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【 CASE REPORT 】

A Novel *De Novo* Variant in *KCNH5* in a Patient with Refractory Epileptic Encephalopathy

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Abstract:

We herein report a novel *de novo KCNH5* variant in a patient with refractory epileptic encephalopathy. The patient exhibited seizures at 1 year and 7 months old, which gradually worsened, leading to a bedridden status. Brain magnetic resonance imaging (MRI) showed cerebral atrophy and cerebellar hypoplasia. A trio whole-exome sequence analysis identified a *de novo* heterozygous c.640A>C, p.Lys214Gln variant in *KCNH5* that was predicted to be deleterious. Recent studies have linked *KCNH5* to various epileptic encephalopathies, with many patients showing normal MRI findings. The present case expands the clinical spectrum of the disease, as it is characterized by severe neurological prognosis, cerebral atrophy, and cerebellar hypoplasia.

Key words: epileptic encephalopathy, whole-exome sequencing, KCNH5, *de novo* variant

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Introduction

Refractory epileptic encephalopathy has various genetic causes, including alterations in neuronal activity, transcriptional regulation, and cell metabolism. *De novo* variants are the most frequently detected causative variants in patients with refractory epileptic encephalopathy (1, 2). *KCNH5* has recently been identified as a cause of this condition (3).

We herein report a patient with refractory epileptic encephalopathy carrying a novel *de novo* variant of *KCNH5*.

Case Report

The patient was born to healthy parents with no family history of neurological disease or parental consanguinity. At six months old, he could roll over but failed to achieve further developmental milestones. Developmental delay was suspected at nine months old, and he was referred to another hospital at one year and four months old.

A cytogenetic examination with G-banding revealed normal results. The patient experienced his first seizure at one year and seven months old, which progressed to refractory generalized tonic-clonic and myoclonic seizures. Despite treatment with immunoglobulin and adrenocorticotropic hormone therapy, seizures persisted, and the patient became bedridden at 14 years old. Brain magnetic resonance imaging (MRI) at 14 years old indicated diffuse cerebral atrophy and cerebellar hypoplasia (Fig. 1). He was referred to our hospital for the exploration of genetic causes at 24 years old when he showed akinetic mutism, hypotonus, hyporeflexia, and positive Babinski signs. A comparison of brain MRI findings obtained at 14 and 24 years old showed the progression of cerebral atrophy over time (Fig. 1).

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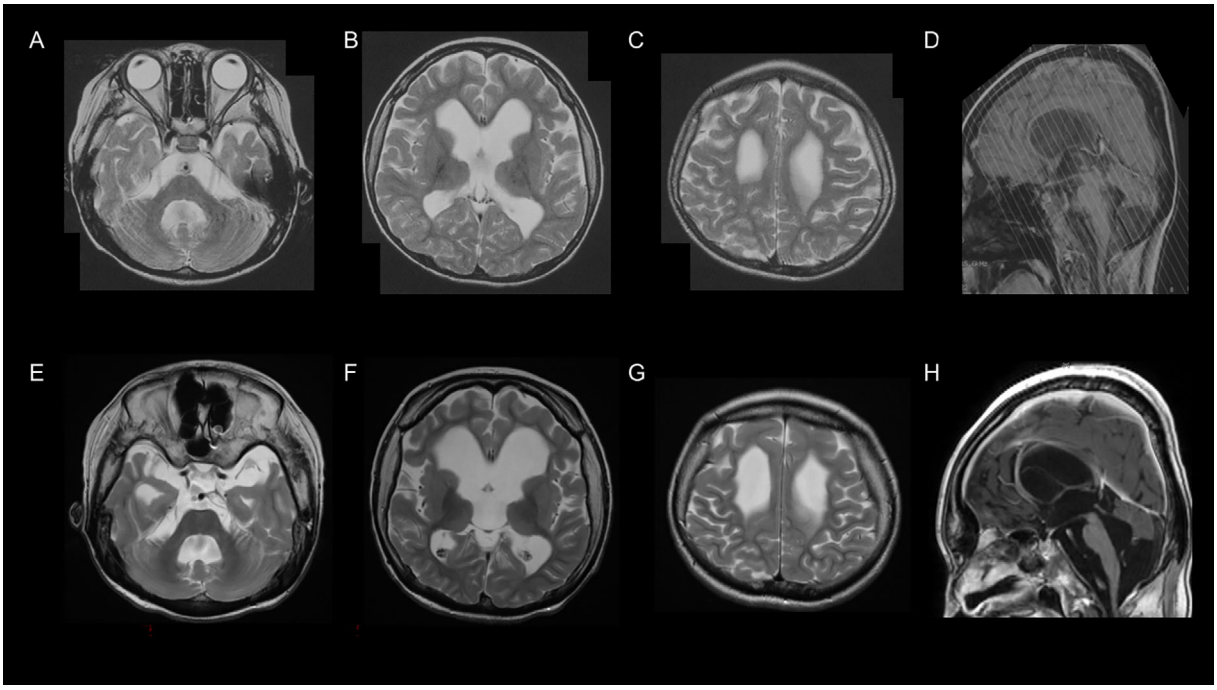

