CASE REPORT

Psychomotor development and attention problems caused by a splicing variant of CNKSR2

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Abstract

Background: Mutations in CNKSR2 have been described in patients with neurodevelopmental disorders characterized by childhood epilepsy, language deficits, and attention problems. The encoded protein plays an important role in synaptic function.

Case presentation: Whole-exome sequencing was applied to detect pathogenic variants in a patient with clinical symptoms of psychomotor development, attention deficit, poor logical thinking ability, and an introverted personality, but without epilepsy or any significant electroencephalogram changes. Genetic study revealed a splicing mutation (c.1904 + 1G > A) and RT-PCR revealed aberrant splicing of exon 16, leading to a reading-frame shift and a truncated protein in the PH domain.

Conclusions: This is the first report of a splicing variant of CNKSR2, and the unique clinical features of this pedigree will help extend our understanding of the genetic and phenotypic spectra of CNKSR2-related disorders.

Keywords: CNKSR2, Neurodevelopmental disorder, Splicing variant, Whole exome sequencing, Attention deficit

Background

Neurodevelopmental disorders including intellectual disability, attention-deficit/hyperactivity disorder, and language deficits are extremely heterogeneous, both clinically and genetically. Underlying pathogenic variants have been identified in genes involved in different neurodevelopmental processes, including cell proliferation, neuron migration, synapse formation, and myelination [1].

CNKSR2—a gene encoding postsynaptic density proteins—plays an important role in neuronal proliferation, migration, differentiation, and death, as well as Ras-mediated synaptogenesis [2]. Impaired synaptic function caused by loss of CNKSR2 has been indicated in patients with seizures, and intellectual, attention, and language deficits [3]. Herein, we report a patient with clinical symptoms including attention deficit, poor logical thinking ability, and an introverted personality (but without epilepsy or electroencephalogram changes) caused by an out-of-frame exon deletion due to a splicing variant of CNKSR2. This is the first reported case with a splice variant in CNKSR2, which could enhance our understanding of the genotypic and phenotypic spectra of CNSKR2 in patients with neurodevelopmental disorders.

Case presentation

The proband of the family was a six-year-old boy who sought help with attention deficit in school. He had an unremarkable prenatal history, with a birth weight of 2.95 kg and length of 50 cm at full term. The boy started walking alone at 18 months, and was diagnosed with a motor developmental delay in a local hospital. He started school at the normal age, but showed poor performance, especially in mathematics, and attention deficit in class.
Language development was systematically evaluated as normal in the Department of Pediatric Developmental Behavior in Shanghai Children’s Medical Center using the Peabody picture vocabulary test-revised. The proband’s general cognitive ability, as estimated by the Wechsler Intelligence Scale for Children-Revised (WISC-R), is slightly below average indicating mild cognitive defects (Full scale IQ = 75). He had normal EEG and brain MRI results. He was raised by his grandparents and was described as very introverted in the presence of unfamiliar adults. The patient’s mother was a 33-year-old female with normal appearance. She worked in an office and completed her college education, although she exhibited poor performance in mathematics. The parents of the mother and father of the proband were all normal.

The patient’s peripheral blood DNA was subjected to WES to screen for causal variants. Details of WES was described in additional file (Additional file 1). A hemizygote splicing variant (c.1904 +1G > A) of CNKSR2 (NM_014927.4) in intron 17 (21 exons in total) was identified through WES in the patient and was considered as the possible disease-causing variant. Sanger sequencing was applied to confirm the variants (Fig. 1).

The primers for amplification were designed using UCSC Exon Primer online software (http://genome.ucsc.edu/index.html) and synthesized. The primer sequences for the truncating variant to be confirmed were forward 5’-TTACAGATATCATACCTTCACACC-3’ and reverse 5’-TGATTGACCTAGAACTTCAGTGAC-3’. Further pedigree investigation revealed the splicing variant was heterozygous in the mother, but wild type in both parents of the mother. According to the ACMG/AMP 2015 guidelines, the variant is categorized as pathogenic. As “G” in the position of CNKSR2 1904 +1 is a consensus sequence at the splice-site, a mutation may induce abnormal splicing. Total RNA was extracted from peripheral blood of the patient and reverse transcription and subsequent PCR were performed to investigate alternative splicing products. RT-PCR was performed using primers spanning exons 13 through 20 (Fig. 1) in

