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Case Report

Novel *de novo* mutation substantiates *ATP6V0C* as a gene causing epilepsy with intellectual disability

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Abstract

Background: In approximately half of patients with epilepsy and intellectual disability (ID), the cause is unidentified and could be a mutation in a new disease gene.

Patient description: To determine the discovery of disease-causing mutation in a female patient with epilepsy and ID, we performed trio whole-exome sequencing, reverse transcription polymerase chain reaction (RT-PCR) followed by Sanger sequencing.

Results: Trio whole-exome sequencing was performed and revealed a novel de novo heterozygous stop-loss c.467A > T (p.*156Leuext*35) mutation in the *ATP6V0C* gene. Using RNA from leukocytes, RT-PCR followed by Sanger sequencing showed the existence of the mutant RNA, and real-time PCR demonstrated that the patient's *ATP6V0C* RNA level was approximately half of that in her parents, suggesting haploinsufficiency as a pathomechanism.

Conclusion: These findings, along with previous reports of individuals with similar phenotypes and variants in the same gene, substantiate *ATP6V0C* as a gene causing epilepsy with ID.

Keywords: *ATP6V0C*; Epilepsy; Intellectual disability

1. Introduction

Epilepsy and intellectual disability (ID) are two distinct neurological disorders, although they are occasionally present in the same individual. Approximately 30% of patients with epilepsy exhibit ID [1], while 20–30% of patients with ID also exhibit epilepsy [2]. Both disorders are etiologically heterogeneous. There are 1,525 and 1,400 genes known to be related to ID (HP:0001249) and epilepsy (HP:0001250), respectively. However, the causes of epilepsy and ID in approximately half of the patients with these disorders remain unidentified [3,4]. Alterations in new disease genes could be responsible for some of these cases.

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Recently, there have been reports of variants in the *ATPase H + Transporting V0 Subunit C* gene (*ATP6V0C*, MIM 108745) that are associated with epilepsy and ID. *ATP6V0C* encodes membrane-bound subunit C of the eukaryotic multisubunit vacuolar ATPase (V-ATPase) enzyme involved in the acidification of various intracellular organelles [5]. Two previous reports of studies of large cohorts of patients with epilepsy identified one patient in each cohort harboring point mutations in *ATP6V0C* [6,7]. Since no details of the two patients were provided, the identification of the etiologic role of *ATP6V0C* awaits more evidence. Here, we report a 16-year-old girl with intractable epilepsy and moderate ID with a novel *de novo* mutation in *ATP6V0C*, substantiating the notion that *ATP6V0C* is a gene responsible for epilepsy and ID.

2. Materials and method

2.1. Patient

The patient was the first child born at term to non-consanguineous healthy parents. The father and mother were 34 and 39 years old, respectively, when the patient was born. Her birth weight was 3,200 g. Her length and head circumference at birth and her growth and development during the first two years after birth were reportedly within normal ranges. She was able to stand and say her first word at 11 months of age. At the age of two years, she presented with generalized tonic-clonic seizures during a febrile illness. Subsequently, she developed multiple episodes of afebrile seizures that were refractory to multiple antiepileptic drugs. She attended kindergarten but did not start primary school. Her speech was at its best at the age of 5 years, when she could tell stories. Tonic-clonic seizures continued, and at age ten, atonic seizures began. Her intellectual development slowly regressed and then plateaued. At her last visit at the age of 16, she could communicate in short sentences and feed herself, was toilet trained but could not do simple math and needed help with grooming. A family history of epilepsy or intellectual disability was denied. She was 146 cm tall ($<3^{rd}$ centile), while the paternal and maternal heights were 165 cm and 150 cm, respectively. Her head circumference was 58 cm ($>97^{th}$ centile), while the father's and mother's head circumferences were 59 and 52 cm, respectively. There was no distinct dysmorphism. Neurological examination showed no focal neurological deficits. She was right handed. Her hearing test, vision evaluation and electrocardiogram were normal. Serial magnetic resonance imaging (MRI) of her brain performed at ages of seven, nine, and 16 years revealed a slightly smaller size of the left hippocampus, which was unchanged over time (Fig. 1A). Sleep electroencephalography (EEG) performed at the age of seven years revealed generalized spike-wave complexes maximum at the bifrontal area. An interictal sleep EEG performed at the age of 16 years showed frequent runs of spike-slow waves arising from the left frontal region (Fig. 1B). Despite the administration of multiple anti-epileptic drugs, including topiramate, clonazepam, levetiracetam, and lamotrigine, the patient still experienced approximately six seizures per month.

