



Identification of de novo *CSNK2A1* and *CSNK2B* variants in cases of global developmental delay with seizures

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Abstract

Casein kinase 2 (CK2) is a serine threonine kinase ubiquitously expressed in eukaryotic cells and involved in various cellular processes. In recent studies, de novo variants in *CSNK2A1* and *CSNK2B*, which encode the subunits of CK2, have been identified in individuals with intellectual disability syndrome. In this study, we describe four patients with neurodevelopmental disorders possessing de novo variants in *CSNK2A1* or *CSNK2B*. Using whole-exome sequencing, we detected two de novo variants in *CSNK2A1* in two unrelated Japanese patients, a novel variant c.571C>T, p.(Arg191*) and a recurrent variant c.593A>G, p.(Lys198Arg), and two novel de novo variants in *CSNK2B* in Japanese and Malaysian patients, c.494A>G, p.(His165Arg) and c.533_534insGT, p.(Pro179Tyrfs*49), respectively. All four patients showed mild to profound intellectual disabilities, developmental delays, and various types of seizures. This and previous studies have found a total of 20 *CSNK2A1* variants in 28 individuals with syndromic intellectual disability. The hotspot variant c.593A>G, p.(Lys198Arg) was found in eight of 28 patients. Meanwhile, only five *CSNK2B* variants were identified in five individuals with neurodevelopmental disorders. We reviewed the previous literature to verify the phenotypic spectrum of *CSNK2A1*- and *CSNK2B*-related syndromes.

Introduction

Neurodevelopmental disorders (NDDs) are a group of neuropsychiatric deficits diagnosed during early childhood.

They include intellectual disability, autism spectrum disorder, attention deficit/hyperactivity disorder, specific learning disorders, communication disorders, motor disorders, and tic disorders [1]. It is estimated that approximately 2–5% of children may be affected by NDDs [2–4]. Recent trio-based whole-exome sequencing (WES) analyses have revealed that de novo variants in loss-of-function intolerant or haploinsufficient genes are important causative

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factors of NDDs [5–9]. However, the genetic causes of NDDs are heterogeneous and have not been fully elucidated.

Protein kinase CK2 is a pleiotropic serine/threonine kinase phosphorylating hundreds of substrates and participating in diverse cellular processes, including cell cycle regulation [10, 11], DNA replication and repair [12], development and differentiation [13], transcription [14], cell signaling [15], carcinogenesis [16], and apoptosis [17]. CK2 acts as a tetrameric complex comprising two catalytic (CK2 α and/or CK2 α') and two non-catalytic (CK2 β) subunits, and CK2 holoenzyme comprises identical (α/α or α'/α') or non-identical (α/α') combinations of catalytic subunits [18–22]. Recent WES studies have reported that de novo variants in *CSNK2A1* and *CSNK2B*, which encode CK2 α and CK2 β subunits, respectively, cause NDDs [23–29]. The disorder caused by *CSNK2A1* variants is known as Okur-Chung neurodevelopmental syndrome (MIM#617062), and is characterized by intellectual disabilities, developmental delays, behavioral problems, and other multisystemic abnormalities [23–26, 29]. To date, 26 individuals with de novo variants in *CSNK2A1* have been reported. In contrast, only three individuals harboring de novo variants in *CSNK2B* have been identified with intellectual disabilities, developmental delays, and myoclonic epilepsy [27, 28].

Here, we describe four individuals exhibiting intractable epilepsy, mild to severe psychomotor retardation, and intellectual disability. Using WES, we identified two de novo *CSNK2A1* variants and two *CSNK2B* variants in these patients. We will describe detailed clinical features of patients with *CSNK2A1* and *CSNK2B* variants to delineate the phenotypic spectrum of CK2-related syndromes.

Materials and methods

Patients

A total of 1230 individuals with early childhood-onset epilepsy and their parents were investigated in this study. Written informed consent was obtained from all participating families and the genomic DNA were extracted from blood leukocytes. Detailed clinical information was obtained from corresponding clinicians. Experimental protocols were approved by the Institutional Review Board of Yokohama City University School of Medicine, Showa University Faculty of Medicine, and Hamamatsu University School of Medicine.

Whole-exome sequencing (WES)

Trio-based WES of 337 families and case-only WES of 893 individuals was performed using the SureSelectXT Human

All Exon v4, v5 or v6 (Agilent Technologies, Santa Clara, CA). Captured libraries were sequenced using Illumina HiSeq 2000 or 2500 (Illumina, San Diego, CA) with 101-base paired-end reads. Exome data processing, variant calling, and variant annotation were performed as previously described [30–32]. Final variants were annotated with Annovar [33] for predictive value of functional impact of the coding variants and assessing allele frequency: in-house database of 575 control exomes, 1KJPN [34] 1000 genome [35] and ExAC database [36]. Variant pathogenicity was predicted by SIFT, Polyphen-2, CADD [37] and M-CAP [38] software. Nucleotide conservation prediction was performed using GERP and PhastCons. Candidate variants were confirmed by Sanger sequencing using ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