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**Fig. 1**  
**a** Pedigree and Sanger sequencing confirmation of the splicing variant (c.1904 +1G > A) of the CNKSR2 gene. **b** Schematic map of variant location and domains of the CNKSR2 gene. **c** Exon 16 skipping transcript detected from the patient caused by splicing variant.
both the proband and control. The primer sequences were forward 5'-caagacatcatgggcactcc-3' and reverse 5'-actccgtaggctgatcttc-3'. Electrophoresis of the RT-PCR products did not show a significant difference in size. Direct sequencing analysis of the products revealed the induction of an exon 16-skipping product compared with the control, and the shift in reading-frame led to a termination of the protein after eight codons.

**Discussion and conclusions**

Houge type of X-linked syndromic mental retardation is thought to be associated with hemizygous or heterozygous variants in **CNKSR2** on chromosome Xp22. Patients with pathogenic **CNKSR2** variants exhibit delayed development, major intellectual disability, speech and language delay, and early-onset seizures; continuous spike-wave activity or centrottemporal spikes are observed in EEGs. Since the first case of a patient lacking **CNKSR2**—due to a deletion of the initial 15 exons of the gene—was reported, seven more pedigrees with a similar phenotype and different **CNKSR2** variants have been reported [3–7]. These mutations include deletions covering all or part of the gene, frameshift-premature termination mutations, or stop-gain mutations, leading to loss-of-function of **CNKSR2**.

All previously reported male patients exhibited onset of seizures from neonatal stage to 3.5 years of age [3, 4] and most had characteristic frequent or continuous spike and wave EEG patterns. Language impairment was a cardinal feature of the reported patients; speech delay was noticed from onset of seizures and persisted indefinitely, leading to the absence of speech [3]. Developmental and behavioral challenges including severe attention deficit and hyperactivity were also recognized in patients. Other common and uncharacteristic features include intellectual disability and psychomotor delay (Table 1). There is only one female carrier in all reported literatures and exhibit with only mild learning disability or completely normal intellectual state.

In the present case, the de novo occurrence of the splicing variant in the boy’s mother strongly supports a causative role of this mutation. RT-PCR of the coding sequence of the gene confirmed out-of-frame deletion of exon 16 and a premature termination of **CNKSR2** in the PH domain. The patient and his mother showed very mild cognitive defects, which could be considered

| Pedigree no | Publication | CNKSR2 variant | Language defect | Attention problems | Seizure | Sleep EEG | Psychomotor delay | Female carrier |
|-------------|-------------|----------------|----------------|-------------------|--------|----------|-----------------|----------------|
| 1 (two siblings) | Vaags et al. | arr[hg19] Xp22.12(20,297,696–21,471,387) × 0[mat] | Yes | Yes | Yes | Continuous spike-and-slow-waves | Yes | Mild learning disability |
| 2 | Vaags et al. | arr[hg19] Xp22.12(21,375,312–21,609,484) × 0[mat] | Yes | Yes | Yes | Continuous spike-and-slow-waves | Yes | N/A |
| 3 (two siblings) | Vaags et al. | arr[hg19] Xp22.12(21,193,947–21,707,169) × 0[mat] | Yes | Yes | One sibling without seizure | No | Yes | N/A |
| 4 (three siblings) | Vaags et al. | c.452insA p.D152Rfs*8 | Yes | Yes | Yes | N/A | Yes | N/A |
| 5 (three siblings) | Damiano et al. | c.2314 C>T; p.Arg712* | Yes | Yes | Yes | Centrottemporal or frontal spike and wave activity | Yes | Febrile seizures |
| 6 | Aypar et al. | arr[hg19] Xp22.12(21,328,677–21,670,497) × 0[mat] | Yes | N/A | Yes | Frequent and continuous centro-temporal spike and wave | Yes | Normal |
| 7 | Houge et al. | arr[hg19] Xp22.12(21,285,233–21,519,405) × 0[mat] | Yes | Yes | Yes | N/A | Yes | Normal |
| 8 | Sun et al. | c.2185C>T; p.Arg729* | Yes | Yes | Yes | Continuous spike-and-wave pattern | Yes | N/A |
| Our case | | c.1904+1G>A | No | Yes | No | No | Mild | Mild learning disability |
subclinical, such as attention deficit, poor logical thinking ability, and introverted personality.