2.2. Whole-exome sequencing (WES)

The study was approved by the Institutional Review Board of the Faculty of Medicine of Chulalongkorn University, Thailand. After informed consent was obtained from the patient and her family members, peripheral blood samples were obtained. DNA was extracted from leukocytes and sent to Macrogen Inc. (Seoul, Korea) for WES. Data analysis was performed by filtering the variants located in exons or flanking introns and those that were nonsynonymous or rare, with a minor allele frequency of less than 1% in the Genome Aggregation Database (gnomAD) and the 2,166-Thai exome database. The variants were considered novel if they were not listed in the Human Gene Mutation Database (HGMD; www.hgmd.cf.ac.uk/ac/index.php) or gnomAD.

2.3. Sanger sequencing of genomic DNA

To verify the existence of c.467A > T (p.*156Leuext*35) in *ATP6V0C*, genomic DNA was extracted from peripheral blood leukocytes and PCR amplified using the following primers: 5′-TCAGGCCT GCTGTGTGGCTG-3′ and 5′-AACAGACGATGGG CACTAGGACA-3′, covering the beginning of exon 3 and the termination codon of *ATP6V0C* (NM_001198569.1). The product was cleaned and sent to Macrogen Inc. for standard sequencing with the internal sequencing primer 5′-AGGCTGCTGATGT CAGTCCT-3′.

2.4. RNA extraction, reverse transcription, and sequencing of the complementary DNA

To determine the existence of the mutant RNA, total RNA was extracted from blood leukocytes of the patient and her parents using an RNA blood mini kit (Qiagen, Hilden, Germany). The ProtoScript® II First Strand cDNA Synthesis Kit (NEB, Ipswich, MA, USA) was used for cDNA synthesis. cDNA-PCR was performed using the primers 5′-CTCTACAA GAGCTTCCTCCAGC-3′ and 5′-CATCCGCATACA CAGAGCAC-3′. The purified PCR products were then subjected to Sanger sequencing.

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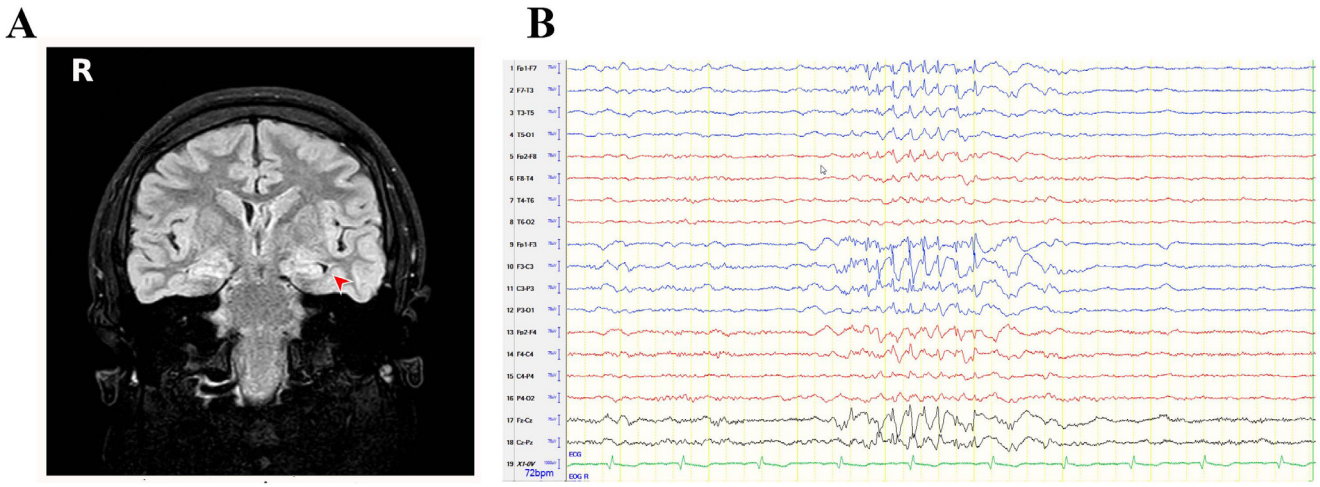

Fig. 1. Neurological studies. A. MRI of the brain performed at the age of 16 years with the coronal oblique fluid-attenuated inversion recovery (FLAIR) sequence reveals a slightly smaller size of the left hippocampus (arrow), suggesting left hippocampal sclerosis. B. An interictal electroencephalography (EEG) recorded during sleep, shows runs of spike-slow wave complexes arising from the left frontal head region.

