**CNKSR2 mutation causes the X-linked epilepsy-aphasia syndrome: A case report and review of literature**

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**Abstract**

The mutation in CNKSR2 leads to a broad spectrum of phenotypic variability and manifests as an X-linked intellectual disability. However, we reported that the male patient in this study not only had intellectual disability but also epileptic seizures. In addition, there were progressive language impairment, attention deficit hyperactivity disorder and autism. Electroencephalograms showed continuous spike-and-wave during sleep. Genetic testing revealed a *de novo* mutation of the CNKSR2 gene (c.2185C>T, p.Arg729Ter) in the child that was not detected in the parents. Therefore, the child was diagnosed with X-linked epilepsy-aphasia syndrome. Deletion of the CNKSR2 gene has been rarely reported in epilepsy-aphasia syndrome, but no *de novo* mutation has been found in this gene. This report not only adds to the spectrum of epilepsy-aphasia syndrome but also helps clinicians in diagnosis and genetic counseling.

**Key words:** Epilepsy; Language impairment; Mental retardation; *De novo* mutation of CNKSR2; X-linked epilepsy-aphasia syndrome

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CNKSR2 mutation causes the X-linked epilepsy-aphasia syndrome

**INTRODUCTION**

Atypical epilepsy-aphasia syndrome is caused by Landau-Kleffner syndrome (LKS) and epileptic encephalopathy, with a continuous spike-and-wave pattern during sleep[4]. The synapse is the core component of brain operations and executive functions, and its function plays an important role in brain neuron function[2]. CNKSR2 is located on the X chromosome, and as a synaptic protein, it is involved in RAS/MAPK signal transduction[3]. It is highly expressed in the brain[5], and its mutation or deletion causes a wide range of neurodevelopmental defects[6]. Currently, CNKSR2 deletion or mutation has been shown to induce symptoms that are part of the EAS spectrum[6-9]. In this paper, we review clinical data and genetic test results of a child with epilepsy and aphasia and have identified a de novo mutation: CNKSR2 (c.2185C>T, p.Arg729Ter). We reviewed the literature and analyzed the clinical features of X-linked epilepsy-aphasia syndrome in order to assist clinicians in their diagnosis of this condition and to help provide genetic counseling.

**CASE REPORT**

An 8-year and 8-mo-old boy from China was admitted to the hospital due to paroxysmal unconsciousness for more than 6 years. The performance of paroxysmal loss of consciousness was associated with brief jerks of the limbs, eye staring, lip bruisings, spitting foam from the mouth, and sometimes with urinary incontinence; however, fever was absent. Each episode lasted 2-3 min and resolved on its own. The episodes of epileptic seizures occurred in varying lengths of times; sometimes, the episodes occurred once every six months, and sometimes only once a month. The form of each seizure can be predicted from the protein database (PDB) (Figure 3). RaptorX (http://raptorx.uchicago.edu)[11-13] can predict protein tertiary structures. After inputting the sequence, the 3D structure of the protein sequence can be predicted from the protein database (PDB) (Figure 4). Compared with the wild type, the patient’s CNKSR2 gene did not fold completely in its spatial structure, thus affecting protein function.

CNKSR2 (also known as CNK2, KSR2, MAGUIIN)[8] interacts with synaptic scaffold molecules (S-SCAM) and the postsynaptic density (PSD)-95/synaptic-associated protein (SAP) 90 to form a complex[14]. The complex is involved in RAS/MAPK signaling and mediates neuronal proliferation, migration, differentiation and death, as well as RAS-mediated synapse formation[9-9]. It also connec-
ts N-methyl-D-aspartate (NMDA) receptors to neuronal cell adhesion molecules[14]. The NMDA subunit encoded by GRIN2A is the first gene associated with EAS[2]. GRIN2A mutations reduce NMDA receptor trafficking and agonist potency–molecular profiling as well as functional rescue[15]. GRIN2A gene is a rare causative gene in Chinese patients with EAS, suggesting the possibility of other genes being involved in the pathogenesis[16]. Hence, we speculate that a mutation or deletion of CNKSR2 may result in changes to the NMDA receptor activity and might affect downstream signaling cascades. Abnormal NMDA receptor will potentially damage the cortical thalamus network during sleep[17]. CNKSR2 is highly expressed in the brain (especially in the hippocampus, amygdala, and cerebellum), and mutations result in loss of specificity and might also affect brain function[7], leading to seizures and neurodevelopmental disorders that especially affect the patient’s speech expression[2]. CNKSR2 is a gene located on the X chromosome, and its mutations or deletions lead to X linkage intelligence disorder (XLID)[8]. The main features of XLID are: (1) intellectual disability; (2) highly restrictive speech (especially expression of language); (3) ADHD; (4) transient childhood epilepsy; and (5) epilepsy with continuous spike waves of slow-wave sleep (CSWS) in early childhood[5].

Before experiencing seizures, our patient suffered from developmental delays and ADHD, which is consistent with the performance of X-linked intellectual disability. After seizure occurrence, the patient’s speech expression gradually decreased, the EEG continued to show abnormal wave patterns during sleep, and a de novo mutation of the CNKSR2 gene was identified. Therefore, we diagnosed this patient as X-linked epilepsy-aphasia syndrome. After definite diagnosis, patients were given immunoglobulin (400-500 mg/kg per day, 3-5 d for 1 course) and oral prednisone (from 1-3 mg/kg per day, and after one month, changed to 1 mg/kg per day), with a total course of 6 to 12 mo. Meanwhile, lamotrigine (75 mg/qd) and sodium valproate oral solution (6 mL/bid) were continued for antiepileptic treatment. At telephone follow-up one year later, the child had fewer epileptic seizures than before as well as partial improvement in verbal ability and an ability to repeat speech; however, the patient had no improvement in intelligence. The disease duration was more than 6 years. If diagnosed early and actively treated, the patient’s intelligence, seizures, and language may have been better mitigated.

