Parkinsonism and Related Disorders 62 (2019) 236–238

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

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Craniocervical dystonia with levodopa-responsive parkinsonism co-segregating with a pathogenic *ANO3* mutation in a Taiwanese family

ARTICLE INFO

Keywords:
Dystonia
Parkinsonism
Myoclonus
Young-onset
Anoctamin 3
ANO3

Variants in the *anoctamin 3* (*ANO3*, OMIM 610110) gene have been identified to cause autosomal dominant cervical dystonia (DYT24) [1]. *ANO3* mutation carriers have been reported to present with young-onset craniocervical dystonia combined with posture tremor and/or myoclonic jerks. To our knowledge, Parkinsonism has not been reported previously in patients with *ANO3* mutations [1–4]. Recently, disturbances of basal ganglia glucose metabolism have been found in a patient having a novel *ANO3* mutation presenting with dystonia and jerky movements [5]. We identified a known pathogenic variant in *ANO*3, NM_031418.2 c.2053A > G p.(Ser685Gly, rs587776923), in a family with autosomal dominant craniocervical dystonia where one of the mutation carriers also had myoclonus and levodopa-responsive early-onset parkinsonism.

A 24-year-old woman had slowly progressing intermittent jerky movements of the arms since the age of 15. In subsequent years, she showed head tilting to the right, right hand dystonia with difficulty in writing, and foot dystonia. Slowness of left hand movements and left leg dragging gait were also noted during the same time period. Her symptoms showed no sensory trick, diurnal change, or sleep benefit. Torticollis, myoclonic jerks and dystonia over hands, as well as rigidity and bradykinesia over the left side limbs were found on neurological examination (Supplementary Video 1). There were no Kayser Fleischer rings, or ataxia. Deep tendon reflexes and sensory systems were normal. Neuropsychological testing revealed normal general cognitive function. Results from a laboratory work-up including serum ceruloplasmin level and immune and metabolic profiles were all within normal limits, and brain MRI was unremarkable. The results of Tc-99m TRODAT SPECT scan showed asymmetrically decreased uptake in right putamen (standardized uptake value of region of interest: caudate: right/left: 99.5%; putamen: right/left: 75.3%; Fig. 1A). Her left-hand bradykinesia and rigidity improved significantly following levodopa treatment with a total dose of 300mg/day. Trihexyphenidyl and clonazepam were maintained as they mildly alleviated hand dystonia and reduced jerky movements. The patient's mother also had cervical dystonia and mild abnormal dystonic posturing of the hands since the age of 5 years, with a very slowly progressing course. She is currently 50 years of age. Notably, she had prominent spasmodic dysphonia during the same time period but no feeding dystonia (Supplementary Video 2). She did not have myoclonic jerks or features of parkinsonism.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.01.020.

Genetic analysis of copy number variations in common PD-causative genes including *SNCA*, *Parkin*, *PINK1*, *DJ-1*, *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, *GCH-1* and *LRRK2* using the salsa multiplex ligation-dependent probe amplification (MLPA) kit P051-c1/P52-c1 (MRC-Holland, Amsterdam, The Netherlands) yielded normal results. A targeted next generation sequencing (NGS) movement disorder gene panel including 69 genes related to PD, dystonia and related neurodegenerative disorders (Supplementary Table 1), revealed a pathogenic variant in exon 21 of *ANO3*, c.2053A > G (p.Ser685Gly), which was previously reported in a European family [2]. The target gene capture sequencing covered 1171 exons in 69 genes representing a total coding region of 238,073 bp. The average coverage was 147 folds, with 93.1% of sequences having coverage greater than 30 folds, and 88.2% greater than 50 folds. The identified mutation, c.2053A > G (p.Ser685Gly) in *ANO3* gene, was validated with Sanger sequencing and was found to be co-segregated within the family (Fig. 1B and C). This variant was not found in the Taiwan Biobank exome database (https://taiwanview.twbiobank.org.tw/index) (n = 1517 exomes), nor in over 250,000 alleles in gnomAD (http://gnomad.broadinstitute.org/). To test the potential effect of this variant allele on transcriptional activity, we performed a q-PCR analysis. We found that the *ANO3* mRNA expression level in the proband was comparable to the age- and gender-matched healthy control (Fig. 1D, controls: 1 ± 0.09 fold; the proband: 1.37 ± 0.15 fold; $P = 0.11$ by Mann–Whitney test), suggesting this variant did not significantly alter the transcriptional activity.

In conclusion, we report a family with a known pathogenic variant of *ANO3* that presents variable degrees of cranio-cervical dystonia and myoclonus, with one patient combining with mild parkinsonism. As *ANO3* encodes a calcium-activated chloride channel protein called anotacmin-3 in vertebrates, which is highly expressed in the striatum [1], it is not surprising that patients with *ANO3* mutations may present with features of parkinsonism. Individuals with pathogenic variants in *ANO3* manifest various phenotypes, including dystonia involving the neck, pharynx, upper limbs, and rarely lower limbs [4], as well as tremor and myoclonus of the upper extremities [2] with an age of onset varying

https://doi.org/10.1016/j.parkreldis.2019.01.020
Received 10 August 2018; Received in revised form 18 January 2019; Accepted 22 January 2019

