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SHORT COMMUNICATION

Sudden unexpected death with rare compound heterozygous variants in *PRICKLE1*

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Abstract

Progressive myoclonus epilepsy-ataxia syndrome (EPM5) is an autosomal recessive form of progressive myoclonus epilepsy that has been associated with a homozygous missense mutation in *PRICKLE1*. We report a 23-year-old male who died shortly after refractory convulsion and respiratory failure. Autopsy showed unilateral hippocampal malformation without significant neuronal loss or gliosis. Genetic analysis that targeted both epilepsy and cardiac disease using next-generation sequencing revealed two variants of *PRICKLE1*. Additional investigation showed that the patient's father (p.Asp760del) and mother (p.Asp201Asn) each had a mutation in this gene. The present case shows that EPM5 can also be caused by compound heterozygous mutations.

Keywords Compound heterozygous mutation · Hippocampus · Neuropathology · *PRICKLE1* · Progressive myoclonus epilepsy · Sudden death

Introduction

Progressive myoclonus epilepsy (PME) is a complex of neurodegenerative diseases that show action myoclonus, epileptic seizures and progressive neurologic decline as core clinical features. The genetics and presentation of PME are varied, making the diagnosis of specific forms of PME without genetic investigation particularly challenging [1]. One of the PMEs, progressive myoclonus epilepsy-ataxia syndrome type 5 (EPM5), is an autosomal recessive form that has been associated with a homozygous missense mutation in *PRICKLE1* [1, 2]. Neuropathological reports of PMEs are rare, and our literature survey has not found any such reports for EPM5.

Here we report the autopsy of a young man with a complex heterozygous mutation in *PRICKLE1* who died suddenly without medical treatment antemortem.

Case description

At midnight, a 23-year-old, male construction worker experienced a sudden loss of consciousness with convulsions while driving home after a karaoke party. His colleagues called the emergency services, and he was transferred to a general hospital. On admission, his Glasgow Coma Scale score was 100. He continuously experienced convulsions and unstable respiratory conditions despite being in intensive care, and he died 5 h after admission. The absence of structural abnormalities and disease was confirmed by biochemical examination of the blood and radiological examination that included a computed tomography scan of his general body during intensive care.

He had neither been diagnosed nor received any medical treatment of any kind throughout his life; however, his colleagues and friends had witnessed him having convulsive attacks several times over the last year, and these convulsions were witnessed three times in the last month. According to his friends, all the previous convulsions they had witnessed disappeared spontaneously within a short interval. Furthermore, the convulsions occurred at around midnight when the proband was in a fatigued state or had consumed alcohol. His parents

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have no significant clinical history. They lived with him until 4 years ago but had never witnessed these convulsive attacks.

Medicolegal autopsy showed some mild bruising and abrasion of the face and extremities. Bone fractures and injuries of the muscle and internal organs were not found. Dextromethorphan and diprophylline, which are present in over-the-counter cough medicine, were detected in his blood at non-lethal concentrations [3] of 0.63 and 5.5 μg/mL, respectively. Alcohol was not detected in the blood and urine.

The brain weighed 1451 g. Besides mild symmetrical oedematous swelling, the left-sided hippocampus showed a more upright and globular appearance with vertical alignment of the subiculum (Fig. 1a–c) when compared with two age-matched male autopsy cases (Fig. 1d, e). In the present case, the density of neurons in the CA1 region of the hippocampus was slightly lower than in the age-matched control cases (Fig. 1f–h). Gliosis was not evident in the cerebrum, cerebellum, or brain stem based on Holzer's staining (Fig. 1i) and immunohistochemistry for glial fibre-associated protein (clone ZCG 29, Nichirei Tokyo, Japan). The heart and other organs did not show any abnormal findings.

After obtaining written informed consent from his parents, next-generation sequencing (NGS) of 146 epilepsy-related genes and 73 inherited cardiac disease-related genes was performed using an Ion PGM system (Life Technologies, Carlsbad, CA, USA) for the patient and his parents. Detailed methods are shown in our previous report [4]. Variants were called within the coding exons and ± 2 bp into the introns using Ingenuity Variants Analysis (QIAGEN, California, USA). All variants with a minor allele frequency (MAF) ≥ 1.0% among the East Asian population were filtered using the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org). All the examined genes are listed in Supplemental Table 1.

