Review

Pathophysiological Roles of Abnormal Axon Initial Segments in Neurodevelopmental Disorders

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Abstract: The 20–60-μm axon initial segment (AIS) is proximally located at the interface between the axon and cell body. AIS has characteristic molecular and structural properties regulated by the crucial protein, ankyrin-G. The AIS contains a high density of Na^+ channels relative to the cell body, which allows low thresholds for initiation of action potential (AP). Molecular and physiological studies have shown that the AIS is also a key domain for the control of neuronal excitability by homeostatic mechanisms. The AIS has high plasticity in normal developmental processes and pathological activities such as injury, neurodegeneration, and neurodevelopmental disorders (NDDs). In the first half of this review, we provide an overview of the molecular, structural, and ion-channel characteristics of AIS, AIS regulation through axo-axonic synapses, and axo-glial interactions. In the second half, to understand the relationship between NDDs and AIS, we discuss the activity-dependent plasticity of AIS, the human mutation of AIS regulatory genes, and the pathophysiological role of the abnormal AIS in the NDD model animals and patients. We propose that AIS may provide a potentially valuable structural biomarker in response to abnormal network activity in vivo as well as a new treatment concept at the neural circuit level.

Keywords: axon initial segment (AIS); action potential (AP); ankyrin-G; spectrins; plasticity; neurodevelopmental disorders (NDDs)

1. Introduction

Neurons in the vertebrate nervous system generally possess several primary features. The cell body contains the nucleus and gives rise to two types of processes: axons and dendrites. Axons are the AP-transmitting element of neurons, and AP is initiated at the initial segment of the axon (AIS) and propagates to the synapse. In myelinated axons, AP is regenerated at the nodes of Ranvier, with small gaps in the myelin sheath. Na^+ channels are located at high density in the AIS as well as at the nodes of Ranvier (Figure 1). In addition to regulating AP initiation, the AIS maintains axon-dendrite polarity [1-4]. To focus on the physiological and pathological excitability-dependent plastic change of the AIS structure, we emphasize mainly on the role of the AIS in AP initiation in this review rather than polarity regulation.

The AIS of a neuron comprises the proximal 20–60 μm at the interface between the axon and cell body. Palay and Peters first described the characteristic structures using electron microscopy [5,6]. The internal structure of the AIS is characterized by specific features segregated from the axon hillock and neuronal cell body. Interestingly, different types of neurons have distinct locations, AIS lengths, and ion channel properties [7,8]. It is well accepted that the density of Na^+ channels is higher in the AIS than in the body. The AIS is not only the beginning of the axon, but also a key domain in the control of neuronal excitability [9-11].

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In this review, we first summarize the molecular, structural, ion-channel characteristics, and cell-cell interactions of the AIS. We then describe the activity-dependent plasticity of AIS, primarily by developmental activity. To link the physiological and pathological roles of the AIS in neurodevelopmental disorders (NDDs), we investigate recent advances in human mutation studies of AIS regulatory genes that are highly related to NDDs. The anatomical properties of AIS can be plastic in response to pathological activity; therefore, we discuss recently discovered abnormal pathophysiological changes in the AIS in NDD-model animals and human patients with NDDs. Future study of the AIS may provide potentially valuable tools for determining structural and functional neuronal plasticity in response to abnormal network activity in vivo.

