Absent CNKSR2 Causes Seizures and Intellectual, Attention, and Language Deficits

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Synaptic function is central to brain function. Understanding the synapse is aided by studies of patients lacking individual synaptic proteins. Common neurological diseases are genetically complex. Their understanding is likewise simplified by studies of less common monogenic forms. We detail the disease caused by absence of the synaptic protein CNKSR2 in 8 patients ranging from 6 to 62 years old. The disease is characterized by intellectual disability, attention problems, and abrupt lifelong language loss following a brief early childhood epilepsy with continuous spike-waves in sleep. This study describes the phenotype of CNKSR2 deficiency and its involvement in systems underlying common neurological disorders.

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Intellectual disability, attention-deficit/hyperactivity disorder (ADHD), epilepsy, and developmental language impediments are common genetically complex disorders. Cases caused by single gene defects are invaluable in elucidating pathogenic processes, which might be shared with the common forms of these diseases.

The postsynaptic density (PSD) consists of ~1,400 diffusible and structural proteins. The latter are organized into 3 layers: a membrane-anchored layer (cell-adhesion, ion-channel, and receptor proteins), a deep layer (SHANK and guanylate-kinase–associated proteins), and between the two a large intermediate layer of numerous scaffold and adaptor proteins, including CNKSR2.[1,2] CNKSR2 physically links with the major PSD proteins densin-180, PSD95, and S-SCAM and regulates Ras signaling, which controls neuronal proliferation, migration, differentiation, and death,[3–7] as well as Ras-mediated synaptogenesis.[8,9] A first report of a patient lacking CNKSR2 was recently published. The patient had developmental delay, well-controlled epilepsy, and microcephaly.[10] We now present comprehensive descriptions of 8 patients with this defect, including the original case (Patient 3). This work establishes the neurodevelopmental and neurological syndrome of absent CNKSR2.

Patients and Methods

The study was approved by the Hospital for Sick Children research ethics board. Informed consent was obtained from

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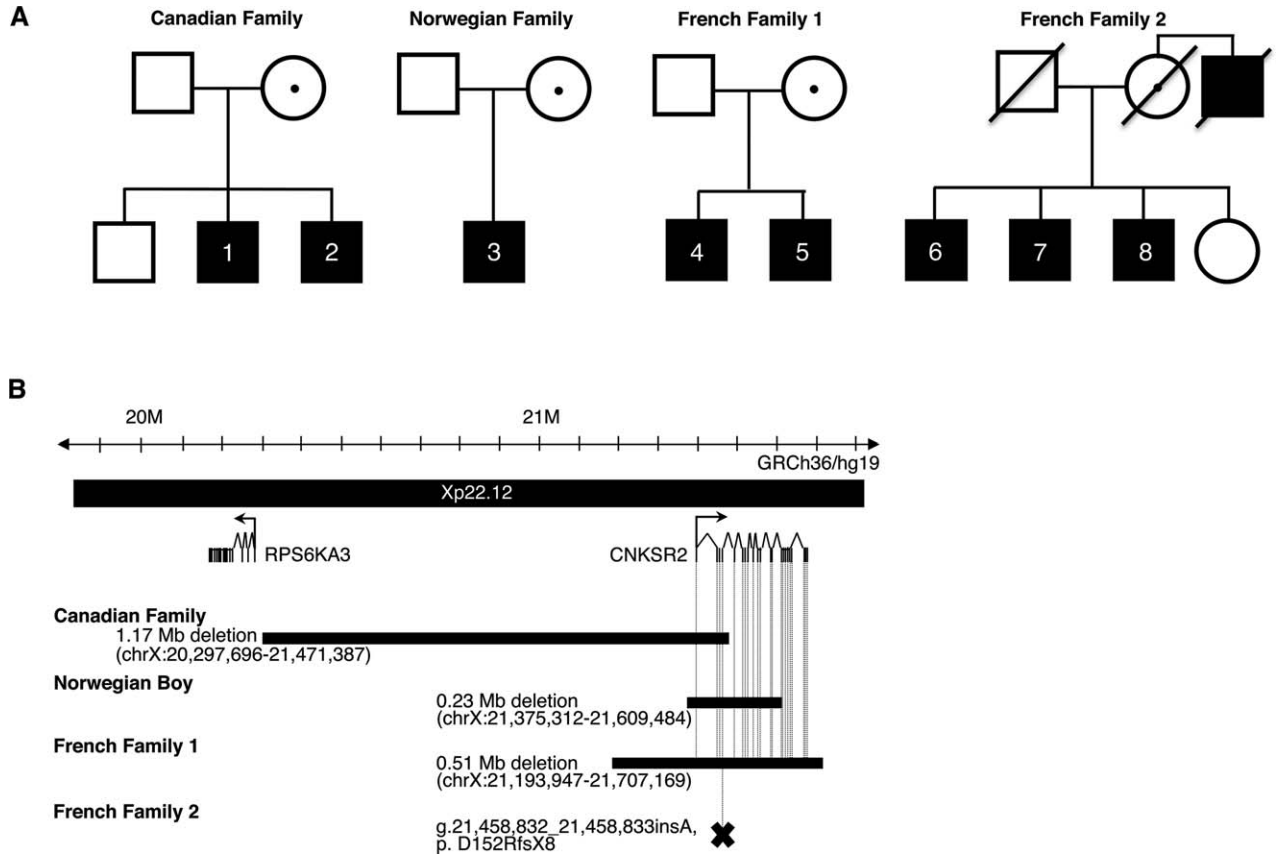

