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Case Report

Rapid progression of a walking disability in a 5-year-old boy with a *CLN6* mutation

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Abstract

Introduction: Neuronal ceroid lipofuscinoses (NCLs; CLN) are mainly autosomal recessive neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigments in neuronal and other cells. Symptoms include visual disabilities, motor decline, and epilepsy. Causative genes are *CLN1*, *CLN2*, *CLN3*, *CLN5*, *CLN6*, *CLN7*, *CLN8*, *CLN10*, *CLN11*, *CLN12*, *CLN13*, and *CLN14*. We present the fourth Japanese case with a *CLN6* mutation.

Case presentation: At 3 years of age, our patient became clumsy and fell down easily. He developed focal seizures with impaired consciousness and was started on carbamazepine. He showed ataxic walking and dysarthria with increased deep tendon reflexes. Interictal electroencephalogram revealed slow waves in the left temporal and occipital areas. Brain magnetic resonance imaging showed cerebellar atrophy and ventriculomegaly. In optical coherence tomography (OCT), the inner layer of the retina was thick and highly reflective. Exome sequencing revealed a known homozygous mutation, C.794_976del, p. (Ser265del) in *CLN6*.

Discussion: A total of 130 cases of NCL with *CLN6* mutations have been reported globally, of which only four were from Japan including the current patient. The deletion of serine at position 265 has been reported in six cases. Ser265 is located in a region of short repeated sequences that is susceptible to mutation. Clinical trials of gene therapy using adeno-associated virus serotype 9 have started for NCL6, making early diagnosis crucial. OCT examination might be helpful in achieving a diagnosis.

Keywords: Neuronal ceroid lipofuscinoses (NCL); *CNL6*; Regression

1. Introduction

Neuronal ceroid lipofuscinoses (NCLs) are mainly autosomal recessive neurodegenerative disorders caused by mutations in the following genes: *CLN1*, *CLN2*, *CLN3*, *CLN5*, *CLN6*, *CLN7*, *CLN8*, *CLN10*, *CLN11*, *CLN12*, *CLN13*, and *CLN14* [1]. They are characterized by the accumulation of autofluorescent lipopigments in neuronal cells and other tissue cells [1].

NCL 6, a late infantile variant, has an onset at 3 to 8 years of age, although there is also an adult onset type. Symptoms include visual disability, motor decline, and epilepsy. It typically develops as epilepsy, movement disorders, myoclonus, dysarthria, and cerebellar ataxia.

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Although visual impairment is slower than in NCL2, vision and the ability to exercise are lost from the age of 4 to 10 years [1].

We report a boy who presented with neurological regression and focal seizures with impaired consciousness from 3 years of age, which resulted in the rapid development of a walking disability. He is the fourth case of NCL6.

2. Patient report

The patient is a 5-year-old boy. He was the first child born to nonconsanguineous healthy parents. He was born by spontaneous delivery at 35 weeks and 6 days of gestation. At birth, his height and weight were 45.6 cm (0 standard deviations [SD]) and 2445 g (−0.2 SD), respectively. His occipitofrontal circumference was 29.5 cm (−1.6 SD). He underwent phototherapy because of hyperbilirubinemia. He could control his head at 4 months of age and spoke individual words at 12 months of age. He walked at 18 months of age. At 3 years of age, he became clumsy and fell down easily.

At 4 years of age, he developed focal impaired awareness seizures (FIAS) and was started on carbamazepine. However, he developed generalized seizures in addition to FIAS. Hypotonus and muscle weakness on the right side were noted. Hyperreflexia was observed in both legs, and he showed ataxic walking and dysarthria. Light reflexes were normal. Eye movements were smooth and nystagmus was not detected. Other cranial

