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CNKSR2 Deletions: A Novel Cause of X-Linked Intellectual Disability and Seizures

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TO THE EDITOR

X-linked intellectual disability (XLID) accounts for approximately 10% of intellectual disability in males and contributes to the excess of males in the intellectually disabled population (male to female ratio 1.3–1.4 to 1) [Ropers and Hamel, 2005]. Underlying causes of XLID have been extensively studied in recent years, and as a result mutations causing XLID have been described in about 106 genes [Piton et al., 2013]. Recently, Houge et al. [2012] described a maternally inherited 234 kilobase deletion on the X chromosome, which removed the majority of the *CNKSR2* gene (OMIM #300724; 21,375,312 – 21,609,484, genome build hg19; Fig. 1E) in a male with developmental delay, epilepsy, and microcephaly. Since *CNKSR2* gene is highly expressed only in the brain [Nagase et al., 1998], Houge et al. [2012] suggested that the phenotypic effects of loss of function mutations in the *CNKSR2* gene may be restricted to the brain. They concluded that the *CNKSR2* gene is a novel candidate gene for nonsyndromic X-linked intellectual disability.

A recent report by Piton et al. [2013] on XLID-causing mutations reassessed the implications of 106 genes in their involvement in XLID and classified them into five groups: genes with known mutations, genes with questionable involvement, those that never been replicated, those awaiting replication, and some with likely involvement. The *CNKSR2* gene was included in the awaiting replication category since its association with intellectual disability has not been replicated. We report on a deletion in the *CNKSR2* gene in a boy with intellectual disability and seizures, therefore replicating the findings of Houge et al. [2012].

The proband was a 7-year-old boy who presented to the Neurology Department for a second opinion regarding his medically intractable focal seizures and developmental delay. He was the first child born to a 24-year-old mother. Aside from occasional alcoholic beverages (one to two drinks at a time at most twice a week) and smoking until approximately five months of gestation, the pregnancy was not complicated by maternal illnesses or exposure to other teratogens. The mother did not take any medications during gestation. Birth was at term and via spontaneous vaginal delivery without complications. Birth weight was 6 pounds, 14 ounces (3118 g, 25th centile), and no postnatal complications were reported.

Concerns regarding the patient's psychomotor development arose within the first year of life, particularly regarding his speech. The patient was slow in achieving milestones but had no regression.

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He sat alone at approximately one year and walked alone at approximately two years. Initially, he was diagnosed with seizures at age two, which presented initially with typical events such as staring, smacking his lips, and having episodes of whole-body tremulousness without any response that would last between 10 and 15 minutes. His longest seizure lasted about 20 min. The mother did not believe that he had any febrile seizures; however, prior to the seizures, the patient had some intermittent clonic jerking affecting both legs that would last up to two minutes. Seizures had persisted despite trials of six antiepileptic drugs and a trial of vagal nerve stimulation.

Currently, at age 7 (125.1 cm height, 75th centile; 23.7 kg weight, 60th centile; 53 cm head circumference; 75th centile), he is able to ride a bike without training wheels; however, he still has balance problems. He has bilateral pincer with left-hand dominance. He can draw a circle but not a face and mostly colors out of the lines. During this visit it was noted that the patient enjoys social interaction and comes to mother for comfort and sharing. The patient still has speech delay but most (approximately 75%) of his speech is intelligible. He mostly speaks in single words but occasionally uses two word phrases such as "help me". He is able to follow single step commands but not two-step commands. In school, the patient receives both occupational and speech therapy. He is enrolled in the special needs classroom and does not know his

Disclosure: Our institution does not consider a case report as human subjects research and this falls in the realm of routine clinical care.

