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Case Report

Single-fiber electromyography-based diagnosis of *CACNA1A* mutation in children: A potential role of the electrodiagnosis in the era of whole exome sequencing

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Abstract

Introduction: A loss-of-function mutation in *CACNA1A*, which encodes P/Q-type Ca channels, causes various diseases. As most of the Ca channels at neuromuscular junctions are of the P/Q type, patients with loss-of-function *CACNA1A* mutations exhibit disturbed neuromuscular transmission. The associated jitters and blocking in such patients can be detected by single-fiber electromyography (SFEMG).

Cases: We report two cases with different phenotypes, which were predicted to harbor loss-of-function mutations of *CACNA1A*, by using axonal stimulation SFEMG. One case involved a 2-year-old boy with episodic ataxia type 2. The other case involved a 7-year-old girl diagnosed with epileptic encephalopathy. SFEMG results revealed jitters and blocking in both cases. Moreover, whole exome sequencing (WES) revealed a heterozygous *CACNA1A* mutation, c.5251C>T, p.Arg1751Trp, in the former case and a novel *de novo CACNA1A* mutation, c.2122G>A, p.Val708Met, in the latter.

Conclusions: Our cases indicate that SFEMG is a potentially useful diagnostic tool for patients with *CACNA1A* mutation, especially in pediatric cases where trio analysis is difficult or novel mutations are present.

Keywords: Episodic ataxia type 2; Epileptic encephalopathy; *CACNA1A*; Single-fiber electromyography (SFEMG); Neuromuscular transmission

1. Introduction

CACNA1A encodes the pore-forming alpha 1A-subunit of P/Q-type Ca channels. The P/Q-type Ca channels are found in the central nervous system and neuromuscular junctions; they play an important role in synaptic transmission. It is reported that 90% of Ca

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channels in neuromuscular junctions are of the P/Q-type. Thus, loss-of-function mutations in *CACNA1A* may disturb neuromuscular transmission [1], thereby causing various diseases, such as episodic ataxia type 2 (EA2), epileptic encephalopathy (EE), and learning disorders.

Single-fiber electromyography (SFEMG) is a technique to examine the end-plate jitter of single muscle fibers in response to voluntary activation or axonal stimulation. Voluntarily activated SFEMG, which demonstrates jitter and blocking in EA2 patients is generally used in adults [1,2]. Conversely, axonal stimulation SFEMG, recently reported as a potentially sensitive and safe diagnostic tool for evaluating neuromuscular junction disorders, can even be used in young children [3,4]. Therefore, we performed axonal stimulation SFEMG for two pediatric patients suspected with loss-of-function mutation in *CACNA1A* to determine pathogenicity.

2. Patients

2.1. Patient 1

The patient was a 2-year-old boy who was the only child of non-consanguineous parents. He had no family history of migraine or other neurological disorders, however, had experienced one febrile convulsion.

The patient's first episode of paroxysmal tonic upward gaze and horizontal nystagmus manifested in the first week of life but the eye movement abnormality disappeared by the time he was one year old However, paroxysmal gait disturbance started at age 14 months which subsequently became a weekly occurrence. At the age of two years, patient 1 was referred to our hospital. At the time of presentation, he was only able to speak few meaningful words, however, his motor development and growth parameters appeared normal. His developmental quotient was assessed as 85 on the Kinder Infant Development scale. He showed no oculomotor abnormalities and interictal activity was also normal. However, during the gait disturbance episodes, which lasted from several hours to a day, his gait was wide-based and unsteady resulting in frequent falls. His laboratory test results, including serum lactate, pyruvate acid, cerebrospinal fluid lactate, and glucose were normal. Brain MRI and electroencephalography (EEG) revealed no abnormalities, however, positron emission tomography revealed cerebellar hypometabolism. We suspected EA2 based on these findings and his clinical course, and thus performed axonal stimulation SFEMG. We examined 20 pairs of muscle fibers.

The mean value of consecutive differences (MCD) and blocking rate were analyzed. Both mean MCD and mean blocking rate were increased (68.7 μs, 9.7% respectively; The upper limit for mean MCD is reported to be 26 μs in children over age 2 years, and blocking is never observed in normal subjects [3,4]) (Fig. 1A, B, Table 1). These results confirmed a decrease in neuromuscular transmission function, which indicated the necessity of genetic analysis. His father was estranged and thus a trio-based exome study was not possible. The whole exome sequencing (WES) of the patient's DNA revealed a heterozygous mutation, c.5251C>T, p.Arg1751Trp, in *CACNA1A*, which was confirmed by Sanger sequence method. The WES was performed as published by Hamanaka et al [5]. The variant was not found in the exome aggregation consortium (ExAC) database and was predicted *in silico* to be deleterious by SIFT and PolyPhen2. The variant is classified as "likely pathogenic" according to the American College of Medical Genetics (ACMG) guidelines (Sue Richards et al., Genetics in Medicine. 2015) and is reportedly associated with EA2 [6].

