**Case report**

**CLN6 유전자 변이에 의한 진행근간대뇌전증 1예**

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**CLN6 Mutation in a Patient with Progressive Myoclonus Epilepsy**

Neuronal ceroid lipofuscinoses (NCLs) are inherited neurodegenerative disorders, which are caused by the accumulation of lipopigment in lysosomes. Variant forms of late infantile NCLs (vLINCLs) characterized by a later onset of seizures and visual impairment (3–8 years) than in the classic form (2–4 years) are caused by mutations of the gene encoding ceroid lipofuscinosis neuronal protein 6 (CLN6). In a girl with progressive myoclonus epilepsy, we found heterozygous variants of CLN6 (NM_017882.2; NP_060352.1): c.296A>G (p.Lys99Arg) and c.307C>T (p.Arg103Trp). They were identified with whole-exome sequencing and verified with Sanger sequencing. At 7 years and 9 months, our patient had developed multiple types of seizures, prominent myoclonus with photosensitivity, regression in motor and language skills, pyramidal and extrapyramidal signs, and brain atrophy in brain images, all of which were progressive and were compatible with vLINCLs. However, this first Korean report shows no visual impairment, which resembles the previously reported Japanese case.

**Key Words:** Neuronal ceroid-lipofuscinoses, Progressive myoclonus epilepsy, Regression, Child, Whole-exome sequencing

**Introduction**

Neuronal ceroid lipofuscinoses (NCLs) are an important cause of progressive myoclonus epilepsy (PME), and are themselves caused by an accumulation of autofluorescent lipopigments in various tissues. The clinical features shared by NCLs are progressive seizures with myoclonus, psychomotor deteriorations with regression, and blindness; these features are diagnostic clues. Various genes encode the ceroid lipofuscinosis neuronal (CLN) enzymes and proteins. NCLs are classified based on onset age of symptoms: 1) infantile (INCL: due to mutations in CLN1/PPT1); 2) late infantile (LINCL: due to mutations in CLN2/TTP1); 3) juvenile (JNCL: caused by variants in CLN3); and 4) adult (ANCL: Kufs’ disease). Atypical subtypes have also been reported with variable onset, severity, and progression of distinct clinical phenotypes. The causative genes of variant forms of LINCLs (vLINCLs) include CLN5, CLN6, CLN7 and CLN8. Congenital CLN is caused by variants of CLN10.

Patients with vLINCL caused by CLN6 mutations experienced a later onset of...
seizures and visual impairment than those with the classic form. Mutations in CLN6 were originally identified in Costa Rican and Venezuelan patients and in limited populations elsewhere initially, but have more recently been documented in many other countries: Croatia, the Czech Republic, Portugal, Central and South America, the Indian subcontinent, and, less frequently, in Central and Northern Europe. However, concerning Asian countries, one Chinese case of unknown phenotype was mentioned in 2011 and one Japanese case of vLINCLs without visual deficit was described in 2016.

The proband in this first Korean report for vLINCL by mutations of CLN6 became symptomatic after age 7, had typical PME and showed progressive brain atrophy in serial brain magnetic resonance imaging (MRI). Although most symptoms and signs were typical findings of vLINCLs, she had no visual problems, which is resembles the previously reported Japanese case.

Case Report

The proband, a girl aged 17 years and 10 months at the time of the report, was born at a gestational age of 38 weeks weighing 2.3 kg (corresponding to the 10th percentile) after an uneventful pregnancy to healthy and unrelated parents. Her 5-year-old brother developed recurrent seizures starting at 8 years of age, followed by regression and intellectual disability. He could walk and speak and did not show any visual deficit after age 20, although his clinical data and genetic tests were limited as he was not raised by his family (Fig. 1).

