Short Report

Molecular Syndromology

Mol Syndromol 2011;2:60–63
DOI: 10.1159/000335159

Accepted: November 14, 2011
by A. Rauch
Published online: December 20, 2011

Loss-of-Function *CNKSR2* Mutation Is a Likely Cause of Non-Syndromic X-Linked Intellectual Disability

G. Houge[a, b] I.H. Rasmussen[c] R. Hovland[a]

[a]Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, and [b]Department of Clinical Medicine, University of Bergen, Bergen, [c]Department of Child Neurology, Stavanger University Hospital, Stavanger, Norway

Key Words
CNK2 · *CNKSR2* · X-linked mental retardation

Abstract
In a non-dysmorphic 5-year-old boy with developmental delay, well-controlled epilepsy, and microcephaly, a 234-kb deletion of Xp22.12 was detected by copy number analysis. The maternally inherited deletion removed the initial 15 of the 21 exons of the connector enhancer of KSR-2 gene called *CNKSR2* or *CNK2*. Our finding suggests that loss of *CNKSR2* is a novel cause of non-syndromic X-linked mental retardation, an assumption supported by high gene expression in the brain, localization to the post-synaptic density, and a role in RAS/MAPK-dependent signal transduction.

The connector enhancer of kinase suppressor of Ras-2 (Ksr2) gene *CNKSR2* was first identified in a genetic screen for modifiers of RAS-dependent photoreceptor development in *Drosophila* [Therrien et al., 1998]. At the same time the gene was isolated from a brain cDNA library and found to be highly expressed only in the brain [Nagase et al., 1998]. The encoded protein, called MAGUIN in rats [Yao et al., 1999] and CNK2 in humans, is a multi-domain scaffold/adaptor protein that stimulates MAPK (mitogen-activated protein kinase) signaling [Roy et al., 2002]. CNK2 may also integrate MAPK activation with e.g. membrane and cytoskeletal remodeling [Therrien et al., 1999; Bumeister et al., 2004]. Of particular interest is the localization of CNK to the postsynaptic density in dendritic spines [Yao et al., 2000; Ohtakara et al., 2002].

Mutations in *CNKSR2* have not previously been linked to disease or developmental disorders. Since the gene is located on the X chromosome, involved in signal transduction, and highly expressed in the brain (especially in the hippocampus, amygdala, and cerebellum), it is a good candidate gene for X-linked intellectual disability (ID). Here we describe a boy with ID and a deletion of the initial 15 of the 21 exons of the *CNKSR2* gene. This deletion was inherited from his mother whose deletion occurred de novo.

Patient, Findings, and Methods

The patient is a 5-year-old boy with ID of mild to moderate degree. He was born at term after a normal pregnancy, his Apgar score was 9 after 1 and 5 minutes, and he was small for gestation-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1661–8769/11/0022–0060$38.00/0

Accessible online at:
www.karger.com/msy

Gunnar Houge
Center for Medical Genetics and Molecular Medicine
Haukeland University Hospital
NO–5021 Bergen (Norway)
Tel. +47 5597 5444, E-Mail gunnar.houge@helse-bergen.no

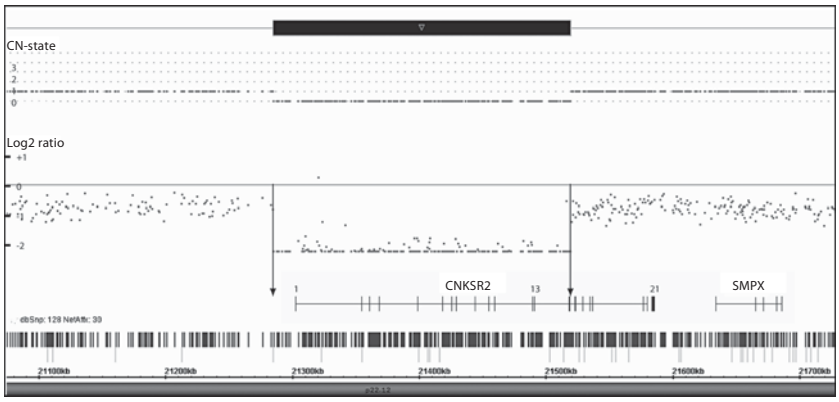

Fig. 1. The Affymetrix SNP-array result of the patient. An Xp22.12 deletion removed a major part of the *CNKSR2* gene. *CNKSR2* exons 1, 13, and 21 are numbered, and the stippled arrows indicate the deletion breakpoints.

