Brain & Development 45 (2023) 445–450

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case Report

Developmental and epileptic encephalopathy in a young Italian woman with a *de novo* missense variant in the *CLCN4* gene: A case report

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Received 15 March 2023; received in revised form 27 April 2023; accepted 15 May 2023

Abstract

Introduction: Raynaud-Claes syndrome is a very rare X-linked condition, characterized by intellectual disability, impaired language development, brain abnormalities, facial dysmorphisms and drug-resistant epilepsy. It is caused by loss-of-function variants in the *CLCN4* gene, which encodes the 2Cl−/H + exchanger *ClC-4*, prominently expressed in the hippocampus and cerebellum. Different genotypic variants have been described, each exhibiting specific phenotypic characteristics. The loss-of-function variant p.Gly544Arg in the *CLCN4* gene has been described in only two male probands, but there are no reports on phenotypic characterization in females.

Case presentation: We present a 30-year-old Italian woman with early-onset drug-resistant epilepsy, developmental and epileptic encephalopathy, developmental delay, absence of verbal language development, behavioral impairment with autistic features, and clusters of seizures during catamenial periods. The interictal EEG showed slight inconstant slowing of the background rhythm, with abnormal frontal predominant mu like rhythm and generalized spike and polyspike wave discharges, which increased in frequency during drowsiness. A brain MRI showed slight cranio-encephalic asymmetry and a smaller size of the left hippocampus. The whole exome sequencing (WES) revealed a *de novo* heterozygous c.1630G > A variant in the *CLCN4* gene, resulting in the amino acid substitution p.Gly544Arg (rs587777161), consistent with Raynaud-Claes syndrome.

Discussion and conclusion: Our patient is the first case of a de novo p.Gly544Arg variant of the *CLCN4* gene in a female proband, confirming that female patients with Raynaud-Claes syndrome can be as severely affected as the male counterparts. Our case expands the phenotypic characterization of different genotypic *CLCN4* variants, which can become crucial in the future for early diagnosis if targeted therapy becomes available.

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https://doi.org/10.1016/j.braindev.2023.05.004
0387-7604/

Keywords: *CLCN4*; Raynaud-Claes syndrome; Developmental Encephalopathy with Epilepsy; Developmental and Epileptic Encephalopathy; Whole exome sequencing (WES); Phenotypic characterization

1. Introduction

Raynaud-Claes syndrome is a very rare X-linked intellectual developmental disorder caused by variants in the *CLCN4* gene on chromosome Xp22.2, which encodes the voltage-dependent $2Cl-/H+$ exchanger ClC-4 [1]. The physiological role of ClC-4 is not fully understood, but probably includes ion homeostasis of endosomes and intracellular trafficking [1]. ClC-4 is expressed in skeletal muscle, liver, kidney, intestine, and heart, but is also found at high concentration in the hippocampus and cerebellum [1]. Recent studies performed on cultured hippocampal neurons confirm that ClC-4 plays an important role in the development of the nervous system [2]. Pathogenic *CLCN4* variants have been reported in hemizygous male and heterozygous female patients with X-linked intellectual disability, epilepsy, behavioral disorder, dysmorphic features, and progressive ataxia. Both truncating and missense variants have been described, acting through either a loss-of-function or a gain-of-function mechanism. In particular, a de novo missense variant, p.Gly544Arg, has been recently reported in two male patients, but the phenotype is not well characterized [3,4]. Here we report the clinical, EEG and neuroradiological features of a young female with Raynaud-Claes syndrome due to the de novo heterozygous NM_001830.4: c.1630G > A variant in the *CLCN4* gene, resulting in the amino acid variation p.Gly544Arg (rs587777161rs5877161).

2. Material and methods

The probands' parents have provided written informed consent for molecular analyses.