FIGURE 1: Genetic details. (A) Pedigrees of the 4 families; solid squares = affected males; open circles with black dots = carrier mothers. (B) Genomic map of chromosome Xp22.12 with extent of deletions (black bars) or position of frameshift premature termination codon (black X). The deletion in the Canadian family approximated the *RPS6KA3* gene, deletions of which cause the Coffin–Lowry syndrome, which our patients do not have. However, fine-mapping the breakpoints of the 1.17Mb deletion showed that it does not involve this gene, and Western blot analysis confirmed that expression of the *RPS6KA3* protein product, RSK2, is unaltered (data not shown).

parents or caregivers. Five boys, aged 13 years and younger, with developmental delay, language deficits, seizures (in 4), and unremarkable magnetic resonance imaging (MRI) were tested for copy number variation (CNV) and found to have deletions of the X-linked *CNKSR2* gene (Fig 1). Examination of the Database of Genomic Variants revealed no CNV of *CNKSR2* in healthy control individuals.[11] We describe their neurological, neurodevelopmental, and neuropsychological features, the latter with standardized psychometric testing adapted to culture and language (Supplementary Table). We also describe 3 brothers, aged 56 years and older, who share a frameshift-premature termination mutation in *CNKSR2* (see Fig 1) identified through a multinational intellectual disability X-chromosome exome sequencing project (in preparation).

Results

The Table 1 summarizes the genetic and clinical features of all 8 patients. The Supplementary Table details the psychometric testing of all 8 patients and the parents of Patients 1 and 2.

Patient 1, a 6-year-old boy, walked at age 2 years and spoke multiple words by that time. He had a generalized nonfebrile sleep-related seizure soon after he walked and lost most of his words. Overnight electroencephalogram (EEG) showed frontotemporal-dominant continuous spike-and-slow-waves (CSWS; Fig 2A). Valproic acid prevented further seizures. At age 3 years 6 months, testing showed poor attention, hyperactivity, and intellectual, especially language delay (verbal intelligence quotient [IQ] $\leq$ 3rd percentile; receptive vocabulary $\leq$ 5th percentile). At age 4 years, his CSWS were treated with high-dose diazepam. Baseline overnight EEG showed CSWS at 80 to 100% of non–rapid eye movement (REM) sleep. Diazepam (0.5 mg/kg) was administered intravenously at 8 and 9 PM, and on the subsequent night CSWS were reduced to 0 to 50% of non-REM sleep. An overnight EEG 3 months later showed CSWS at 50 to 75%. With no improvement in language, at 4 years 10 months he was treated with prednisone, 40 mg daily over 6 weeks followed by a 7-week weaning. Repeat

