Commentary

The Splice Is Right: *ANK3* and the Control of Cortical Circuits

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Bipolar disorder (BD) and schizophrenia (SZ) affect approximately 2–4% of the general population and are among the world's top 10 most disabling diseases. Current pharmacological therapies for BD and SZ are ineffective at targeting the underlying cause of these diseases and solely aid in reducing the severity of the psychotic symptoms. We need to identify the cellular and molecular mechanisms that contribute to the pathophysiology of complex neuropsychiatric disorders in order to advance the therapeutic potential for treatment.

Epidemiologic studies suggest that the etiology of BD and SZ is largely caused by genetic factors, with approximately 60–80% of phenotypic variation in BD and 70–90% in SZ based on genetic heritability (1). Genome-wide association studies have identified multiple risk loci involved in the development of BD; the most consistent hits include *DGKH*, *ZNF804A*, *SYNE1*, *ODZ4*, *CACNA1C,* and *ANK3* (2). One of the strongest and most replicated associations with BD has been the *ANK3* gene, which encodes the ankyrin-G protein. In addition, *ANK3* single nucleotide polymorphisms (SNPs) have been found to be associated with SZ; however, the significance threshold is not as compelling as that seen with BD. Although the genetic link between *ANK3* and neuropsychiatric disease is strong, there are still no conclusive family or animal data that represent a "smoking gun" proving the role of *ANK3* in BD or SZ.

In this issue of *Biological Psychiatry*, Hughes *et al.* (3) uncovered a SNP (rs41283526) in *ANK3* with a strong protective effect against BD and SZ. Their experimental data indicate that the presence of this SNP is associated with a decrease in the splicing of a small 54-nucleotide exon of *ANK3*, resulting in an odds ratio of 0.31 for BD and 0.21 for SZ. These findings strengthen the link between *ANK3* and normal neuronal function; however, how alternative splicing of the *ANK3* gene is involved in disease pathophysiology remains elusive. To answer this question, we need to evaluate how the splice variants of ankyrin-G regulate distinct cellular and neuronal circuit functions and how abnormalities in these processes contribute to neuropsychiatric disease.

Ankyrins are a family of scaffolding proteins that organize ion channels, transporters, and cell adhesion molecules into discrete domains at the plasma membrane and link these protein complexes to the spectrin-actin cytoskeleton. Three ankyrin genes are present in vertebrates: *ANK1* (encoding ankyrin-R), *ANK2* (encoding ankyrin-B), and *ANK3* (encoding ankyrin-G) (4). The canonical ankyrins consists of a membrane-binding domain at the N-terminus with 24 consecutive ankyrin repeats folded into a solenoid structure, followed by two ZU5 domains (initially found in ZO-1 and UNC5),[1] a UPA domain (conserved in UNC5, PIDD, and ankyrins), a death domain, and an unstructured C-terminal regulatory domain (4). Alternative splicing of the *ANK3* gene gives rise to three main classes of ankyrin-G isoforms—the canonical 190-kDa isoform described above, and the 270-kDa and giant 480-kDa isoforms, which arise from alternative splicing of a 7.8-kb vertebrate-specific exon (Figure 1A, B) (5). In addition, there is evidence for extensive splice variation within these three main classes of transcripts, including the "little exon" described in Hughes *et al.* (3). The canonical 190-kDa isoform of ankyrin-G is expressed in most tissues and cell types throughout the body, including the brain, heart, skeletal muscle, kidney, and retina, and plays a critical role in the localization of key signaling proteins to the cardiac intercalated disc and transverse tubule, epithelial lateral membrane, and photoreceptor outer segment. In contrast, the 270- and 480-kDa isoforms of ankyrin-G are found predominantly in the nervous system, where they localize ion channels and transporters to the axon initial segment (AIS), nodes of Ranvier, and the somatodendritic membrane (5).

