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CASE REPORT

EXPERIENCE WITH THE KETOGENIC DIET IN A BOY WITH *CLCN4* RELATED NEURODEVELOPMENTAL DISORDER

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ABSTRACT

Raynaud-Claes syndrome is rare condition characterized with intellectual disability and is caused by X-linked pathogenic variants in *CLCN4* gene. Hemizygous missense variant NM_001830.4: c.1597G>A (p.V533M) was detected in a 6-year-old male followed up with intellectual disability, dysmorphism, and epileptic encephalopathy. The mother and one sister of the patient were also carrying the same variant. The clinical picture of the patient was significantly more severe, and the patient exhibited nonconvulsive status. Tonic status was observed with benzodiazepine treatment and the patient was successfully treated with a ketogenic diet. Many types of seizures can be seen in Raynaud-Claes syndrome, some of which can be life-threatening. *CLCN4* variants can be investigated in patients who exhibit an increase in tonic seizures with benzodiazepine treatment. However, ketogenic dietary therapy as first-line treatment can be lifesaving in resistant epilepsy cases caused by the *CLCN4* gene.

Key words: ClC-4, epilepsy, lennox gastaut, ketogenic diet, Raynaud-Claes

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INTRODUCTION

Raynaud-Claes syndrome is a rare syndrome linked to the X chromosome. People with this syndrome exhibit facial dysmorphism (long face, prominent chin, flat midface, downslanting palpebral fissures, strabismus), hypotonia, mild to severe intellectual disability, epilepsy, epileptic encephalopathy, behavioral problems, and cerebral atrophy [1]. Some heterozygous females are unaffected; however, mild to severe intellectual disability can be seen in some heterozygous girls. As expected, male patients exhibit a more severe clinical picture [2].

The chloride channel (CLC) gene family comprises nine different channel proteins in mammals, four of which encode plasma membrane CLCs (ClC-1, ClC-2, ClC-Ka, ClC-Kb) and the other five encode intracellular 2Cl−/H+ exchangers (ClC-3–7). The ClC-4 channel protein encoded by the *CLCN4* gene in chromosome Xp22.2 is a voltage-dependent 2Cl−/H+ exchanger. Pathogenic variations in the *CLCN4* gene cause Raynaud-Claes syndrome (MIM:#300114) listed in the Online Mendelian Inheritance in Man database. ClC-4 is expressed in the brain as well as in striped muscle tissue, heart, intestine, and kidney. CIC-4 is probably involved in the ion homeostasis of endosomes and intracellular trafficking, but its physiological function is still unknown [3].

A study conducted with a meta-analysis revealed that neurodevelopmental disorders associated with *CLCN4* have been identified in 122 individuals from 67 families so far [4].

The traditional ketogenic diet is characterized by its high-fat, adequate-protein (1 gram/kg), and low-carbohydrate composition, inducing metabolic alterations reminiscent of a state of starvation. Shifts in plasma ke-

tones, insulin, glucose, glucagon, and free fatty acids may manifest within hours of initiating the diet, exhibiting significant and rapid changes [5].

The effectiveness of ketogenic diet therapy extends to patients with epilepsy across various ages and seizure types, solidifying its status as a beneficial treatment option [6].

Here, we report on three cases of Raynaud-Claes syndrome in a family with a missense variant in the *CLCN4* gene.

CASE PRESENTATIONS

Case 1

A 6-year-old male was evaluated in the pediatric neurology department due to multidrug resistance epilepsy. Perinatal history was uneventful (caesarean section; birth weight of 2850 g) and was born from a non-consanguineous marriage.

The patient exhibited mild hypotonia during the first year of life. Eye contact was partial and social interaction was poor. The patient learned to walk at the age of 24 months and started speaking with few single words at the age of 3.5 years but could not form a sentence. The patient had severe intellectual disability and was receiving special training for the same. The patient's height was 110 cm, in the 10 percentile (p), weight was 20 kg (50p), and head circumference was 49.6 cm (3-10p). Dysmorphological examination revealed a round face, bitemporal narrowing, depressed nasal bridge, narrow and downslanting palpebral fissures, and strabismus. Cerebellar examination revealed intentional tremor and ataxia; the extrapyramidal system examination was normal. Moreover, the cranial nervous system examination was normal, but strabismus was present.