TABLE 1. Genetic and Clinical Details of 8 Males with Disrupted *CNKSR2*

	Canadian Boy 1	Canadian Boy 2	Norwegian Boy	French Teenage Boy 1	French Teenage Boy 2	French Adult Man 1	French Adult Man 2	French Adult Man 3
Patient ID	1	2	3	4	5	6	7	8
Genetic findings								
CNKSR2 mutation	arr[hg19] Xp22.12 (20,297,696–21,471,387)x0	arr[hg19] Xp22.12 (20,297,696–21,471,387)x0	arr[hg19] Xp22.12 (21,375,312–21,609,484)x0	arr[hg19] Xp22.12 (21,193,947–21,707,169)x0	arr[hg19] Xp22.12 (21,193,947–21,707,169)x0	g.21,458,832_3insA, p.D152RfsX8	g.21,458,832_3insA, p.D152RfsX8	g.21,458,832_3insA, p.D152RfsX8
Clinical findings								
Age, yr	6	8	8	12	13	56	58	62
Seizures	Yes, onset at age 2 years	Yes, onset at age 2 years	Yes, onset at age 2.5 years	Yes, onset at age 8 days	No	Yes, onset unclear, but treated with phenobarbital until age 8 years	Febrile seizures	Yes, onset in neonatal period
Sleep disorder	None reported	Nocturnal wakening, difficulty sleeping thereafter	Nocturnal wakening with screaming	Frequent nocturnal wakening from 7 months	None reported	ND	ND	ND
MRI	Normal	Normal	Normal	Nonspecific periventricular white matter hyperintensity	Normal	ND	Minor cortical atrophy	ND
Sleep EEG	CSWS	CSWS	CSWS at age 7 years	No CSWS at age 12 years	No CSWS at age 12 years	ND	ND	ND
Language defect	Multiple words prior to seizure onset with loss of most of his speech with seizure onset	Multiple words prior to seizure onset with complete loss of speech with seizure onset, absent speech at age 8 years	Short, mostly echolalic phrases at age 8 years	Single words at age 12 years	Multiple words at age 1 year with abrupt loss of speech at age 15 months; now has single words at age 13 years	Near-absent speech, few words	Very limited speech, but does have multiple words	Multiple words at age 2 years with subsequent loss; now has absent speech
Attention problems	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hyperactivity	Yes	Yes	Yes	Yes	Yes	Yes in childhood, now calm	Yes in childhood, now calm	Yes in childhood, now calm
Psychomotor delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Age of walking, mo	21	27	18	24	20	Unknown	Unknown	18

CSWS = continuous spike-and-slow-waves; EEG = electroencephalography; MRI = magnetic resonance imaging; ND = not done.