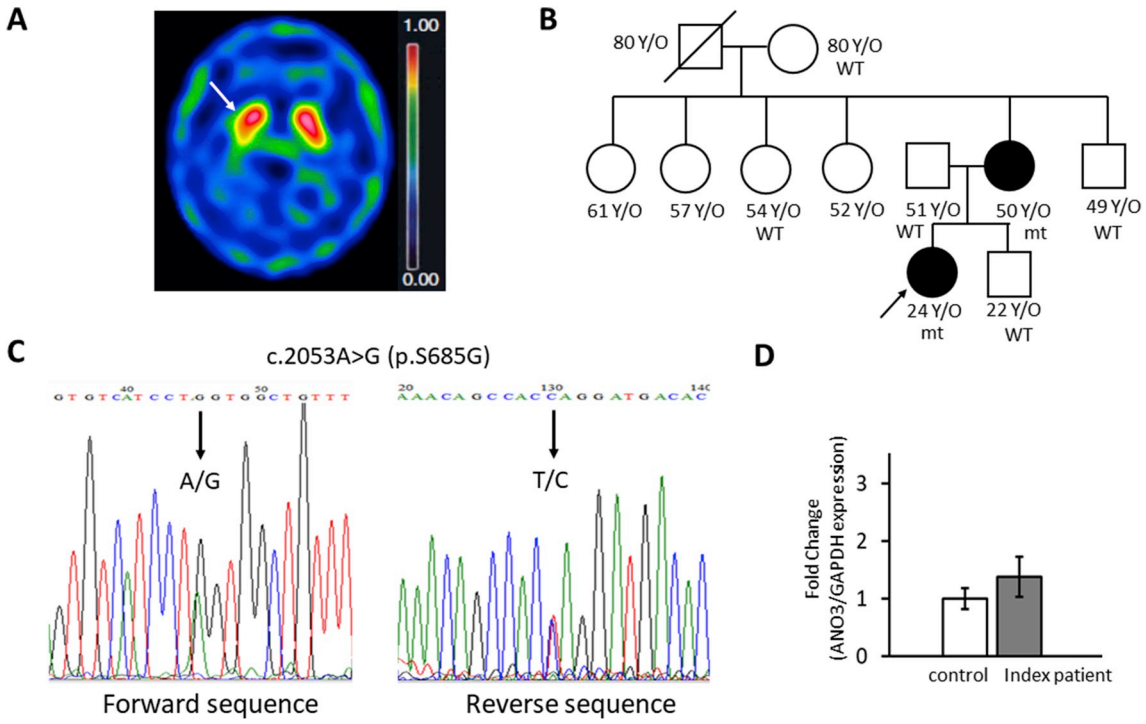

Fig. 1. Tc-99m TRODAT SPECT scan, family pedigree and genetic analysis of the index patient.

from 3 to 69 years [1–4]. The combined dystonia syndrome comprising dystonia with another movement disorder, such as parkinsonism, has also been reported in patients harboring mutations in other isolated dystonias including DYT5, DYT6, DYT12, or in *SLC63*-associated dopamine transporter deficiency syndrome [6–8].

This is the second family where the same, extremely rare mutation in *ANO3* co-segregates with early-onset dystonia affecting the neck, larynx and upper extremity, clearly supporting the disease-causing role of *ANO3* variants [1]. The levodopa-responsive Parkinsonism in this family expands on the current knowledge of the phenotypic spectrum of DYT24 caused by *ANO3* mutations. *ANO3* mutations should be considered for patients with craniocervical dystonia and myoclonus combined with early-onset parkinsonism.

(A) The Tc-99m TRODAT SPECT scan of the proband showed asymmetrically decreased uptake in right putamen (arrow). (B) Index family pedigree. Black symbols denote affected family members. The proband described in the current study is marked with an arrow. mt, mutated alleles; WT, normal alleles. (C) Chromatograms of Sanger sequencing confirmation of the *ANO3*, c.2053A > G (p.Ser685Gly) mutation. Genetic analysis revealed forward (left panel) and reverse sequencing (right panel) of the denoted mutation. The mutations identified in this study were located in the indicated position. (D) A quantitative q-PCR analysis the *ANO3*, c.2053A > G (p.Ser685Gly) substitution in the proband and age/gender-matched control. *ANO3* cDNA were amplified from the whole venous blood of the proband and age/gender-matched control. The cDNA expression of *ANO3* relative to the expression of GAPDH was shown as folds relative to those of the control, averaged from three independent experiments.

Conflicts of interest

All authors report no competing interests.

Authors' contributions

Study concept and design: MC Kuo.
Acquisition of data: MC Kuo, Han-I Lin and CH Lin.
Analysis and interpretation of data: MC Kuo, Han-I Lin and CH Lin.
Drafting of the manuscript: MC Kuo and CH Lin.
Critical revision of the manuscript and study supervision: CH Lin.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of National Taiwan University Hospital and all study participants gave their informed consent before inclusion in the study.

Acknowledgements

We thank the patient in this case study and her family for participating in the study. We are also grateful to the staff of the Second Core Lab, Department of Medical Research, National Taiwan University Hospital, for technical support during the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.01.020.

Funding

This work was supported by the Ministry of Science and Technology (grant number: MOST 106-2314-B-002-072-MY3), and National Taiwan University Hospital (grant number: 106C101-83).

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