Figure 1. **Brain MRI of the present case at 14 and 24 years old. Brain MRI performed at 14 years old (A-D) shows frontal dominant ventricular and sulcal enlargement on T2-weighted axial images (A-C). The presence of cerebellar hypoplasia was deduced because the cerebellum was extremely small in size, and the prominence of cerebellar folia was not evident (A). Enlargement of the fourth ventricle and Blake's pouch cyst formation were also observed. Note that the brainstem is relatively well preserved on T1-weighted sagittal images (D). Brain MRI performed at 24 years old showed progression of cerebral atrophy (E-H).**

Epilepsy was managed with 850 mg valproic acid, 70 mg phenobarbital, and 200 mg zonisamide.

Genetic analyses

The patient's parents provided their written informed consent, as the patient was unable to make decisions for himself. Genomic DNA samples were prepared from peripheral blood leukocytes obtained from the patient and his parents.

We conducted a whole-exome sequence analysis of the patient using the SureSelect Human All Exon V6+UTRs kit (Agilent Technology, Santa Clara, USA) with HiSeq 2500 (Illumina, San Diego, USA). The sequences were aligned to GRCh37/hg19 using the Burrows Wheeler Aligner, and variants were called using SAMtools (4, 5). We searched for rare variants with a quality score of over 20 and minor allele frequencies less than 0.01 utilizing our in-house exome database of 1,194 Japanese controls and identified 405 variants. We then searched for rare variants in genes related to epileptic encephalopathy known in 2021 (Supplementary material), but we did not detect any candidate variants.

Next, we conducted whole-exome sequence analysis of the parents to identify *de novo* variants. To estimate the identity-by-descent of the proband and parents, PI_HAT was calculated using PLINK (v1.90). This calculation was based on 167,512 variants extracted from the autosomal chromosomes of the merged vcf files obtained from the exome sequence data after filling missing calls as homozygous reference alleles. The variants selected for calculation met the criteria of QUAL>40, DP>10, and GQ>20 (6). The PI_HAT values between the patient and his father and between the patient and his mother were 0.4970 and 0.5020, respectively, confirming paternity and maternity. Candidate *de novo* variants were extracted such that the number of alternative allele reads in the proband was at least 30% compared to reliable homozygous references in each parent (7). Heterozygous c.640A>C, p.Lys214Gln in *KCNH5* (NM_139318.5), c.595T >C, p.Phe199Leu in *OR2L3* (NM_001004687.2), and c.6997 C>T, p.Leu2333Phe (NM_004667.6) in *HERC2* were extracted as candidate *de novo* variants. Direct nucleotide sequence analysis of the polymerase chain reaction products confirmed that the heterozygous c.640A>C, p.Lys214Gln variant in *KCNH5* was a *de novo* variant. c.595T>C, p.Phe 199Leu in *OR2L3* and c.6997C>T p.Leu2333Phe in *HERC 2*, which were not confirmed by direct nucleotide sequence analysis, did not fulfill the strict QC conditions of MAPQ (> 20) and GQ (>30).

The *KCNH5* variant has not been registered in HGMD, ClinVar, jMorp 38KJPN, or gnomAD v3.1.2. This variant codes an amino acid conserved across vertebrates and was predicted to be deleterious, with a combined annotation-dependent depletion v1.6 Phred score of 26.0. According to the American College of Medical Genetics and Genomics guidelines, this variant is classified as likely pathogenic (PS 2, PM2, and PP3) (8).