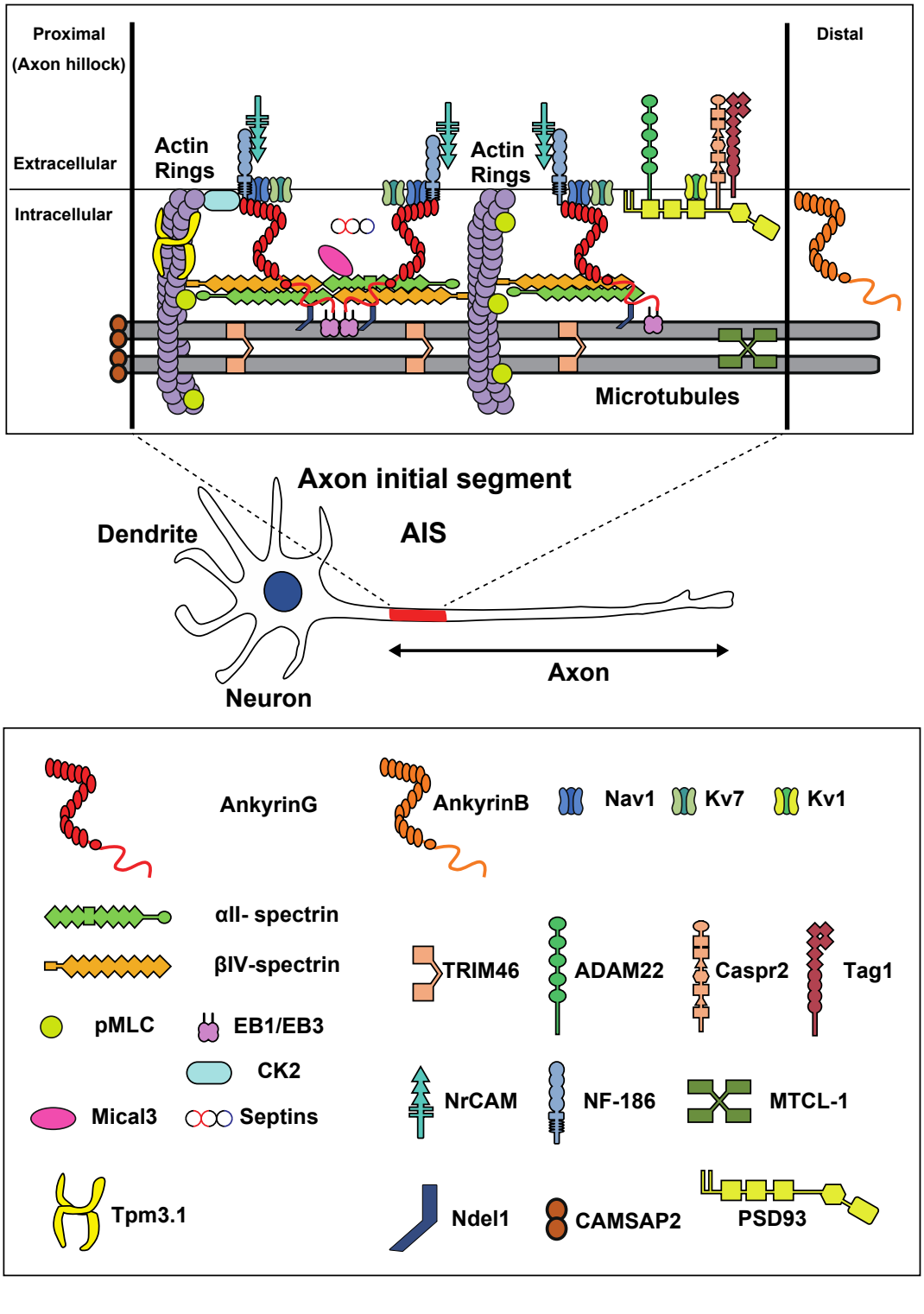

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Figure legend

Figure 1. The molecular organization of the AIS and diagram of a multipolar neuron. Membrane proteins (Nav/Kv7 channels, NF-186, NrCAM) are anchored by Ankyrin-G (red). Ankyrin-G inserted into the αII (green)/βIV (orange) spectrin tetramers. Actin rings (purple) are connected with spectrin tetramers. In the distal part, Kv1 channels, ADAM22, Tag1, and Caspr2 are present. Ankyrin-G binds to microtubules via EB1/EB3 proteins and Ndel1. Bundles of microtubules are crosslinked by TRIM46 and MTCL-1. Actin rings appear as twisted ropes possessing two long, intertwined actin filaments connected by a dense mesh of aligned spectrins. CK2, MLC, and Tpm 3.1 bind to actin rings. Mical3 and septins are present at the AIS and control the AIS structure.