The underlying mechanism for EAS disorders occurrence remains unknown, although environmental factors such as thalamic injury[18] and immunity disorders[19], with evidence of onconeural antibodies that can cause the EEG phenotype, have been reported. Studies have shown that the antibodies of brain endothelial cells and nuclei in children were elevated[20]. Additionally, inflammatory markers of children with electrical status epilepticus in sleep (ESES) may be increased[21]. Some researchers have proposed a potential autoimmune reaction secondary to blood-brain-barrier disruption from a thalamocortical uncoupling secondary to the spike-wave activation seen in slow-wave sleep[22]. Furthermore, few genetic causes of ESESS/CSWSS/epilepsy aphasia spectrum have been reported, where the common underlying pathway is channelopathy[23]. The different forms of seizures in EAS include partial seizure, generalized tonic-clonic seizure, atypical absence seizure, myoclonic seizure, atonic seizure, etc. Aphasia can occur before or after epilepsy. Moreover, 70% of patients with epilepsy-aphasia syndrome have epileptic seizures with EEG features that reveal spike-and-wave patterns in the unilateral or bitemporal lobes during the waking period.

Figure 1  Electroencephalogram of the patient. It showed generalized continuous spike-and-wave patterns in the bitemporal and frontal lobes, noticeable on the left side. Abnormal discharge was more pronounced during the sleep-electroencephalogram. Slower on background activity.
Generalized continuous spike-and-wave is seen in all leads during sleep, and bilateral synchronous discharge accounts for more than 85% of stage in abnormal discharge\(^1\). The other 30% of children do not seizures, but show EEG abnormalities (which does not meet the EEG standards of CSWS). Their EEG’s showed the following during sleep: Induced focal epileptiform discharges were identified mainly at the center (but may also might be involved in other areas); or there was no bilateral synchronous activity; or synchronous activity accounted for less than 85% of NREM (non-rapid eye movement) sleep. These cases are all called intermediate epilepsy aphasia (IEADs). The speech recovery ability of IEADs patients is better than that of EAS.

Patients with epilepsy and speech disorders should be advised to undergo EEG monitoring and genetic testing to confirm the diagnosis. Currently, there are no specific medications for the treatment of X-linked epilepsy-aphasia syndrome.

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**Figure 2** Gene sequences of three members in the family. A: De novo mutation of the CNKSR2 gene (c. 2185C>T, p.Arg729Ter) in the patient. B, C: No mutation was observed at the same locus in the parents (arrows).
The early diagnosis and early use of antiepileptic drugs as well as hormone therapy can recover speech comprehension to different degrees and improve abnormal discharge. Therefore, the overall prognosis of patients is good. Clinical seizures should be treated with antiseizure drugs, and barbiturates, carbamazepine, and phenytoin should be avoided as they can potentiate spike wave discharges during sleep \[24,25\].

Although there is evidence that mutations or deletions of CNKSR2 lead to neurological development defects, such as epilepsy and intellectual disability, the pathogenesis remains unclear. Therefore, the next step is to screen a large number of epileptic encephalopathy individuals to delineate the phenotypic spectrum of the CNKSR2 mutation. Second-generation gene sequencing can assist in the identification of hereditary etiology and discovery of new mutations while expanding on the early epilepsy encephalopathy clinical phenotype and genetic spectrum. Simultaneously, the pathogenesis of X-linked epilepsy-aphasia syndrome should be studied to assist clinicians in diagnosis and genetic counseling.

**ARTICLE HIGHLIGHTS**

**Case characteristics**

Before experiencing seizures, our patient suffered from developmental de-
lays and attention deficit hyperactivity disorder, which is consistent with the performance of X-linked intellectual disability. After seizure occurrence, the patients’ speech expression gradually decreased, the electroencephalogram (EEG) continued to show abnormal wave patterns during sleep, and a de novo mutation of the CNKSR2 gene was identified.

**Clinical diagnosis**
X-linked epilepsy-aphasia syndrome.

**Differential diagnosis**
Hystera and childhood autism.

**Laboratory diagnosis**
A de novo mutation of the CNKSR2 gene.

**Imaging diagnosis**
EEG continued to show abnormal wave patterns during sleep.

**Treatment**
Immunoglobulin, oral prednisone, lamotrigine and sodium valproate oral solution.

**Related reports**
Frequency of CNKSR2 mutation in the X-linked epilepsy-aphasia spectrum has been reported in the journal of Epilepsia.

**Term explanation**
Epileptic encephalopathy with continuous spike-and-wave during sleep.

**Experiences and lessons**
This case will contribute to improvements in our understanding of X-linked epilepsy-aphasia syndrome. Patients with epilepsy and speech disorders should be advised to undergo EEG monitoring and genetic testing to confirm the diagnosis. The early diagnosis and early use of antiepileptic drugs as well as hormone therapy can recover speech comprehension to different degrees and improve abnormal discharge.

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**Figure 4** Tertiary structures of wild-type and mutated CNKSR2 proteins predicted by RaptorX. The spatial structures of CNKSR2 proteins are significantly different between the wild-type (A) and the patient (B).
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