Plasmid construction

Human WT *CSNK2A1* (NM_177559.2) and *CSNK2B* (NM_001320.5) cDNAs were prepared by polymerase chain reaction using a cDNA library transcribed from mRNA derived from human brain tissues as a template, and verified by Sanger sequencing. WT *CSNK2B* cDNA was cloned into a pCAGGS-IRES2-nucEGFP (pCIG) vector to express N-terminal HA-tagged *CSNK2B*, as well as nuclear-localized EGFP. Site-directed mutagenesis using a KOD-Plus-Mutagenesis kit (TOYOBO, Osaka, Japan) was used to generate mutant *CSNK2B* (p.Thr37Tyrfs*5, p.His165Arg, and p.Pro179Tyrfs*49) vectors in accordance with the manufacturer's instructions. WT *CSNK2A1* cDNA was cloned into a pEF1 α -1xFLAG vector to express N-terminal FLAG-tagged *CSNK2A1* under an EF1 α promoter.

Cell culture, transfection, immunoprecipitation, and western blotting

HEK293T human embryonic kidney cells were grown in DMEM supplemented with 4.5 g/l glucose, L-glutamine (Wako, Osaka, Japan, 043-30085), 10% FBS, and penicillin/streptomycin at 37 °C and 5% CO₂.

WT or mutant HA-tagged *CSNK2B* vectors with/without FLAG-tagged *CSNK2A1* vectors were transfected in HEK293T cells using Polyethylenimine MAX (Polysciences, Warrington, PA, USA) in accordance with the manufacturer's instructions. After 24 h incubation, cells were lysed with 0.1% NP-40 lysis buffer (500 mM Tris base, 500 mM NaCl, 0.1% NP-40) containing protease inhibitor cocktails (cOmplete Mini EASYpack, Roche Diagnostics, Mannheim, Germany). The lysates for co-immunoprecipitation (IP) assay were incubated with 250 ng anti-DYKDDDK (TransGenic, Fukuoka, Japan, 2H8) and Dynabeads Protein G (Thermo Fisher Scientific) at 4 °C for 1 h. The beads were washed three times with lysis buffer

Table 1 Summary of clinical features of de novo *CSNK2A1* and *CSNK2B* variants

Individuals	Patient 1	Patient 2	Patient 3	Patient 4
Age	15 y	Died at 1 y 7 m	15 y	7 y
Gender	Female	Male	Female	Male
Variant	<i>CSNK2A1</i> :c.593A>G, p.Lys198Arg	<i>CSNK2A1</i> :c.571C>T, p.Arg191*	<i>CSNK2B</i> :c.533_534insGT, p.Pro179Tyrfs*49	<i>CSNK2B</i> :c.494A>G, p.His165Arg
Gestation/weeks	34	40	41	40
Birth WT/g (S.D.)	2194 (−2.0)	N.A.	3,092 (+0.2)	3460 (+1.1)
Birth BL/cm (S.D.)	46 (−1.1)	N.A.	49.4 (+0.5)	50 (+0.5)
Birth OFC/cm (S.D.)	33.5 (+0.4)	29 (−2.8)	32 (−0.6)	35 (+1.5)
Growth	At 15 y	At 1 y 4 m	At 14 y	At 4 y
WT /kg (S.D.)	30 (−2.7)	N.A.	22 (−3.6)	15 (−0.6)
HT/cm (S.D.)	144.3 (−2.5)	N.A.	145 (−2.2)	95 (−1.9)
OFC (cm)	52.0 (+0.7)	44 (−1.5)	49 (−3.7)	50 (0.2)
Seizure	Onset at 4 y 7 m	Onset at 5 m	Onset at 2 m	Onset at 3 d
Seizure type at onset	Febrile seizures	Tonic-clonic seizure	Facial clonic seizures	Focal seizures
<i>Development</i>				
Age at sitting	10 months	7 months	Not acquired	Not acquired
Age at walking	2 y 6 m	1 y 6 m	Not acquired	Not acquired
Language	Short sentences	One word	Not acquired	Not acquired
<i>Neurologic</i>				
Hypotonia	+	+	—	—
Dystonia	—	—	—	+
Intellectual disability	Mild	N.A.	Profound	Profound
Brain image	Volumetric loss in cerebral white matter around posterior horn of the lateral ventricular	Normal	Cerebellar atrophy	Mega cisterna magna
Facial dysmorphism	Broad nasal bridge, short upturned nose, arched eyebrows	None	None	Inverted mouth shape
Immunological	Bronchial asthma	N.A.	N.A.	Frequent acute pneumonia
Other	Congenital biliary dilatation, pharyngeal dysphagia, respiratory failure, easy fatigability, progressive muscle weakness, sleep apnea	N.A.	Precocious puberty	Gastro esophageal reflux disease

WT weight, BL body length, OFC occipitofrontal circumference, HT height, N.A. not assessed or not available, y year(s), m month(s), d day(s)

and proteins were eluted with 1X SDS sample buffer. Samples were separated using SDS polyacrylamide gel electrophoresis and analyzed via western blotting with rabbit polyclonal antibodies against HA tag (Roche Diagnostics, 11 867 431 001), DYKDDDK tag (TransGenic) and GFP (GE Healthcare, 27457701).