al age (birth weight 2,589 g and length 45 cm). Later, his growth has been along the 5–10th centile which has been within expectations based on mean parental heights (173 cm for both parents). At the age of 5.5 years he was 109.5 cm tall (10th centile) with a normal body weight. He is borderline microcephalic with a head circumference that has followed the 2.5th centile, e.g. 48.3 cm at the age of 33 months. Upon clinical examination no dysmorphic signs were found, neither facial nor on his hands or feet. Psychomotor development has been clearly delayed, especially concerning language. At the age of 5 years, language development was on the level of a 1.5–2 year old, but at the age of 5.5 years, he was able to speak in short sentences. He managed to walk unsupported at the age of 18 months, but initially very unsteady. He has epilepsy with absence seizures and one recorded grand mal seizure which is well controlled by treatment with lamotrigin. His attention span is short, and he also has an ADHD diagnosis. He rather prefers to be with adults than other children. There were no neonatal feeding problems. Sleeping pattern, hearing, and vision have all been considered to be normal. Cerebral MRI at the age of 2.5 years was also normal (online suppl. fig. 1, www.karger.com/doi/10.1159/000335159), but the presence of a possible Chiari malformation type 1 was questioned. There are no other individuals known to have developmental delay in the family.

At the age of 2 years, routine blood karyotyping and fragile X testing were normal. More recently, copy number analysis by an Affymetrix 6.0 SNP array revealed a 234-kb deletion on the X chromosome removing a major part of *CNKSR2* (fig. 1). His ISCN karyotype is 46,XY.arr Xp22.12(21,285,233–21,519,405)×1 (positions according to hg18/NCBI Build 36). Family follow-up revealed that this deletion was inherited from his mother, but none of the mother's parents had the deletion. The mother had a balanced X-inactivation pattern in her blood DNA (57/43), and she did not report on learning difficulties at school age or later.

Copy number analysis was performed according to the manufacturer's instruction using Affymetrix genome-wide human SNP array 6.0. Primary data analysis was done by Affymetrix GeneChip® Genotyping Console v 4.0 using an in-house reference file for normalization (DNA from 44 healthy blood donors), and further data analysis was performed in the Affymetrix Chromosome analysis suite with a threshold of 15 kb and 5 markers.

The deletion in *CNKSR2* was verified by PCR using the primer pairs 5′-GAGAGGGTGGTGGGTAGTGA-3′ (forward) and 5′-AGCCCCATTCCTTCTAGGTC-3′ (reverse) for the telomeric deletion breakpoint, 5′-ATGATTGCATGTTGGGGTTC-3′ (forward) and 5′-TATGTGCCAGGCATTGATTC-3′ (reverse) for the centromeric deletion breakpoint, and 5′-TAGCCTGGGTTCTTTCTGGA-3′ (forward) and 5′-CATACCCACTCAGCCCACTT-3′ (reverse) for the middle part of the deletion. As expected, none of the primer pairs gave a PCR product from the patient's DNA sample, unlike his mother's DNA sample and normal control DNA samples where the PCR product sizes were as expected. Using the deletion-flanking primer pair 5′-GAGAGGGTGGTGGGTAGTGA-3′ and 5′-TATGTGCCAGGCATTGATTC-3′, a 1.5-kb PCR product was amplified from the mother's and son's DNA but not the control DNA. Taken together, this confirms that the centromeric deletion breakpoint was within intron 15 of *CNKSR2*, and sequencing using the same primers suggested the breakpoint to be quite close to exon 15. DNA FISH with BAC probe RP11-16H4 gave normal signals from the boy's as well as his mother's metaphases, probably because about 30 kb of this BAC probe hybridizes to 3′-flanking genomic sequences.

