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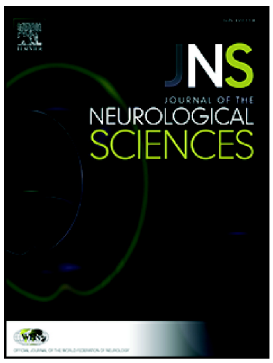

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PII: S0022-510X(19)30111-X
DOI: https://doi.org/10.1016/j.jns.2019.02.040
Reference: JNS 16239

To appear in: *Journal of the Neurological Sciences*

Received date: 3 January 2019
Revised date: 27 February 2019
Accepted date: 27 February 2019

Please cite this article as: Y. Ogawa, K. Nakamura, N. Ezawa, et al., A novel CACNA1A nonsense variant in a patient presenting with paroxysmal exertion-induced dyskinesia, Journal of the Neurological Sciences, https://doi.org/10.1016/j.jns.2019.02.040

A novel *CACNA1A* nonsense variant in a patient presenting with paroxysmal exertion-induced dyskinesia

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Word count: 1009 (excluding the References section, figure caption, and back matter information)

Abbreviations:

CACNA1A: calcium voltage-gated channel subunit alpha 1A gene; EA2: episodic ataxia type

2; GLUT1-DS2: glucose transporter type 1 deficiency syndrome 2; PED: paroxysmal exertion-induced dyskinesia; *SLC2A1*: solute carrier family 2 member 1 gene.

Key words: paroxysmal exertion-induced dyskinesia, channelopathy, *CACNA1A*, *SLC2A1*, phenotypic overlap

Highlights:

- We report a novel nonsense variant in the *CACNA1A* gene.
- Our patients showed paroxysmal exertion-induced dyskinesia with mild ataxia and intellectual disability.
- The present study might broaden the clinical overlap between *SLC2A1*- and *CACNA1A*-related disorders.

Dear Editor,

Paroxysmal dyskinesias are a group of genetically and clinically heterogeneous movement disorders that manifest as episodic dyskinesias lasting for a brief duration [1]. Among those, paroxysmal exertion-induced dyskinesia (PED) is induced by prolonged exercise [1]. Interictal examination shows normal in some patients; however other patients show various degrees of neurological manifestations, including intellectual disability, disturbance of smooth eye movement, nystagmus, cerebellar ataxia, spasticity, and epilepsy. Various conditions, including GLUT1 deficiency, dopa-responsive dystonia, juvenile parkinson's disease and various mitochondrial disorders, can present as PED; however, the pathophysiology of PED is still unknown [2]. GLUT-1 deficiency is rather a proteiform disorder than an allelic disorder as there is no clear link between one variant on one particular clinical form.

CACNA1A encodes the voltage-dependent P/Q-type calcium channel subunit alpha 1A protein [3]. Variants of this gene can result in various phenotype including spinocerebellar ataxia 6 (OMIM 183086), familial hemiplegic migraine 1 (OMIM 141500), early infantile epileptic encephalopathy 42 (OMIM 617106), and episodic ataxia type 2 (EA2, OMIM 108500) [4]. EA2 is the most common form of episodic ataxias, and is characterized by debilitating spells of recurrent paroxysmal unsteadiness, incoordination, vertigo, and slurring of speech that last for hours to days, with variable baseline progressive ataxia [4].

Herein, we report the case of a 22-year-old Japanese man carrying a novel nonsense *CACNA1A* variant (NM_001127221:c.4294C>T, p.R1432*).

Case Report

The patient (II-1), a 22-year-old Japanese man, was the first child of non-consanguineous healthy parents (Fig. 1A). He showed normal development up to the age of 6 years when he

was noticed as having difficulty in reading and writing. At age 9 years, he developed paroxysmal episodes of involuntary movements that occurred under stress or while prolonged (e.g., few to 30 min) exercising, such as playing baseball or running. Episodes started with spastic gait or mild dysmetria (e.g., mistimed jumping or difficulty in catching a ball) lasting for 5–30 min, followed by involuntary flexion, extension, and twisting movements of the upper and lower limbs, lasting for a few minutes. Neither vertigo nor dysarthria were seen during the abnormal limb movement episodes. His episodes of involuntary movement showed gradual attenuation upon reaching adolescence. At age 17 years, he also had an episode of loss of consciousness that lasted for approximately 30 min.