Trio-based whole-exome sequencing (WES) was performed on genomic DNA by using the Twist Human Core Exome Kit (Twist Bioscience) according to the manufacture's protocol on a NovaSeq6000 platform (Illumina). The reads were aligned to human genome build GRCh37/UCSC hg19. The Dragen Germline Enrichment application of BaseSpace (Illumina) and the Geneyx Analysis (Knowledge-Driven NGS Analysis tool powered by the GeneCards Suite) were used for the variant calling and annotating variants, respectively. Sequence data were carefully analyzed, and the presence of all suspected variants was checked in the public databases dbSNP, 1000 Genomes Project, EVS, ExAC, gnomAD [5–8]. Exome sequencing data filtering was performed to identify protein-altering, putative rare recessive homozygous, compound heterozygous, and pathogenic or likely pathogenic heterozygous variants with an allele frequency < 1%, according to ExAC's overall frequency, that result in a change in the amino acid sequence (i.e., missense/nonsense), or that reside within a canonical splice site. The variants were evaluated by VarSome [9] and categorized in accordance with the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) recommendations [10]. Variants were examined for coverage and Qscore (minimum threshold of 30) and visualized by the Integrative Genome Viewer (IGV). The determination of the X chromosome inactivation pattern was performed by analyzing 4 loci as previously described [11]. Briefly, 100 ng of DNA was digested with 0.1U Hpa II restriction enzyme (Life Thecnologies) in a total reaction volume of 20 μL and incubated at 37 °C for 16 h and then 15 min al 85 °C for enzyme inactivation. PCR amplification was performed using 100 ng of undigested DNA and 3 μL of Hpa II digested DNA under standard conditions. Forward primers were labeled with a FAM-tag and PCR products were analyzed on an ABI 3500 Dx Genetic Analyzer (Life Thecnologies).

3. Clinical case

A female patient came to our department when she was 24-year-old for a seizure disorder characterized by developmental and epileptic encephalopathy which started with brief noise-induced myoclonic seizures at six months of age. After one year, she developed spontaneous myoclonic seizures, tonic-clonic seizures, atypical absences, and focal impaired awareness seizures. Her antenatal and birth history were unremarkable. She was the first child of non-consanguineous parents. She had two maternal cousins with a diagnosis of unspecified epilepsy. According to her medical records, she was born by spontaneous delivery after 41 weeks' gestation, with birth weight of 3,400 g and Apgar score of 10/10. She was able to sit without support at the age of 7 months. She presented with hypotonia at the age of 10 months old but was able to walk independently at 18 months. She acquired sphincter control at 4 years. Between 18 and 24 months, together with the worsening of the epilepsy, the patient experimented an autistic regression, with loss of early social communication skills (it was also thought to be a Landau Kleffner variant syndrome); once this phase has been overcome, her behavior and social communication improved, as well as motor functions and gait stability, without however acquiring verbal communication ability: she had

significant speech delay and did not produce any meaningful words. However, she showed good eye contact and interest in her surroundings, with sufficient non-verbal communication when seizures were controlled. Her behavior was hyperactive with rare episodes of aggressiveness towards others. She displays numerous motor stereotypies, placing her hands one above the other. At the time of our first evaluation, she was in polytherapy with valproate 1250 mg/day, topiramate 200 mg/day, clonazepam 1 mg/day, perampanel 4 mg/-day (which was subsequently stopped for behavioral concerns: transient catatonic dissociative psychosis lasted a couple of years before remitting), with poor seizure control (1 seizures cluster/week) and persistent behavior and communication disorder, worsened by antipsychotic drugs (in turn, quickly suspended). After the introduction of carbamazepine up to 1200 mg/day, tapering off topiramate and clonazepam increase up to 4.5 mg/day, seizure frequency decreased to monthly clusters, often with catamenial pattern. During clusters, the patient presented sudden awakenings from nocturnal sleep, with abnormal behaviors and confusion. Moreover, during the day she displayed frequent atypical absences and seizures with head and eye deviation to the right, as well as eyelid and right hemiface myoclonic seizures. Additionally, she experienced persistent confusion, psychomotor slowing, and non-verbal communication worsening. During clusters, clonazepam dosage was increased to 6 mg/day, with improvement in behavior and good seizure control. A complete physical examination showed obesity, short stature (weight: 80 kg, >90th percentile; height: 150 cm, <3rd percentile; head circumference 56.5 cm, 97th percentile; pubertal stages: A++P4B4; body mass index 35.5 kg/m2), round face with regular forehead, round and flat nasal tip, fleshy lips, and posteriorly rotated ears. She showed small hands and feet, without significant skin or distal ligamentous laxity. The patient and her family gave consent to the publication of the photographs shown in Fig. 1. Her gait had a slightly widened base. Her blood count, liver and renal function tests, immunoglobulins, creatine phosphokinase, transaminases, thyroid profile, and meta-