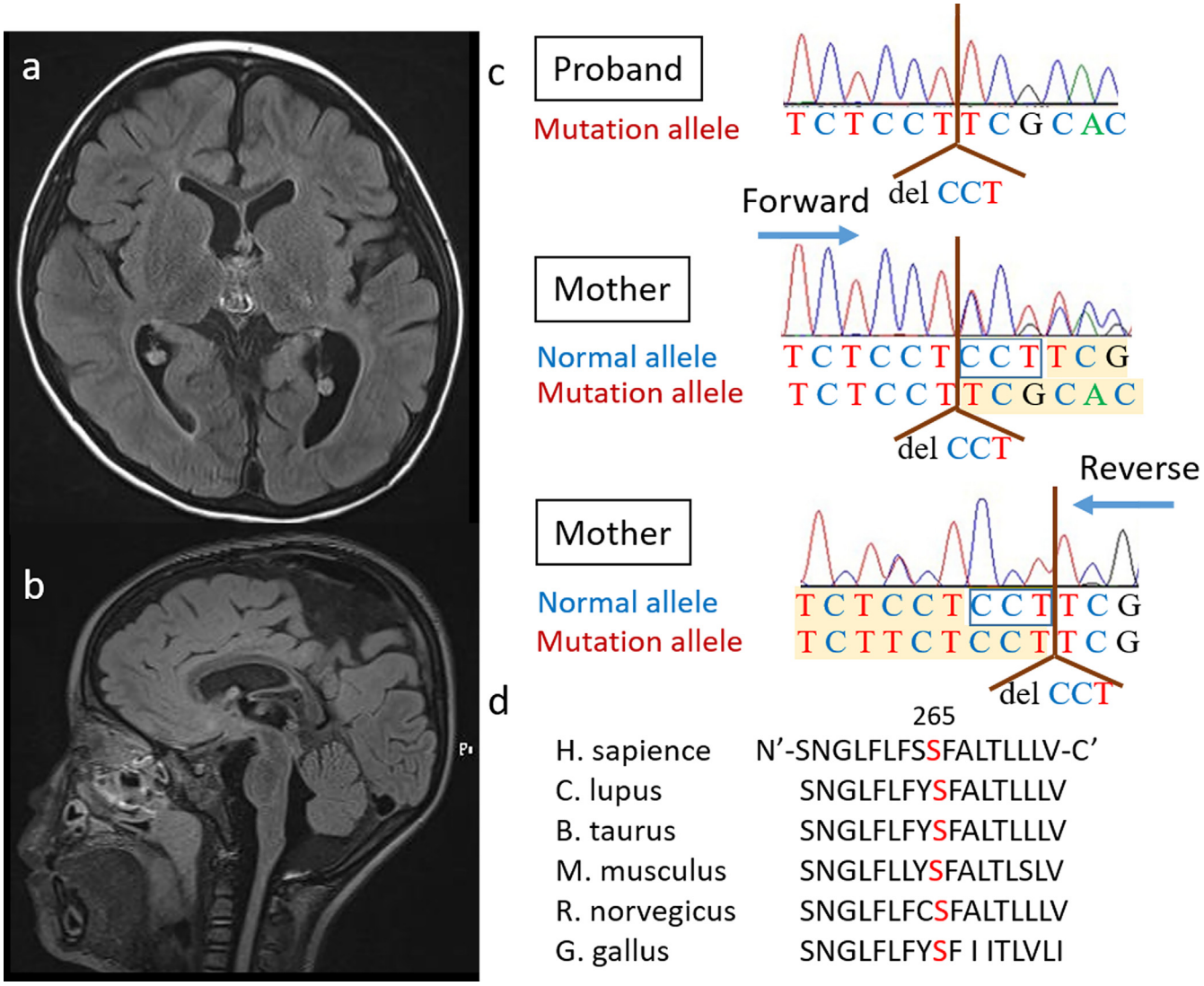

Fig. 1. Cranial MRI findings at 4 years of age and Electropherograms of direct sequencing. Axial (a) FLAIR image showing ventriculomegaly and brain atrophy. Sagittal (b) FLAIR image showing brainstem and cerebellar atrophy. (c) Electropherograms of the proband and mother. Closed square indicates the *known* mutation, c.794_796del, p.(Ser265del), in *CLN6* (based on NM_017882.2). (d) p.S265 residue shown in red is evolutionarily highly conserved from *Gallus gallus* to humans.