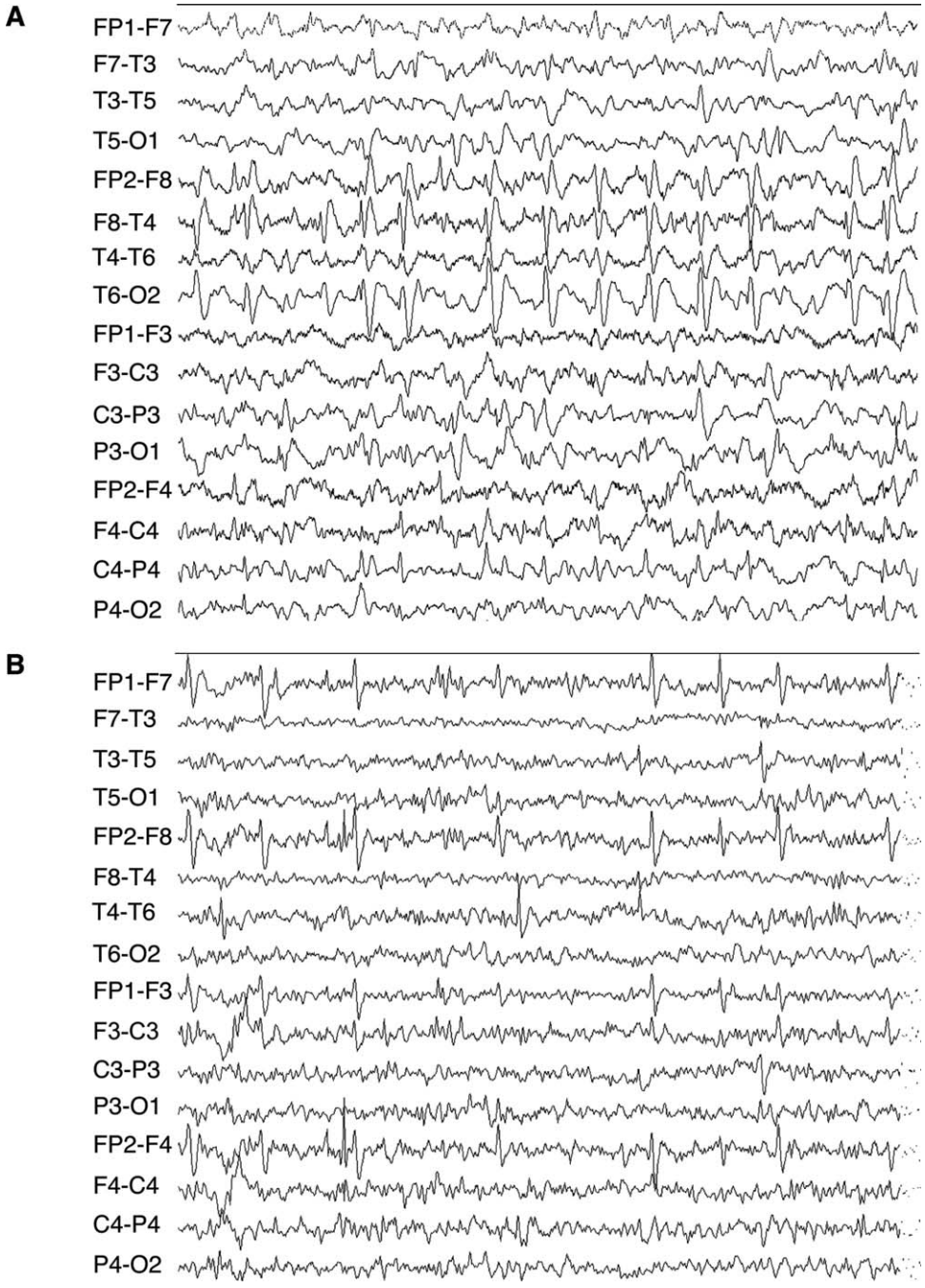

FIGURE 2: Sleep electroencephalogram (EEG) longitudinal bipolar montage. (A) Sample from Patient 1 (sleep stage N2). Throughout non–rapid eye movement sleep continuous spike-and-slow-waves were mostly symmetrically bifrontotemporal, although they were sometimes unilateral dominant, in either hemisphere, as in the right-sided example shown here. Wakeful EEG had normal posterior alpha rhythm, with infrequent symmetric or independent frontotemporal spikes. (B) Sleep EEG sample from Patient 3 (early stage N1 sleep). Wakeful EEG showed infrequent symmetric or independent frontally dominant spikes.

EEG showed CSWS occupying approximately 90% of non-REM sleep. Neuropsychological assessment at age 5 years indicated a lack of developmental gains, with major delays in intellectual function ($\leq$1st percentile), including language function (verbal IQ $\leq$ 0.1 percentile; receptive vocabulary $\leq$ 1st percentile).

Patient 2 is the 8-year-old brother of Patient 1. He walked at 2 years, having acquired 10 words. At 27 months, he experienced a prolonged nonfebrile sleep-related generalized tonic–clonic seizure, and stopped speaking. He had several self-limited generalized nonfebrile seizures on carbamazepine. Overnight video-

EEG at age 4 years showed rhythmic frontotemporal-dominant CSWS exceeding 80% of the record during non-REM sleep. On valproic acid, he had no further seizures. Neuropsychological testing showed global developmental delay with no language, impaired attention, and hyperactivity.

The mother, a carrier of the deletion, had mild learning disability in childhood affecting reading and mathematics. Neuropsychological assessment at age 41 years indicated normal overall intellectual function, with a deficit in completing numerical operations. Her overnight EEG was normal, as was that of her unaffected third son, who did not have the *CNKSR2* deletion, and the father of the 3 boys.

Patient 3, 8 years old, babbled in the first year of life and walked at 18 months. At 2 years 6 months, he developed staring spells and a single generalized tonic–clonic seizure, controlled by lamotrigine. At age 6 years, he had single words or echolalic phrases (eg, when asked, "Do you go to kindergarten?" he would not respond, but repeat, "Go kindergarten."). He was diagnosed with ADHD and treated with methylphenidate. Sleep EEG at age 7 years showed CSWS (see Fig 2B), and neuropsychological assessment revealed significant intellectual disability ($\leq$1st percentile), inattention, and impulsivity.

Patient 4, 12 years old, was born at 31 weeks gestational age with no etiology determined for his prematurity. He required treatment for hyaline membrane disease, developing seizures on day 8 of life with right-sided hemiparesis. The latter resolved, and he now has a normal neurological examination. He had several complex partial and generalized seizures, controlled with carbamazepine and valproic acid. He has frequent nocturnal awakenings and severe hyperactivity, treated with the dopamine antagonist tiapride. MRI showed nonspecific bilateral periventricular hyperintensities. EEGs during wakefulness showed active spiking from the left temporal lobe. Overnight EEG at age 12 years did not reveal CSWS. Formal psychometric assessment confirmed major intellectual disability and inattention.

