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Case Report

# A novel homozygous mutation of *CLCN2* in a patient with characteristic brain MRI images – A first case of *CLCN2*-related leukoencephalopathy in Japan

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**Abstract**

Chloride channel 2 (ClC-2) is one of nine ClC family proteins and is encoded by *CLCN2*. We report the first patient with a *CLCN2* mutation in Japan. A 22-month-old female had generalized tonic-clonic convulsions at the age of 3 months. Brain MRI showed high signals in the bilateral cerebellar white matter including the dentate nucleus, dorsal midbrain, and posterior limbs of the internal capsules in diffusion-weighted images, and apparent diffusion coefficient values were low in the same areas. Antiepileptic drugs were effective, and she had neither intellectual disabilities nor motor disturbance.

A homozygous frameshift mutation (c.61dup, p.Leu21Profs*27) of *CLCN2* was identified in the patient. Homozygous mutations of *CLCN2* are known to be associated with *CLCN2*-related leukoencephalopathy (CC2L). The clinical findings of this patient were different from other patients with CC2L. Therefore, mutations in *CLCN2* may cause various phenotypes. Further accumulation of cases with *CLCN2*-mutations is required to explore the clinical spectrum of CC2L.


*Keywords:* MRI; Chloride channel 2 (ClC-2); *CLCN2*; Homozygous frameshift mutation; *CLCN2*-related leukoencephalopathy (CC2L)

## 1. Introduction

ClC-2 is a chloride channel in the plasma membrane. Although the roles of ClC-2 are still unclear, it is considered to regulate brain ions and water homeostasis in humans [1]. *CLCN2* encodes ClC-2, and various mutations of *CLCN2* had been reported. Biallelic mutations are usually found in patients with *CLCN2*-related leukoencephalopathy (CC2L).

Here, we describe the first patient with a *CLCN2* mutation in Japan. The patient has a novel homozygous mutation in *CLCN2* and shows atypical clinical findings compared with previously reported CC2L patients.

## 2. Case report

A 22-month-old female was the first child of non-consanguineous healthy Japanese parents. Their family history was unremarkable.

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Pregnancy and perinatal history were uneventful. Automated auditory brain stem response (aABR) showed normal response in her infancy. The first seizure occurred at the age of 3 months. She had frequent and daily generalized tonic-clonic seizures; however, interictal and ictal electroencephalograms were normal. Muscle tone and tendon reflex in the extremities showed normal range. Signs of hearing loss and visual loss were not detected. Brain MRI at the age of 3 months showed high signals in the bilateral cerebellar white matter including the dentate nucleus, dorsal midbrain, and posterior limbs of the internal capsules in diffusion-weighted images (DWI) and apparent diffusion coefficient (ADC) values were low in the same areas (Fig. 1).

Follow-up MRI studies were performed at the age of 7 and 13 months. Abnormal MRI signal regions had spread at the age of 7 months; however, those in the dorsal midbrain and posterior limbs of the internal capsules were disappearing at the age of 13 months (Fig. 2).

Magnetic resonance spectroscopy was performed at the age of 4 months, and metabolites showed normal scores in those regions (data not shown). To screen for various metabolic diseases, laboratory tests including serum very long-chain fatty acids and fluorescence-activated cell sorting analysis of granulocytes were performed; however, they revealed normal scores (data not shown).

Phenobarbital and valproate were effective in controlling the seizures at the age of 4 months. Kinder Infant Development Scale was performed at the age of 17 months, and the developmental quotient was 106. Developmental milestones were almost within the normal range. Auditory brain stem response (ABR) was performed at the age of 2 years. V wave was recognized in both sides from 40 dB stimulation. I–V latent time showed normal range in both side at 90 dB stimulation (data not shown).

Whole exome sequencing (WES) was performed as previously described [2]. This analysis was approved by the Committee for Ethical Issues at Yokohama City University School of Medicine and informed consent was obtained from the parents. A novel homozygous frameshift mutation in *CLCN2* of the patient was identified: c.61dup, p. (Leu21Profs*27). The father and mother were both heterozygous carriers (Fig. 3). This variant has been observed in 3 out of 1209 normal Japanese control exome data with no homozygotes in