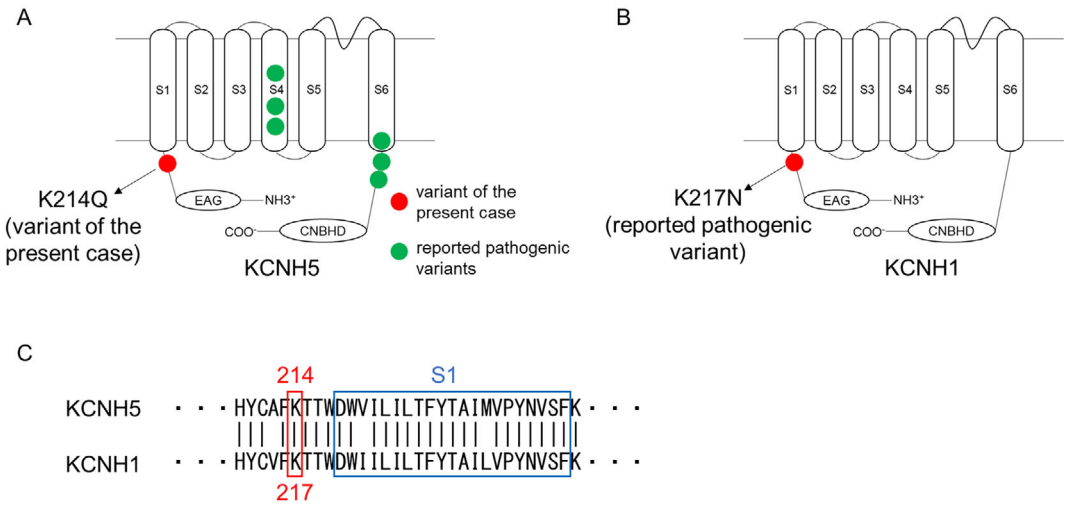

Figure 2. ***KCNH5*** **variant of the patient. A schematic presentation of Kv10.1 (KCNH1) is shown here. The p.Lys217Asn variant (red circle) was located near the junction of the S1 transmembrane domain. This variant has been identified as pathogenic in Temple-Baraitser syndrome (A). A schematic presentation of Kv10.2 (KCNH5) is also presented. The p.Lys214Gln variant (red circle) was located near the junction of the S1 transmembrane domain (B). Kv10.1 and Kv10.2 have a high degree of sequence similarity, with the Lys217 residue in Kv10.1 corresponding to the Lys214 residue in Kv10.2 (C).**

Discussion

In this study, we identified p.Lys214Gln as a likely pathogenic variant of *KCNH5*. The first report linking *KCNH5* to epileptic encephalopathy was published by Veeramah et al. They identified a heterozygous p.Arg327His variant in a patient with epileptic encephalopathy with mild developmental delay and regression (9). Functional analysis using voltage-clamp recordings revealed a hyperpolarizing shift and accelerated activation (10). Recently, Happ et al. reported 17 patients with *KCNH5* variants (3). Phenotypic variability ranged from self-limited epilepsy to severe developmental delay and epileptic encephalopathy. Brain MRI was performed in 15 patients, with normal findings found in 11. Cerebral atrophy was present in three patients, but severe atrophy, as seen in the present case, was not documented. Hu et al. reported three patients with *KCNH5*-related epileptic encephalopathy with normal brain MRI findings (11).

The ether-a-go-go voltage-gated potassium channel family consists of Kv10.1 encoded by *KCNH1* and Kv10.2 encoded by *KCNH5*. *KCNH1* variants have been associated with Temple-Baraitser syndrome and Zimmermann-Laband syndrome, both associated with severe intellectual disability and epilepsy (12, 13). Both Kv10.1 and Kv10.2 have a high degree of sequence similarity (72% identity at the amino acid level) and consist of 6 transmembrane domains (S1-S6). Three of the six reported *KCNH5* variants (p.Lys324Glu, p.Arg327His, and p.Ile463Thr) correspond to those reported in *KCNH1*-associated epilepsy. Of note, the Lys214 residue in Kv10.2, where the variant was found in the patient, corresponds to Lys217 in Kv10.1, where a pathogenic variant (p.Lys217Asn) has been reported (Fig. 2).

The phenotype-genotype correlation is unclear in *KCNH5*-related epilepsy. Two recurrent variants, p.Arg327His and p.Arg333His, are in the S4 domain, but their phenotypes differ: p.Arg327His causes infantile-onset refractory epileptic encephalopathy, while p.Arg333His causes drug-responsive seizures. However, the location of the variant may not definitively determine the phenotype. It is unclear why p.Lys214Gln caused the severe phenotype in the present case.

Despite the limited number of cases, recent studies have linked *KCNH5* to various epileptic encephalopathies, ranging from mild to severe, with many cases showing normal MRI findings. However, the present case is unique compared with the reported MRI findings, expanding the clinical spectrum of *KCNH5*-related epileptic encephalopathy.

This study was approved by the Institutional Review Board of the University of Tokyo. The parents provided written informed consent for publication because the patient was unable to provide consent.

The authors state that they have no Conflict of Interest (COI).

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