2. Molecular characteristics of AIS

The axon initial segment (AIS), 20–60 μm in length, is located at the proximal interface between the axon and cell body. Many studies have shown that the AIS contains a wide array of proteins, including microtubules and the plasma membrane (Figure 1) [1-4]. Among these, ankyrin-G (ANK3), a neuron-specific protein located at the AIS and nodes of Ranvier, is thought to be the most critical component of the scaffold structure of the AIS.

Ankyrin-G has a membrane-binding domain at the N-terminus, as well as a spectrin-binding domain, a serine-rich domain, and a C-terminal domain [3]. Ankyrin-G has two main isoforms, 270 and 480 kDa, respectively, that are the major isoforms specifically localized at the AIS and nodes of Ranvier [12,13]. Shortly after axon specification, ankyrin-G begins to cluster, recruiting AIS components, such as spectrin tetramers, at the proximal axon [14,15]. Most AIS protein components interact with Ankyrin-G either directly or indirectly, and knockdown or knockout of ankyrin-G completely disrupts the localization of AIS proteins [16-18]. Therefore, ankyrin-G has been proposed as a master regulator of AIS structure and polarity [1-4].

In addition to ankyrin-G, spectrins are essential components of the AIS structure. Spectrin tetramers consisting of two α and two β subunits are widely expressed in neurons. Vertebrate axons possess α-spectrin and five types of β spectrins: βI–βV [19]. Spectrin tetramers contain the βIV spectrin subunit at the AIS and nodes of Ranvier (Figure 1) [20]. The 280 kDa full-length isoform of βIV-spectrin contains an actin-binding domain, 17 triple-helical spectrin repeats, and a specific pleckstrin-homology domain [3]. Recently, the αII-spectrin subunit has been identified as the partner of the βIV-spectrin at the AIS and nodes of Ranvier [21,22]. Spectrin tetramers link adjacent actin rings, as shown in Figure 1. Knockout of αII-spectrin results in embryonic lethality as well as cardiac, craniofacial, and neural tube malformations in the embryo [23]. The conditional deletion of αII-spectrin leads to the disruption of the AIS structure; therefore, αII-spectrin is also an important AIS protein, even though it is expressed throughout neurons, rather than only in the AIS [22,24]. Similarly, the loss of βIV-spectrin in the brain induces disruption of AIS structure in vivo [25].

This modular structure allows ankyrin-G to organize the AIS scaffold (Figure 1) [3]. It anchors AIS-specific membrane proteins, including voltage-gated sodium (Nav) and potassium (Kv) channels, initiating the AP (Figure 1). Ankyrin-G knockdown prevented the localization and clustering of AIS proteins, including sodium channels [16-18].

As described in Section 4 below, the major subtypes of Na^+ channels at the AIS are Nav 1.1, 1.2, and 1.6 [9-11]. In addition, K^+ channels at the AIS are also important for modulating the AP. Kv7.2 and Kv7.3 (KCNQ2/3) channels can form homomeric or heteromeric complexes and accumulate at the AIS without binding to Ankyrin-G [9-11].

Conversely, the mechanism of ankyrin-G clustering at the AIS is strongly related to the complex of Ankyrin-B, αII-spectrin, and βII-spectrin. This complex excludes Ankyrin-G from the distal axon, confining it to the proximal axon [15]. Neuronal polarity and the developmental process of the AIS is well discussed in an excellent review [26]. The dynein regulator Ndel1 was also stably anchored at the AIS by its interaction with Ankyrin-G. Ndel1 activates the retrieval of vesicles transported by dynein in the AIS [27].

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Additionally, two cell adhesion molecules, neurofascin 186 (NF-186) and NrCAM, were found to exist in the AIS; however, an initial study showed that these adhesion molecules are not necessary for Nav channel clustering [18,28]. Recent studies have also shown that NF-186 is not required for AIS assembly, but is required for AIS maintenance in vivo [29]. Other studies have also shown that NF-186 knockdown induces AIS structural abnormalities [30,31]. Therefore, further investigation is necessary to fully understand the molecular functions of NF-186.

