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TRIO gene segregation in a family with cerebellar ataxia☆

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ABSTRACT

Aim of the study: To report a family with a novel *TRIO* gene mutation associated with phenotype of cerebellar ataxia.

Materials and methods: Seven family members of Caribbean descent were recruited through our ataxia research protocol; of the family members, the mother and all 3 children were found to be affected with severe young-onset and rapidly progressive truncal and appendicular ataxia leading to early disability. Array comparative genomic hybridization, mitochondrial DNA analysis, and whole-exome sequencing were performed on 3 of the family members (mother and 2 daughters).

Results: While the maternal grandmother, great uncle and great aunt were unaffected, the mother and 3 children displayed cognitive dysfunction, severe ataxia, spasticity, and speech disturbances. Age of onset ranged between 3 and 17 years, with average current disease duration of 21 years. Whole-exome sequencing showed a variant p.A1214V in exon 22 of the *TRIO* gene in 3 of the family members. Array comparative genomic hybridization and mitochondrial DNA analysis were normal. The same variant was later discovered in all but one family member.

Conclusions and clinical implications: The *TRIO* p.A1214V variant is associated with cerebellar ataxia in the studied family; it was present in all affected and unaffected family members. Phenotype is severe and broad. Anticipation seems to be present (based on 2 affected generations). It is warranted to screen additional familial early-onset and rapidly progressive ataxia cases for this genotype. *TRIO* gene mutations may well represent a novel spinocerebellar ataxia subtype.

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1. Introduction

Spinocerebellar ataxia (SCA) is a form of autosomal-dominant cerebellar ataxia involving the spinal cord and cerebellum [1,2]. SCA occurs in 1 to 5 per 100,000 individuals worldwide [3]. Over 40 genetic subtypes of SCA have been identified thus far [1,2]. There is a clinical heterogeneity that accompanies genetic heterogeneity [4,5]. The wide spectrum of phenotypic manifestations in SCA subtypes include cognitive deficits; oculomotor disturbances; and neurologic dysfunction involving bulbar, cerebellar, extrapyramidal, and spinal system impairment. Retinal abnormalities, intellectual disability (ID), and peripheral neuropathy have been associated with genetic forms of cerebellar ataxia [1]. Oculomotor disturbances, such as impaired pursuit and saccades and nystagmus, have been documented in SCA patients [6,7]. Genetic studies have shown that certain classes of SCA are caused by CAG or other trinucleotide repeat expansions, while others are due to missense mutations and deletions [1,2,4,8].

1.1. *Clinical rationale for the study*

TRIO gene mutations have previously been correlated with ID [9]. Gait ataxia was only detected in a single familial case harboring a p.Asn1080lle variant on exon 19 of the *TRIO* gene [10]. We describe a 3 generation family of Caribbean descent presenting with ID, schizophrenia, behavioral symptoms, speech disturbances, cerebellar ataxia, and spasticity carrying a novel *TRIO* gene mutation.

2. Materials and methods

2.1. *Standard protocol approval, patients consent, clinical scales/assessments, sample collection*

Our study was approved by the Institutional Review Board. Seven family members (3 unaffected, 4 affected) were recruited into the study (Fig. 1). Written informed consent was obtained from all participants.

Neurologic evaluations included the Scale for Assessment and Rating for Ataxia and Mini-Mental State Examination, in addition to video recordings. We retrieved and reviewed all available medical records dating back to childhood (over 20 years ago). Individuals III-1, III-2, and III-3 (discussed in more detail below) were followed clinically by two of the co-authors (Z.K.W. and R.J.U.), and office visits/notes were reviewed.

2.2. *Magnetic resonance imaging*

Neuroradiologic imaging was obtained for members of the third generation, and involved MRI scans. We reviewed all MRI sequences available that had been obtained for members of the third generation for clinical purposes.