Results

Clinical features

The clinical features of the four individuals with *CSNK2A1* and *CSNK2B* variants are summarized in Table 1. Two

patients with *CSNK2A1* variants shared global developmental delays, hypotonia, and intractable seizures, but each exhibited interesting features. Patient 1 showed late-onset and less-frequent seizures (Fig. 1a) and exhibited various abnormalities, including facial dysmorphisms (Supple. Fig S1), brain abnormalities (Fig. 2a, b), short stature, congenital biliary dilatation, and pharyngeal dysphagia. This patient also showed distinct easy fatigability and muscle weakness. These manifestations progressively worsened and led to gait difficulty and respiratory failure. Subsequently, the patient needed a wheelchair for moving and needed continuous mechanical ventilation support. Meanwhile, patient 2 presented a severer course, with seizures resembling those with Dravet syndrome. This patient began

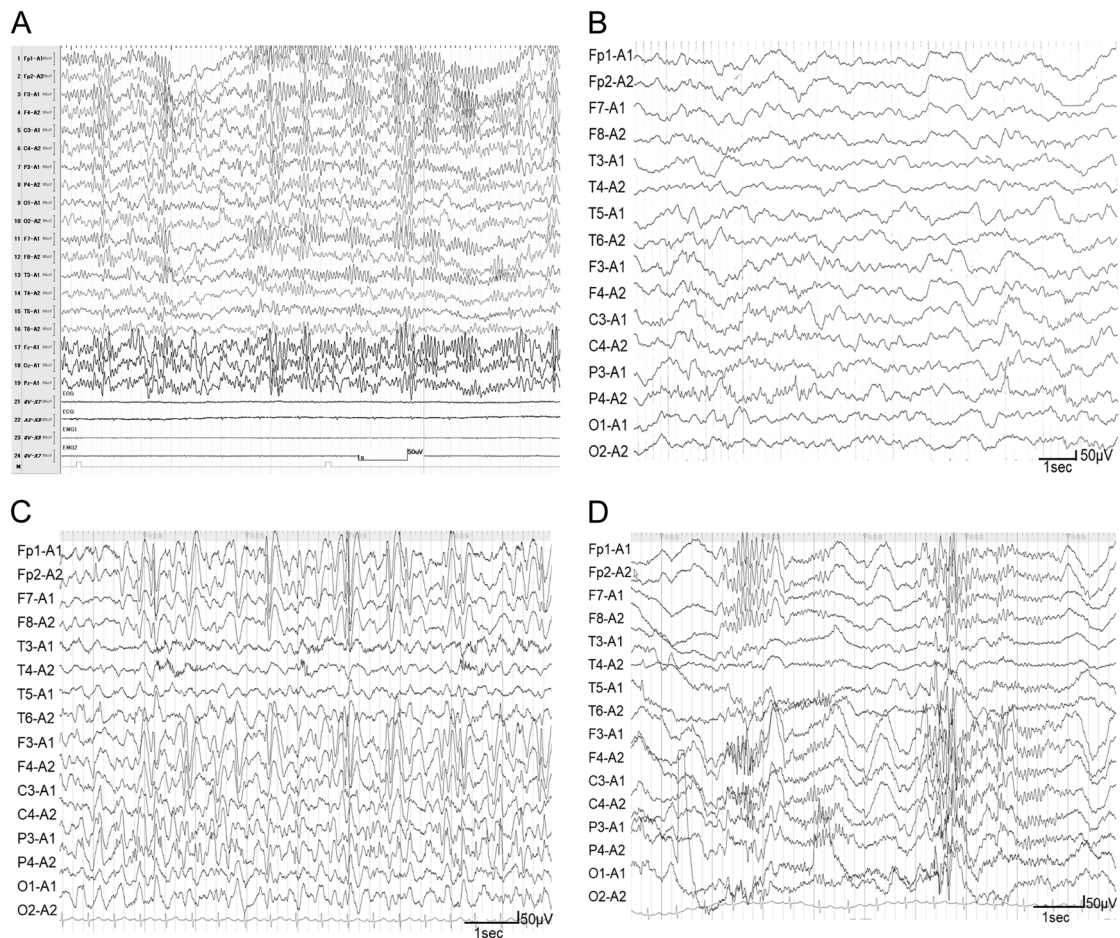


Fig. 1 Electroencephalogram (EEG) of patient 1 **a** and patient 3 **b–d**. **a** EEG of patient 1 at 12 years. **b** Frequent right parietal spikes were present during

sleep. **c** Frequent semi-rhythmic generalized bifrontally predominant 2 c/s spike-and-wave complex were seen during wakefulness. **d** Occasional generalized 10-Hz fast rhythms were seen during sleep

to show seizures at the early infantile period, with acute encephalopathy resulting in brain death at the age of 1 year and 7 months. He showed no dysmorphic features or brain abnormalities. These two patients suggested that some cases of *CSNK2A1*-related disorders could show progressive and serious clinical conditions.