Among microtubule-associated proteins, the C-terminus of Ankyrin-G connects to microtubules via interactions with EB1/3. EB3 knockdown alters Nav localization [32]. TRIM46 was shown to be uniquely enriched in the proximal AIS and does not regulate AIS maintenance [33] (Figure 1). Cerebellar Purkinje cell-specific microtubule cross-linking factor 1 (MTCL1) knockout mice exhibit the loss of axonal polarity and mislocalization of Ankyrin-G [34].

In addition to these proteins, several other adhesion molecules, such as Tag1, Caspr2, and disintegrin and metalloproteinase domain-containing protein 22 (ADAM22), have been shown to localize in distal AIS, although no knockout mice studies of these molecules have demonstrated disruption of the AIS compartment [35].

Among actin-associated proteins, CK2 (Casein kinase2), the actin ring-related proteins, myosin light chain (MLC), and tropomyosin（Tpm）3.1 are important for AIS structural maintenance [36-38]. Recent studies have also shown that Mical3 (microtubule associated monooxygenase, calponin, and LIM domain containing 3), an oxidoreductase that depolymerizes F-actin, co-localizes with spectrins in the insoluble fraction of AIS, regulating the AIS assembly transiently and via actin patches [39]. The same research team also showed that septins, small GTP binding proteins, are also localized in the insoluble fraction of AIS, and that knockdown of septins induces disruption of AIS assembly [39].

Ultimately, these results show that regulatory proteins for microtubules and the actin cytoskeleton and AIS-specific components such as ankyrin-G or spectrins are alk important components in the regulation of the structure of the AIS.

3. Structural characteristics of AIS

Palay and Peters initially described the characteristic structure of the AIS using electron microscopy [5,6]. The internal structure of the AIS is characterized by three special features: (*a*) scattered clusters of ribosomes, (*b*) a dense layer of fine granular material beneath the plasma membrane, and (*c*) the fascicles of microtubules.

Subsequent investigations using a super-resolution imaging method called stochastic optical reconstruction microscopy (STORM), have shown the unique structure of the axon [40]. Actin forms ring-like structures wrapped around the surface of the axons. These rings are evenly spaced every 190 nm. This structure is not observed in dendrites [40]. In the AIS, a periodic assembly of actin rings, ßIV-spectrin, and ankyrin-G have been observed [41,42].

Simultaneously, the actin structure in the AIS has been shown using platinum replica electron microscopy (PREM). In the center of the AIS cytoskeleton exist bundles of microtubules coated with a dense, fibrillar–globular actin [43]. Immunogold PREM has shown that the actin coat contains AIS proteins such as neurofascin, NrCAM, or Nav [43].

Finally, a 2019 study by Vassilopoulos et al. unroofed the dorsal part of cultured neurons and used PREM and STORM to observe that the actin rings are likely to be in the form of twisted ropes containing two long, intertwined actin filaments connected by a dense mesh of aligned spectrins [44]. As discussed in their 2021 review article, similar techniques conducted without unroofing the dorsal part of neurons will help us to understand the structure whole actin rings at the ultrastructural level [45].

4. Ion channel properties of AIS

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Physiological studies have shown direct evidence that APs are initiated at the distal end of the AIS in many types of neurons [9-11]. It has also become clear that the AIS is not simply a trigger zone for AP generation but also plays a key role in regulating the integration of synaptic inputs, intrinsic excitability, and transmitter release, as has been intensively discussed [9-11].

Na^+ channels are involved in rapid depolarization of APs. Of the four Na^+ channel α-subunits (Nav 1.1, Nav 1.2, Nav 1.3, and Nav 1.6) expressed in the brain, three subtypes (Nav 1.1, Nav 1.2, and Nav 1.6) are localized in the AIS according to their developmental diversity. Immunocytochemical studies have shown that the major Na^+ channel isoform in the AIS of adult CNS neurons is Nav 1.6 [46,47]. Interestingly, tight coupling of Na^+ channels to the actin cytoskeleton was found to prevent access to the Na^+ channels in the AIS by the patch-clamp technique [48]. Furthermore, a method was established to unbiasedly match the properties of a wide range of APs in a morphologically realistic model to accurately determine the distribution of Na^+ channels [48]. Using this approach, the number of Na^+ channels in the hippocampal pyramidal neuronal AIS was estimated to be 50 times greater than in the cell body [48], but only 5 times greater than that in dentate granule cells [49]. In addition to their high density, the properties of Na^+ channels in the AIS are unique, probably because they facilitate the initiation of APs in AIS [9-11].