2.3. *Genetic analysis*

Whole-exome sequencing was performed on 4 family members. Genomic DNA was extracted from blood and buccal samples. DNA was sequenced using Illumina HighSeq system with 100 bp paired-end reads, and mapped to the human genome build GRCh37/UCSC hg19. The exonic regions and

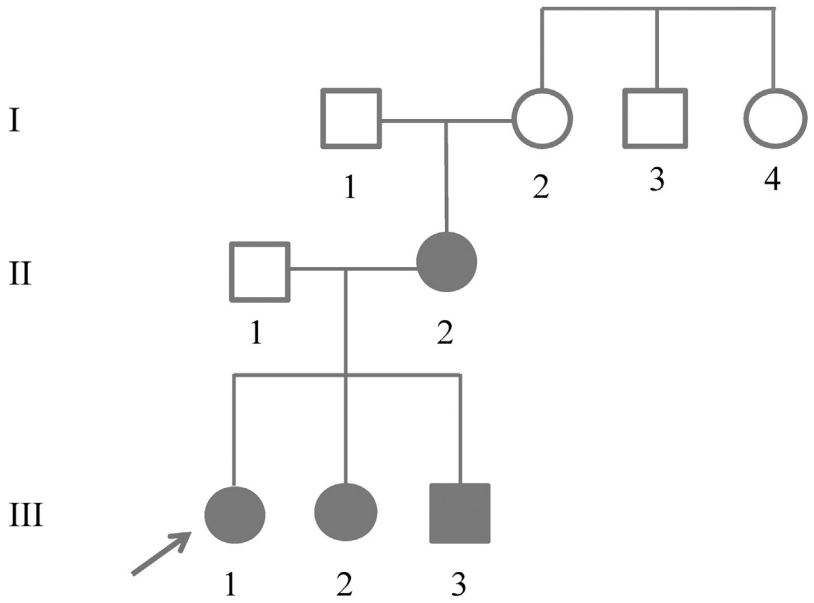

Fig. 1 – Family pedigree drawn according to standard procedures. Squares indicate men and circles, women. Fully darkened symbols indicate affected individuals. I-2, I-3, II-2, and all members of the third generation possess a p.Ala1214Val in exon 22 of the TRIO gene.

flanking splice junctions were analyzed for variants using GeneDx's XomeAnalyzer. Pathogenic variants were confirmed using capillary sequencing and other appropriate methods. Human Genome Variation Society (HGVS) and International System for Human Cytogenetic Nomenclature (ISCN) guidelines were utilized when stating the sequence and copy number of variations.

In addition, the entire mitochondrial genome was amplified and sequenced via a solid state sequencing by-synthesis process. DNA sequence was constructed and scrutinized in comparison with the revised Cambridge Reference Sequence (rCRS) and the reported variants and polymorphisms listed in the MITOMAP database (http://mitomap.org). The presence of a disease associated sequence variant, if present, was confirmed by conventional dideoxy sequence analysis or other methods. A reference library of more than 6000 samples from different ethnic groups and online databases for mtDNA variations is used to evaluate variants of unknown clinical significance.

We tested for the presence of CAG trinucleotide expansion in *ATXN3* in patients and controls using Taq DNA Polymerase and a primer set containing a FAM labeled primer. PCR products were diluted, combined with GeneScan 500 LIZ dye Size Standard and analyzed on the ABI 3730 Genetic Analyzer. GeneMapper Software was used to size and genotype the alleles.

The p.Ala1214Val mutation in the TRIO gene was amplified from genomic DNA from patients and controls using AmpliTaq Gold 360 (Thermo Fisher Scientific Inc.) in sequentially nested polymerase chain reactions (PCRs). The following primers were used: external primer pair 5′-CTACCATCAGCCGGTTTTCC-3′ and 5′-GGGCGTTAGGAAGAAGACC-3′, and internal primer pair 5-′CCGGTTTTCCTGTTCAGAGC-3′ and 5′-GTTTGCACAAGGCCATTTACCG-3′. Samples from the second PCR were run on a 2% NuSieve Agarose gel (Lonza, Supplementary Fig. 1), purified and quantified. Approximately 50 ng of PCR product was used in a sequencing reaction employing the BigDye Terminator v3.1 Cycle Sequencing Kit (according to manufacturer's instructions, Applied Biosystems) and 1 of the following sequencing primers: forward 5′-TTGAGGTTTCTGGCAGTCGG-3′ or reverse 5′-CGATTTGTTGGAATCTGAAG-3′. Sequencing products were purified using the Montage Seq_{96} Sequencing Reaction Cleanup Kit (according to manufacturer's instructions, Millipore Sigma), and analyzed on the Applied Biosystems ABI 3730 Genetic Analyzer. The sequence was analyzed using Sequencher® version 4.8 DNA sequence analysis software (Gene Codes Corporation).