2.5. Real-time PCR

Real-time PCR was performed using the hs00733757_g1 TaqMan kit from Thermo Scientific, Inc. (Waltham, MA, USA). The probe sequence was 5′-GACATCAGCCTCTACAAGAGCTTCC-3′, spanning the junction of exons 2 and 3 of the *ATP6V0C* gene. ACTB (beta-actin; provided by the manufacturer in the hs02458991_g1 kit) was used as an internal control.

2.6. Evolutionary conservation

Protein alignment of the orthologous *ATP6V0C* gene sequences (Ensembl; ENSG00000185883) of 10 different species, including human, chimpanzee, mouse, dog, chicken, goldfish, lamprey, tropical clawed frog, *D. melanogaster*, and *C. elegans,* was performed.

3. Results

3.1. WES and Sanger sequencing

Through trio-WES analysis, the proband was found to harbor a novel heterozygous c.467A > T (chr16:2569745A > T, GRCh37/hg19) mutation in the *ATP6V0C* gene. The mutation was absent in her parents. Sanger sequencing confirmed the findings (Fig. 2A). The nucleotide substitution is expected to change the termination codon (TAG; Ter) at position 156 to leucine (TTG; Leu), resulting in an abnormal extension of 35 residues at the carboxyl terminus of the protein, p.*156Leuext*35 (Fig. 2A, the top panel). This mutation is absent in gnomAD, HGMD, and our own in-house 2,166-exome database. Filtering criteria for variant selection are shown in Supplementary material. The *ATP6V0C* c.467A > T variant has been submitted to the ClinVar database (accession number VCV000816939.1).

3.2. Sanger sequencing of complementary DNA

Sanger sequencing using cDNA reverse transcribed from the RNA extracted from leukocytes demonstrated the existence of the c.467A > T mutant RNA in the patient but not in her parents, as shown in Fig. 2A.

3.3. Real-time PCR

The expression level of the *ATP6V0C* gene of the unaffected mother was used for normalization. The patient was found to exhibit a 44% to 56% decrease in *ATP6V0C* expression compared to her mother (Fig. 2B).

3.4. Evolutionary conservation

The termination codon at residue 156 of human *ATP6V0C* is highly conserved among species, as shown in Fig. 2C.

4. Discussion

The etiology of approximately half of patients with epilepsy and ID is unknown. Here, we used WES to identify a *de novo* heterozygous c.467A > T (p.*156Leuext*35) variant in *ATP6V0C* in a Thai female patient with epilepsy and ID. Several lines of evidence indicate that the variant is pathogenic. First, it is a termination codon mutation. Stop-loss mutations have been shown to be likely pathogenic [8]. Second, the c.467A > T mutation is absent in our in-house Thai