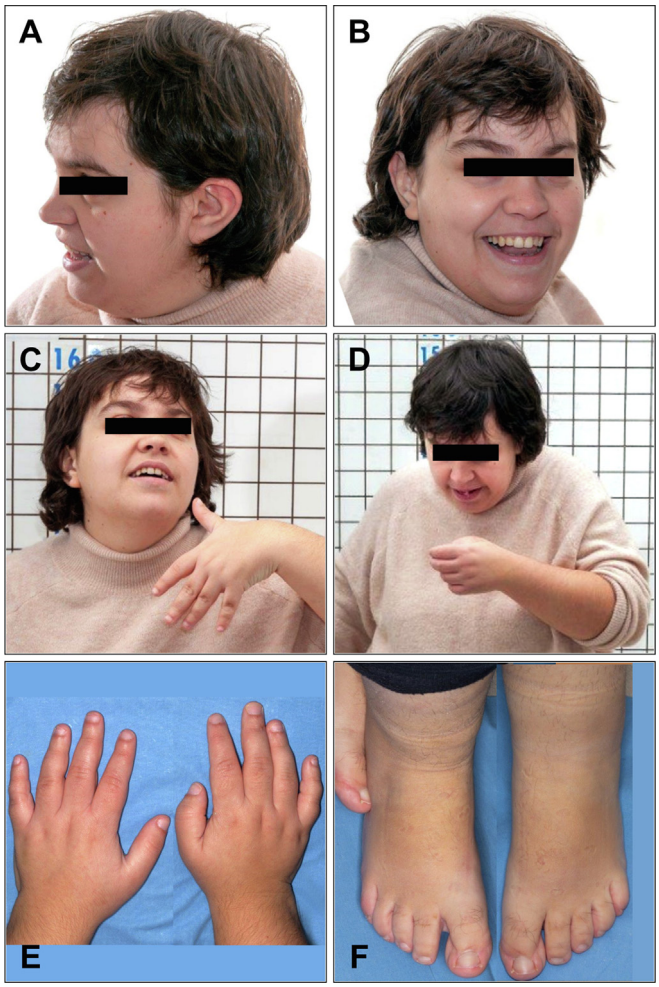

Fig. 1. General examination of the patient showing obesity, short stature, round face with regular forehead (A, B), round and flat nasal tip (A-D), fleshy lips, and posteriorly rotated ears (A, B), as well as small hands and feet (E, F).