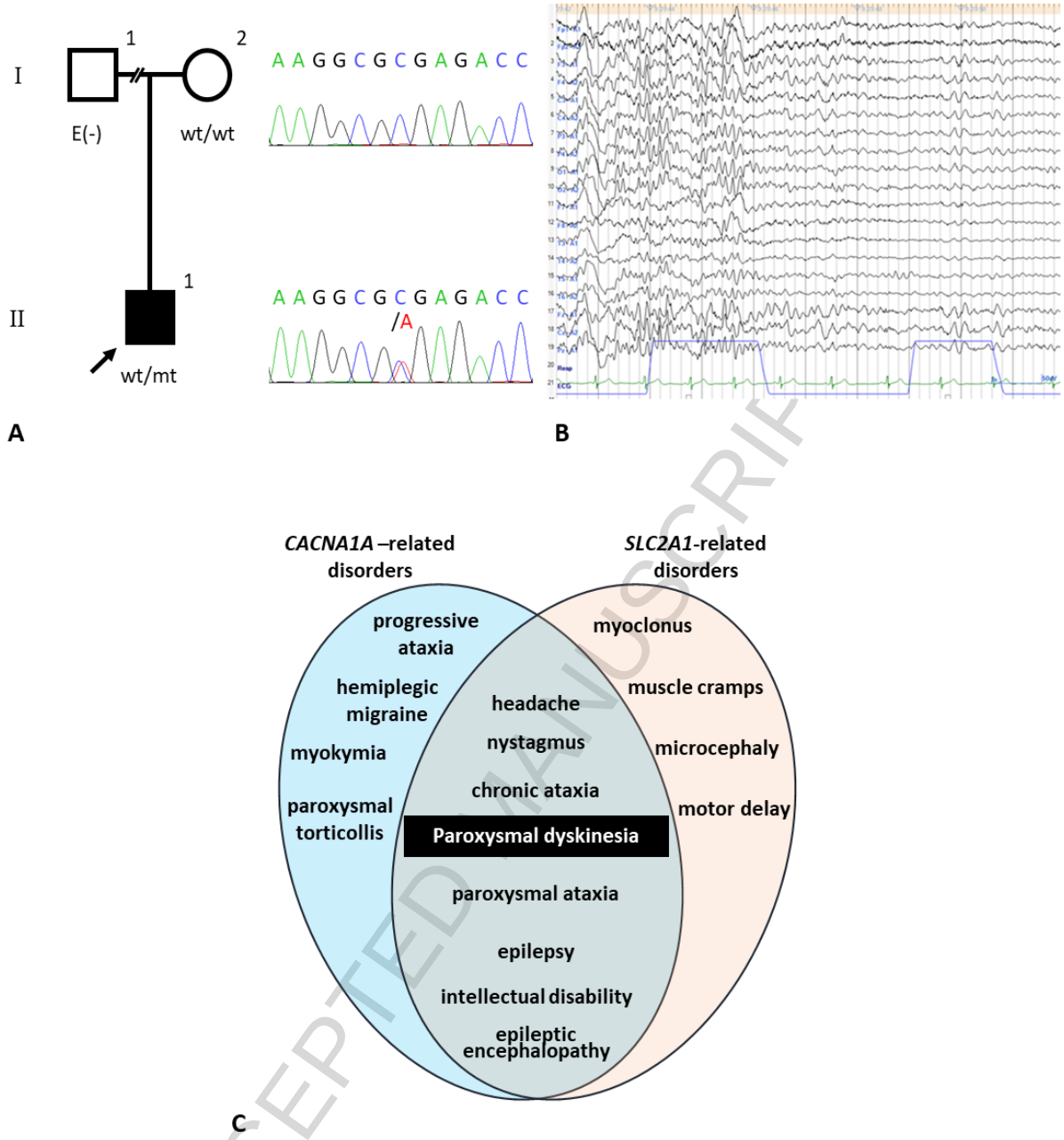

Fig. 1. (A) Pedigree of the family. ■/● = affected individuals; → = proband. (B) Electroencephalogram (EEG) findings. The EEG (performed during the non-dyskinesia period) showed frequent bursting generalized slow wave. (C) Clinical characteristics of *CACNA1A* mutation-related disorders and *SLC2A1* mutation-related disorders. Adapted from [4, 5].

The patient had his first extensive medical examination at age 20 years. The neurological examination showed horizontal and vertical gaze nystagmus, and disturbance of smooth pursuits; however, there were no abnormal findings in the cranial nerves. Mild limb ataxia in his lower extremity was evident. The muscle tonus in the lower extremities was spastic, with ankle clonus. The tendon reflexes were increased without laterality. Babinski and Chaddock signs were negative on both sides. Based on the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III), he showed a full-scale IQ of 69, verbal IQ of 75, performance IQ of 68, verbal comprehension index of 82, perceptual organization index of 75, working memory index of 88, and processing speed index of 57. Findings of routine blood examination, cerebrospinal fluid and nerve conduction studies, brain magnetic resonance imaging (MRI), and I^{123}-IMP single-photon emission computed tomography (SPECT) were all normal. His electroencephalogram (EEG), obtained in the clinical interval, showed frequent bursting in the generalized slow wave (Fig. 1B).