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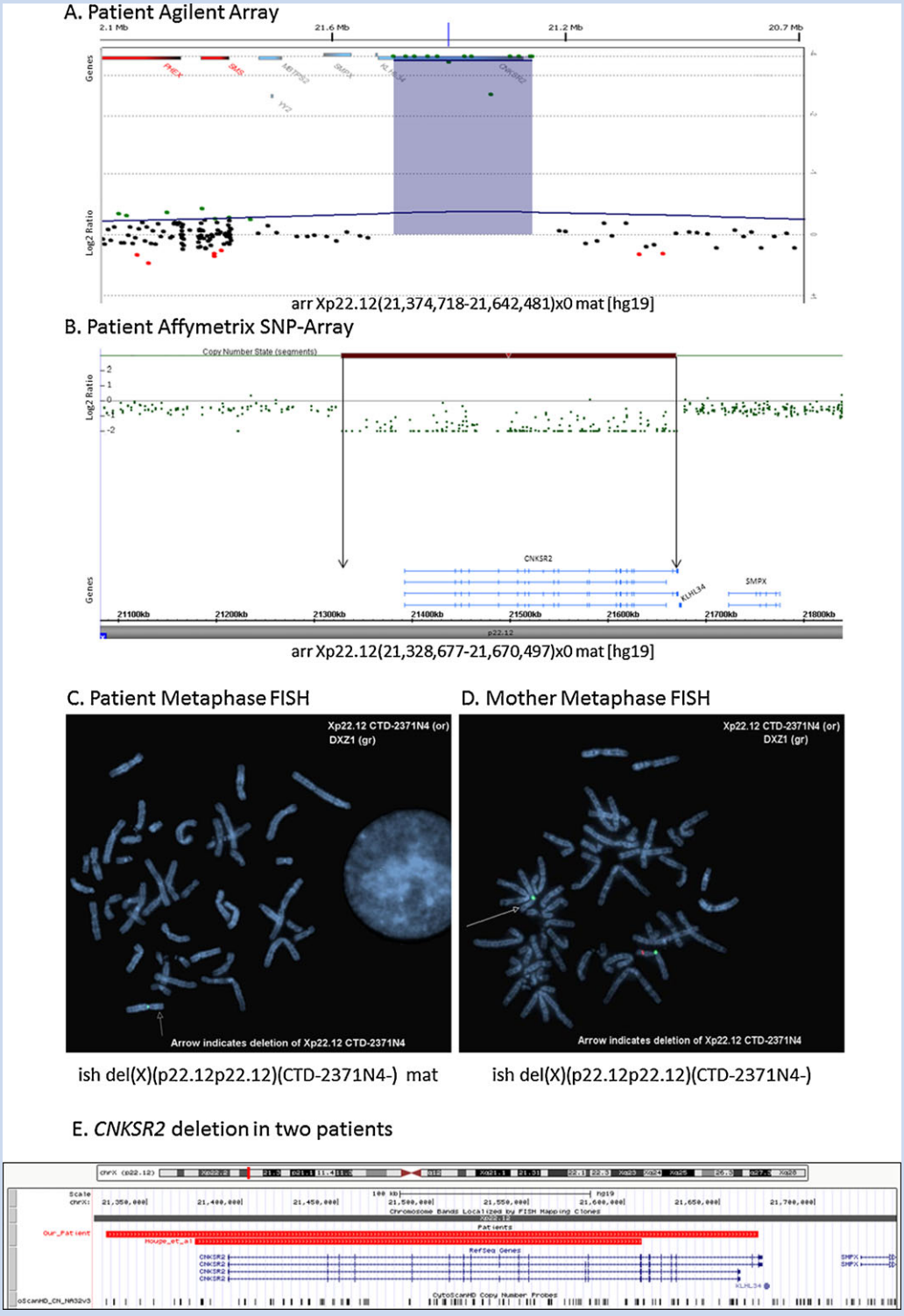

Fig. 1. Microarray results of the patient showing deletion of the *CNKSR2* gene at Xp22.12 both on the Agilent array software (A) and the Affymetrix array software (B). Metaphase FISH results of (C) the patient and (D) the mother showing the maternally inherited deletion on Xp22.12. The chromosome X centromere is labeled in green (DXZ1) and the *CNKSR2* gene region is labeled in orange (CTD-2371N4). (E) *CNKSR2* deletions seen in the first patient reported in the literature and in the present patient, as shown on the UCSC genome browser.