The patient was neurologically normal until age 7. At 7 years and 9 months she experienced the first seizure accompanied by mild cognitive problems. The first brain MRI was normal (Fig. 2A and 2B). At 8 years and 7 months, she had mild ataxia, apraxia, and dysarthria. Interictal electroencephalography (EEG) showed frequent bursts of bifrontal high-voltage spike and slow wave discharges (Fig. 3A). After age 9, she could not run and her motor and language skills deteriorated rapidly. The total IQ (intelligence quotient) score was 35. At 9 and a half years, she developed dys-
phagia with drooling. Brain MRI at 10 years and 8 months showed diffuse atrophy involving supra- and infra-tentorial brain parenchyma (including brainstem and cerebellum) with passive dilations of ventricles (Fig. 2C and 2D), which became aggravated at age 15 (Fig. 2E and 2F). At 11 years of age, severe ataxia and limb weakness started to disturb her walking. At 12 years and 9 months, she had regressed to be wheelchair-bound and then bed-ridden. EEG showed rhythmic bifrontal and right occipital spike and slow waves to photic stimuli at more than 9 Hz (Fig. 3B).

The frequency and intensity of multiple types of seizures increased in progression: generalized tonic or tonic-clonic seizures, and focal motor or non-motor seizures with unawareness. Perioral twitching and limb myoclonus appeared at age 11, which were provoked by light and somatosensory stimuli and were ameliorated during sleep. Motor seizures were relatively reactive to levetiracetam and valproate, and myoclonus was slightly alleviated with clonazepam. At age 16, she was non-verbal and being fed with a gastric tube. Ophthalmologic exams until 16 year and 3 months found no problems in the cornea, retina, and optic nerve head. A thorough examination, however excluding whole-exome sequencing (WES), identified no positive results for PME.

Molecular Genetic analysis

WES was performed on the proband. The TruSeq Exome Kit (Illumina) was used on a HiSeq2000 (Illumina) platform. The variants were prioritized in different inheritance models, using our bioinformatics workflow and referring to SNP databases including the Korean Reference Genome Database (622 Korean controls: http://152.99.75.168/KRGDB/menu Pages/intro.jsp). Two pathogenic rare variants of CLN6 (NM_017882.2; NP_060352.1) were found: 1) c.296A>G (p.Lys99Arg); and 2) c.307C>T (p.Arg103Trp). They were verified with direct sequencing (Fig. 4). These rare variants were deleterious in silico (Table 1). A segregation study on her biological parents and siblings could not be completed, as blood samples were not available.

This study was approved by the Human Research Ethics Committee of Chonnam National University Hospital (IRB number CNUH-2017-167). Informed consent for participation was obtained from the patient’s parents. The biospecimens were pro-

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**Fig. 3.** Electroencephalography (EEG) findings. EEG showed frequent bursts of bifrontal high-voltage spike and slow wave discharges at 8 years and 7 months (A) and demonstrated rhythmic bifrontal and right occipital spike and slow waves to photic stimuli at a 12 Hz frequency at 12 years and 9 months (B).

**Fig. 4.** Electropherogram showing the sequence of CLN6 mutations.
vided by the Chonnam National University Hospital Biomedical Research Institute Biobank with informed consent, under institutional review board–approved protocols.

Discussion

The genetically–heterogeneous NCLs are differentiated by the age of onset, ultrastructural morphology, and genetic analysis. Most NCLs are inherited in an autosomal recessive pattern. Eight genes are well known in humans: genes encoding lysosomal enzymes (CLN1/PPT1, CLN2/TTP1, and CLN10/CTSD), a soluble protein (CLN5), and transmembrane proteins (CLN3, CLN6, CLN7/MFSD8, and CLN8). Although most of the mutations are associated with typical disease phenotypes with complete functional loss, some result in variant forms. Additionally, mutations in the same gene can cause diverse phenotypes, from mild forms to severe ones. In the past, NCLs were diagnosed with electron microscopic findings in skin specimens and enzymatic activities in blood before genetic confirmation. At present, molecular genetic testing is more easily available than ever and has broadened a clinical spectrum of the diseases.