As the results, NGS newly identified two variants of *PRICKLE1*, and subsequent analysis of the family members showed that the father (p.Asp760del) and mother (p.Asp201Asn) each had a mutation in this gene (Fig. 2a, b; Table 1) [6]. In the commonly used gnomAD, 15 alleles in over 282,752 were identified for pAsp760del and only three in 251,342 were identified for p.Asp201Asn. p.Asp201Asn and p.Asp760del in *PRICKLE1* are in the LIM zinc-binding 2 domain and just before the serine-rich domain, respectively. These amino acids are completely conserved among vertebrate species (Fig. 2c) [5]. We applied four different in silico predictive algorithms (CADD, Polyphen-2, SIFT, and Mutation Taster), which revealed that both variants may be pathogenic as shown in Table 1. Possible pathogenic variants related to cardiovascular disease-related genetic variant were not detected.

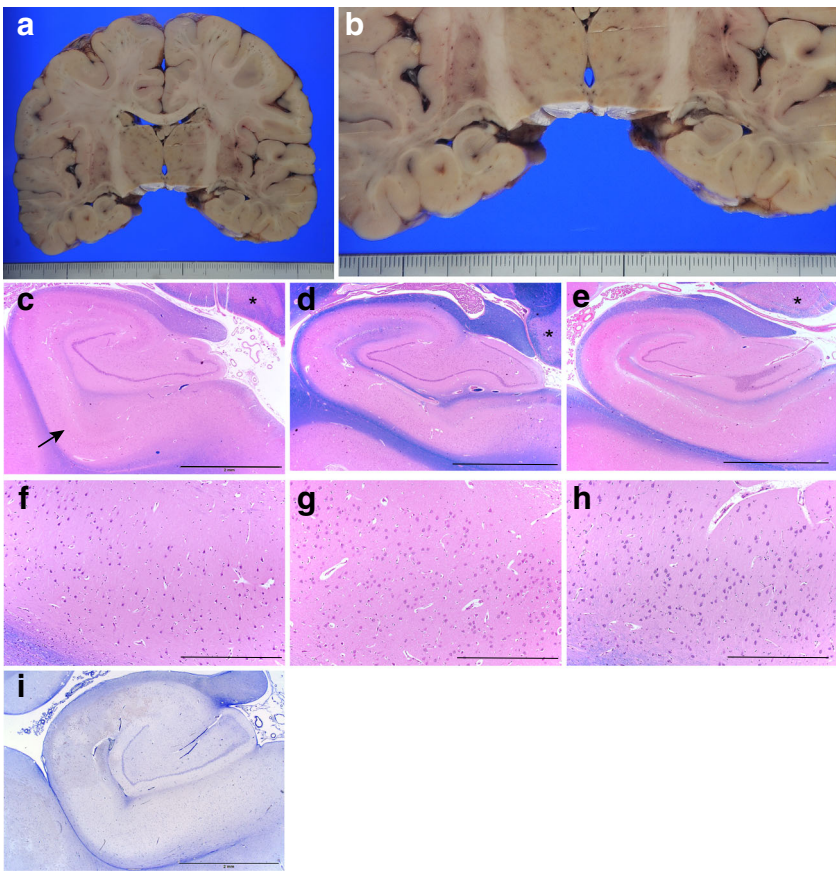

Fig. 1 Pathological findings for the present case. **a**, **b** Gross appearance showing deformity of the left hippocampus. **c**–**e** Low-power view of the hippocampus of the present case (**c**) and the control cases (**d**, 25-year-old suicide case; **e**, 22-year-old traffic accident case). The arrow indicates the vertical alignment of the subiculum. **f**–**h** Moderate-power view of the CA1 region of (**c**–**e**). The asterisks indicate medial geniculate nuclei. **i** Holzer's staining of the hippocampus. Significant gliosis was not evident. Scale bars, 2 mm (**c**–**e**, **i**), 200 μm (**f**–**h**)