2.4. *Computational modeling*

Computer-assisted modeling was completed using a sequence of human triple functional domain protein (*TRIO*) taken from the National Center for Biotechnology Information Reference Accession Sequence, NP_009049 version NP_009049.2, focusing solely on domains 4, 5, and 6, and the inter-domain regions spanning between Spectrin 3, Spectrin 4, and DH1 domains (residues 878 through 1467), which forms a segment of 589 amino acids of the larger sequence.

Monte Carlo (MC) simulations were performed on the altered proteins to observe local and regional changes for 589 amino acids for the *TRIO* protein. A variant was modeled at p. A1214V and subsequently compared to the wildtype.

YASARA is linux-based, modeling and simulation program interface, which was utilized for biomolecular calculations for the secondary-structure prediction (SSP) using a position-specific scoring matrix (PSSM) from related sequences. Scanning of depositories of PDBs for potential modeling templates, which used with our programming codes forms composite models for the full-sequence protein structure. Final molecular refinement for MC was built using the YASARA SSP/PSSM method [11–16]. Relaxation of the structure was completed via the YASARA/Amber force field using YASARA knowledge-based potentials utilizing YASARA's refinement protocol [17], we also allowed for the filling in of any gaps or unresolved portions of both full-length protein structures beyond what was available in the crystallographic structure using ab initio methods. Supplementary Fig. 2A shows the wildtype structure, and Supplementary Fig. 2B shows the *TRIO* domain schematic.

Starting with the super positioning the YASARA-generated initial model and mutant protein [11–13,15], then using the Schrodinger's MC program (*low modes* type algorithm) coupled with NAMD2 protocols was used to further refine and generate the finalized the models [13]. Review and development of the overlapping regions yielded a complete protein model. Energy optimization though a Polak–Ribière conjugate gradient with an R-dependent dielectric was run on the final model.

This confirmation helped to verify correctness of chain name, dihedrals, angles, torsions, non-bonds, electrostatics, atom typing, and parameters using Maestro (Macromodel, Schrodinger) and YASARA [18].

Conformational sampling was done through MC dynamics searching (LCMOD-MC) [19–22]. Steepest descent or Polak–Ribière conjugate gradient was used to minimize all systems; and then the systems then proceeded to the MC search [19–22].

3. Results

3.1. *Clinical case descriptions*

3.1.1. *I-2 (63 years old)*

I-2, the grandmother, is asymptomatic. *TRIO* gene p.A1214V variant in exon 22 was apparent upon genetic analysis.

3.1.2. *I-3 (62 years old)*

I-3, the great-uncle, is asymptomatic. He was found to harbor the same *TRIO* gene p.A1214V variant in exon 22 upon genetic analysis.

3.1.3. *I-4 (59 years old)*

I-4, the great-aunt, is unaffected. She does not possess the *TRIO* gene variant present in the other family members.

3.1.4. *II-2 (44 years old)*

II-2, the mother, had a history of seizures and began experiencing falls at 17 years of age. At 19, she was diagnosed with postpartum depression after her first pregnancy. She progressively deteriorated cognitively, and physically was confined to a wheel chair following the birth of her third child. Upon evaluation, she had right-sided torticollis with hypertrophy of the sternocleidomas-

toid muscle. Her feet were fully inverted, and she was no longer able to stand or move her legs. Severe spasticity with sustained ankle clonus was present bilaterally; weakness in the lower extremities was noted. She had dysmetria and dysdiadochokinesia. Reflexes were exaggerated. No Babinski sign was present. Superficial sensation was normal. Speech was intelligible, one word at a time. She was suffering from dysphagia and bowel and bladder incontinence.

3.1.5. *III-1 (24 years old)*

III-1, a woman with a past medical history of developmental delay, schizophrenia, and depression, presented for an evaluation of gait impairment. She was delivered at term following an uncomplicated pregnancy. She began to display muscle weakness and spasticity at age 9, attended regular elementary school, and was enrolled in special education during high school. Her loss of balance and falls began at 22 years of age; she has been ambulating with the assistance of a walker since then and she requires assistance with most daily activities. She had no history of seizures. She was diagnosed with schizophreniform disorder with moderate ID after neuropsychologic testing. It was also noted that she was experiencing signs and symptoms of autistic spectrum disorder; her cognitive screening score was in the very low range. The patient has previously undergone commercial genetic testing for SCA1, SCA2, SCA3, SCA5, SCA6, SCA7, SCA8, SCA10, SCA12, SCA13, SCA14, SCA17, SCA18, Marinesco-Sjogren syndrome (SIL1), Ataxia with isolated Vitamin E deficiency (TTPA), Friedreich's Ataxia (FXN), Mitochondrial recessive ataxia syndrome (MIRAS-POLG), Ataxia, early-onset, with oculomotor apraxia and hypoalbuminemia (APTX), and Senataxin (SETX). Array comparative genomic hybridization and mitochondrial DNA analysis results were normal.