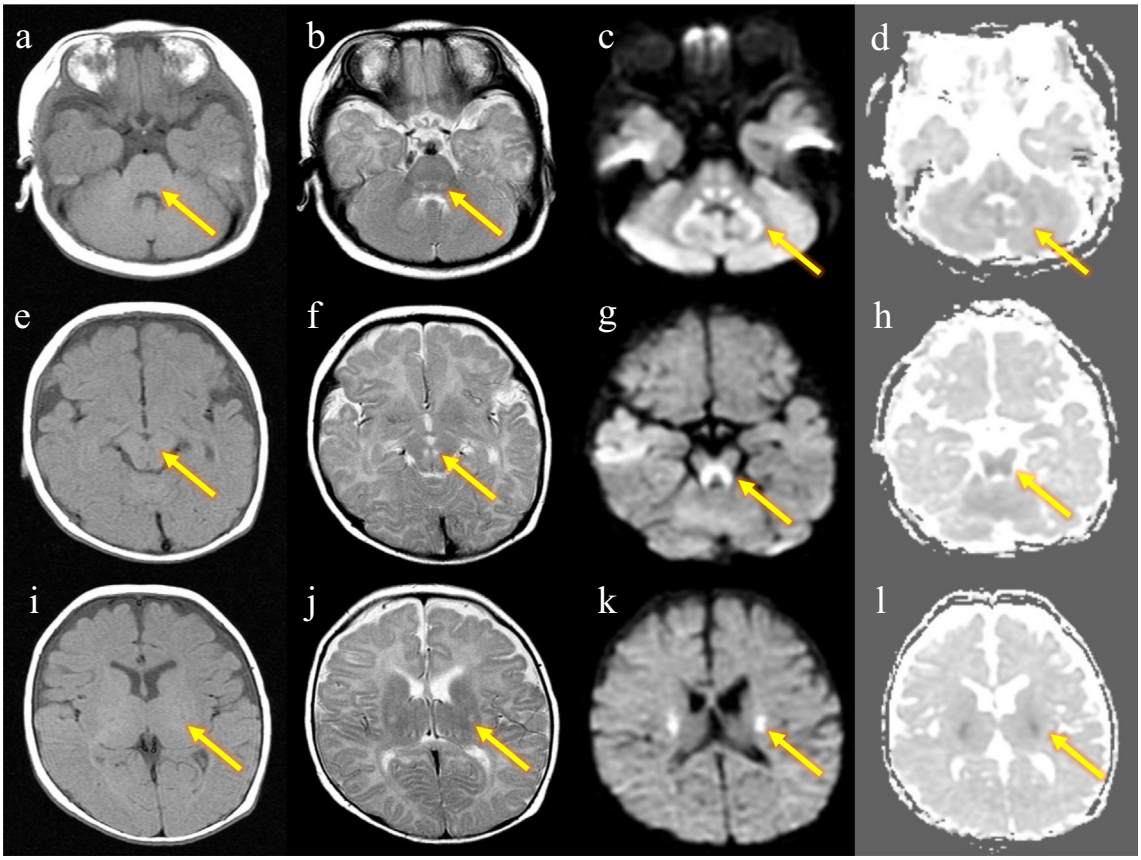


Fig. 1. Brain MRI of the patient at the age of 3 months. Hypointensities on axial T1-weighted images (a, e, i), hyperintensities on axial T2-weighted images (b, f, j), DWI (c, g, k), and corresponding low signals in axial ADC maps (d, h, i). Abnormal signals in the cerebellar white matter including the dentate nucleus (a–d), dorsal midbrain (e–h), and posterior limbs of the internal capsules (i–l) are shown.