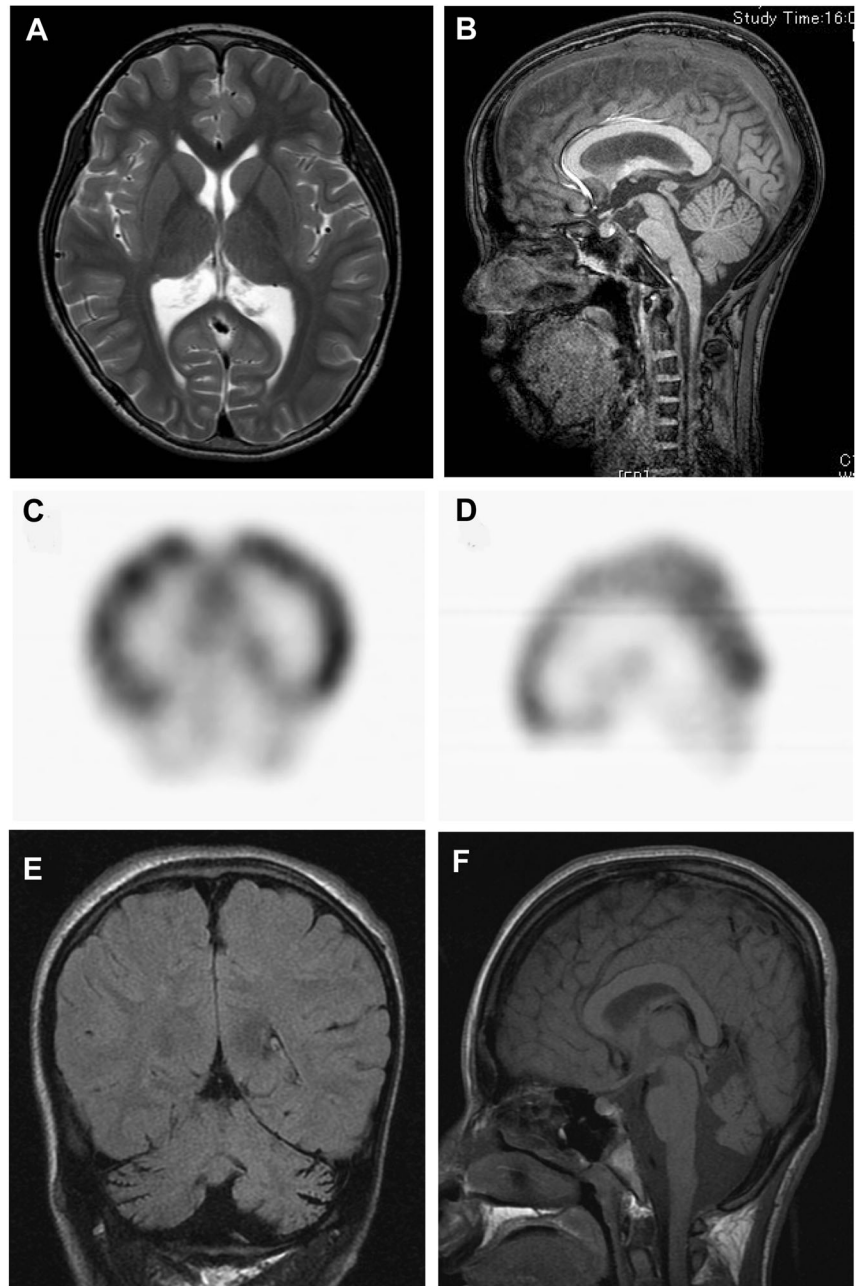
Clinical outcomes of our two cases with *CSNK2B* variants were both severe. They had seizures within 2 months of age, which were refractory to multiple antiepileptic drugs. Patient 3 showed facial clonic seizures evolving into generalized tonic-clonic seizures at 2 months of age, which developed into Lennox-Gastaut syndrome at 9 years. Patient 4 showed focal seizures on the 5th day after birth; these seizures currently occur 2 or 3 times per day. Severe psychomotor impairment was recognized. Both patients 3 and 4 are bedridden and not able speak any meaningful words. Brain image analysis showed cerebellar atrophy and mega cisterna magna in patients 3 (Fig. 2e, f) and 4, respectively. Patient 3 was diagnosed with precocious puberty at 4 years. Patient 4 was frequently hospitalized due to acute

gastroenteritis and pneumonia caused by gastroesophageal reflux disease. Case reports are available in supplementary note.