In addition to tissue-specific distribution, the different isoforms of ankyrin-G have unique cellular functions (Figure 1C). A recent study found that the 190-kDa isoform of ankyrin-G plays a critical role in the regulation of dendritic spine morphology and *N*-methyl-D-aspartate (NMDA) receptor trafficking to glutamatergic excitatory synapses (6). The 270-kDa splice variant is likely important for oligodendrocyte function and the enrichment of myelin sheaths surrounding the nodes of Ranvier (7). The 480-kDa isoform of ankyrin-G has recently been characterized as the master organizer of the AIS and plays a critical role in formation of the nodes of Ranvier. The 480-kDa isoform of ankyrin-G directly interacts with and localizes all known components of the AIS, including voltage-gated sodium channels, *KCNQ2/3* channels, β4 spectrin, and the cell adhesion molecule neurofascin (5). Recent work has shown a new role for the 480-kDa splice variant of ankyrin-G in the stabilization of gamma-aminobutyric acid type A (GABA$_A$) receptors at somatodendritic sites and the formation GABAergic inhibitory synapses (8). Therefore, splice variants of ankyrin-G contribute to the proper formation of both excitatory and inhibitory synapses.

The coordinated balance between excitatory and inhibitory circuits is necessary for normal brain function, and neural oscillations enable the rhythmic timing and communication

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[1]Underscore indicates letters used in domain abbreviation; that is, UPA is (conserved in) UNC5, PIDD, and ankyrins, with each of the first letters of the three proteins represented in the abbreviation. ZU5 is (initially found in) ZO-1 and UNC5.

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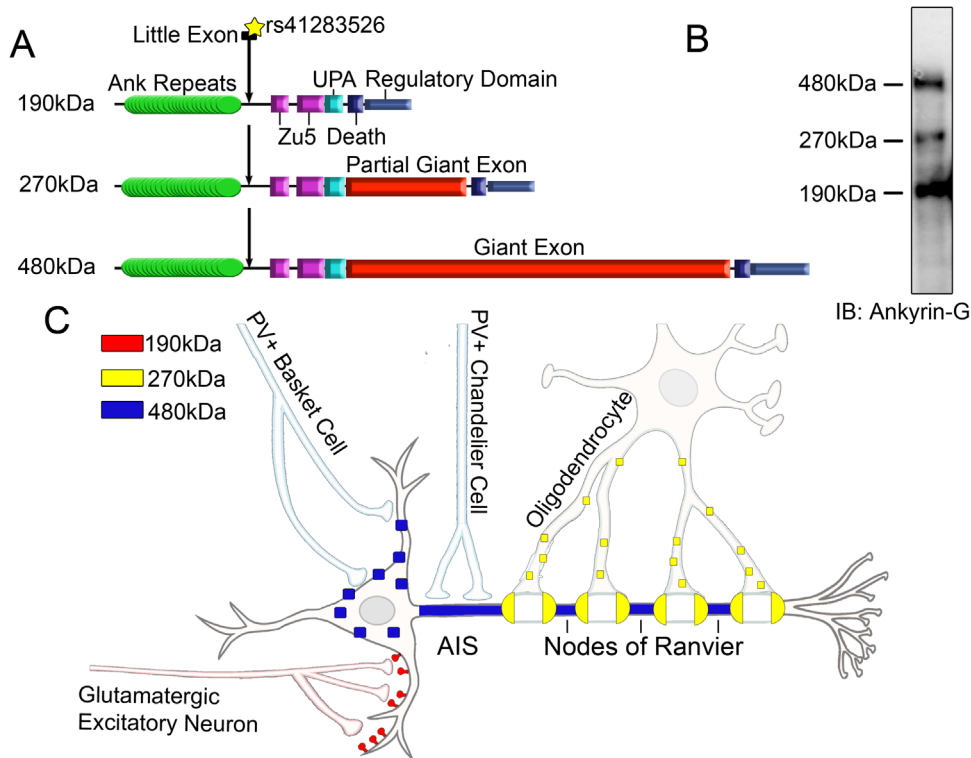
Figure 1. (A) Representative domain structure of the main classes of splice variants of ankyrin-G. The position of the little exon containing the loss-of-function single nucleotide polymorphism (rs41283526) identified by Hughes *et al.* (3) is depicted by the arrow. **(B)** Western blotting of whole brain lysate from adult wild-type mice probed with anti–ankyrin-G antibodies. **(C)** Schematic showing the different localization of the 190-kDa (red), 270-kDa (yellow), and 480-kDa (blue) variants of ankyrin-G. AIS, axon initial segment; IB, immunoblot; PV, parvalbumin.

