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

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**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-
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.

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), ECA-RUCA (www.ecaruca.net), and ISCA (www.iscasonsortium.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-motif), 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 nonsyndromic X-linked intellectual disability.

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62 Mol Syndromol 2011;2:60–63

Houge/Rasmussen/Hovland