Patient 5, 13 years old, is the brother of Patient 4. He walked at 20 months. He had cessation of language development at 15 months, and has attention problems, hyperactivity, and aggressive behavior, treated with methylphenidate. He has had no seizures. Overnight EEG at age 12 years revealed no CSWS but showed infrequent left hemispheric and generalized spike-waves. Formal psychometric assessment confirmed major intellectual disability, with marked improvement in attention and behavior.

Patients 6 to 8, aged 56, 58, and 62 years, live in an institution and have either no or limited speech. Data on these patients' childhood was obtained in large part from their lifelong caregiver sister. The eldest spoke in short phrases in early childhood, which he then lost. Early speech histories in the other brothers were unavailable. All had major attention deficit and hyperactivity throughout childhood, but are presently calm. Febrile seizures were present in childhood in the middle brother and afebrile seizures in the other two. There have been no seizures beyond age 10 years in any, and none is presently on antiepileptic medications. The middle brother had a recent MRI, which showed minor cortical atrophy.

Discussion

The cardinal features of CNKSR2 deficiency are: (1) intellectual disability, (2) highly restricted speech (especially expressive language), (3) attentional problems/hyperactivity, and (4) brief childhood epilepsy. A fifth feature may be CSWS in early childhood. Of 8 patients studied, 3 who were younger than 10 years (from 2 separate families) had CSWS, whereas the 5 who were older did not. CSWS in encephalopathy related to electrical status epilepticus during sleep (ESES) and in Landau–Kleffner syndrome (LKS) are developmental stage limited and disappear in the teenage years.[12] Whether this is what we are observing in the spread of ages in our set of patients remains to be determined with future cases and with follow-up of the 3 youngest boys. If it is, one would ask whether CSWS are contributing to the developmental and language disorder in this disease, as it does in ESES and LKS.[12] It is notable that half our patients had a clear history of some language prior to their first seizures, which they then lost and never reacquired. The CSWS in the 3 young children were resistant to standard antiepileptic management, and also to aggressive therapies with high-dose diazepam and corticosteroids, as they are in most cases of ESES and LKS.[12] The seizures themselves readily responded to standard therapy, reminiscent of most cases of ESES and LKS.[12]

Our patients had developmental delay prior to the onset of seizures. This is consistent with the finding that *CNKSR2* is expressed prenatally, suggesting a role in neurodevelopment. In the adult brain, it is expressed ubiquitously, indicating a general synaptic or synaptogenic function.[13,14]

LKS and ESES, with benign rolandic epilepsy, are a continuum of related diseases, sharing sleep-enhanced epileptic discharges and associated behavioral and cognitive deficits[15] and male predominance.[15,16] Gene mutations are considered to play an important, albeit complex, role in this spectrum of diseases. Currently, one genetic cause is known (Online Mendelian Inheritance in Man database [OMIM] 138253, *GRIN2A*, encoding the

N2RA subunit of the N-methyl-D-aspartate receptor), explaining a fraction of these patients. Whether *CNKSR2* mutations, milder than the deleting mutations reported here, might be additional causes awaits future studies.

ADHD is among the most heritable neuropsychiatric diseases, and is genetically complex.[17] ADHD is a common feature of many single gene disorders including tuberous sclerosis, fragile X syndrome, and neurofibromatosis type 1, as well as microdeletion syndromes such as Smith–Magenis syndrome and 22q11.2 deletion syndrome.[18] Combinations of genome-wide association and CNV studies have revealed a small portion of the disorder's gene risk.[19,20] ADHD is also 4-fold more common in boys than girls,[21] suggesting substantial contributions from X-chromosome loci. However, genome-wide association studies, for methodological reasons, have not considered the X chromosome.[22] The 8 males lacking *CNKSR2* due to CNV or nonsense mutations have prominent attentional problems/hyperactivity. Whether milder *CNKSR2* mutations might be found in other ADHD cohorts remains to be seen.

CNKSR2 joins the growing list of genes encoding PSD proteins, which, when mutated, cause a broad range of neurodevelopmental disorders. Notable examples include *SHANK3* (OMIM 606230), which is associated with autism, schizophrenia, and intellectual disability, and *DLG3* (OMIM 300189), which is associated with intellectual disability. Further delineation of the molecular pathogenesis of this group of conditions will allow the development of individualized treatments on the basis of molecular diagnosis, replacing current empiric approaches.

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Authorship

A.K.V. and S.B. contributed equally to this work.

Potential Conflicts of Interest

Nothing to report.

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