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Absence of CNTNAP2 Leads to Epilepsy, Neuronal Migration Abnormalities, and Core Autism-Related Deficits

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SUMMARY

Although many genes predisposing to autism spectrum disorders (ASD) have been identified, the biological mechanism(s) remain unclear. Mouse models based on human disease-causing mutations provide the potential for understanding gene function and novel treatment development. Here, we characterize a mouse knockout of the *Cntnap2* gene, which is strongly associated with ASD and allied neurodevelopmental disorders. *Cntnap2*$^{-/-}$ mice show deficits in the three core ASD behavioral domains, as well as hyperactivity and epileptic seizures, as have been reported in humans with *CNTNAP2* mutations. Neuropathological and physiological analyses of these mice before the onset of seizures reveal neuronal migration abnormalities, reduced number of interneurons, and abnormal neuronal network activity. In addition, treatment with the FDA-approved drug risperidone ameliorates the targeted repetitive behaviors in the mutant mice. These data demonstrate a functional role for *CNTNAP2* in brain development and provide a new tool for mechanistic and therapeutic research in ASD.

INTRODUCTION

Autism spectrum disorders (ASD) form a heterogeneous neurodevelopmental syndrome characterized by deficits in language development, social interactions, and repetitive behavior/restricted interests (APA, 2000). Although not necessary for diagnosis, a number of other neurological or behavioral abnormalities are frequently associated with ASD, including hyperactivity, epilepsy, and sensory processing abnormalities (Geschwind, 2009).

Research into the genetic basis for ASD has identified many genes, including common and rare variants (Sebat et al., 2007; Glessner et al., 2009; Weiss et al., 2009). Association, linkage, gene expression, and imaging data support the role of both common and rare variants of *contactin associated protein-like 2* (*CNTNAP2*) in ASD. Originally, a recessive nonsense mutation in *CNTNAP2* was shown to cause a syndromic form of ASD, cortical dysplasia-focal epilepsy syndrome (CDFE), a rare disorder resulting in epileptic seizures, language regression, intellectual disability, hyperactivity, and, in nearly two-thirds of the patients, autism (Strauss et al., 2006). Several reports have since linked this gene to an increased risk of autism or autism-related endophenotypes (Alarcón et al., 2008; Arking et al., 2008; Bakkaloglu et al., 2008; Vernes et al., 2008). Recently, we have shown that the same *CNTNAP2* variant that increases risk for the language endophenotype in autism leads to abnormal functional brain connectivity in human subjects (Scott-Van Zeeland et al., 2010), consistent with emerging theories of ASD pathophysiology based on altered neuronal synchrony and disconnection (Belmonte et al., 2004).

Cntnap2 (also known as *Caspr2*) encodes a neuronal transmembrane protein member of the neurexin superfamily involved in neuron-glia interactions and clustering of K^+ channels in myelinated axons (Poliak et al., 1999; 2003). However, the fact that the gene is expressed embryonically (Poliak et al., 1999; Abrahams et al., 2007; Alarcón et al., 2008) and myelination takes place postnatally, together with the increasing number of reports that link the gene to ASD, suggest an additional role for *CNTNAP2* in early brain development. This is supported by the imaging and pathology data in patients with CDFE, in whom nearly half manifest presumed neuronal migration abnormalities on MRI, confirmed by histological analysis of brain tissue resected from patients who underwent surgery for epilepsy (Strauss et al., 2006).

The generation of valid animal models is critical for understanding the pathophysiology of ASD and to assess the potential of proposed treatments, as well as developing new, effective interventions. Ideally, mouse models should be based on a known genetic cause of the disease (construct validity), reflect key aspects of the human symptoms (face validity), and respond to treatments that are effective in the human disease (predictive validity) (Chadman et al., 2009; Nestler and Hyman, 2010). Here, we demonstrate that the *Cntnap2* knockout mouse exhibits striking parallels to the major neuropathological features in CDFE and the core features of ASD. We observe defects in the migration of cortical projection neurons and a reduction in the number of GABAergic interneurons, as well as accompanying neurophysiological alterations. These data show that CNTNAP2 is involved in the development of cortical circuits and further support alterations in brain synchrony or connectivity in ASD pathophysiology. In addition, treating *Cntnap2*$^{-/-}$ mice with risperidone rescues the repetitive behavior, but not the social deficits, a dissociation parallel to what is seen in human patients. These data demonstrate the validity of the *Cntnap2* KO as a mouse model for ASD and provide initial insight into the underlying mechanisms by which CNTNAP2 affects brain development and function.