between excitatory and inhibitory neuronal populations. Neural oscillations in the high frequency ranges, known as beta (13–30 Hz) and gamma (30–200 Hz) oscillations, are responsible for the precise synchronization of local cortical circuits. Gamma oscillations are of particular importance because impairments in these oscillations are seen in patients with SZ and BD (9). Clinical studies revealed that cortical gamma oscillations are decreased during sensory stimulation or cognitive tasks in individuals with SZ compared with neurotypical patients (9). In addition, recent studies have suggested that these alterations in gamma oscillations could act as potential biomarkers for abnormal neural network activity and behavior associated with BD (9). GABAergic inhibitory interneurons are of particular importance for the proper synchronization of neuronal networks, and multiple studies suggest that BD and SZ involve abnormal GABAergic neurotransmission. Interneurons that express the Ca^{2+}-binding protein parvalbumin (PV) are thought to play a crucial role in gamma oscillations because of their unique fast-spiking electrophysiological characteristics. PV-positive basket cells synapse on to the proximal dendrites and soma of pyramidal neurons, and PV-positive chandelier cells synapse into the AIS to precisely control neuron firing and cortical circuit synchronization (Figure 1C). Alterations in GABAergic neurotransmission seen in individuals with BD and SZ are supported by findings of significantly reduced expression of glutamic acid decarboxylase (GAD_{67}), the 67-kDa isoform of glutamate decarboxylase that synthesizes GABA, decreased GABA transporter expression, and increased postsynaptic $GABA_A$ receptor expression. These deficits in GABAergic signaling are also significantly pronounced in the PV-positive interneurons of patients with BD and SZ (9). These changes in GABAergic circuits have been predominately observed in layers II and III of the prefrontal cortex and CA2/3 regions of the hippocampus, which are key brain regions affected in psychiatric disease (10). Recent studies have determined that the 480-kDa splice variant of ankyrin-G is critical for the stabilization of GABAergic synapses at somatodendritic sites of cortical neurons (Figure 1C). Future work should focus on how the loss of ankyrin-G–dependent inhibitory synapses affects GABAergic circuits, gamma oscillations, and behavior.

Abnormalities in glutamatergic synapse plasticity and dendritic spine morphology have also been implicated in the pathogenesis of SZ and BD. Dysfunction of NMDA receptor signaling at glutamatergic synapses located on GABAergic interneurons has been shown to reduce neuronal synchronization (9). In addition, Smith *et al.* found that the 190-kDa isoform of ankyrin-G plays a key role in NMDA receptor trafficking and dendritic spine morphology (6). These findings suggest a second mechanism by which ankyrin-G impacts GABAergic neurotransmission and gamma oscillations. Therefore, mutations in the different splice variants of ankyrin-G may have a profound effect on the maintenance and function of mature cortical circuitry and disrupted neural oscillations seen in patients with BD and SZ.

Studies evaluating human neuroanatomy suggest that the precise refinement and pruning of excitatory and inhibitory synapses occur throughout adolescence, highlighting the developmental nature of these diseases. Emerging data suggest that SNPs associated with psychiatric diseases affect the intricate calibration of excitatory-inhibitory balance in the cortex (9). Interestingly, the accumulation of 480-kDa ankyrin-G on the pyramidal cell soma in mice occurs at the start of adolescence (8), which correlates with the formation of inhibitory synapses and the onset of the symptoms of BD in

humans. Consistent with these molecular findings, Hughes *et al.* present evidence that the splice variants containing the protective ankyrin-G SNP (rs41283526) display a clear upward shift in expression from infancy and childhood to adolescence and adulthood (3). These data suggest that the timing of expression of ankyrin-G splice variants changes with human development and may be a key factor involved in the pathophysiology of these disorders.

Exciting new evidence has pointed to key roles for different splice variants of ankyrin-G in both excitatory and inhibitory synapses. Hughes *et al.* have found that a SNP in *ANK3* (rs41283526) that prevents normal splicing of a "little exon" in ankyrin-G is actually protective against BD and SZ (3). It will be important in future work to determine which of the three main classes of ankyrin-G isoforms are affected by rs41283526 and to understand the mechanisms by which loss of expression of those splice variants provides protection against neuropsychiatric disease. Elucidation of the cellular and molecular mechanisms by which splice variants of ankyrin-G control neuronal circuits and how mutations affect ankyrin-G expression and function will hopefully provide insight into the mechanisms by which *ANK3* mutations contribute to the etiology of BD and SZ.

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