

CNKSR2 gene mutation leads to Houge type of X-linked syndromic mental retardation

A case report and review of literature

Qingyun Kang¹, Liming Yang*, Hongmei Liao, Liwen Wu, Bo Chen, Sai Yang, Xiaojun Kuang, Haiyang Yang, Caishi Liao

Abstract

Rationale: Mutations of connector enhancer of kinase suppressor of Ras-2 (CNKSR2) gene were identified as the cause of Houge type of X-linked syndromic mental retardation. The mutations of CNKSR2 gene are rare, we report a patient carrying a novel nonsense mutation of CNKSR2, c.625C>T(p.Gln209*) and review the clinical features and mutations of CNKSR2 gene for this rare condition considering previous literature.

Patient concerns: We report a case of a 7-year and 5-month-old Chinese patient with clinical symptoms of intellectual disability, language defect, epilepsy and hyperactivity. Genetic study revealed a novel nonsense variant of CNKSR2, which has not been reported yet.

Diagnosis: According to clinical manifestations, genetic pattern and ACMG classification of mutation site as Class 1-cause disease, the patient was diagnosed as Houge type of X-linked syndromic mental retardation caused by CNKSR2 gene mutation.

Interventions: The patient was administrated with a gradual titration of valproic acid (VPA).

Outcomes: On administration of valproic acid, he had no further seizures.

Lessons: This is the first time to report a nonsense variant in CNKSR2, c.625C>T(p.Gln209*), this finding could expand the spectrum of CNKSR2 mutations and might also support the further study of Houge type of X-linked syndromic mental retardation.

Abbreviations: CNKSR2 = connector enhancer of kinase suppressor of Ras-2, CSWS = continuous spike-and-slow-waves, EEG = Electroencephalogram, MRI = Magnetic resonance imaging, WES = Whole-exome sequencing.

Keywords: CNKSR2, intellectual disability, seizure, whole exome sequencing, X-linked

1. Introduction

The connector enhancer of kinase suppressor of Ras-2 (CNKSR2) gene, located on chromosome Xp22.12, encodes connector enhancer of KSR2 (CNK2). CNK2 is a multidomain protein playing vital role in synaptic function.^[1,2] The mutation in CNKSR2 leads to a broad spectrum of phenotypic variability

and manifests as Houge type of X-linked syndromic mental retardation.^[3] Here we report a patient with clinical phenotype including global developmental delay, hyperactivity, severe language impairment, and epileptic encephalopathy caused by a nonsense mutation of CNKSR2 (c.625C>T(p.Gln209*)). This nonsense mutation had never been reported, in order to enhance

Editor: Maya Saranathan.

This work was supported by the Scientific Research Project of Hunan Health and Family Planning Commission(B20180528). Hunan Health and Family Planning Commission supervised this study.

The authors have no conflicts of interest to disclose.

Ethics approval and consent to participate:

This study was approved by the ethics committee of Hunan Children's Hospital. Written informed consent was obtained from the proband's parents with the agreement to share the clinical and genetic information for research analysis.

Consent for publication: Written informed consent was obtained from the proband's parents for the publication of proband's clinical and genetic information included in this manuscript. Written consent is available for review, please contact the corresponding author if necessary.

Availability of data and materials: If you need more data or materials about this case, please contact the corresponding author Dr. Liming Yang.

The datasets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

Department of Neurology, Hunan Children's Hospital, Ziyuan Road, Changsha, Hunan, P.R.China.

* Correspondence: Liming Yang, Department of Neurology, Hunan Children's Hospital, Ziyuan Road 86th, Changsha 410007, Hunan, P.R. China (e-mail: 158632670@qq.com).

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How to cite this article: Kang Q, Yang L, Liao H, Wu L, Chen B, Yang S, Kuang X, Yang H, Liao C. CNKSR2 gene mutation leads to Houge type of X-linked syndromic mental retardation: A case report and review of literature. *Medicine* 2021;100:23(e26093).