Cases involving deletion of the entire CNKSR2 or the N-terminal of the gene [3, 4] are thought to lead to loss of the CNK2. A frameshift mutation in the N-terminal of CNKSR2 leads to an early stop-codon, possibly producing a non-functional protein product of 160 amino acids. The stop-codon variant detected in the 712 codon retained the SAM, PDZ, and PH domains of CNKSR2, but lacked the C-terminal of the gene. The out-of-frame deletion and reading-frame shift detected in our pedigree resulted in a truncated PH domain. The PH domain, located in the C-terminal of the gene, is known to stimulate the MAPK pathway [8] and both isoforms of CNK2 are located synthetically through the PH domain [9]. A possible reason for the relatively mild symptoms observed might be that the skewed PH domain of the truncated CNK2 protein gained a new function. As this variant is the first CNKSR2 splicing variant detected, and aberrant splicing was confirmed from peripheral blood not from neuron tissues, it is possible that localized splicing and transcription of CNKSR2 was different and maintained some level of functioning CNK2 protein.

One reason why CNKSR2 variants are rarely detected may be that exonic deletions or small deletions encompassing CNKSR2 are neglected by exome sequencing or targeted gene panel sequencing, which are widely used for identifying the genetic background of patients with neurodevelopmental disorders. Thus, evaluating exonic copy number variants with additional tests such as sensitive exon-level copy number arrays is worth considering for the relatively mild symptoms observed might be that the skewed PH domain of the truncated CNK2 protein gained a new function. As this variant is the first CNK2 variant detected, and aberrant splicing was confirmed from peripheral blood not from neuron tissues, it is possible that localized splicing and transcription of CNKSR2 was different and maintained some level of functioning CNK2 protein.

In conclusion, a c.1904 + 1G > A variant in the CNKSR2 is the first to be identified pathogenic splicing variant in patients, which broadens the spectrum of genetic variants of this gene.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12920-020-00844-4.

Additional file 1. Details of whole exome sequencing. Whole exome sequencing details for pathogenic variants detection.

Abbreviations
WES: Whole-exome sequencing, RT-PCR: Reverse transcription-polymerase chain reaction, EEG: Electroencephalogram, MRI: Magnetic resonance imaging.

Acknowledgements
We would like to express our sincere gratitude to our patient and his family for their cooperation.

Authors’ contributions
RY and JW2 designed and organized the study. JW1, YG, and NL sampled the family members and acquired the clinical data. RY and YZ carried out the molecular testing. JW1 and NL carried out the transcriptional experiment and analyzed the data. TY and JW2 analyzed and interpreted the genetic testing and clinical data. YZ wrote the manuscript, which was then edited by RY and YG. All authors have read and approved the final version of the manuscript submitted by RY.

Funding
This work was supported by the National Natural Science Foundation of China under Grant 82001371, the Project of Shanghail Municipal Science and Technology Commission under Grant 16ZR1421700, and the young talents training program of Shanghai Municipal Health Commission under Grant 2018YQ24. These funding bodies contributed to design of the study and collection, analysis and interpretation of data.

Availability of data and materials
The datasets (whole-exome sequencing and Sanger sequencing files) used and/or analysed during the current study are available in NCBI Sequence Read Archive (SRA), SRR13105481.

Ethics approval and consent to participate
This study was approved by the Committee on Ethics of the Shanghai Children’s Medical Center (SCMCRB-K2016013) and was performed in accordance with the Declaration of Helsinki. After explanation of the possible consequences of the study, written informed consent was obtained from both of the patient’s father and mother, and from both of the mother’s parents.

Consent for publication
Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Competing interest
The authors declare that they have no competing interests.

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Received: 26 November 2020 Accepted: 1 December 2020
Published online: 09 December 2020

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