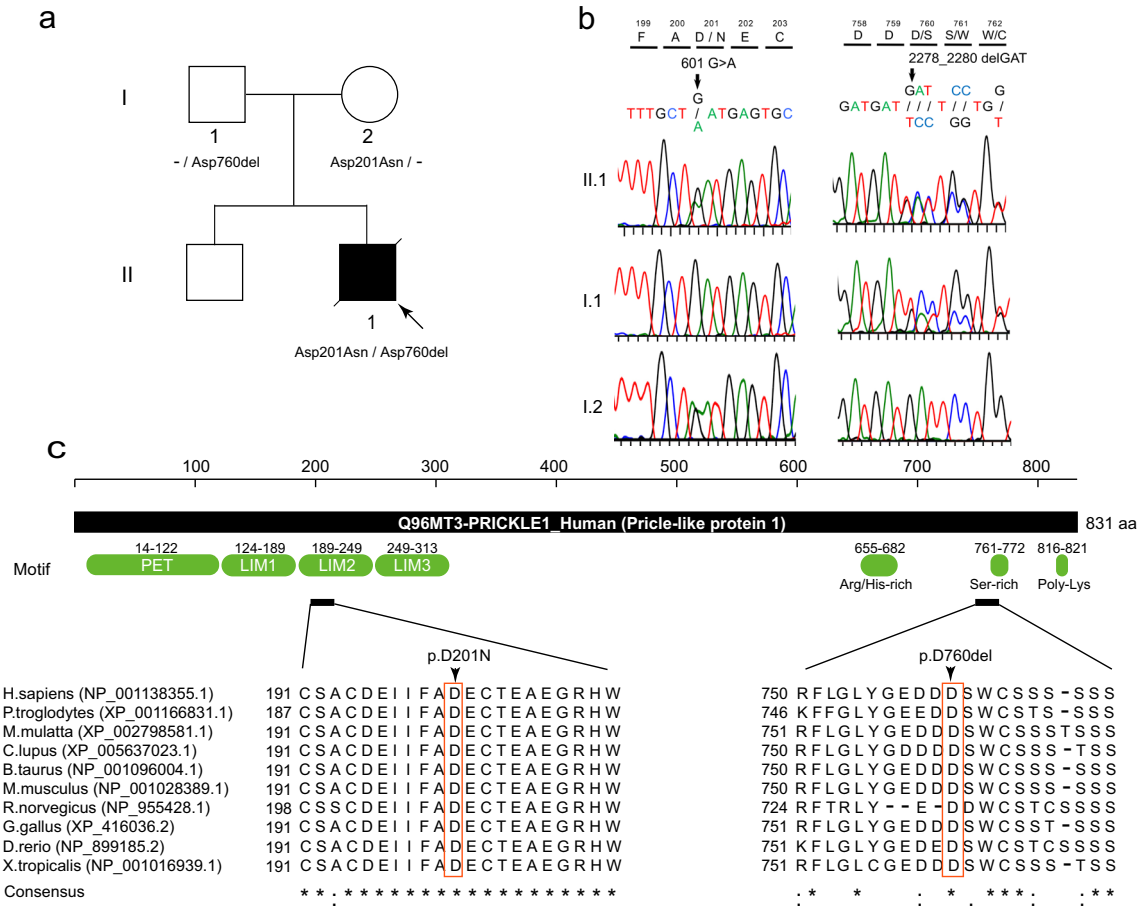

Fig. 2 Pedigree chart, electropherograms and evolutionary comparison. **a** Pedigree of family. Squares and circle indicate males and female, respectively; the hatched square indicates the proband. Subjects who participate in DNA analysis are labelled with numbers. **b** Electropherogram of c.601G>A (p.Asp201Asn) and c.2278_2280 delGAT (Asn760del) of the *PRICKLE1* gene in individuals of the family, displaying compound heterozygous status in the affected subject (II.1), and heterozygous status in his parents (I.1 and I.2). **c** A multiple species protein alignment of the region surrounding the amino acid changes caused by the variants in *PRICKLE1*. We selected a multiple sequence alignment of PRICKLE1 in NCBI HomoloGene site (https://www.ncbi.nlm.nih.gov/homologene) and then aligned with M-Coffee, a meta-multiple sequence alignment programme [5]. The number before the sequence indicates the poison of the first residue within the region. Arrows indicate each substitution site. The key below denotes conserved sequence (*), conservative mutations (:), semi-conservative mutations (.) and non-conservative mutations ( )

Discussion

The function of PRICKLE1 has been closely linked with Wnt/Planar cell polarity (PCP) signalling in the development of, for example, apical spiral ganglion neurons, facial motor neurons, the limbs and palate [7]. Additional studies have shown that PRICKLE1 regulates neuron morphogenesis, including neuron migration and neurite growth during maturation of the central nervous system neurons [7, 8]. Previously, a mutation in *PRICKLE1* was identified in three pedigrees of individuals with PME and ataxia, where either the clinical features or linkage mapping excluded known PME loci, and mutated *PRICKLE1* was found to be expressed in neurons in the thalamus, hippocampus, cerebral cortex and cerebellar cortex of these individuals' brains and was thought to play a role in their seizures and ataxia [2].