Received: 3 March 2021 / Received in final form: 26 April 2021 / Accepted: 6 May 2021

<http://dx.doi.org/10.1097/MD.0000000000026093>

our understanding of the phenotypic and genotypic spectra of CNKSR2 in patients with neurodevelopmental disorders, we report a new patient and retrospectively summarize the 15 previously reported pedigrees.

2. Case presentation

2.1. Clinical information

A 7-year and 5-month-old Chinese boy was the second child of non-consanguineous, healthy parents. The family history of the patient was unremarkable. The patient was born at 40 weeks of gestation with a birth weight of 3.5 kg, body height of 51 cm and head circumference of 32 cm. His motor development was delayed from birth. He began to gain head and neck stability at 7 months old and rolled over at 11 months old, he can walk alone at the age of 2 years. His language development was severely delayed. At the age of 2 years, he had first epileptic episode lasting for minutes, with eye staring, cyanosis of lip, spitting foam from the mouth, and jerks of the limbs. He was admitted to our department, a four-hour sleep deprived electroencephalogram (EEG) revealed continuous spike-and-slow-waves (CSWS) (Fig. 1C). Cranial magnetic resonance imaging (MRI) and

multiple auditory brainstem responses were normal, and no further abnormal findings were noted. As the seizures stopped spontaneously, anti-epileptic therapy was refused by his parents. But four months later, seizures recurred in this patient and then a gradual titration of valproic acid (VPA) was administered at an initial dose of 10 mg/kg.d increasing to 30 mg/kg.d within one month. On valproic acid of 30 mg/kg.d, he had no further seizures. However, he couldn't take care of himself or speak until now, and neuropsychological testing show global intellectual disability and hyperactivity.

2.2. Molecular genetic analysis

WES and Sanger sequencing were performed by Running Gene Inc. (Beijing, China) using their standard process, which is available in the previous report.^[4] In our case, the variant c.625C>T (p.Gln209*) of CNKSR2 was identified (Fig. 1B), that was inherited from his unaffected carrier mother. The mutation located toward the N-terminal of the gene alter the codon sequence of the no. 209 amino acid Gln into a termination codon, resulting in a truncated protein lacking the last 825 of 1034 residues, that leads to a truncated protein without the PDZ, DUF1170, PH, and C-terminal ETHV domains (Fig. 1A). we