3.1.6. *III-2 (22 years old)*

III-2 has been struggling with fine-motor coordination and cognitive impairment since birth. She received speech therapy during childhood, is independent in all daily living functions, and has completed a 2-year training program as a patient care technician. Her grandmother reported that the patient experiences anger episodes; however, she does not display signs of physical aggression during those episodes. She sometimes displays obsessive compulsive behavior. The patient reported that her balance has been impaired and she has suffered near falls recently (over the past 3 months). Her gait was spastic, speech was ataxic, and lateral nystagmus was present in both eyes. She had hyperactive deep tendon reflexes in her lower extremities more so than in her upper extremities, with sustained clonus present in her ankles. Ataxia was present in all 4 extremities. She was able to regain her balance with difficulty during a pull test. Her Mini-Mental State Examination score was 27 out of 30 points. Patient's psychological manifestations have increased in severity since last follow up and will be undergoing neuropsychological testing, she has also reported weakness and increase in incoordination since last follow up.

3.1.7. *III-3 (20 years old)*

Cognitive and learning delays began around 3–4 years of age in III-3. He has struggled with clumsiness since childhood, and his grandmother also noted fine motor abnormalities. He had been experiencing difficulty with speech, for which he received therapy; upon presentation, his speech was dysarthric in nature. In addition, he had experienced unprovoked anger outbursts since age 17. Although present in all 4 extremities, ataxia was more pronounced in the lower extremities and spasticity was more pronounced in the upper extremities. On Mini-Mental State Examination, he scored 25 out of 30 points. It was determined that he has mild ID upon neuropsychologic examination. He was found to harbor the same variant in exon 22 of the *TRIO* gene as his grandmother, great-uncle, mother, and 2 sisters. It has been noted that the patient is experiencing more pronounced psychological symptoms including anger outbursts since the last visit.

3.2. *Magnetic resonance imaging*

Loss of volume in the superior portion of the cerebellar vermis was evident on MRI neuroradiologic imaging of patient III-1, but brainstem atrophy was not apparent. Of note, the patient also displayed mild bilateral parietal sulcal enlargement. Patient III-2 displayed mild cerebellar vermian atrophy upon imaging. Patient III-3 had a normal study (Fig. 2).

3.3. *Genetic analysis*

Array comparative genomic hybridization (aCGH), mitochondrial DNA (mtDNA) and whole exome sequencing were performed on patients II-2, III-1, and III-2 prior to their enrollment in the study. Results for aCGH and mtDNA were normal. Whole-exome sequencing for the same family members showed a variant (p.A1214V (GCG/GTG): c.3641 C > T) in exon 22 of the *TRIO* gene (NM_007118.2) and all generations except I-4 were heterozygous for this variant on chromosome 5 p15.2. The father of III-1, III-2, and III-3 was also tested prior to enrollment, and he does not harbor this same variant. The prevalence of this variant is 1:66,364, and occurs at a rate of 0.0015% in alleles from individuals of European (non-Finnish) descent, according to the ExAC dataset [23].

3.4. *Computational modeling*

TRIO forms internal domain interactions important to function. The structural modeling drawn from radiographic structural datasets demonstrated a strong effect from the respective variants. The in silico results represent a complementary technique utilized to support the findings and functions as a sorting tool to assist with variant classification.

When compared to wildtype, the stability of the object from energetic calculations for ΔG per amino acid is higher for the *TRIO* variant p.A1214V. Wildtype and p.A1214V were 224.62 and 638.35 kcal/mol Å^2, respectively [19,20,22,24–27]. Per amino acid, wildtype measured 0.382 kcal/mol Å^2 and p.A1214V 1.084 kcal/mol Å^2. Correspondingly, the p.A1214V protein domains modeled (4–6) had worse total object stability when not examined per amino acid distribution of energies.