Discussion

Our finding of a 234-kb Xp22.12 deletion removing the N-terminal 15 of the 21 *CNKSR2* exons in a boy with non-syndromic intellectual disability (fig. 1) pointed to *CNKSR2* as a novel candidate gene for non-syndromic X-linked ID. In addition, copy number variants in *CNKSR2* have not been reported in normal healthy individuals in the Database of Genomic Variants or our in-house copy number variation database containing data from over a thousand Norwegians, both normal and with ID. The de novo occurrence of the deletion in the boy's mother strongly supported a causative role of our finding.

In the internet-based copy number aberration databases DECIPHER (www.decipher.sanger.ac.uk), ECARUCA (www.ecaruca.net), and ISCA (www.iscaconsortium.org), there are no males with similar deletions. In

DECIPHER there is a female reported with a 0.91-Mb deletion removing the whole *CNKSR2* gene (patient 250431), but she also has a 0.83-Mb duplication on chromosome 7, and it is unknown if any of the aberrations are de novo. This female has ID, epilepsy, hyperactivity, and dysmorphic signs (small ears, short palpebral fissures with epicanthus, wide nasal bridge, and clinodactyly), i.e. phenotypic traits not found in our carrier mother. In their multicenter next-generation sequencing study of >240 families with suspected X-linked intellectual disability, Kalscheuer et al. [pers. commun., manuscript in preparation] found 1 family with a truncating *CNKSR2* mutation in 3 affected brothers and their mother.

In theory transcription of the C-terminal 6 exons (16–21) of *CNKSR2* could restore some CNK2 function in our family, especially since such a transcript would be in-frame, starting with amino acid 611 (of 1,034), the first potential start codon being an AUG at position 657. There are, however, no records of transcript variants not including the N-terminal part of the gene (4 transcript variants are known, and they all include exon 1), and the C-terminal 6 exons of *CNKSR2* contain none of the known functional domains (see below). Nevertheless, this does not exclude that transcription of the C-terminal part of *CNKSR2* may take place, stimulated by a putative promoter region upstream of the natural promoter (which is deleted), and restoring some CNK2 function.

The multidomain and rather large (899–1,034 amino acids) CNK2 protein appears to link different signal transduction pathways. The N-terminal half, containing a SAM (sterile α-motive), CRIC (conserved region in CNK), and PDZ (PSD-95, DLG1, and ZO-1) domain, may stimulate the RalGEF-RAL effector signaling network [Therrien et al., 1999]. The C-terminal half, containing a PH (pleckstrin homology) domain, stimulates the MAPK pathway [Therrien et al., 1999]. So far the latter function seems most important. CNK2 also facilitates RAF1 and BRAF activation [Iida et al., 2002; Lanigan et al., 2003; Bumeister et al., 2004]. In *Drosophila*, CNK together with its partner hyphen (HYP) stimulates the RAS-dependent RAF-activating property of KSR (kinase suppressor of RAS), i.e. activation of downstream MEK (mitogen-activated protein kinase kinase, MAP2K) [Douziech et al., 2006]. In this CNK/HYP/KSR regulatory complex, CNK and HYP interact through their SAM domains [Rajakulendran et al., 2008]. KSR stimulates RAF-signaling by opening up RAF phosphorylation sites in MEK. This is induced by RAF binding to KSR and causes an allosteric change that affects MEK [Liu et al., 2009; Brennan et al., 2011]. In this respect, CNK probably functions as an assembly platform for KSR and RAF [Douziech et al., 2006].

Based on the above and data from *Drosophila*, where the phenotype of different CNK loss-of-function mutations is comparable to hypomorphic alleles of the RAS pathway [Therrien et al., 1998], it can be expected that loss of CNK2 makes RAS/MAPK-dependent signal transduction less efficient. Since expression of *CNKSR2* appears to be high only in the brain in various gene expression arrays (see the UCSC genome browser and links therein) and upon specific testing [Nagase et al., 1998], phenotypic effects of such CNK2 loss-of-function mutations could be restricted to the brain. This is consistent with lack of other dysmorphic features than microcephaly in our proband and the presence of mild/moderate ID and epilepsy. The localization of CNK2 to the dendritic spines, where it forms a complex with other proteins of the postsynaptic density (e.g. Densin-180, PSD-95, and RAF), is of particular relevance for trying to understand the consequences of such a CNK2 loss [Ohtakara et al., 2002].