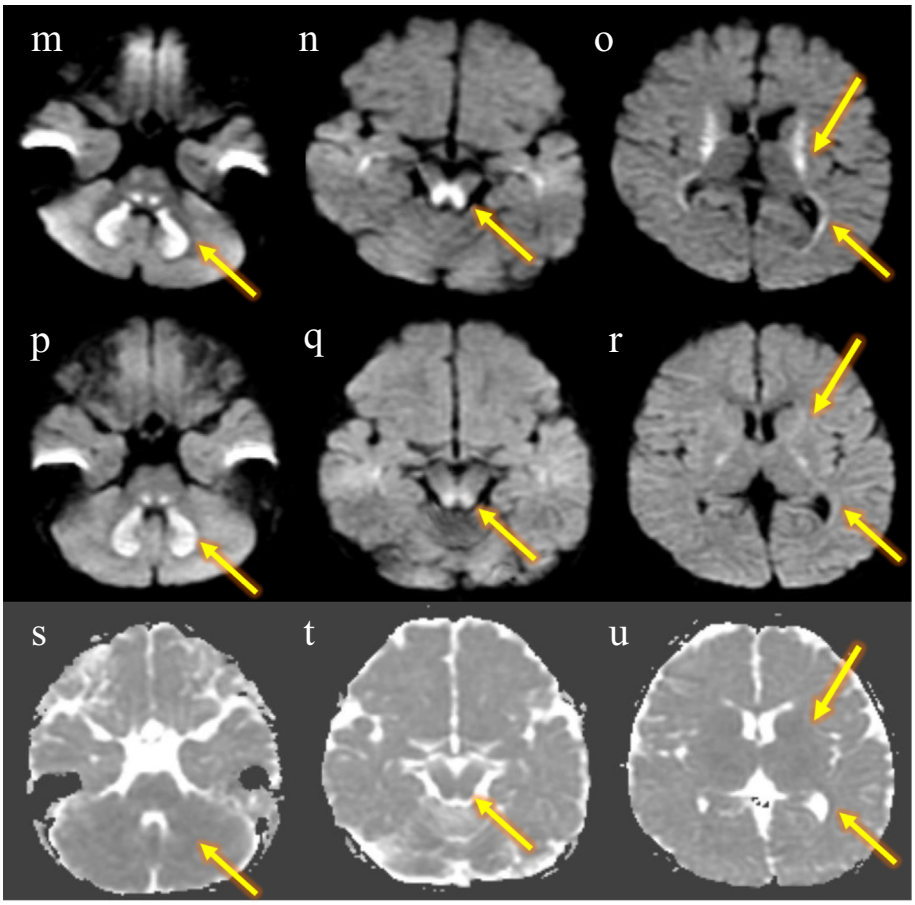


Fig. 2. Serial DWI of the patient at the age of 7 and 13 months and ADC maps at the age of 13 months. Abnormal signals in the cerebellar white matter including dentate nucleus (m, p, s), dorsal midbrain (n, q, t), posterior limbs of the internal capsules, and cerebral white matter (o, r, u) are shown. Abnormal regions on DWI had spread at the age of 7 months (m, n, o); however, those in the dorsal midbrain (q) and posterior limbs of the internal capsules (r) were disappearing at the age of 13 months. There was no change in the cerebellar white matter including dentate nucleus on DWI at the age of 13 months (p). ADC values were slightly low in the same areas at the age of 13 months (s, t, u).

the Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB/) but not in the NHLBI Exome Sequencing Project (ESP) Exome Variant Server (http://evs.gs.washington.edu/EVS/) and ExAC Browser (http://exac.broadinstitute.org/).

## 3. Discussion

Chloride channels (ClCs) are a type of permeable channels for chloride and other anions and are located on the cell membrane. ClCs have 9 mammalian family members of which ClC-2 is a member that is encoded by *CLCN2*. ClC-2 is almost ubiquitously distributed; however, it is highly expressed in the brains of humans. It regulates neuronal cell volume and post-synaptic responses to GABA and glycine [3,4].

Patients with *CLCN2* mutations have been previously reported [1,5–8]. Heterozygous *CLCN2* mutations may cause IGE since they have been detected in patients with this condition; however, this remains controversial. Haug et al. retracted their previous report because they later revealed both major differences in two of the three published pedigrees and the existence of several asymptomatic *CLCN2* mutation carriers in their patients with IGE [9].

Biallelic *CLCN2* mutations are identified as the cause of *CLCN2*-related leukoencephalopathy (CC2L) and phenotypes include ataxia, learning disabilities, headaches, mild visual impairment, and male infertility [6].

Major criteria for CC2L are T2-weighted images that show high signal intensity in the posterior of the internal capsules, midbrain cerebral peduncles, and middle cerebellar peduncles as well as T1-weighted images that show low signal intensity in the same areas. Minor criteria for CC2L are MRI that indicates abnormal signals in the central tegmental tracts in the medulla, pons, midbrain, superior cerebellar peduncles as well as decussation of the superior cerebellar peduncles in the midbrain and cerebellar white matter [6]. In our patient, the MRI findings were consistent with the major criteria except for midbrain cerebral peduncles and minor criteria for CC2L. In addition, DWI clarified that the