RESULTS

Expression of *Cntnap2* in Mouse Brain

Mutant mice lacking the *Cntnap2* gene (*Caspr2* null mice) were generated by Dr. Elior Peles (Poliak et al., 2003). We backcrossed the original ICR outbred strain onto the C57BL/6J background for 10–12 generations. *Cntnap2*$^{-/-}$ mice on the C57BL/6J background had a normal appearance; no differences in weight or growth rate were observed when compared with WT littermates. In WT brain, expression of CNTNAP2 was first detected by western blot around embryonic day 14 (E14). As expected, CNTNAP2 was completely absent in the brain of homozygous mutant animals (Figure S1A available online). In situ hybridization demonstrated *Cntnap2* expression in multiple adult brain regions, primarily cerebral cortex, hippocampus, striatum, olfactory tract, and cerebellar cortex (Figure S1B). Embryonic expression was also broad, including the ventricular proliferative zones of the developing cortex and ganglionic eminences (where excitatory projection neurons and inhibitory interneurons arise, respectively) overlapping with regions containing migrating neurons and postmigratory cells, indicating a possible role in neuron development and/or migration (Figure S1C).

Cntnap2$^{-/-}$ Mice Exhibit Epileptic Seizures and Abnormal Electroencephalogram Pattern

One of the major phenotypes of CDFE syndrome is the presence of epileptic seizures, which is associated with dense hippocampal astroglyosis (Strauss et al., 2006). In *Cntnap2*$^{-/-}$ mice, spontaneous seizures were commonly observed in animals older than 6 months of age. Seizures were consistently induced by mild stressors during routine handling (Movie S1). A behavioral study of the frequency and severity of the seizures (Racine, 1972) is presented in Table S1. Histological analysis of the hippocampal formation in these animals did not show any gross structural abnormalities, although a reduction in parvalbumin-positive interneurons was found (see below and Figure S3C). Reactive astrocytosis as indicated by an enhanced expression of glial fibrillary acidic protein (GFAP) was observed throughout the hippocampus of mutant mice after the onset of seizures. Reactive astrocytosis was especially dense in the hilus but was not accompanied by neuronal loss in this structure as indicated by neuronal nuclei (NeuN) staining (Figure 1A). Electroencephalogram (EEG) recordings from freely moving mutant animals implanted with cortical electrodes at 8 months of age showed generalized interictal spike discharges during slow-wave sleep, whereas no electrical abnormalities were found in the EEG of mutant mice at times before the onset of seizures (Figure 1B). To avoid any confounding effect due to the presence of epileptic seizures, the following neuropathological, physiological, and behavioral characterization of *Cntnap2* mutants was performed at an age before the onset of seizures.

Cntnap2$^{-/-}$ Mice Show Neuronal Migration Abnormalities

We performed detailed histological analyses of *Cntnap2* KO brain and found no gross morphological changes in the brain structure of mutant animals by conventional staining techniques (cresyl violet staining), consistent with previous reports (Poliak et al., 2003). NeuN immunohistochemistry (IHC) revealed the presence of ectopic neurons in the corpus callosum of mutant mice at postnatal day 14 (P14), after neuronal migration is completed, which persisted through adulthood (Figure 2A). Interestingly, ectopic neurons of unknown origin in white matter were also reported in CDFE syndrome patients (Strauss et al., 2006).

Patients with CDFE syndrome also show neuronal migration abnormalities, such as abnormal arrangements of neurons in clusters or migratory rows in the deep layers of cortex (Strauss et al., 2006). We assessed laminar positioning of cortical projection neurons in WT and *Cntnap2* KO mice with antibodies against CUX1, a marker for upper-cortical layers, and FOXP2, a marker for deep cortical layers (Molyneaux et al., 2007). *Cntnap2*$^{-/-}$ mice show significantly higher numbers of CUX1+ cells in deep cortical layers (V and VI; Figure 2B). In contrast, deeper-layer FOXP2+ cells show the same pattern in both genotypes (Figure S2).

We performed BrdU neuron birthdating at E16.5, after the birth of layer V neurons (Angevine and Sidman, 1961) to confirm that the CUX1+ ectopic neurons observed in deep cortical layers were being born concomitant with superficial cell identity and their presence was not due to changes in cell fate. Sections of E16.5-labeled animals were analyzed at P7, when cortical lamination is essentially complete. As shown in Figure 2C, the distribution of BrdU+ cells is significantly different between WT and KO littermates, the latter showing a shortage of cells in upper-cortical layers that are redistributed to lower cortical layers. These data indicate that CNTNAP2 is necessary for the normal migration of cortical projection neurons.

Reduced Number of Interneurons in *Cntnap2*$^{-/-}$ Mice

Since the identification of CNTNAP2 (CASPR2) in 1999 (Poliak et al., 1999), its expression has been reported mainly in