nerve findings were unremarkable. Laboratory tests were normal. An interictal electroencephalogram revealed slow waves in the left temporal and occipital areas (Supplementary Fig. 1), but no spikes or waves on photic stimulation and no abnormalities on the auditory brainstem response or somatosensory evoked potential (SEP). The peripheral nerve conduction velocity amplitude was decreased, indicating axon disturbance. Brain magnetic resonance imaging showed brainstem, cerebellar, and progressive cerebral atrophy (Fig. 1a, b). Although the patient was unable to be examined by Snellen charts, his both visual acuity was 20/1400 by Teller Acuity Cards. He had no strabismus. Cherry red spot was suspected in the fundus examination (Fig. 2a). The inner layer of the retina was thick and highly reflective (OTC; Fig. 2b) compared with healthy image (OTC; Fig. 2c) on optical coherence tomography.

Exome sequencing was performed as previously described [2] and identified a known homozygous mutation, c.794_796del, p.(Ser265del), in *CLN6* (based on NM_017882.2). The homozygous mutation of the proband and the heterozygous mutation of the mother were confirmed by Sanger sequencing (Fig. 1c). We couldn't check the mutation analysis of father because we could not gain his consent.

This research was approved by the bioethics committee for human gene analysis at Jichi Medical University (approval number 18-07), and written informed consent was obtained for the genetics analyses.

3. Discussion

NCL reportedly constitutes around 18% of cases of cerebellar atrophy in children, second to mitochondrial disease worldwide [3]. However, NCL is a relatively rare disease in Japan, with only 27 cases reported in a nationwide survey conducted in 2001 (www.shouman.jp/disease/details/08_06_101/). Although 130 cases of NCL *with CLN6* mutations have been documented worldwide, ours is the third family to come from Japan [4,5].

The CLN6 protein is formed of 311 amino acids and is predicted to be a 7-transmembrane protein. It is thought to be involved in the selective transport of proteins and lipids with acidification and lysosomal functions from the endoplasmic reticulum to the lysosome [6]. The first reported Japanese NCL patient harboring