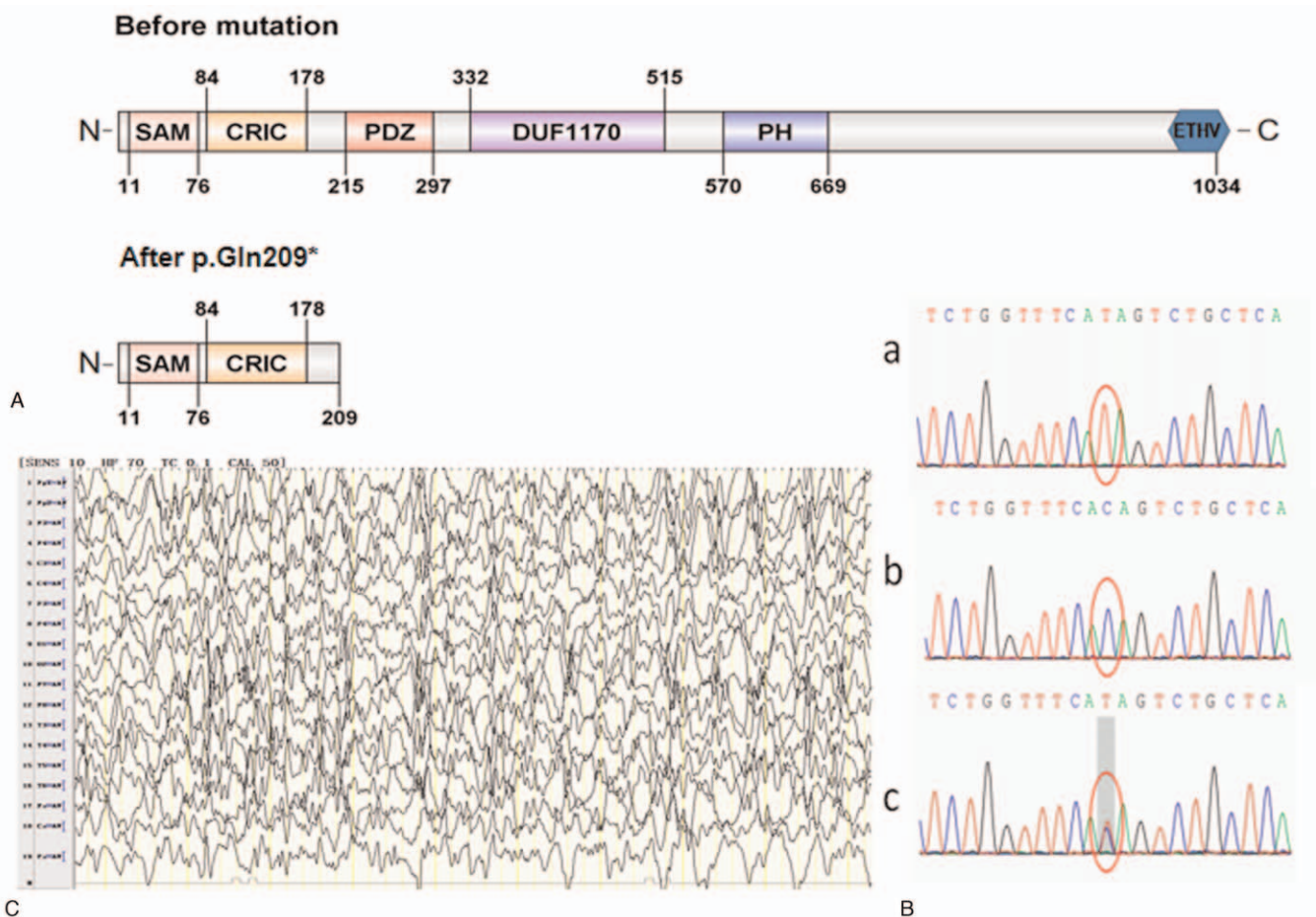


Figure 1. (A). The variant c.625C>T (p.Gln209*) of CNKSR2 leads to a truncated protein without the PDZ, DUF1170, PH, and C-terminal ETHV domains. CRIC = connector enhancer of kinase suppressor of ras, DUF1170 = domain of unknown function, PDZ = PDZ domain, PH = Pleckstrin homology domain, SAM = sterile alpha motif. ETHV motif = Glu-Ser/Thr-Xaa-Val, or E-S/T-X-V in single letter amino acid code (where Xaa/X is any amino acid residue) motif at the COOH terminus. (B). Sequencing of the CNKSR2 gene: c.625C>T (p.Gln209*). The child has a half zygote mutation inherited from his mother, and his father is normal. (a: patient; b: the father; c: the mother). (C). EEG revealed continuous spike-and-slow-waves (CSWS).

speculate that the absence of multiple CNKSR2 domains leads to a more detrimental effect on residual protein function(PVS1). Computer-aided analysis predicts that this mutation is harmful (PP3). So far, this mutation has not been reported in our reference population gene database (PM2), so it was classified as ‘pathogenic’ according to the ACMG guidelines. This mutation has been recorded in the Sequence Read Archive (SRA) database and the accession number is SRR14321539.