Classic LINCL is the second most common form of NCL, and is caused by mutations of CLN2. The seizures and ataxia predominating at the early stage appear between age 2 and 4: cognitive and motor skills rapidly deteriorate over the next 6–12 months. In ultrastructural analysis, curvilinear bodies are detected. Limb spasticity and truncal hypotonia become so prominent as to result in gaitstrophy by age 6 and cause death usually within the first decade (or at the latest by the mid-teens). Mutations in CLN6, which maps to chromosome 15q21–q23, cause vLINCLs. About 70 types of mutations have been reported, of which some mild ones were identified in adult–onset NCLs (Kufs’s disease type A) lacking visual impairment. Symptom onset occurs later than in LINCLs (18 months–9 years; outside Finland, 5–6 years). Visual deficits also appear at a later age (between 3 and 8 years), and electron microscopic analysis reveals mixed curvilinear and fingerprint profiles in addition to a rectilinear complex. Unlike CLN1 or CLN2 disease due to deficit of the enzyme of palmityl–protein thioesterase 1 or tripeptidyl–peptidase 1 respectively, CLN6 disease does not have any distinctive features in blood. Multiple types of seizures present not in early stage but in the intermediate or late stages, which are associated with or followed by progressive myoclonus, speech impairment, ataxia, and regression to being bedridden. Patients typically die in the middle of their second decade, although some did after age 6. Early EEG recordings may show spikes in the occipital region in response to photic stimulation. On the brain MRI of patients with vLINCLs, progressive cerebral and cerebellar atrophy are observed with changes of signal intensity in the periventricular white matter (high) and the basal ganglia and putamen (low), whereas brain atrophy in LINCL is more pronounced in the cerebellum.

Visual impairment in vLINCLs caused by CLN6 mutations is observed in 50% of patients at an early stage, although it is reported in most cases at later stages. However, some recent studies reported cases with preserved visual function. In the Japanese case with vLINCL, visual function was preserved until age 12. In our patient, visual problems were not observed on ophthalmologic exams until age 16. It remains to be determined until when visual function can be preserved and how frequently it can be preserved even until the last stages of the disease. Although the electroretinogram (ERG) could be useful to elucidate the existence of retinal impairment in our case, compliance of the patient was too poor to get the ERG examination.

In our case report, the one variant of c.307C>T (p.Arg103Trp) was reported before in the patient with vLINCLs, while the other variant of c.296A>G (p.Lys99Arg) has not been reported yet in the patient group. To confirm the trans–heterozygous variants, a trio study including parental blood samples is recommended. However, a segregation study in the family of our proband could not be completed. Functional studies in vivo and in vitro can be useful to confirm the functional deficit of these variants of CLN6 in our case.

Herein, we report the first Korean case of vLINCL caused by...
CLN6 mutations. Seizure and myoclonus were somewhat reactive to levetiracetam, valproic acid, and clonazepam. The clinical course of the disease without visual impairment until age 16 resembles that of the Japanese case. Although NCLs by CLN6 mutations are rare in Asian countries, WES can broaden diagnostic possibilities to delineate the clinical characteristics in Asian people.

요약

NCLs는 선천성 대사 이상 질환의 하나로, 림프소포에 자가 형광물질인 lipopigment가 축적되어 발생하는 신경퇴행성 질환이다. 2세에서 4세 사이에 발병하는 전형적인 영아 후기형 NCL에 비해 더 늦은 나이인 3세에서 8세 사이에 경련과 점진적인 시력 소실이 시작되는NCLs를 변이 영아 후기형 NCLs (Variant forms of late infantile NCLs, vLINCLs)이라고 하며, 이는 CLN6에 의해 생긴다고 알려져 있다. 저자들은 진행성 근간대 뇌전증으로 진단된 여아에서 whole-exome sequencing을 통해서 CLN6 유전자 (NM_017882.2; NP_060352.1)의 c.296A>G(p.Lys99Arg)와 c.307C>T(p.Arg103Trp) 이형접합체 변이를 발견하였고 이를 Sanger sequencing으로 확인하였다. 환아는 7세 이후에 여러 형태의 경련을 보였고, 지명한 광과일성 근간대 경련, 진행성 운동 및 언어능력 퇴행, 추체로 및 추체외로 징후, 뇌자기공명 검사에서 진행성 뇌위축 등을 vLINCLs의 전형적인 특징을 보였다. 본 증례는 한국인에서 첫번쨰로 보고되는 CLN6 유전자 변이에 의한 vLINCLs보고이며, 일본에서 보고된 증례와 같이 청소년기에도 시력 소실을 보이지 않았다.

References

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