Mutations in *PRICKLE* genes can cause seizures in humans, zebrafish, mice, and flies, suggesting that the seizure suppression pathway is evolutionarily conserved [2]. Previous studies suggested that interactions between PRICKLE1 and other proteins could explain the pathogenesis of EPM5 [9, 10]. Paemka et al. defined the mammalian PRICKLE-interactome and showed that inhibition of the substrate-specific de-ubiquitinase USP9X could suppress *Prickle*-mediated seizure activity, and that *USP9X* variants may predispose individuals to seizures [10].

De novo *PRICKLE1* mutations were previously identified in various neurological diseases, such as myoclonic epilepsy with autism or agenesis of the corpus callosum and polymicrogyria [11, 12]. The present case did not have any history of medical attention; however, we concluded that he developed PME associated with a pathogenic compound heterozygous mutation of *PRICKLE1*, and the cause of death was status epilepticus due to EPM5. Asssenza et al. and Dibbens et al. respectively showed that compound heterozygous *cystatin B* and *SCARB2* mutations were causative of Unverricht–Lundborg disease (EPM1) and

Table 1 Summary of the variants of *PRICKLE1* identified in the proband and his parents

Transcript	Protein	Coding	dbSNP	ACMG variant classification	gnomAD frequency (%)	HGVD (%)	CADD Score	PolyPhen-2	SIFT	Mutation Taster
NM_0011448-81.1	p.Asp760del	c.2278_2280del-GAT	rs557205452	Uncertain significance	<Exomes+Genomes>: ALL:0.0058% - AFR:0.0042% - EAS:0.074% - NFE:0.00079%	0.79	11.39	NA	NA	Disease-causing
NM_0011448-81.1	p.Asp201Asn	c.601G>A	–	Uncertain significance	<Exomes>: ALL:0.0012% - EAS:0.017%	ND	33	Probably damaging	Damaging	Disease-causing

dbSNP The Single Nucleotide Polymorphism Database (https://www.ncbi.nlm.nih.gov/projects/SNP/), *ACMG* American College of Medical Genetics and Genomics [6], *CADD* combined annotation dependent depletion (https://cadd.gs.washington.edu), *gnomAD* The Genome Aggregation Database (http://gnomad.broadinstitute.org), *HGVD* Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp.), *NA* not available, *ND* not described

EPM4, which are subtypes of PMEs usually inherited in an autosomal recessive manner [13, 14]. Although heterozygous variants of *Prickle1* were identified in animals with seizures [8], as far as we can determine, the present case is the first reported case with a symptomatic pathogenic compound heterozygous mutation of *PRICKLE1*. Based on the comments of people who knew the proband, the onset time of EPM5 for the present case was about a year before death. Because of this late onset, the severity of the pathogenicity of this compound heterozygous mutation in *PRICKLE1* is likely lower than that of previously reported homozygous cases; however, such a mutation leads to an increased risk of status epilepticus in the absence of appropriate medical treatment. Fatigue, alcohol intake or waking from a drunken state might have been triggers of the epileptic seizures in EPM5 from the investigation of the present case.

The role of hippocampal malformation in the development of epilepsy is extensive and complex [15, 16]. While some studies suggest that hippocampal malformation is more prevalent in patients with epilepsy [15], others show that it is frequent in healthy volunteers [16]. Although the proband's hippocampal malformation was not associated with significant hippocampal sclerosis, which is known to be the most common pathology of temporal epilepsy [17], the present case showed that hippocampal malformation may be a significant pathological finding, suggesting the possible pathomechanism of the compound heterozygous *PRICKLE1* mutations in the development of EPM5. Additional pathological investigation of EPM5 cases will likely be essential to explore whether it has pathological significance for EPM5.

Criscuolo et al. indicated that routine screening of the Italian population for *PRICKLE1* mutations in association with PMEs is of limited clinical value because of the rarity of such cases [18]. The present case showed that comprehensive genetic analysis using NGS and subsequent analysis of the family members may increase the chances of diagnosing this compound heterozygous mutation of *PRICKLE1*, particularly in the event of a sudden, unexpected death with convulsions of unknown aetiology and no clear family history of PME.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Written consent was obtained from family members before the genetic study. The ethical committee of Toyama University approved this study, which was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

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