3. Discussion and conclusion

CNKSR2, expressed highly in the brain (especially in the amygdala, cerebellum and hippocampus), plays an important role in Ras signaling-mediated neuronal proliferation, migration and differentiation.^[2] The variants or deletions of CNKSR2 affect brain function, leading to seizures and neurodevelopmental disorders.^[5] Owing to the patient with CNKSR2-related seizures

and neurodevelopmental disorders was first reported by Houge et al,^[3] the CNKSR2-related disorders was named as Houge type of X-linked syndromic mental retardation. This genetic syndrome is characterized by intellectual disability, language defect (especially expression of language), epilepsy; attention deficit/hyperactivity (ADHD), and (CSWS) in early childhood.^[1]

Since the first patient with X linkage intelligence disorder associated with CNKSR2 deletions was reported,^[3] fourteen more pedigrees with a similar phenotype and different CNKSR2 mutations have been reported. So far, seven kinds of deletions of CNKSR2, either partially or complete, have been reported in a total of nine patients from seven families,^[1,3,6,7] there are Xp22.12 (21,285,233–21,519,405),Xp22.12(20,297,696–21,471,387), Xp22.12(21,375,312–21,609,484), Xp22.12(21,193,947–21,707,169), Xp22.12(21,328,677–21,670,497), Xp22.12 (21523673–21558329) and Xp22.12 (21609392–21619786) (Fig. 2A). In addition, eight kinds of variants in CNKSR2 had