2.2. Patient 2

The patient was a 7-year-old girl. The patient was the only child of non-consanguineous parents with no family history of neurological disorders. She was suspected to have developmental delay when she was 6 months old as she could not keep her head steady. At the age of one year, she gained eye tracking and stable head and neck, but she could not roll over, sit by herself, nor say any meaningful word. EEG revealed non-convulsive status epilepticus. She presented horizontal nystagmus and hypotonia, however, other neurological findings and laboratory test results including screening for metabolic disorders, and examination of cerebrospinal fluid was normal. Brain MRI showed mild cerebellar atrophy. There was no abnormality was detected by brain single photon emission computed tomography imaging. Based on these findings, we could not identify the cause of her disease. After the treatment of non-convulsive status epilepticus, here interictal EEG showed poly-spike and wave. Moreover, she had some episodes of epileptic spasms and absence seizures exhibiting refractory to antiepileptic drugs and her cognitive and motor development did not progress. Therefore, we had diagnosed her condition as EE. At the age of five years, WES revealed heterogenous *CACNA1A* mutation, c.2122G>A, p.Val708Met, which was confirmed by Sanger sequence method. Although, the variant has not been reported as a disease-causing variant and is not found in the ExAC database, we confirmed its *de novo* occurrence (parentage was confirmed with microsatellite makers). Additionally, multiple computational predictions support its deleterious effect (SIFT score 0.001, PolyPhen2 HumVar score 0.944, and GERP score 4.96). Thus, this variant was considered to be "likely pathogenic" according to the ACMG guidelines (PS2, PM2, PP3). Axonal stimulation

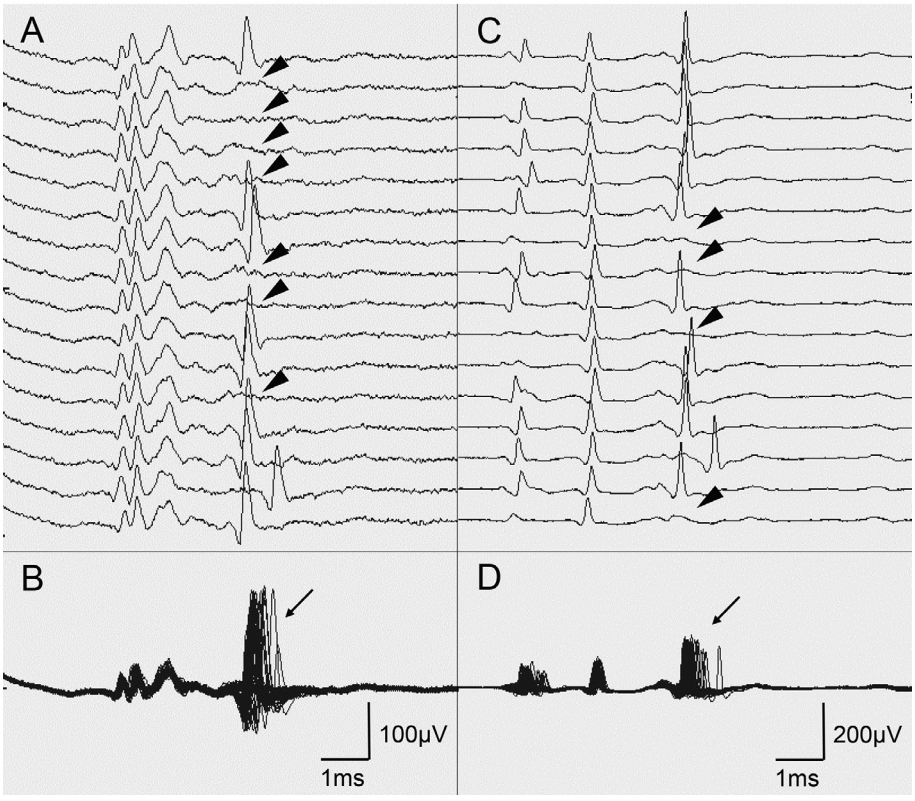

Fig. 1. Axonal stimulation SFEMG from frontal muscle of our patients: The mean value of consecutive differences (MCD) and blocking rate in one of the 20 muscle fibers with each sweep from same single fiber (arrow: jitter, arrowheads: blocking fibers), and sweep accumulation; patient 1 (A, B): MCD = 136.3 μs, Blocking rate = 35.3%, patient 2 (C, D): MCD = 127.0 μs, Blocking rate = 22.2%.

Table 1
Jitter and blocking values in our patients revealed by axonal stimulation SFEMG.

Values	Patient 1	Patient 2
Number of fibers	20	20
With increased jitter	16	7
With blocking	8	3
Mean MCD, μs (range)	68.7 (18.9–160.6)	41.6 (14.5–169.8)
Mean blocking rate, % (range)	9.7 (0–88.7)	3.8 (0–28.3)

SFEMG revealed that both the mean MCD and mean blocking rate were increased (41.6 μs, 3.8% respectively) (Fig. 1C, D, Table 1). These results strongly suggested Ca channel dysfunction and pathogenic nature of the mutation.