TABLE I. The Phenotype Information of the Present Patient in Comparison to the First Patient Published in the Literature

			Phenotype information					
Patient	Age	Gender	developmental delay	speech delay	intellectual disability	epilepsy/ seizures	microcephaly	ADHD
Present patient	7y	M	yes	yes	apparent	yes	no	n/a
Houge et al. [2012]	5y	M	yes	yes	yes	yes	borderline	yes

ADHD, attention deficit and hyperactivity disorder; n/a, not available.

alphabet, colors, or shapes. He is capable of feeding himself and his vision and hearing are normal.

Cranial magnetic resonance imaging (MRI) was normal, and no structural lesions were identified to account for his seizures; however electroencephalography (EEG) was severely abnormal. Frequent and nearly continuous independent discharges were noted from the right, predominantly central and temporal as well as bifrontal and bitemporal regions. In addition, there was diffuse slowing over the right posterior head region. Prior EEGs showed electrographic seizures from the right temporal and independently from bifrontal regions.

The patient's family history on the paternal side is unremarkable. Mother suffers from high cholesterol and sleep apnea. He is the only child. The maternal grandfather has diabetes and had strokes. The maternal great-aunt has some type of epilepsy. The patient had normal test results for the following: CBC, vitamin D, electrolytes, liver function, ammonia, aclycarnitine analysis, very long chain fatty acids, cerebrospinal fluid (CSF) lactate, CSF glucose, CSF plasma/glucose ratio, and CSF protein. A peripheral blood G-banded chromosome study, as well as Fragile X testing, was negative. His lactate was slightly elevated and the mucopolysaccharide was abnormal. The CSF methyltetrahydrofolate level was borderline low but the rest of the neurotransmitters were normal.

Chromosomal microarray (CMA; Agilent 180 K) detected an interstitial deletion of 268 kilobases at Xp22.12 (human genome build hg19 coordinates 21,374,718 – 21,642,481, Fig 1A). CMA using Affymetrix Cytoscan HD SNP array was later performed to further define the breakpoints and a 342 kilobases deletion was detected (human genome build hg19 coordinates 21,328,677 – 21,670,497, Fig 1B). Affymetrix CMA better defined the breakpoints with most proximal normal probe coordinates 21,322,029 and the most distal normal probe coordinates 21,678,137. The minimal deleted interval contains the entire *CNKSR2* gene isoforms 3 and 4, and the majority of the *CNKSR2* gene isoforms 1 and 2 with the most distal deleted probe located within the last exon of these isoforms. The interval between the most distal deleted probe, 21,670,497, and the most distal normal probe, 21,678,137, contains a hypothetical gene, *KLHL34*. Maternal fluorescent *in situ* hybridization (FISH) studies demonstrated that the deletion observed in the patient was inherited from the mother, who is reportedly unaffected (Fig 1C-D).

In summary, we identified a maternally inherited 342 kilobase deletion at Xp22.12 in a non-dysmorphic male with seizures and developmental delay (Fig 1E). A similar, maternally inherited deletion of 234 kilobases, also containing a portion of *CNKSR2* and no additional genes, was recently reported by Houge et al. [2012] in a male patient with very similar phenotypic features, including developmental delay, speech delay, intellectual disability, and seizures/epilepsy (Table I). As discussed in this report, copy number variants in *CNKSR2* have not been reported in healthy individuals suggesting a causative role for deletions in this gene. Gene expression studies identified *CNKSR2* expression highly in the brain [Nagase et al., 1998], therefore, Houge et al. suggested that loss of function mutations in the *CNKSR2* gene could specifically affect the brain. This is consistent with both patients being non-dysmorphic and both having phenotypic features that involve the brain such as seizures and intellectual disability. Following the report by Houge et al., a role for *CNKSR2* in spine morphogenesis in hippocampal neurons was described [Lim et al., 2014]. In this study, the interaction between CNKSR2 and other regulators such as Vilse was disrupted, resulting in spine defects. This finding lends further evidence that loss of *CNKSR2* would result in an abnormal phenotype.

A recent report by Piton et al. classified XLID-causing mutations into at least five groups, and *CNKSR2* gene was included in the "awaiting replication" category since it was only described by Houge et al. [Piton et al., 2013]. The identification of our current patient further defines the phenotype resulting from loss of function of this gene in males. In addition, this also suggests that the *CNKSR2* gene can be moved to the likely involvement category. Additional case reports are necessary to further define the phenotype of mutations in this gene.

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