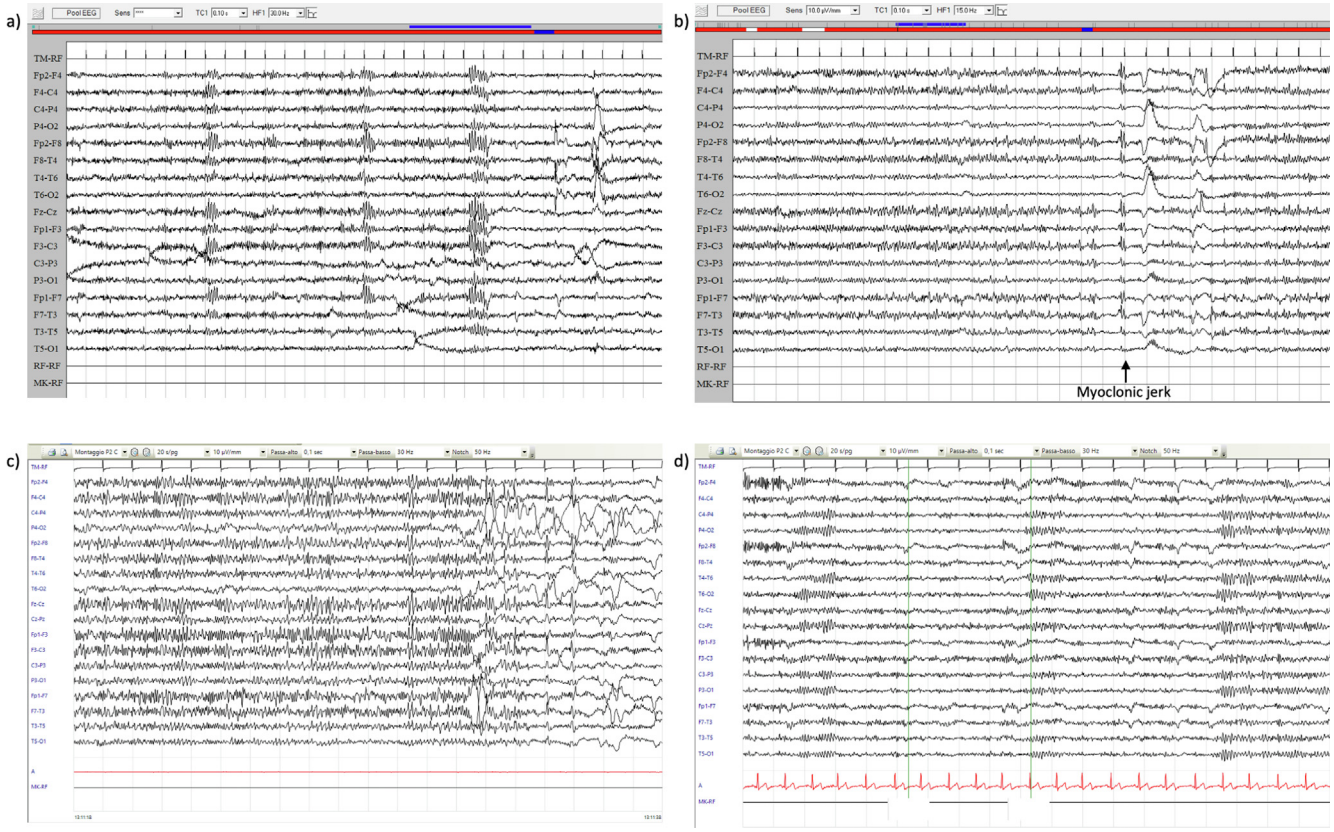

Fig. 2. Interictal (a, c) and ictal (during myoclonic jerk, b) EEG of the patient before (a-c) and after (d) the introduction of carbamazepine up to 1200 mg/day, showing abnormal frontal predominant, arceaux-like activity at 7–8 Hz (better seen during drowsiness, c) with generalized, frontal predominant spike and polyspike discharges. This activity disappeared during noncatamenial periods after the introduction of carbamazepine (d).

bolic screening were all normal. Brain MRI performed over time showed slight cranio-encephalic asymmetry, as well as asymmetry of the mesial temporal lobes and lateral ventricles due to a smaller size of the left hippocampus (Supplementary Fig. 1). The EEG showed slight inconstant slowing of the background rhythm (6–7 Hz), with presence of abnormal, frontal predominant, arceaux-like activity with generalized, frontal predominant, spike and polyspike wave discharges, which increased in frequency during drowsiness (Fig. 2).

4. Results

At first karyotype, comparative genomic hybridization (CGH) array and 15q11.2-q13 methylation and *SCN1A* analysis were performed and were non-conclusive. To identify the underlying disease, trio-based WES was performed, which revealed the de novo heterozygous nucleotide transition c.1630G > A in the *CLCN4* gene (NM_001830.4), resulting in the amino acid substitution p.Gly544Arg (rs587777161). This missense variant is absent from control populations (gnomAD v3.1.2, accession 2022/12), affects an evolutionarily conserved amino acid residue (Supplementary Fig. 2) in the helical transmembrane domain of the protein and is predicted in silico to be damaging (Varsome). It has already been reported in curated databases (human gene mutation database, HGMD, ClinVar) and described in two male individuals affected with Raynaud-Claes syndrome [4,12], and can be classified as pathogenetic.

X-inactivation studies in blood lymphocytes from our patient showed a random inactivation pattern (Supplementary Table 1).

5. Discussion and conclusion

Our patient presented with early-onset drug-resistant epilepsy with developmental and epileptic encephalopathy, developmental delay, absence of verbal language development, and behavioral impairment, all features consistent with Raynaud-Claes syndrome. Currently, around 45 patients and 23 distinct *CLCN4* variants have been identified [1,3,13–15], all determining loss of function of the *CLCN4* gene, with reduced current amplitude in 2Cl−/H + exchanger ClC-4. As expected for an X-linked disease, females are affected less often. However, the affected females can develop as severe phenotypes as males [4]. The underlying complexity of X-chromosome inactivation in cells and tissues hinders a

Table 1
Summary of phenotypic characteristics of male hemizygotes and female heterozygotes with CLCN4 p. Gly544Arg variant.