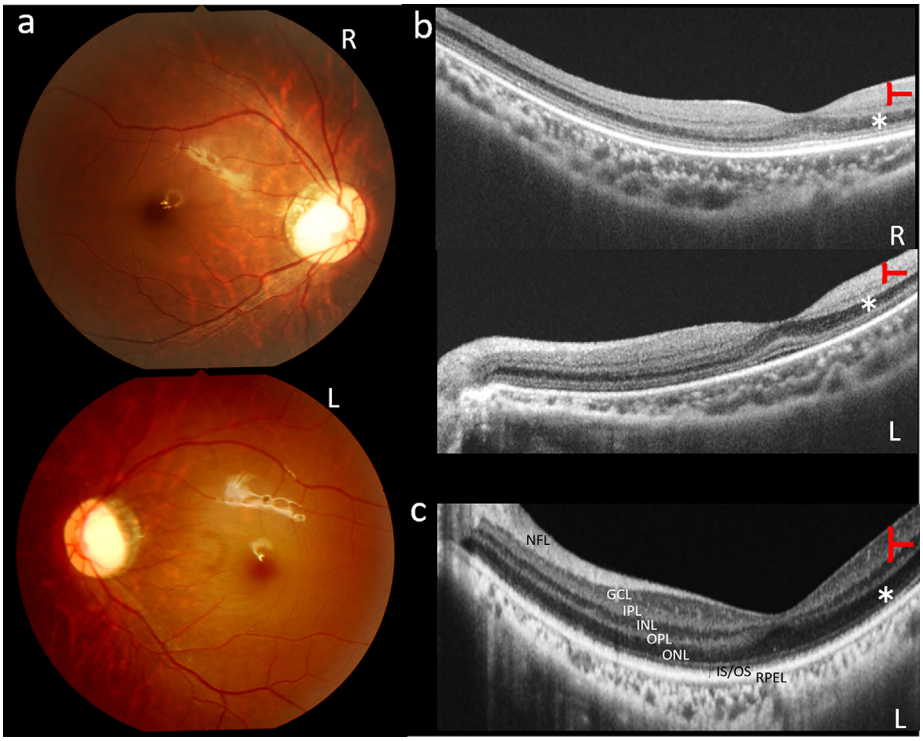

Fig. 2. Fundus examination and optical coherence tomography (OCT). (a) Cherry red spot was suspected in the fundus examination. (b) In OCT, GCL and IPL show high reflection and thickness (right brace) compared with healthy images (c). The INL has a normal density (*). NFL: Nerve fiber layer, GCL: Ganglion cell layer, IPL: Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, IS/OS: Inner segment–outer segment junction, RPEL: Retinal pigment epithelium layer.

a *CLN6* mutation presented with intellectual disability, hyperactivity, and myoclonus at 6 years of age, and developed hypotonia and ataxia at 7 years of age. He could not sit or walk unaided. The visual activity was preserved, and cherry red spot was not observed [5]. He had the compound heterozygous mutation, c.917_918dup, p.(Val307Thrfs*44) and c.348C > A, p.(Ser116Arg) in *CLN6*. His clinical course, EEG, and SEP presented typical findings of NCL. The second case is 20-year-old female. She developed myoclonus, ataxia and generalized tonic clonic seizure at 4 years of age. She became bedridden. Cherry red spot was not detected. She has an affected younger brother. His clinical course is almost same. These second and third case were detected homozygous 12.4-kb deletion (chr15: 68518038-68530471) involving *CNL6* [4].

In our case, the deletion of serine at position 265 in CLN6 (Fig. 1c) has already been reported in six cases [1,7,8]. Because Ser265 is situated in a region of short repeated sequences, this amino acid is thought to be susceptible to mutation [7]. The location of this residue in the 7th transmembrane (Supplementary Fig. 2) indicates that this mutation might affect the transport of protein and lipid to the lysosome [1,6]. The detailed clinical course is only known for one of the six cases. This male patient started generalized tonic-clonic convulsions when he was 4 years of age. He became ataxic and lost his ability to walk by the age of 6. This severe case presented a very similar clinical course to our own patient, suggesting that this mutation causes a severe type of NCL.

OCT examination of our patient showed high reflective findings in the inner layer of the retina, possibly because of the abnormal accumulation of metabolic products by lysosomal dysfunction (Fig. 2b, c). Retinal cherry red spots are thought to be formed as follows: 1) the deposition of abnormal accumulation of metabolic products in the ganglion cell layer in the perifoveal areas, which results in a white patch around the fovea, and 2) the foveal pit lacks ganglion cells, and thus continues to maintain its reddish appearance [9]. The absence of a typical cherry red spot in CLN6 cases may be a result of atrophy that has already progressed to some extent, potentially at the observed point [4,5]. OCT has been reported to be useful in detecting NCL2 and NCL3, with previous documentation of unremarkable retinal nerve fiber layers and a thin irregular hyperreflective inner layer of the retina suggestive of atrophy [10,11]. Vision is reportedly worse in NCL2 and NCL3 than in NCL6, and we believe that OCT findings of NCL6 have not been reported previously. Furthermore, we would like to follow this patient if the thick high reflective inner layer of his retina is atrophied, as reported in NCL2 and NCL3.

Gene therapy using adeno-associated virus serotype (AAV) 9 has previously been shown to be effective for dog and mouse models of NCL2 [12]. Moreover, a clinical gene therapy trial using AAV9 is currently underway for NCL6 (https://clinicaltrials.gov/ct2/show/NCT02725580). An early diagnosis of disease would therefore be prudent for such therapy.

In conclusion, we describe the fourth Japanese case of NCL6 in a patient who showed regression and FIAS at 4 years of age, resulting in the development of rapid walking disability. OCT examination might be helpful in disease diagnosis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.04.009.

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