The p.A1214V variant for the TRIO protein results in a large conformational shift in the domain arrangement between domains 5 (Spectrin 4) and 6 (DH1), likely altering protein function due to location of inter-helical bundling within the

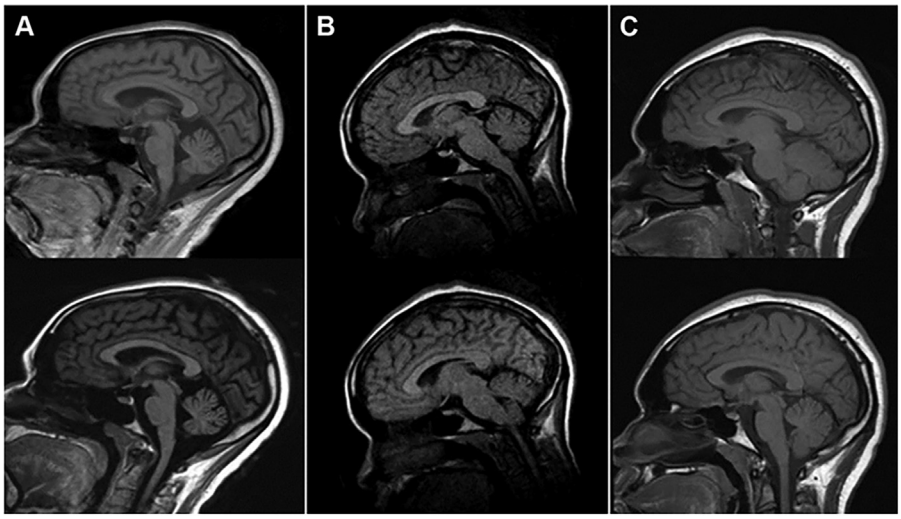

Fig. 2 – Sagittal cranial sections using MRI. (A) III-1 T1 sagittal flair; (B) III-2 T1 sagittal flair; and (C) III-3 sagittal SE T1 image.

spectrin domains, which eliminates partnering and protein structural integrity. The actual residue mutation, p.A1214V, induces a +0.408 kcal/mol Å^2 change in free energy, which is disruptive to the local region. The molecular model for the full structure and its variant form are displayed in Supplementary Fig. 2 [19–22,27–34]. Local structural changes between wildtype and p.A1214V is shown in Supplementary Fig. 2C and F.

Examination of the domain regions affected by the *TRIO* variant consists of residues within the Spectrin 4 domain (residues 1109–1216), and important domain-domain binding sites are reduced or removed completely (residues C1041, L1047, H1055, V1056, T1057, R1133, Q1136, C1137, Y1140, V1141, E1144, Q1139, F1143, L1199, I1200, D1204, F1206, H1211, H1213, I1217, K1218, K1219, and A1223) (Supplementary Fig. 2C–F). All of the important domain contacts are lost over the duration of simulation, allowing for the large rearrangement within domain 4 and the spacer region adjacent, which has a profound effect on the rest of the domains (domain 6 through C-terminus domain) (Supplementary Fig. 2A and E). This is also pronounced in our electrostatic surface calculation for TRIO, which shows how the helical bundling breaks down and an extra lobe appears, which could frustrate partner protein binding (Supplementary Fig. 3).

4. Discussion

TRIO is a diverse protein with many domains, including guanine nucleotide exchange factor for RHOA and RAC1 GTPases [10,35]. TRIO is also utilized with the coordination of actin remodeling, which is critically necessary for cell migration and growth [36]. TRIO is found in developing hippocampal neurons and can limit dendrite formation without causing axon polarity. After dendrites are developed, TRIO remains involved in synaptic function control via regulation of the endocytosis of AMPA-selective glutamate receptors at CA1 excitatory synapses.