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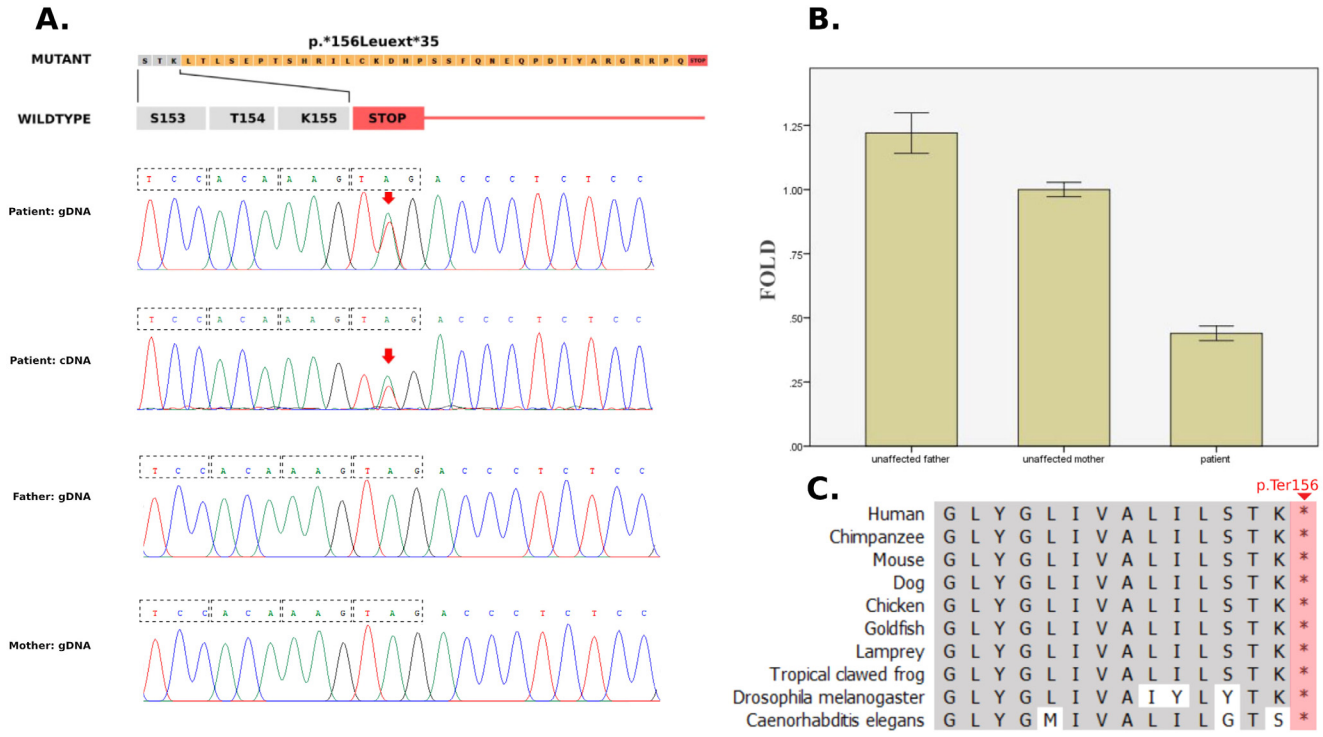

Fig. 2. Molecular studies. The uppermost panel shows the DNA sequence encoding the predicted elongated 35 amino acid residue sequence resulting from c.467A > T (p.*156Leuex*35) in *ATP6V0C*. The second and third panels are electropherograms of the patient's genomic DNA and complementary DNA (cDNA) showing the c.467A > T mutation in *ATP6V0C* (arrows). The fourth and fifth panels are electropherograms of the father's and mother's genomic DNA, respectively, showing only the wild-type sequences. B. The bar graph represents the fold difference in the RNA expression of *ATP6V0C* normalized to that of the unaffected mother. C. Protein alignment of orthologous *ATP6V0C* gene sequences shows that the stop codon is evolutionarily conserved among the 10 species.

exome database (N = 2,166), gnomAD, and HGMD. Third, it is a *de novo* mutation, which is suggestive of pathogenicity in neurodevelopmental and psychiatric disorders [9]. Fourth, it is evolutionarily conserved (Fig. 2C).

Ten patients with epilepsy and ID have previously been reported to harbor *ATP6V0C* variants. Two harbored point mutations, one of whom exhibited Dravet syndrome and a heterozygous 2-bp deletion, c.134_135delTC, p. Ser45Cysfs*37 [6], while the other presented a developmental disorder and a heterozygous 16-bp deletion, c.344_359del, p.Asp115Alafs*12 [7]. The other eight patients, who displayed syndromic microcephaly, developmental delay, intellectual disability, and epilepsy, harbored large chromosomal deletions with a 112-kb overlap encompassing three genes, *TBC1D24*, *ATP6V0C*, and *PDPK1* [10]. A previous experiment focusing on atp6v0c2 in zebrafish suggested that the ATP6V0C2 protein shows neuron-specific expression and is involved in neurotransmitter storage and/or secretion within presynaptic vesicles [11]. Heterozygous Atp6v0c knockout mice exhibited no defects, but homozygous knockout mice showed embryonic lethality [12]. Although these findings suggest that *ATP6V0C* is a potential candidate gene for epilepsy and ID, its etiologic role remains inconclusive [10].

Our patient and these 10 patients with variants in *ATP6V0C* all exhibited epilepsy and ID. The majority (8/10) presented generalized tonic-clonic seizures. Among these 11 patients, 10 underwent MRI of the brain, and six showed normal results, while four exhibited nonspecific brain lesions [10], including the smaller left hippocampus seen in our patient.

Although the mutant *ATP6V0C* RNA was present in our patient, as shown by RT-PCR, real-time PCR revealed that the expression level in the patient was less than half of that in her parents (Fig. 2B). This suggests that the mutant RNA, although expressed, is mostly degraded, and haploinsufficiency is the likely pathomechanism in this patient.

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.

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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.2020.10.016.

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