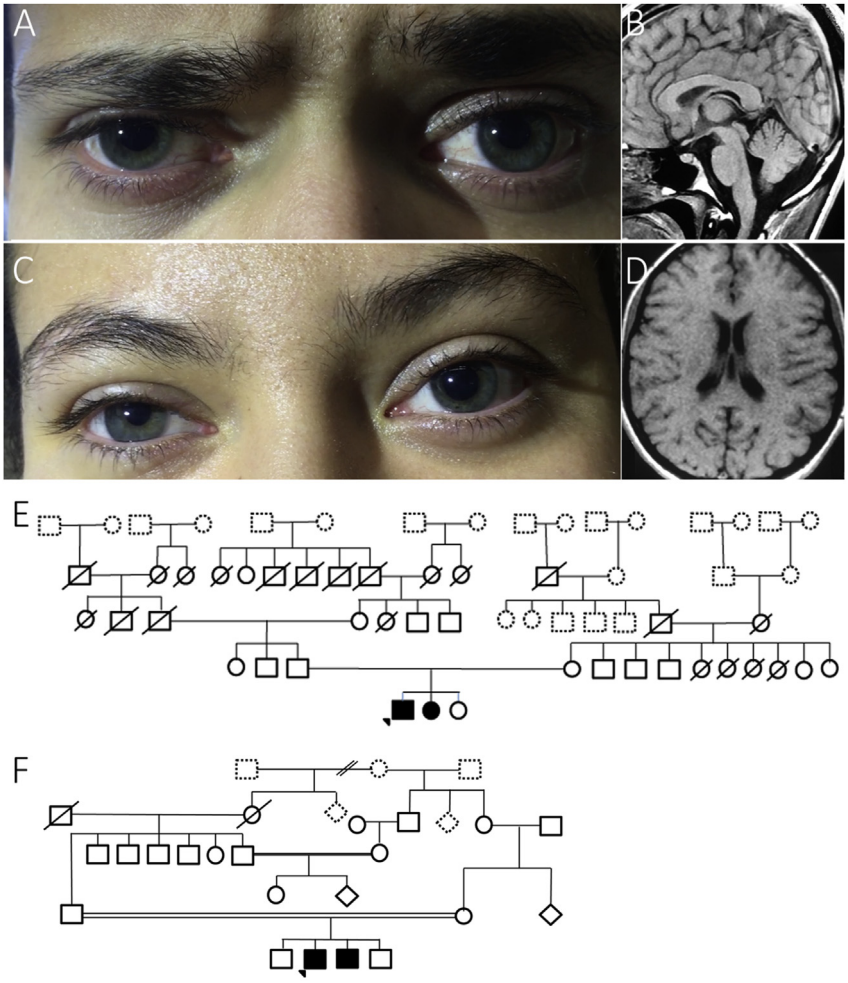

Fig. 1. Iridoplegia and anisocoria (A) and mild vermian cerebellar atrophy as seen at T1w brain MRI (B) of CASE 1. Iridoplegia and anisocoria (C) and normal brain MRI (in T1w cavum velum interpositum is seen) (D) in CASE 2. The pedigree of Cases 1 and 2's family and of Cases 3 and 4's family are depicted in panel E and F, respectively.

by age 11, anxiety by age 13, drooling by age 16, memory problems, insomnia, REM sleep behavior disorder (RBD), depression and restless legs syndrome (RLS) by age 18, and excessive sweating and unprovoked vomiting by age 20. Different tests were performed with unremarkable results. Brain MRI showed slight dorsal cerebellar atrophy (Fig. 1B). Whole exome sequencing (WES) depicted two compound heterozygous mutations of the *SQSTM1* gene (c.257_259delins35 and c.301 + 1G > T); no other relevant mutations were detected. Treatment with amantadine 150 mg BID mildly improved dystonia.