Mutations involving the *TRIO* gene are associated with ID, behavioral manifestations, and certain physical features, such as microcephaly, as demonstrated by Ba et al. [9]. Our study provides evidence that the *TRIO* gene p.A1214V variant in exon 22 is not only associated with ID and autism, it is also linked to cerebellar ataxia. There is obvious heritability among the family members in this pedigree (Fig. 1); the mutation is transmitted in an autosomal dominant fashion among the family members. The *TRIO* gene mutation is segregated in all except one family member (I-4). Members of the second and third generations share a similar phenotype. The age of onset ranged from 3 to 17, with an average of 8.25 years. The *TRIO* gene plays a role during neuronal development, assisting in neurite outgrowth and basal synaptic transmission; thus, loss of function inhibits both processes from taking place [35]. Seipel et al. [36] reported that the TRIO protein was associated with cellular growth, migration, and spreading via orchestration of the function of the actin cytoskeleton. TRIO-deficient mice possess features of severe ataxia and multiple developmental defects of the cerebellum [37], providing a plausible explanation for the developmental delay and cognitive impairment present in 4 of the family members in our study.

Cerebellar atrophy is evident upon imaging for the proband (III-1) and her sister III-2 (Fig. 2). III-1 exhibits a considerable amount of cerebellar vermian volume loss for her age, along with an increase in the size of the 4th ventricle. Meanwhile brainstem atrophy which is present in a number of spinocerebellar ataxia patients is not apparent in III-1 and III-2. MRI for III-3 was shown to be normal. Neuroradiology findings have never been discussed in prior cases harboring *TRIO* gene mutations, thus indicating that these findings prove to be novel.

Anticipation is demonstrated when symptoms occur at an early age of onset and with higher level of severity than in the previous generations [38]. It is evident in the family we studied. While patients I-2, I-3 and I-4 remain unaffected, II-2 began to present with symptoms at 17 years of age, and III-1 was 3 years old at symptom onset. Early onset of symptoms and delay in speech have been noted among other patients with the *TRIO* gene mutation [10], similar to all 3 siblings (III-1, III-2, and III-3), who began speech therapy at or around 3 years of age. Psychiatric manifestations are also present among family members of the

second and third generations. Patient II-2 was diagnosed with postpartum depression following her first pregnancy. At the age of 22, patient III-1 developed signs and symptoms of schizophrenia. Patient III-2 currently encounters frequent mood fluctuations and often displays obsessive compulsive behavior, while patient III-3 has anger outbursts that are medically managed. Psychological manifestations have worsened in two members of the third generation since initial evaluation and III-2 is scheduled to undergo neuropsychological testing upon the next visit. These features fit the phenotype described by Pengelly et al. [10]. The same study reported one case with gait ataxia. In this family all 4 affected individuals display clinical features of cerebellar ataxia. Thus, we can safely infer that this TRIO gene mutation is indeed associated with ataxia.

Using established techniques, energetics-based and modeling examinations reveal conclusive defects in the p.A1214V TRIO protein [19–22,24–27]. Models for the TRIO structure reveal a striking loss of structural secondary and tertiary contacts needed between the domains for proper enzymatic function. The energetic calculations from measurements of the TRIO structure show, in great detail, how the A1214V variant (mutation) disrupts the wildtype protein in such a manner that the variant protein would likely have a notable disadvantage competing for binding partners compared to the wildtype. A loss of function or a substantially decreased amount of activity for the TRIO protein is expected given this huge conformational change and breakdown between the domain-domain interactions of the inter-protein sites, which are critical for stability.

Similarly, energetic calculations from measurements of the structure show greater instability for the variant than the wildtype protein. Losing the domain interactions could account for loss of or a considerable decrease in function for this protein. The structural model of this variant concurs with the clinical data. Protein modeling can be precisely employed as a useful tool for the arduous task of variant categorization. Further studies of including participants with similar phenotype and mutation are warranted in order to solidify the involvement of cerebellar ataxia among patients with *TRIO* gene mutations.

5. Clinical implications/future directions

The *TRIO* p.A1214V variant is associated with cerebellar ataxia in the family we studied; it was present in all affected family members and two unaffected members. The phenotype is severe and broad. Anticipation seems to be present (based on 2 affected generations). Screening of more familial early-onset and rapidly progressive ataxia cases for this genotype is warranted. The *TRIO* gene mutations may well represent a novel SCA subtype.

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Conflict of interest

RHA receives funding from the ataxia grant gift of Donald G. and Jodi P. Heeringa. TC receives funding from NIH grant L30 CA209803-01. LP receives funding from NIH grant P01 NS084974 and a gift from Donald G. and Jodi P. Heeringa. ZKW is partially supported by Mayo Clinic Center for Regenerative Medicine, and a gift from Donald G. and Jodi P. Heeringa.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pjnns.2018.09.006.

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