Identification of de novo *CSNK2A1* and *CSNK2B* variants

Using trio-based WES data of patients 1 and 3, we searched variants consistent with autosomal dominant (including de novo) and autosomal or X-linked recessive models. We found three candidate de novo variants, *ATAD2B* (NM_017552.2:c.1699G>A, p.Glu567Lys), *TOPORS* (NM_005802.4:c.298G>A, p.Asp100Asn), and *CSNK2A1* (NM_177559.2:c.593A>G, p.(Lys198Arg) in patient 1 (Table 2 and S1). These variants were predicted to be deleterious and were absent in 575 in-house control exomes, public databases, including dbSNP137 data, 1KJPN, the 1000 Genomes Project, and ExAC. We evaluated the pathogenicity of these variants based on the ACMG variant classification guideline [39]. Both variants in *ATAD2B* and

Fig. 2 Brain images of patient 1 **a, b** and patient 3 **c–f**. Axial T2-weighted **a** and sagittal T1-weighted **b** brain magnetic resonance imaging (MRI) images of patient 1 at 12 years show volumetric loss in cerebral white matter around the posterior horn of the lateral ventricular. Coronal **c** and sagittal **d** ^{99m}Tc -ethyl cysteinate dimer (ECD) brain single-photon emission computed tomography of patient 3 at 8 months show decreased radiotracer uptake in the cerebellum. Cerebellar atrophy of the vermis and hemisphere were observed in coronal FLAIR **e** and sagittal T1-weighted brain MRI images **f** at 14 years



TOPORS were classified as likely being pathogenic, and a variant in *CSNK2A1* was classified as pathogenic because the c.593A>G, p.(Lys198Arg) variant in *CSNK2A1* had been reported in patients with Okur-Chung neurodevelopmental syndrome [23, 24, 26]. Previous studies suggested missense variants in *TOPORS* cause autosomal dominant retinitis pigmentosa [40]. Previous patients with *TOPORS* variants showed no neurodevelopmental disorders, and patient 1 exhibited no manifestations of retinal degenerations. *ATAD2B* encodes a nuclear protein belonging to the AAA ATPase family and may play a role in neuronal

differentiation and tumor progression, though its biological functions and pathogenicity of variants were still uncertain [41]. Considering these findings, we concluded that a de novo *CSNK2A1* variant was the most-plausible causative variant in this patient. On the other hand, we found only one candidate de novo variant, *CSNK2B* (NM_001320.5: c.533_534insGT, p.Pro179Tyrfs*49), in patient 3 (Table 2 and S1), and this variant was classified as pathogenic based on the ACMG guideline. We then searched for possible pathogenic *CSNK2A1* and *CSNK2B* variants using case-only WES data of 893 individuals and identified a novel

Table 2 Predicted pathogenicity of de novo *CSNK2A1* and *CSNK2B* variants

Patient	Gene	Refseq	Variant	SIFT	PolyPhen2 HumVar	CADD phred	M-CAP	GERP	Phast Cons
Patient 1	<i>CSNK2A1</i>	NM_177559.2	c.593A>G, p.(Lys198Arg)	0.001	0.887	30.000	0.019	4.840	1.000
Patient 2	<i>CSNK2A1</i>	NM_177559.2	c.571C>T, p.(Arg191*)	N.A.	N.A.	39.000	N.A.	2.830	1.000
Patient 3	<i>CSNK2B</i>	NM_001320.5	c.533_534insGT, p. (Pro179Tyrfs*49)	N.A.	N.A.	N.A.	N.A.	4.780	1.000
Patient 4	<i>CSNK2B</i>	NM_001320.5	c.494A>G, p.(His165Arg)	0.001	0.999	25.100	0.057	5.650	1.000

N.A. not available

CSNK2A1 variant (NM_177559.2:c.571C>T, p.Arg191*) and a novel *CSNK2B* variant (NM_001320.5:c.494A>G, p.His165Arg) in patients 2 and 4, respectively (Table 2). In patients 2 and 4, we also searched for other candidate variants in known causative genes for epileptic encephalopathies, including Dravet syndrome, but could not find any possible pathogenic variants. Altered Arg191 in *CSNK2A1* and His165 in *CSNK2B* residues are evolutionarily highly conserved (Fig. 3a, b). In accordance with the ACMG guideline, *CSNK2A1* (c.571C>T) and *CSNK2B* (c.494A>G) variants were classified as pathogenic and likely pathogenic, respectively. Sanger sequencing confirmed that all four variants occurred de novo (Supple. Fig. S2). In light of these findings, we concluded that their clinical courses were very likely to be related to the *CSNK2A1* and *CSNK2B* variants. The biological parentage of all families was confirmed by analyzing 12 microsatellite markers (data not shown).

Protein stability and binding ability of mutant *CSNK2B*

To examine the protein stability of mutant *CSNK2B*, HA-tagged WT, or mutant *CSNK2B*, along with nuclear-localized enhanced green fluorescent protein was transiently expressed in HEK23T cells. Although the protein level of p.Thr37Tyrfs*5 mutant was extremely low, that of two mutants—p.His165Arg and p.Pro179Tyrfs*49—was equivalent to that of WT (Fig. 3c). This result indicated that truncated *CSNK2B* protein would be degraded in vivo, but prolonged *CSNK2B* protein would escape from protein degeneration. Next, using co-IP assay we examined the protein interaction between the *CSNK2A1* and WT or mutant *CSNK2B* proteins. The three independent co-IP experiments indicated that wild type and p.His165Arg mutant *CSNK2B* showed comparable interaction to the *CSNK2A1* protein; however, p.Pro179Tyrfs*49 mutant *CSNK2B* showed no interaction with the *CSNK2A1* (Fig. 3d). These findings suggested that p.Pro179Tyrfs*49 mutant may lack the binding ability with *CSNK2A1*, which may induce the instability of the protein structure of CK2 holoenzyme.