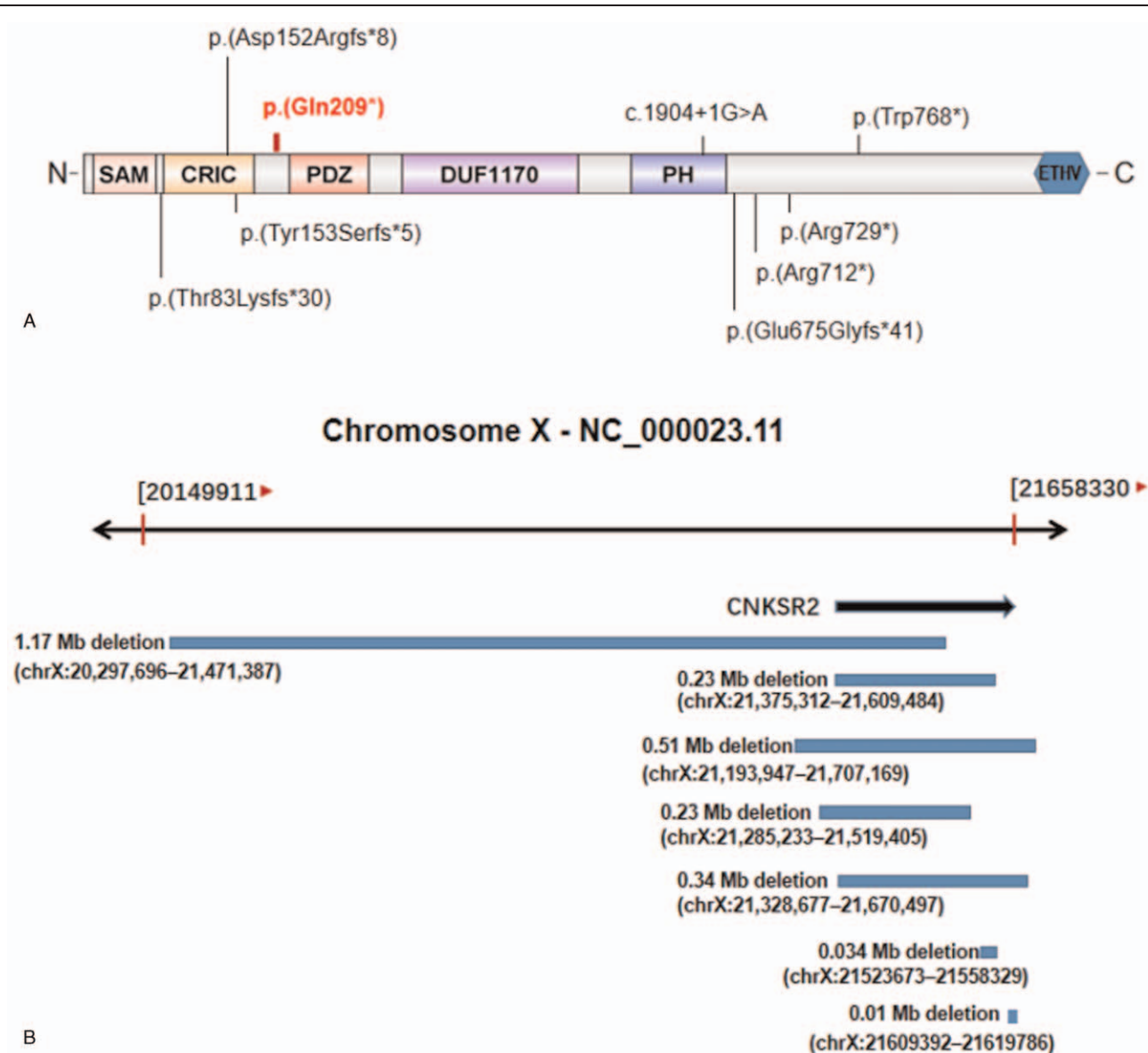


Figure 2. (A). Schematic representation of human CNKSR2 including the positions and the predicted effect of the eight identified variants, p.(Asp152Argfs*8), p.(Arg712*), p.(Arg729*), p.Glu675Glyfs*41, p.Thr83Lysfs*30, p. Tyr153Serfs*5, p.(Trp768*),c.1904+1G>A and p.Gln209* (this report), in CNKSR2 and on protein level. (B). Genomic map of chromosome Xp22.12 with extent of deletions (blue bars).

Table 1
Comparison of reported pedigrees and cases with CNKSR2 gene variants.

| Pedigree no | Publication | Gender | CNKSR2 variant | Occurrence | Intellectual disability | Language defect | Attention problems | MRI Scanning | Seizure | Seizure onset-age | Seizure outcome | CSWS | Female carrier |
|--------------------|-------------------|--------|--|----------------------------------|-------------------------|-----------------|--------------------|---|---------|-------------------|-----------------------------------|------|--------------------------|
| 1 | Houge et al [3] | male | Deletion Xp22.12 (21,285,233–21,519,405) | Maternal | ID of mild to moderate | Yes | Yes | N/A | Yes | N/A | seizure free | N/A | Normal |
| 2 (two siblings) | Vaags et al [1] | male | Deletion Xp22.12 (20,297,696–21,471,387) | Maternal | Yes | Yes | Yes | Normal | Yes | 2y | N/A | Yes | Mild learning disability |
| 3 | Vaags et al [1] | male | Deletion Xp22.12 (21,375,312–21,609,484) | N/A | Yes | Yes | Yes | Normal | Yes | 2y/3m | seizure free | Yes | N/A |
| 4 (two siblings) | Vaags et al [1] | male | Deletion Xp22.12 (21,193,947–21,707,169) | N/A | Yes | Yes | Yes | Nonspecific periventricular white matter hyperintensity | Yes | 8d | seizure free | No | N/A |
| 5 (three siblings) | Vaags et al [1] | male | c.452InsA (p.Asp152Argfs*8) | N/A | Yes | Yes | Yes | Normal | No | / | / | No | N/A |
| 6 | Aypar et al [6] | male | Deletion Xp22.12 (21,328,677–21,670,497) | Maternal | Yes | Yes | Yes | Normal | Yes | N/A | seizure free | N/A | N/A |
| 7 (three siblings) | Damiano et al [5] | male | c.2314 C>T(p.Arg712*) | Maternal | Yes | Yes | Yes | Normal | Yes | 3y/6m | N/A | Yes | febrile seizures |
| 8 | Sun et al [8] | male | c.2185C>T (p.Arg729*) | De novo | Yes | Yes | Yes | N/A | Yes | 3y/6m | N/A | Yes | / |
| 9 | Zhang et al [10] | male | c.1904+1G>A | Maternal | mild | No | Yes | Normal | No | / | / | No | Mild learning disability |
| 10 | Polia et al [9] | female | c.2304G>A (p.Trp768*) | De novo | Yes | No | No | Normal | Yes | NA | N/A | N/A | / |
| 11 | Bonardi et al [7] | male | c.2024_2027delAGAG (p.Glu675Glyfs*41) | De novo | Yes | Yes | N/A | Normal | Yes | 2y | Multiple daily atypical absences | Yes | / |
| 12 | Bonardi et al [7] | male | c.246–247delAG (p.Thr83Lysfs*30) | De novo | Yes | Yes | Yes | Normal | Yes | 3y | seizure free | Yes | / |
| 13 | Bonardi et al [7] | male | c.457_461del(p. Tyr153Serfs*5) | Maternal mosaicism (5/156 reads) | Yes | Yes | N/A | Normal | Yes | 4y | Atypical absences with head drops | Yes | Normal |
| 14 | Bonardi et al [7] | female | Deletion Xp22.12 (21523673–21558329) | Parents unavailable | Yes | Yes | Yes | Normal | Yes | 6y | seizure free | Yes | / |
| 15 | Bonardi et al [7] | male | Deletion Xp22.12 (21609392–21619786) | De novo | Yes | Yes | Yes | Normal | Yes | 3y/2m | 9y8m: 1–2seizures/year | Yes | / |
| 16 | our case | male | c.625C>T(p.Gln209) | Maternal | Yes | Yes | Yes | Normal | Yes | 2y | seizure free | Yes | Normal |