CASE 2. A 24-year-old Mexican right-handed female, sibling of CASE 1, started with writing problems at the age of 7. Two years later, dysarthria became a problem. Facial grimacing and periodic limb movements during sleep were seen when she was 10 years old. Dystonic posturing involving her left foot was seen at age 11. Falls when upstanding and walking were noticed when she was 13. Finally, mydriatic, unresponsive pupils and ophthalmoplegia were evident by age 16 (Fig. 1C). Non-motor symptoms were also referred: excessive sweating and lacrimation starting at 8 years, depression by age 11, orthostatic hypotension by age 15, constipation by age 16, memory problems, dysphagia, insomnia, RLS and RBD by age 18. A low IQ was also evident when she was 18 years old (Suppl. Table 1). Laboratory tests were unremarkable, a brain MRI depicted a cavum velum interpositum only (Fig. 1D). *SQSTM1* sequencing showed the same heterozygous mutation at the *SQSTM1* gene found in CASE 1. Amantadine 100 mg BID almost resolved dystonic features, some cerebellar symptoms improved as well. Greater doses were not tolerated due to gastrointestinal side effects.

CASE 3. A 35-year-old man born in Jordan from consanguineous parents presented with slight cognitive decline around age 9. His speech got slower and he presented with swaying while running. After a severe traumatic brain injury (TBI) at age 16 with need of anterior fossa repair and one-week sedation, he developed involuntary movements of the trunk, feet and hand with abnormal postures as well as frequent falls. Facial grimacing, worsening of cognition, drooling and excessive sweating were also subsequently noted. His abnormal movements progressed over the first year after the TBI and plateaued. Examination at age 17 showed supranuclear gaze palsy, dysarthria, brisk reflexes, generalized choreodystonic movements and wide-based stance. Choking episodes were noted around age 20. Mild constipation has been present for many years, but no other dysautonomic features were noticed. He was treated without benefit with tetrabenazine, trihexyphenidyl and baclofen; ultimately he underwent bilateral deep brain stimulation of the globus pallidus at age 28 with transient mild improvement. Bilateral electrode repositioning after 1 year resulted in minimal improvement for 6 years. Brain MRI revealed a well-defined area of encephalomalacia in the left frontal lobe, few scattered foci of high FLAIR signal in the frontal lobes bilaterally possibly resulting from the TBI. *SQSTM1* sequencing revealed 2 copies of the c.823_824delAG mutation.

CASE 4. A 24-year-old man – brother of CASE 3 – presented with mild

cognitive and behavioral symptoms at age 14. First, social isolation and loss of interest to accomplish homework were noted. He was later transferred to a special class at school due to difficulties learning. Around age 15, wide-based gait, slight inwards posture of the right foot and low speech volume were also observed. Four years later, mild impairment of manual coordination and difficulty arising were present. Involuntary facial movements (eyebrow and mouth) and infrequent mild drooling were seen at age 21. Rare falls were reported in the last 3 years. Slight constipation has been present for many years but no other dysautonomic features were noticed. Examination at age 24 showed dysphonic and mildly dysarthric speech, supranuclear gaze palsy, gaze-evoked nystagmus, eyebrow elevation and occasional mouth pulling. He had mild hand and foot dystonia, mild appendicular ataxia and slightly wide based gait. MRI was reported to be normal. WES showed 2 copies of the c.823_824delAG mutation in the *SQSTM1* gene, no other relevant mutations were detected. His parents were heterozygous carriers of the same mutation. Despite attempts by the family to promote social inclusion, he continues to have marked social isolation. Motor symptoms are overall stable for 9 years and no formal treatment was initiated.

Discussion

We provide video documentation of four SQSTM1 patients with dystonia, ataxia and gaze palsy and describe a new variant related to this phenotype, particularly the occurrence of dysautonomia with the very distinctive features of iridoplegia/anisocoria.

Heterozygous mutations in *SQSTM1* (also known as *p62* gene), localized in chromosome 5 (5q35.3), were initially identified as a cause of Paget disease of bone. *SQSTM1* transcript is p62 protein, also called sequestosome 1, a protein involved in several protein-protein interactions and with multiple functions in receptor-mediated signal transduction, regulation of osteoclast differentiation, activity and survival. Furthermore, p62 has been related to neurodegenerative phenotypes and linked to the ubiquitin-proteasome system and autophagy. Aggregates containing p62 in neurons were found in various neurodegenerative disorders, including frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Heterozygous mutations in *SQSTM1* have been reported in both disorders in keeping with the notion of the ALS/FTD continuum [4–6]. Recently, dominant mutations have been also described in distal myopathy with rimmed vacuoles [2].