Discussion

In recent years, 26 patients with de novo *CSNK2A1* variants have been described [23–26, 29]. CK2 has two types catalytic subunits, CK2 α , and CK2 α' , which are encoded from *CSNK2A1* and *CSNK2A2*, respectively. Although both *CSNK2A1* and *CSNK2A2* are extremely intolerant of single nucleotide variations (Z-score = 3.89 for *CSNK2A1*, 3.49 for *CSNK2A2*) and loss-of-function variants (pLI = 1) [36] in humans, pathogenic variants in *CSNK2A2* associated with NDDs has not been identified. Previous animal models showed that CK2 α knockout mice were embryonic lethal [42, 43]. Conversely, CK2 α' knockout mice showed normal embryonic development and were viable, but the male mice had oligospermia, which led to male infertility [44]. These mutant mice studies suggest that *CSNK2A1* variants may have more severe pathogenicity than *CSNK2A2* variants at least in neuronal development.

Including the two patients mentioned here, patients having de novo *CSNK2A1* variants showed neurodevelopmental disorders with various congenital anomalies (Table 1 and Suppl. Table S2). The most general findings were developmental delay (28/28), intellectual disabilities (26/28), and hypotonia (20/28). In many cases, they accompanied neurological deficits, including motor disorders (11/28), seizures (8/28), autistic traits (8/28), sleep problems (7/28), and attention deficit hyperactivity disorder (3/28). Observed seizures types were febrile (3/8), atonic (1/8), breath-holding spells (1/8), absence (1/8), myoclonic (1/8), and tonic clonic (1/8). Facial dysmorphisms (13/28), short stature (11/28), extremities abnormalities (10/28), and gastrointestinal disorders (10/28) were sometimes noted. As other physical findings, skeletal disorders (8/28), immunological problems (7/28), cardiac abnormalities (5/28), and skin abnormalities (4/28) were occasionally noted (Table S2).

In our study, some characteristic clinical features—congenital biliary dilatation, easy fatigability, and progressive muscle weakness—were noted in patient 1. One previous case (individual 4, Okur et al., Table S2) also presented easy fatigability [23], so this may be one of the minor findings of *CSNK2A1*-related disorders. Progressive easy fatigability