been reported in the literature,^[1,5,7–10] there are c.452insA(p.Asp152Argfs*8), c.2024_2027delAGAG (p.Glu675Glyfs*41), c.246–247delAG(p.Thr83Lysfs*30), c.457_461del(p.Tyr153Serfs*5), c.2314 C>T(p.Arg712*), c.2185C>T(p.Arg729*), c.2304G>A (p.Trp768*) and c.1904+1G>A (Fig. 2B). Among the twenty one cases reported, nine of them have their genotype being inherited from mothers (Table 1). Because of the X-inactivation in females, most of carrier mothers had normal phenotype. Compared with those patients reported, herein we identified a novel CNKSR2 (c.625C>T (p.Gln209*)) truncating variant in a Chinese boy. In our case, this variant c.625C>T (p.Gln209*) was inherited from his unaffected carrier mother.

Except one patient with splicing variant, all previously reported male patients exhibited seizures and language impairment. Continuous spike-and-slow-waves (CSWS) on EEG has been documented in twelve reported cases, consistent with the patient in our study. Intellectual disability and psychomotor delay were also recognized in all of patients reported previously, other common and uncharacteristic features including severe attention deficit and hyperactivity have been frequently observed in the literature (Table 1). The patient in our study had a consistent clinical phenotype with the previous descriptions. He showed intellectual disability, language defect, epilepsy and hyperactivity. Therefore, combined with our patient's clinical manifestations, genetic pattern and ACMG classification of mutation site as Class 1-cause disease, the patient was diagnosed as Houge type of X-linked syndromic mental retardation caused by CNKSR2 gene mutation. He had onset of seizures at 2 years, developmental delay existed prior to the onset of seizures. After the treatment of valproic acid, he had no further seizures. Even though the patient was free from seizures, he still had psychomotor delay and language. The intellectual disability was severe. And the patient couldn't speak until now. This is consistent with the finding that CNKSR2 is expressed prenatally, suggesting a vital role in neurodevelopment.

In conclusion, we reported a case of CNKSR2 c.625C>T (p.Gln209*) nonsense variants in a child with Houge type of X-linked syndromic mental retardation. This site is the first to be identified, which broadens the spectrum of genetic variants of this gene. We hope that our research can improve clinicians' understanding of the disease.

Acknowledgments

We would like to thank the patient and his family for their cooperation. We also appreciate Running-Gene Inc. for professional genetic sequencing.

Author contributions

Conceptualization: Liming Yang.

Data curation: Sai Yang, Xiaojun Kuang.

Formal analysis: Hongmei Liao, Haiyang Yang, Caishi Liao.

Supervision: Liming Yang, Liwen Wu, Bo Chen.

Writing – original draft: Qingyun Kang.

Writing – review & editing: Qingyun Kang.

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