The patient's first seizure, as cyanosis and motor arrest, occurred at 12 months of age. The electroencephalogram (EEG) findings at that time were multifocal and accompanied by generalized spike slow wave activity, slow background activity, and paroxysmal rapid rhythms, which were found to be compatible with epileptic encephalopathy. After 20 months of age, the patient's generalized tonic and atypical absence seizures continued intermittently. From a phenotypical perspective, Lennox–Gastaut Syndrome was considered. Metabolic scans of blood amino acids, organic acid analysis, creatine kinase, lactate, ammonia, and tandem mass spectrometry were normal (amino acids and acyl-carnitine profile). Cerebrospinal fluid examination for amino acid and glucose content were also normal. Past medical history included valproic acid, levetiracetam, and phenobarbital therapy. Topiramate was discontinued due to ineffectiveness, ethosuximide, and clobazam treatment increased tonic seizures in approximately 10 days of use, lamotrigine was discontinued due to an allergic reaction.

The patient presented to our clinic with complaints of continuous absence seizure, non-responsiveness, and inability to walk. Continuous generalized 2.5–3 Hz spike slow wave activity was detected in the patient's EEG (Figure 1a). The patient was admitted to the intensive care unit with the diagnosis of atypical absence status. The patient was taking oral valproic acid, levetiracetam, and phenobarbital medications. IV benzodiazepine infusion was started. However, the patient's seizures assumed a tonic status after benzodiazepine infusion (Figure 1b); therefore, thiopental infusion was initiated. Thiopental infusion reduced the seizures; therefore, rufunamide and cannabidiol (CBD) oil therapy were added to the oral treatment. However, seizure activity increased with the reduction in thiopental infusion. Further, CBD oil therapy was terminated, and ketogenic diet therapy was initiated. Thiopental infusion began to be reduced at the 72nd hour of the ketogenic diet.

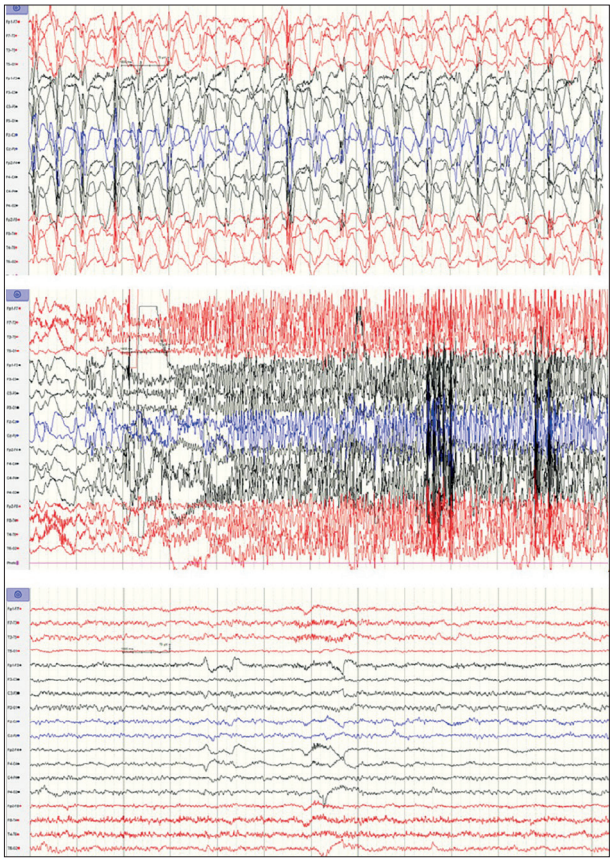

Figure 1. a) Top EEG shows atypical absence status epilepticus with ictal generalized spike-and-wave discharges with a frequency of 3 Hz. b) Middle EEG shows ictal generalized paroxysmal fast activity accompanied by an intense tonic contraction induced by benzodiazepine infusion c) Last EEG shows ,slow background activity superimposed with fast rhythms caused by phenobarbital use without ictal or inter-ictal discharges. Patient was on the 8 months of his ketogenic diet.

The patient did not have any further seizures under the ketogenic diet. The patient was discharged from intensive care after 17 days with oral valproic acid, rufinamide, phenobarbital, levetiracetam, and a ketogenic diet therapy. EEG taken 8 months after discharge from the intensive care unit showed slow background without epileptic discharges. The patient is being followed up without seizures for 8 months (Figure 1c).