As the clinical findings for this patient were suggestive of PED, molecular diagnosis was performed on genomic DNA extracted from blood samples from the patient and his unaffected mother (I-2) after informed consent was obtained. First, we performed direct sequence analysis and multiplex ligation-dependent probe amplification (MLPA) analysis of *SLC2A1*, which showed no variant. Next, to screen multiple genes associated with involuntary paroxysmal movement (i.e. *SLC2A1*, *CACNA1A*, *PRRT2*, *SLC16A2*, *SCN8A*, *SLC20A2*, *GCH1*, *DLAT*, *PARK2*, *MR1*, *KCNMA1*, *KCNA1*, *CACNB4*, *SLC1A3*), we performed panel-based targeted exome sequencing using a TruSight One Sequencing Panel (Illumina, San Diego, CA, USA) and a MiSeq benchtop sequencer (Illumina). For data analysis, we used VariantStudio, version 3.0.12 (Illumina). This study was approved by the ethics committee at Shinshu University.

The Sanger sequencing results revealed a novel heterozygous nonsense variant

(NM_001127221:c.4294C>T, p.R1432*) in exon 27 of the patient's *CACNA1A* gene, whereas the gene was wild type in his unaffected mother (II-2). This variant has not been described in human genome variation databases or in disease-causing mutation databases. In silico prediction programs, including CADD and Mutation Taster predicted this variant to be highly deleterious with a score of 38 and 1, respectively.

Discussion

Although the clinical features of our patient were similar to those of the aforementioned family with PED [1], our patient had a novel heterozygous nonsense variant (p.R1432*) in *CACNA1A*. In most reports of EA2, patients harbor truncating variants in *CACNA1A* [4], and the R1432 residue is situated in an extracellular loop of the third of four homolog domains [3]. Voltage-dependent calcium channels consist of alpha-1, alpha-2, beta, and delta subunits in a 1:1:1:1 ratio; thus, the truncated mutation in our patient may have affected the channel activity, which is directed by the pore-forming and voltage-sensitive alpha-1 subunit [6].

Our case suggests that *CACNA1A*-related disorders could present with the PED phenotype, whereas *SLC2A1* is recognized as one of the causative genes of PED. The clinical overlap between *SLC2A1*- and *CACNA1A*-related disorders is summarized in Figure 1C. Variants in *SLC2A1* encoding GLUT1 affect the brain glucose levels and lead to cerebral energy deficiency [7], which induces intracellular acidosis and abnormal ion channel activity in neuronal cells [8, 9]. On the other hand, *CACNA1A* variants induce abnormal channel responses to intra- or extracellular pH changes [10]. Considering the common mechanism of abnormal neuronal ion channel activities, it is no wonder that both *CACNA1A* and *SLC2A1* variants have overlapping phenotypes.

It is difficult to make a correct genetic diagnosis of paroxysmal movement disorders by using conventional direct sequencing analysis, as each phenotype has high genetic

heterogeneity and each gene has high phenotypic heterogeneity. Recent progress in the development of high-throughput sequencing technologies should provide a definitive molecular diagnosis for these disorders. Although based on a single observation, we suggest that PED might be part of the *CACNA1A*-related clinical spectrum.

Acknowledgements

The authors would like to thank Dr. Y. Ito from the Department of Pediatrics, Tokyo Women's Medical University Hospital, and Dr. S. Takahashi from the Department of Pediatrics, Asahikawa Medical University, for the *SLC2A1* analysis. The authors thank Ms. T. Kojima, Ms. A. Fujikawa, Ms. Y. Takiguchi, Ms. E. Nomura, and Ms. S. Nagasaki for their technical support. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology (Problem-Solving Oriented Training Program for Advanced Medical Personnel: NGSD Project);the Program for an Integrated Database of Clinical and Genomic Information, Japan Agency for Medical Research and Development (AMED) under Grant Number JP18kk0205012h0003; and a Grant-in-Aid for Initiative on Rare and Undiagnosed Diseases (IRUD) from the Japan Agency for Medical Research and Development, AMED under Grant Number JP18ek0109301h0001.

Conflict of interest

The authors have no conflict of interest to declare.

Video legends

Episodes started with dystonic gait which occurred under stress, followed by marked involuntary flexion, extension, and twisting movements of both the upper and lower limbs.

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