K^+ channels are essential for AP repolarization and play a role in setting the AP threshold, interspike intervals, and firing frequencies [9-11]. The predominant K^+ channel in the AIS of most neurons is the low-threshold Kv1 subtype [9-11]. Most neurons possess both Kv 1.1 and Kv 1.2 in the AIS [9-11]. Direct patch-clamp recording from the AIS showed a high density of dendrotoxin (DTX)-sensitive, fast-activating, slow-inactivating Kv1-type K^+ currents in the AIS of cortical pyramidal neurons [9-11]. In addition, the distal part of the AIS of pyramidal neurons contains a high density of Kv 7.2 and Kv 7.3 (KCNQ2/3) channels [9-11].

Taken together, these data indicate that the expression patterns of different ion channels in AIS are highly cell-specific.

5. Non-cell-autonomous AIS regulation through axo-axonic synapse and axo-glial interactions

To the best of our knowledge, the only neurotransmission at the AIS through ligand-gated ion channels uses γ-aminobutyric acid type A receptors ($GABA_AR$s) [50]. $GABA_AR$s containing α2 subunits (α2-$GABA_AR$s) are specifically enriched in the AIS ([50]). The AISes of the pyramidal cells of the forebrain contain inhibitory synapses that are exclusively innervated by chandelier cells (ChCs) [51,52]. Hines et al. showed that the localization of α2-$GABA_AR$s to the AIS is essential for the inhibitory control of pathological excitation [53].

The axons of ChCs are highly branched and characterized by arrays of vertically oriented terminals called cartridges, each of which holds synaptic connections [54]. Tai et al. found that the L1 family member L1CAM is required for ChC AIS innervation and maintenance [55]. Selective innervation of the pyramidal neuron AIS by ChCs requires the anchoring of L1CAM to the AIS by the cytoskeletal Ankyrin-G/βIV-spectrin complex [54].

More recently, Pan-Vazquez et al. found, by in vivo imaging, that ChC axons and their axo-axonic synapses develop rapidly in infant mice from postnatal day (P)12 to P18 [56]. They also found that increasing network activity during this period reduces the number of axo-axonic synapses and that the AIS length changes slightly [56]. In older mice (P40–P46), when ChC synapses switch to inhibitory, they result in an increase in axo-axonic synapses. The depolarizing nature of axo-axonic synapses suggests that this plasticity is homeostatic. In addition, increasing ChC activity may decrease the AIS length of pyramidal neurons that are connected to activated ChCs [56].

In glial cells, Rasband et al. reported an interesting interaction between microglia and AIS [57]. Microglia are immune cells that reside in the brain and are actively involved in

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the regulation of neuronal excitability and function. Rasband et al. found that in the cerebral cortex, a subset of microglia spatially extended a single process that binds to the AIS. The interaction between microglia and AISes appears early in development and persists into adulthood. However, these interactions are reduced after brain injury due to the activation of microglia [57]. Recently, in a hypoglossal nerve injury model, Tamada et al. observed microglia—AIS interaction and the accumulation of mitochondria in the AIS after injury [58].

Astrocytes, the most abundant cells in the CNS, promote synapse formation and help refine neural connectivity. Molofsky et al. showed that loss of astrocyte-encoded semaphorin Sema-3a leads to dysregulated α-motor neuron AIS orientation [59].

Lastly, regarding oligodendrocytes, cuprizone treatment increases axonal excitability in dysmyelinated mouse brains. Membrane repolarization and energy expenditure may be affected by the general misalignment of ion channels in the AIS [60]. The AIS of demyelinated axons begins closer to the cell body than in myelinated axons, but the expression of ankyrin G, βIV-spectrin, and ion channels was maintained [60]. Another study replicated this result using a genetic demyelination model [61]. These results suggest that myelination by oligodendrocytes is important for the maintenance of the AIS by the inhibition of hyperexcitability of pyramidal neurons.