In summary, our finding of a *CNKSR2* deletion in a boy with ID and epilepsy and de novo in his carrier mother points to *CNKSR2* as a novel candidate gene for non-syndromic X-linked intellectual disability.

Acknowledgements

We are most grateful to the index patient and his family. Without their enthusiastic collaboration this study would have been impossible. The initial discovery of the deletion was done by Marion Vallanger, working in the diagnostic SNP-array group at the Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, and Atle Brendehaug has provided valuable technical assistance. We are also most grateful to Dr. Vera M. Kalscheuer in the Chromosome Rearrangement and Disease group at the Max-Planck-Institute for Molecular Genetics, Germany, for sharing unpublished data with us.

References

Brennan DF, Dar AC, Hertz NT, Chao WC, Burlingame AL, et al: A Raf-induced allosteric transition of KSR stimulates phosphorylation of MEK. Nature 472:366–369 (2011).

Bumeister R, Rosse C, Anselmo A, Camonis J, White MA: CNK2 couples NGF signal propagation to multiple regulatory cascades driving cell differentiation. Curr Biol 14:439–445 (2004).

Douziech M, Sahmi M, Laberge G, Therrien M: A KSR/CNK complex mediated by HYP, a novel SAM domain-containing protein, regulates RAS-dependent RAF activation in *Drosophila*. Genes Dev 20:807–819 (2006).

Iida J, Nishimura W, Yao I, Hata Y: Synaptic localization of membrane-associated guanylate kinase-interacting protein mediated by the pleckstrin homology domain. Eur J Neurosci 15:1493–1498 (2002).

Lanigan TM, Liu A, Huang YZ, Mei L, Margolis B, Guan KL: Human homologue of *Drosophila* CNK interacts with Ras effector proteins Raf and Rlf. FASEB J 17:2048–2060 (2003).

Liu L, Channavajhala PL, Rao VR, Moutsatsos I, Wu L, et al: Proteomic characterization of the dynamic KSR-2 interactome, a signaling scaffold complex in MAPK pathway. Biochim Biophys Acta 1794:1485–1495 (2009).

Nagase T, Ishikawa K, Suyama M, Kikuno R, Hirosawa M, et al: Prediction of the coding sequences of unidentified human genes. XII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Res 5:355–364 (1998).

Ohtakara K, Nishizawa M, Izawa I, Hata Y, Matsushima S, et al: Densin-180, a synaptic protein, links to PSD-95 through its direct interaction with MAGUIN-1. Genes Cells 7:1149–1160 (2002).

Rajakulendran T, Sahmi M, Kurinov I, Tyers M, Therrien M, Sicheri F: CNK and HYP form a discrete dimer by their SAM domains to mediate RAF kinase signaling. Proc Natl Acad Sci USA 105:2836–2841 (2008).

Roy F, Laberge G, Douziech M, Ferland-McCollough D, Therrien M: KSR is a scaffold required for activation of the ERK/MAPK module. Genes Dev 16:427–438 (2002).

Therrien M, Wong AM, Rubin GM: CNK, a RAF-binding multidomain protein required for RAS signaling. Cell 95:343–353 (1998).

Therrien M, Wong AM, Kwan E, Rubin GM: Functional analysis of CNK in RAS signaling. Proc Natl Acad Sci USA 96:13259–13263 (1999).

Yao I, Hata Y, Ide N, Hirao K, Deguchi M, et al: MAGUIN, a novel neuronal membrane-associated guanylate kinase-interacting protein. J Biol Chem 274:11889–11896 (1999).

Yao I, Ohtsuka T, Kawabe H, Matsuura Y, Takai Y, Hata Y: Association of membrane-associated guanylate kinase-interacting protein-1 with Raf-1. Biochem Biophys Res Commun 270:538–542 (2000).