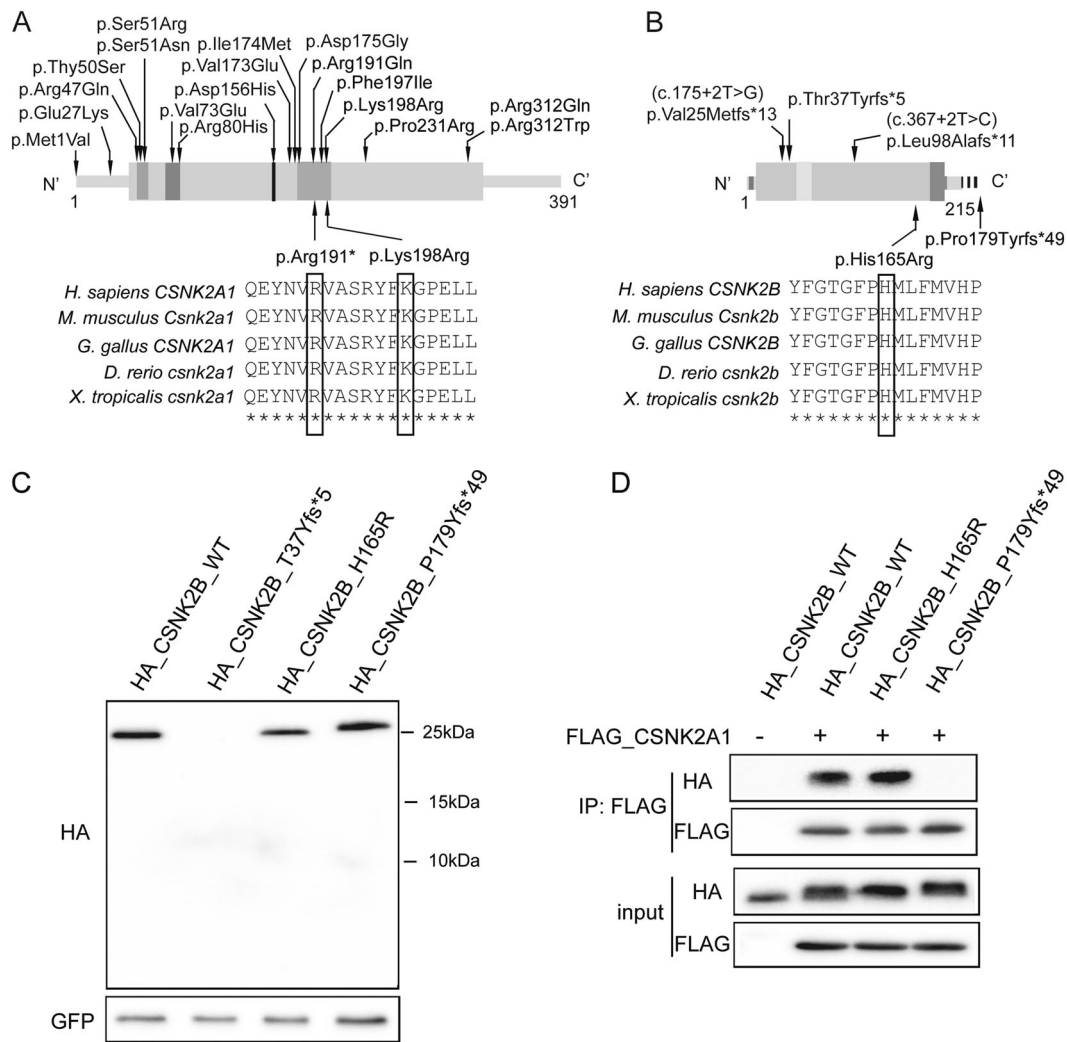


Fig. 3 Locations of de novo variants in *CSNK2A1* **a** and *CSNK2B* **b** are shown with schematic protein structure. Arrows depicted above are previously reported variants. Four variants found in our cases are shown below. All missense variants occurred at evolutionarily conserved amino acids. Multiple amino acid sequences of *CSNK2A1* and *CSNK2B* proteins were aligned with tools available on the CLUSTALW web site (<http://www.genome.jp/tools/clustalw/>). **a** The green box indicates protein kinase domain annotated by Pfam. The protein kinase domain harbors the ATP/GTP binding loop (45–53 aa, pink), basic cluster (68–80 aa, red), active site (156 aa, black) and activation segment (175–201 aa, orange). **b** The blue box indicates CK_II_beta

domain annotated by Pfam. The *N*-terminal autophosphorylation site (2–6 aa, purple) is attached to the CK_II_beta domain, which contains an acidic cluster (55–64 aa, yellow) and interaction domain with CK2α subunit (188–193 aa, brown). Protein expression **c** and interaction **d** of *CSNK2B* and *CSNK2A1*. **c** Representative western blots using extracts from HEK293T cells transiently expressing *CSNK2B*. Normalization was performed with GFP as a loading control. **d** HEK293T cells were co-transfected with WT or mutant HA-tagged *CSNK2B* and FLAG-tagged *CSNK2A1* plasmids. Cell lysates were immunoprecipitated with anti-FLAG antibody beads and then probed with anti-HA for *CSNK2B* and anti-FLAG for *CSNK2A1*

suggested the possibility of myopathies, such as glyco-genosis or myasthenia gravis. Previous studies suggested CK2 interacts and co-localizes with the muscle-specific receptor tyrosine kinase (MuSK) at the neuromuscular junction (NMJ) [45]. CK2 mediates the phosphorylation of serine residues within a specific MuSK epitope, then the signal transduction mediates by MuSK-induced aggregation of the acetylcholine receptor (AChR) at the endplate, leading to increasing of the receptor effect to acetylcholine [46]. The loss-of-CK2 function induced fragmentation of AChR clustering, resulting in disintegration of the muscle

endplate; notably, muscle-specific CK2β knockout mice develop a myasthenic phenotype due to impaired muscle endplate structure and function [45, 47]. These findings imply CK2 may play important roles in the muscular nerve transmission mechanism and loss-of-CK2 function may be involved in the pathogenesis of muscle weakness; however, the examination of myopathology and repeatedly stimulated nerve conduction studies in patient 1 showed no evident abnormal findings. The impact of CKα functional disability in the signal transduction at NMJ was still uncertain; therefore, further research is needed to prove the relevance

of variants in *CSNK2A1* in muscle weakness. Patient 2 incurred the most serious clinical outcome among 28 patients with *CSNK2A1* variants. This patient only showed developmental delays and frequent epileptic attacks, and died from acute encephalopathy at 1 year and 7 months. This case suggested that variants in *CSNK2A1* could cause early onset epileptic encephalopathies.

Patient 1 had a missense variant c.593A>G, p.Lys198Arg, which was hotspot variant of Okur-Chung neurodevelopmental syndrome. Clinical features of eight patients with p.Lys198Arg variants do not entirely match. These variegated phenotypes might be because CK2 holoenzyme comprises identical (α/α or α'/α') or non-identical (α/α') combination of catalytic subunits and its enzyme damaging might be variable, depending on heterozygous or homozygous CK α mutants present in the complex. We also found a novel nonsense variant c.571C>T, p.Arg191* caused lack of C-terminal of CK2 α protein in patient 2. These two variants occurred at the activation segment, which is the unique alignment of the protein kinase domain of CK2 α . The activation segment is fixed in an active conformation and important for CK2 catalytic activity [20]. The amino acid alterations in this region may cause destabilization of this critical conformation and result in impaired kinase activity [48]. Additionally, the alteration in Asp156 residue, referred to as an active site that plays a key role in catalytic activity, had a dominant-negative effect and induced complete loss-of-CK2 α enzyme activity [49]. These findings suggest that variants in *CSNK2A1* might have dominant-negative effects on CK2 α function, but functional confirmation by molecular biological methods is indispensable for this conclusion.