The link between p62 and neurodegeneration has been focused on autophagy, the ubiquitin-proteasome degradation pathway and the mitochondrial quality control process known as mitophagy. For instance, the silencing of the *p62* ortholog in drosophila results in mitochondrial dysfunction, mitochondrial DNA accumulation and is linked to locomotor deficits. Other models such as the knockout mouse and p62-silenced mouse embryonic fibroblast have also shown impaired mitochondrial function with reduced ATP production [4].

In 2016 Haack et al. first described four different families with childhood and adolescence onset of neurodegenerative syndrome manifesting mainly with gait abnormalities and ataxia. Exome sequencing in 9 individuals revealed three different biallelic loss-function variants in *SQSTM1*. The identified variants c.286C > T (p.Arg96*) and c.311_312del (p.Glu104Valfs*48) affect all three predicted *SQSTM1* isoforms; while variant c.2T > A (p.Met1Lys) only affects the start codon of one isoform. RNA sequencing in fibroblasts and whole blood of the later suggested partial expression of the two other isoforms. The authors found evidence of early regulation of mitophagy by this protein, including perinuclear clustering of mitochondria and autophagosome formation upon depolarization [2]. This is in keeping with the aforementioned role of SQSTM1/p62 in autophagy and mitophagy.

Recently, 11 affected individuals from three unrelated families were reported [3]. Truncating variants (c.934_936delinsTGA and c.875_876insT) were found in two families, and the remaining family revealed a splice site substitution (c.301 + 2T > A) that generates aberrant processing of the *SQSTM1* transcript, accelerated degradation of the resulting protein and nearly abolished expression of the mutant protein in primary skin fibroblasts.

Comparing these results with our patients, we also found mutations predicted to cause loss of function [7], novel pathogenic variants that have never been reported in association with neurodegenerative ataxia and dystonia. The c.257_259delins35 (p.Ala86Aspfs*17) variant identified in cases 1 and 2, and the c.823_824delAG variant identified in cases 3 and 4 cause a frameshift, supporting their pathogenicity. On the other hand, the mutation c.301 + 1G > T determines a splice site substitution in a highly conserved part of the gene.

The pivotal features of the 20 affected individuals reported so far were cerebellar ataxia and dysarthria (seen in all), accompanied by cognitive impairment (in 19) and gaze palsy (in 18). Dystonia was present in nine patients, choreoathetotic movements in eight, and hypergonadotropic hypogonadism in two. In keeping with our findings, predominant executive dysfunction and apathy were also noted. Herein we expanded the phenotype of these patients by identifying a series of dysautonomic features along with other non-motor symptoms. The reasons for the difference captured by our series are only speculative at the moment. Limitations of our study included lack of functional studies confirming the pathogenic role of the reported mutations and no genetic testing for non-affected individuals.

DYT-SQSTM1 is a rare ataxic and dystonic syndrome with a broader phenotype than initially thought (hence ATX/DYT-SQSTM1 is probably a better way to define it). We are sure that future case series will shed light on the prevalence of other features, including non-motor signs. In fact, given the relative rarity of these features in other generalized dystonias, these additional signs (and particularly the pupillary abnormalities) will possibly inform the diagnosis on clinical grounds.

Conflicts of interest

None.

Author's contributions

Carlos Zúñiga-Ramírez: Conceptualized and organized the study, role in data acquisition and interpretation, drafted the manuscript for intellectual content.

Lais Machado de Oliveira: Conceptualized the study, role in data acquisition and interpretation, drafted the manuscript for intellectual content.

Mirelle Kramis-Hollands: Conceptualized the study, role in data acquisition and interpretation, drafted the manuscript for intellectual content.

Musleh Algarni: Role in data acquisition and interpretation, revised the manuscript for intellectual content.

Alberto Soto-Escageda: Major role in data acquisition.

Michel Sáenz-Farret: Organized the study, role in data acquisition.

Héctor Alberto González-Usigli: Organized the study, role in data acquisition.

Alfonso Fasano: Conceptualized and organized the study, interpreted the data, revised the manuscript for intellectual content.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2018.12.031.

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