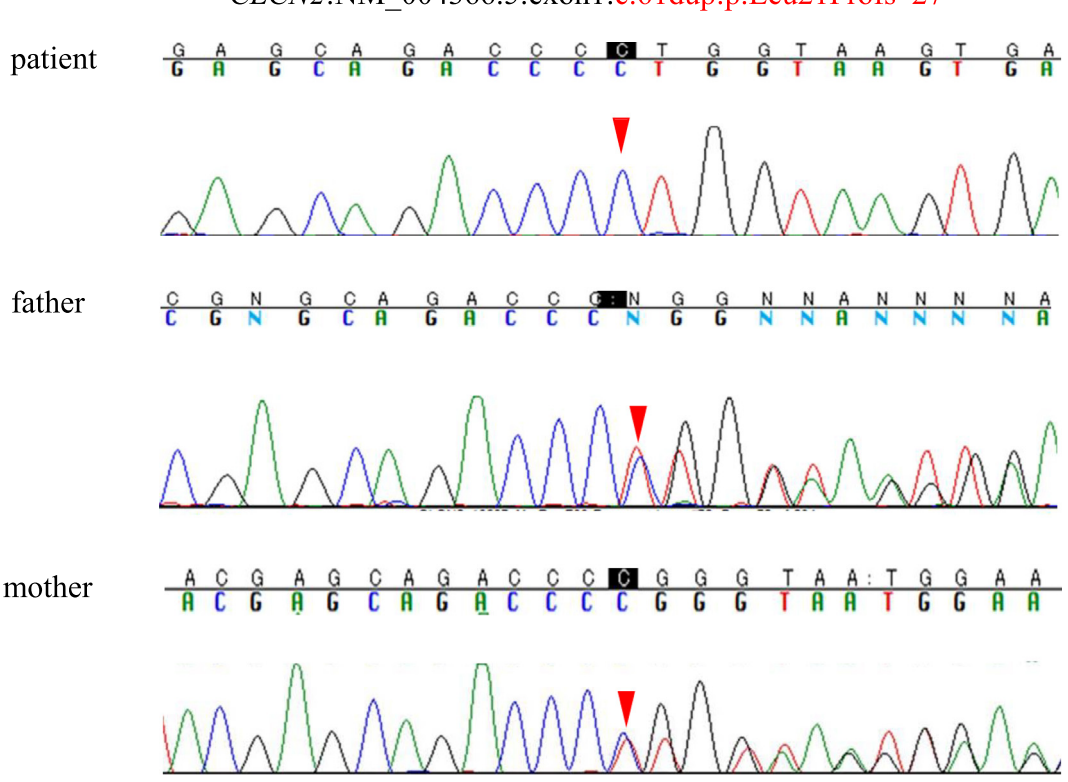


Fig. 3. *CLCN2* sequencing for the family of the patient. Frameshift mutation (c.61dup, p.Leu21Profs*27) is shown in *CLCN2*. The patient has a homozygous mutation.

abnormal high signals are enlarged to bilateral cerebellar white matter including dentate nucleus. Despite these MRI abnormalities, our patient showed neither developmental delay nor deterioration so far except for seizure clusters responsive to antiepileptic drugs (AEDs).

Di et al. suggested that clinical-radiological discrepancies may not be due to axonal degeneration or dysfunction but may be the result of intramyelinic edema [7].

Serial MRI studies showed reversible signal intensity in our patient, which is consistent with the findings in the previous report.

CC2L is rare disease and has been reported or identified in 16 individuals from 15 families to date [6,8]. However, the clinical course of our patient is different from other patients. First, although other patients had no epileptic events, our patient showed AEDs responsive generalized tonic-clonic seizures during initial stage. However, no epileptic discharge was detected in both ictal and interictal EEG. The reasons are still unknown. The activation of ClC-2 leads to hyperpolarizing action of $GABA_A$ receptors. Hyperpolarized $GABA_A$ signaling occurs neuronal excitability and epilepsy. Various non epileptic seizures including febrile seizure are caused by neuronal excitability associated with $GABA_A$ receptors [10]. Our patient's seizures disappeared immediately and had never recurred despite AEDs tapering. Therefore, those might be non epileptic seizures caused by hyperpolarized GABAergic signaling attributed to the *CLCN2* mutation. Second, our patient has shown neither developmental delay nor deterioration to date while other patients showed mild neurological deficits such as ataxia and unstable gait [1,6,8]. The reasons are also still unknown. In CC2L patients with childhood onset, the clinical course showed either stable or slowly progressive [1]. As mentioned above, ClC-2 regulates the function of neuronal cells, therefore *CLCN2* mutations may cause various phenotypes in patients with CC2L.

In our patient, the mutation was located at the N-terminal of *CLCN2* and was assumed to truncate a major section of ClC-2. As mentioned above, our patient expressed a mild CC2L phenotype even while having a homozygous nonsense mutation in *CLCN2*. Further studies are required to explore the function of ClC-2 regarding this mutation.

Here, to our knowledge, we describe the first patient having a *CLCN2* mutation in Japan. The patient had a novel homozygous mutation and showed atypical clinical phenotypes compared to previously reported CC2L patients. The accumulation of more data concerning patients with *CLCN2* mutations will enable the clarification of the clinical spectrum of CC2L.

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