CK2 β proteins form homodimers and each CK2 β subunit binds to either CK2 α or CK2 α' subunit. CK2 β acts as a regulator of CK2 holoenzyme, which contributes to the modulation of the enzyme activity, substrate recognition, and stabilization of the holoenzyme complex [50, 51]. CK2 β may be crucial for normal embryonic development and CK2 β knockout mice were embryonic lethal [52, 53]. *CSNK2B* is also extremely intolerant toward single-nucleotide variants (Z -score = 3.60) and loss-of-function variants (pLI = 0.94); therefore, *CSNK2B* variants may cause neurodevelopmental disorders.

Prior to this study, only three patients with *CSNK2B* variants had been reported [27, 28] and we added two patients with novel *CSNK2B* variants. Regarding the phenotypes, patients with *CSNK2B* variants commonly exhibited developmental delay (5/5), intellectual disability (5/5), intractable seizures (4/5), and facial dysmorphisms (3/5) (Table 1 and Suppl. Table S2). The most frequent seizure type was myoclonic (3/4), while tonic, tonic clonic, and focal seizures were also observed. Brain MRI of four

patients was normal, but that of patient 3 showed cerebellar atrophy. One patient showed autistic features. Our two cases showed the most severe developmental delay because both patients are currently bedridden and are unable to speak any meaningful words. The number of cases with *CSNK2B* variants are much smaller than that of *CSNK2A1* variants. Therefore, a further study of many *CSNK2B* cases would be needed for better understanding of the phenotypic spectrum of *CSNK2B*-related disorders.

Five *CSNK2B* variants identified to date include two canonical splice site variants, two frameshift variants, and one missense variant (Fig. 3b). Both splice site variants, c.175+2T>G and c.367+2T>C, induced the aberrant exon skipping, which resulted in generating premature stop codons (p.Val25Metfs*13 and p.Leu98Alafs*11, respectively) [27]. Along with a frameshift variant (c.108dup, p.Thr37Thyfs*5) [28], mutant transcripts with these variants are likely subjected to the nonsense-mediated mRNA decay mechanism. Therefore, *CSNK2B* haploinsufficiency is the pathomechanism in these patients. Our transient expression analysis demonstrated the protein instability of this frameshift mutant and supported this hypothesis (Fig. 3c). Conversely, the mutant transcript with a frameshift variant (c.533_534insGT, p.Pro179Tyrfs*49) could be escaped from the decomposition through nonsense-mediated mRNA decay, and produced the altered and prolonged C terminus of CK2 β protein (Fig. 3c). The CK2 β tail (Asn181–Arg215) plays key roles in stabilizing the β – β dimer and the β – α contacts [20]. The result of co-IP analysis indicated this prolonged CK2 β protein lost the binding ability to CK α protein (Fig. 3d). Therefore, this frameshift variant is likely to cause the instability of the protein structure of CK2 holoenzyme. We also found the missense variant, c.494A>G, p.His165Arg, on an evolutionarily highly conserved residue at CK_II_beta domain (Fig. 3b). Previous studies suggested that the amino acid sequence between Asp155 and Val170 of CK_II_beta domain may have the important role in tightening the β – β interaction [22, 54]; therefore, this amino acid alteration may lead the instability of the β – β dimer formation. We speculate that these two variants (p.His165Arg and p.Pro179Tyrfs*49) may have a dominant-negative effect and impair the CK2 enzyme activities; however, functional analyses are required to prove these hypotheses.

In conclusion, we have identified four patients with *CSNK2A1* or *CSNK2B* variants. Although our study is limited to individuals with early childhood-onset epilepsy, our study expands the phenotypic spectrum of *CSNK2A1*-related disorders and support the pathogenicity of *CSNK2B* variants in the patients with neurodevelopmental disorders. Further cases, especially with *CSNK2B* variants, are needed to delineate the clinical phenotype of CK2-related disorders and genotype–phenotype correlation.

URLs. 1KJPN (<https://ijgvd.megabank.tohoku.ac.jp/>) 1000 genome (<http://www.internationalgenome.org/>) ExAC (<http://exac.broadinstitute.org>) Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>) CADD (<http://cadd.gs.washington.edu/>) M-CAP (<http://bejerano.stanford.edu/mcap/>) GERP (<http://mendel.stanford.edu/SidowLab/downloads/GERP/index.html>) PhastCons (<http://compugen.cshl.edu/phast/>)

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Compliance with ethical standards

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

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