Cranial MRI of the patient showed a thin corpus callosum, ventriculomegaly, and white matter atrophy (Figure 2). Chromosome analysis and array-CGH analyses of the patient were normal. Furthermore, the patient was evaluated using WES analysis, and a maternal hemizygous missense variant NM_001830.4: c.1597G>A (p.V533M) was detected in exon 11 of the *CLCN4* gene.

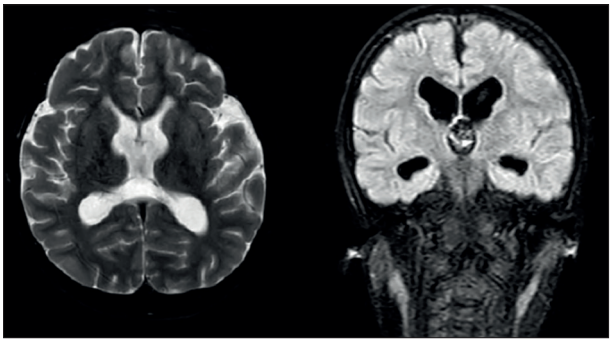

Figure 2. Axiel T2 and Coronal T2 flair Cranial MRI images of the patient showed a thin corpus callosum, ventriculomegaly, and white matter atrophy.

Cases 2-3

Thise index case has two sisters, and the same variant was found as heterozygous in one and wild-type in the other.

The seven years old sister with the heterozygous variant was born with term birth weight of 3200 g. She started walking at 18 months of age and began to speak at 24 months of age. She received special education due to learning difficulties. The patient had no seizures with a normal EEG. Neurological examination of the patient was normal. Her height was 115 cm (10p), weight was 22 kg (25p), and head circumference was 50 cm (3-10p). The Wechsler Intelligence Scale for Children-Revised (WISC-R) evaluation revealed borderline mental retardation. A heterozygous variant was detected in the mother. The neurological evaluation of the mother was normal. The patient's Porteus Maze Test score was 89, her Kent Egy intelligence test score was 64 (the average score was 77), and the mother was diagnosed with borderline mental retardation. The mother had no history of epilepsy. Moreover, it was found that the patient's aunt had poor school achievement and the son of the aunt had epilepsy and learning difficulties.

DISCUSSION

CLCN4 variants have been associated with X-linked dominant intellectual disability and epilepsy phenotype. Epilepsy is seen in 56% of these patients, and 25% of patients suffer from epilepsy-related deaths. Reported epilepsies are mostly drug resistant and range from absence to epileptic encephalopathy [1,7]. Our patient also has drug-resistant epilepsy accompanied by various types of seizures, such as atypical absence and tonic seizures, and exhibited characteristics of Lennox-Gastaut epilepsy phenotype from both an EEG and clinical perspective [8].

The *CLCN4* family of voltage-dependent *CLC* genes comprises nine members (*CLCN-1–7, Ka,* and *Kb*), which demonstrate quite diverse functional characteristics while sharing significant sequence homology. *CLCN4* is a voltage-dependent 2Cl−/H+ exchanger. Its precise physiological function is unclear, but *ClC-4* is probably involved in the ion homeostasis of endosomes and intracellular trafficking. Additionally, *CLCN4* has a significant effect on neuronal differentiation. It was reported that the number and length of dendritic branches decreased significantly in primary hippocampal neurons of *CLCN4*-null mice and hippocampal or cortical neurons of *CLCN4* knock down mice. However, the epilepsy mechanism of *CLCN4* variants is still unclear [1,9,10].

So far, 18 missense, 2 frameshift, 1 splice-site, and 1 exonic deletion mutations have been detected in the *CLCN4* gene in the literature [1,2,11]. The NM_001830.4: c.1597G>A (p.V533M) variant in the *CLCN4* gene detected in this family was previously reported by Fernandez-Marmiesse et al. in a 14-year-old male with Dravet syndrome-like phenotype whose seizures were taken under control by topiramate. This variation in the exon 11 of the *CLCN4* gene is known to be located at helical-intramembrane domains, which play an important role in CLC activity of the *CLCN4* protein. This variation was shown to be co-segregated with the disease in our family. This variation is not currently available in population databases (ExAC, gnomAD, 1000 Genomes Project) and was predicted as disease-causing in in-silico analyses (Mutation Taster, Polyphen2, SIFT, CADD). Comparative amino acid sequence alignment of *CLCN4* across different species at https://www.ncbi.nlm.nih.gov/homologene revealed that the glycine at position 533 is highly conserved. Considering these data, this variation is thought to be responsible for phenotype [12].