3. Discussion

The *CACNA1A* on chromosome 19p13 encodes the alpha 1A-subunit of the $Ca_v2.1$ (P/Q-type) voltage-gated Ca channel. Mutations in this gene cause three allelic autosomal dominant conditions: EA2, spinocerebellar ataxia type 6 (SCA6), and familial hemiplegic migraine type 1. Gain-of-function missense mutations and a third allelic disorder have been reportedly associated with familial hemiplegic migraine type 1 and SCA6, respectively [7]. Conversely, loss-of-function mutations of *CACNA1A* have been known to cause EA2, however, recent reports have revealed that they also cause EE, and cognitive impairment including intellectual disability (ID), attention deficit hyperactivity disorder (ADHD), and autism [8].

P/Q-type Ca channels are mainly expressed at neuromuscular junctions and in the cerebellum, particularly, in the Purkinje cells. They are involved in the control of membrane excitability and neurotransmitter release [9]. Thus, dysfunctional mutant P/Q-type Ca channel subunit may interfere with normal Ca entry into the presynaptic nerve terminal, leading to impaired neuromuscular transmission.

SFEMG is a technique that records nerve-evoked muscle potentials in single muscle fibers. The sweep is

triggered by the first spike of a pair with voluntary activation or by the needle activated stimulant and jitter is measured by evaluating the time delay variation for the second spike. MCD indicates the jitter degree and is calculated by a software, while blocking occurs when the end-plate potential slope is so low that it fails to reach the threshold. Jitters and blocking act as markers of impaired neuromuscular transmission demonstrated by the SFEMG technique [1]. Voluntarily activated SFEMG is the gold standard for assessing the neuromuscular junction in adults, however, can be unsuitable for children. On the other hand, axonal stimulation SFEMG has been recently reported as a safe technique for examining pediatric patients. The upper limit for mean MCD is reported to be 26 μs in children over age 2 years, whereas blocking is never observed in normal subjects [3,4].

According to the previous reports, jitter and blocking were revealed by SFEMG in adult EA2 patients [1,2]. However, SFEMG yields normal results in familial migraine patients which is caused by gain-of-function mutation of *CACNA1A* [10]. Till date, SFEMG has not been used to evaluate an EE patient with a *CACNA1A* mutation. In this study, we used the technique in such a patient and showed that irrespective of the phenotype, SFEMG will always detect Ca channel dysfunction owing to the loss-of-function mutation of *CACNA1A* in pediatric patients.

In our cases, we used axonal stimulation SFEMG, which is suitable for young children, to detect dysfunction of the P/Q voltage-gated Ca channel. We were able to safely complete the procedure in a one-hour period with minimal sedation (triclofos sodium 70 mg/kg, and ketamine hydrochloride 0.5 mg/kg). This approach allowed us to estimate a loss-of-function mutation of *CACNA1A* which was then genetically investigated.

We suspected our first patient to have EA2 as he showed an eye movement abnormality since he was one week old and episodes of paroxysmal gait disturbance since he was 14 months old. Thus, we performed axonal stimulation SFEMG which revealed increased values of MCD and blocking rate, indicating neuromuscular transmission disturbance. WES revealed the loss-of-function mutation of *CACNA1A*. Although, we could not obtain trio samples in this case; we were able to conclude that the mutation was pathogenic owing to the previous reports and the axonal stimulation SFEMG results which indicated Ca channel dysfunction.

In the second case, WES revealed a heterogenous *CACNA1A* variant, which had not been reported as a disease-causing variant. As the mutation was not found in the control database and occurred *de novo*, this variant was considered to be likely pathogenic. In addition, we considered the mutation to be a loss-of-function mutation owing to the phenotype. Thus, we performed axonal stimulation SFEMG to verify the pathogenicity of the *CACNA1A* mutation.

CACNA1A is a large gene and screening of pathogenic sites in this gene is very difficult. Large clinical-genetic surveys have shown that only 20–30% of EA2 patients have detectable mutations. Consequently, the genetic cause for the disease remains unknown in the majority of cases [7]. In addition, it is generally difficult to confirm pathogenic mutation using WES, if the variant is novel or is based solely on the patient's gene study. In such a case, SFEMG can be used to help diagnose patients with loss-of-function *CACNA1A* mutations.

In conclusion, SFEMG findings in EA2 or EE patients with *CACNA1A* mutations suggest disturbed neuronal transmission. Therefore, it may facilitate diagnosis, especially in the pediatric cases where trio analysis is difficult or novel mutations are present.

4. Ethics

This study was approved by the ethics committees of the National Center of Neurology and Psychiatry (NCNP) and Yokohama City University School of Medicine. The parents of the patients provided written informed consent.

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