	Male (2) [1,4,12]	Female (1, our patient)
Seizure onset	4 months (1), <1 year (1)	6 months
Seizures type	Absence, GTC (1), focal impaired awareness, focal to GTC (1)	Atypical absences, GTC, focal impaired awareness, myoclonic
Seizures frequency	Not reported (2)	Clusters of multi-daily seizures during catamenial periods
Epileptic Encephalopathy	Yes (2)	Yes
EEG	Bilateral independent high-amplitude spikes; bursts of generalized spike and polyspike waves (1)	Left temporal sharp waves and generalized, frontal predominant, spike and polyspike wave discharges
Level of ID	Severe (2)	Severe
Infantile Hypotonia	Yes (1), No (1)	Yes
MRI	Corpus callosum hypoplasia, increased FLAIR signal in white matter (1), normal (1)	Asymmetry of the mesial temporal lobes and lateral ventricles due to a smaller size of the left hippocampus
Behavioral issues	Apathy, social disinhibition, motor stereotypies (1), no (1)	Hyperactivity with rare episodes of aggressiveness towards others, motor stereotypies
Other neurological features	Dystonic posturing (1), progressive spasticity, unsteady gait (1)	Slightly widened-base gait
Facial dysmorphism	Round face, down sloping palpebral fissures, open mouth (1), NR (1)	Round face, round and flat nasal tip, fleshy lips, posteriorly rotated ears. Small hands and feet.
Speech abilities	Nonverbal (1), Not Reported (1)	Nonverbal

prediction of the outcome. Most of these variant are inherited, but around 30% of them appear as de novo variants [1]. Notably, patients with multiple seizure types, or missense or de novo variants showed more severe phenotype, whereas single seizure type, frameshift or intragenic deletion, or inherited variants were associated with milder phenotypes [1].

In 2013, Veeramah et al described a de novo loss-of-function variant (c.1630G > A, p.Gly544Arg) of the *CLCN4* gene in a Dutch male proband with early onset epileptic encephalopathy and severe developmental delay (DD) [12]. A few years later, Palmer et al. reported phenotypic similarities in an American male with the same de novo variant found in a mosaic state [4]. Our patient is the third case described, and confirms that female patients harboring the p.Gly544Arg variant can be as severely affected as the male counterparts [1]. Blood DNA-based studies indicated a random X-inactivation, consistent with a partial expression of the mutated allele. However, previous experimental data on the X-inactivation state of the *CLCN4* gene are discordant [16,17]. Palmer et al. suggested that X-inactivation in the blood of the female carriers is not predictive of the manifestation or clinical severity of the disease, but also pointed out that females are more likely to be affected by missense variants than by loss-of-function variants or microdeletions [4,18].

As shown in Table 1, although the patient's clinical phenotype is similar to that previously described for males, the clinical worsening and the presence of seizure clusters during the catamenial period are peculiar to our case. In these phases, together with the recurrence of the seizures, the patient shows a transient neurologic deterioration as observed between the first and second year of life (epileptic encephalopathy). This suggests the need for closer monitoring of antiepileptic therapy in female patients during the fertile period.

Currently, treatment for Raynaud-Claes syndrome is limited to education, family support and symptomatic treatment of seizures, as well as management of behavioral and sleep problems, whereas there is no etiology-targeted treatment [4]. A better understanding of the underlying pathophysiology of *CLCN4*-related disorder may allow targeted treatment in the future. Therefore, a good phenotypic characterization of different genotypic variants of the gene *CLCN4* is important to provide tools enabling early diagnosis. This becomes crucial in the context of target treatment availability, as it allows early treatment of patients, preventing the irreversible consequences of loss of gene function on neurodevelopment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work has been generated within the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN-ITHACA) [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01–2016/739516]. The authors also wish to thank the patients' family members for their cooperation in

providing the medical data and photographs necessary for this publication.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2023.05.004.

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