Literature evidence shows that missense variants are more severe than frameshift and intragenic deletions in terms of epilepsy. Our patient also carried a missense variant and had polytherapy-resistant epilepsy. Two separate studies reported that one patient benefited from carbam-

azepine and one patient benefited from levetiracetam treatment. However, atypical absence seizures were predominant in our patient; therefore, carbamazepine treatment was not initiated. Lamotrigine was reported as beneficial in the literature and treatment could not be continued due to an allergic reaction in our patient. Studies have reported that the effect of valproic acid is limited [1]. Atypical absence status developed under the use of 30 mg/kg/day valproic acid in our patient. It can be concluded that anti-epileptic treatment as first-line therapy is unsuccessful in severe cases. Our patient developed a benzodiazepine-resistant tonic status. There are reports of some Lennox–Gastaut patients developing tonic status with benzodiazepine and the molecular etiopathogenesis of this condition is unclear [13]. This case can lead us to believe that the chlorine channels encoded by *CLCN4* caused this. Our patient prominently benefited from ketogenic diet treatment and showed improvement in interictal discharges on EEG. The patient had no seizures for 8 months.

All benzodiazepines enhance the binding of gamma-aminobutyric acid (GABA) to the (GABA) receptor and increase the threat of CLC conductance triggered by the GABA-GABAa receptor interaction following greater chloride influx mediated by an increased frequency of CLC opening [14]. Interestingly, while benzodiazepines do not directly activate channels but only modify GABA binding affinity, phenobarbital can directly promote channel opening in the presence and absence of GABA [15]. The shift in seizure characteristics into tonic status with benzodiazepine use may give us an opportunity to explain the mechanisms of action of *CLNC4* on the nervous system.

The male patient ad moderate to severe ID. He had no seizures in the past 8 months under a ketogenic diet. Improvement in social interaction skills and gait were observed. The sister of the patient, who carried the same mutation as heterozygous, has mild ID and her clinical picture is significantly better than her brother. The sister has never had epilepsy. Studies in the literature report normal–moderate ID in female cases and epileptic EEG disorders in some cases. EEG was normal in the sister. The patient's mother also had mild ID and did not finish primary school. The mother had no history of epilepsy or febrile seizures [2].

Cranial MRI revealed a thin corpus callosum, ventriculomegaly, and white matter atrophy in our patient. Among the reported cases, ventriculomegaly, cortical atrophy, and white matter lesions were reported in 9 patients and no correlation was found between epilepsy severity and cranial abnormality [16].

Ketogenic dietary therapy emerges as a viable treatment option for patients who have not responded to at least two antiseizure medications. There are several conditions in which ketogenic dietary therapy shows notable effectiveness, and it can be considered early in the treatment process. These conditions encompass Doose syndrome, Dravet syndrome, glucose transporter 1 (GLUT-1) deficiency, infantile spasms, pyruvate dehydrogenase deficiency, and tuberous sclerosis complex. Moreover, ketogenic dietary therapy may prove particularly beneficial for individuals with drug-resistant epilepsy who rely on a gastrostomy tube or formula for nutrition.

According to the existing evidence, a consensus panel of experts in 2018 recommended the consideration of ketogenic dietary therapy for children facing drug-resistant epilepsy when two antiseizure medication trials have proven unsuccessful.

Our case is the first one in which a ketogenic diet was applied and yielded successful results in epilepsy cases associated with *CLCN4.*

In conclusion, many types of seizures can be seen in Raynaud-Claes syndrome, some of which can be life-threatening. *CLCN4* variants can be investigated in patients who exhibit an increase in tonic seizures with benzodiazepine treatment. However, ketogenic dietary therapy as the first-line treatment can be lifesaving in resistant epilepsy cases caused by the *CLCN4* gene mutations.

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AUTHOR CONTRIBUTIONS

Gunes Sager and Ufuk Yukselmis examined and treated the patient, conceiving the idea for the case report, leading the case report writing, Merve Akcay provided data collection, Orkide Guzel provided the ketogenic diet treatment and revised the manuscript. Ayberk Turkyilmaz completed the WES examination and genetic counseling, supervised the entire case report and revised the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to disclose.

CONSENT

Written informed consent was obtained from the legal guardian of the patient (father) for publication of this case report and any accompanying images. We confirm that

we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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