Taken together, these results indicate that the AIS can be controlled either in a cell-autonomous fashion or in a non-cell-autonomous fashion.

6. Activity-responding plasticity of AIS in development and disease models

Different types of neurons differ in the location, length, and ion channel configuration of the AIS [1,2,11,62]. Recent studies have shown that the structural properties of the AIS are plastic in response to normal developmental and pathological activities [1,2,11,62]. Since other rapid inhibition of electrical properties of the neuronal AIS in vitro is well-documented [63,64], this review focuses on the plastic changes of the AIS in vivo.

Kuba et al. strikingly showed that the AIS in chicken nucleus magnocellularis neurons increased after deprivation of auditory inputs and moved closer to the cell body [65]. Physiological recordings also showed that these cells become more excitable [65]. The same research team recently reported that the regulation of cytoskeletal reorganization and sodium channel enrichment in the AIS differs depending on tonotopy (the spatial arrangement of sound frequency processing in the brain), but acts synergistically in the auditory nucleus [66]. These results indicate that neurons adapt to presynaptic activity and maintain neural circuit homeostasis by altering the properties of the AIS.

Expanding evidence of structural changes and remodeling of the AIS has been observed in disease models, such as type 2 diabetes [67], epilepsy [68], cerebral infarction [69], neuroinflammation by LPS [70], traumatic brain injury [71], Alzheimer's disease [72], amyotrophic lateral sclerosis [73] and mutations in the *Tau* gene causing frontotemporal dementia [74]. We will discuss these observations at length in Section 8.

In addition to these studies, cortical neurons undergo dynamic changes in the AIS in the visual cortex during the critical period of visual system development [75]. However, these changes were prevented by visual deprivation for postnatal weeks [75]. They also showed the relationship between the cisternal organelle (CO) and the AIS during visual cortex development. Synaptopodin-deficient mice lack CO and show shortening of the AIS in the dark for three to five weeks [76]. Another study showed observed structural plasticity of the AIS in response to various auditory experiences at varying mouse ages [77].

More interestingly, long-term sensory deprivation alters the AIS length of layer II/III pyramidal neurons in the sensory cortex, and the same cortical neurons rapidly decrease the AIS length in an enriched environment [78]. In addition to visual and somatosensory deprivation, Galliano et al. showed that odor deprivation by naris occlusion produced AIS shortening and a decrease in intrinsic excitability in axon-bearing dopaminergic neurons in the glomerular layer of olfactory bulbs [79].

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Taken together, these results suggest that bidirectional activity-dependent remodeling of the AIS by various types of sensory inputs plays a role in homeostatic adaptation in vivo.

7. Association and mutation studies on AIS-related genes

Molecular studies have shown the importance of AIS proteins, and clinical studies have shown that mutations in AIS regulatory genes are highly related to the etiology of neurodevelopmental disorders.

Table 1. Mutations of *ANK-3*, *SPTAN1*, *SPTBN4* and related symptoms

Intellectual Disability (ID); autism spectrum disorder (ASD); attention deficit hyperactivity disorder (ADHD);

Gene Name Accesion Number	Chromosome Number	Symptoms	Gene Mutation Locus	Mutated Protein
ANK-3 ANKYRIN 3 NM_020987.3	10q21	ASD	c.4705T>G	p.S1569A
			c.11159C>T*	p.T3720M
			c.12763A>C*,	p.T4255P
			c.11068G > A	p.G3690R
		ADHD, ASD, cognitive problems	46,XY,t(2;10)(q11.2;q21.2)	
		ID, hypotonia, spasticity, behavioral problems	c.10995delC	p.T3666LfsX2
		ID, cerebral and cerebellar atrophy, delayed myelination	c.9652C>T;	p.L3218F
		ID, speech impairment, autistic features, macrocephaly,	c.1990G>T	p.G664*
		ID	c.11033del	p.P3678Lfs*45
		Severe intractable seizures With DD	c.4960G>T, c.4465C>T	p.D1654Y, p.P1489S
		BD	Association	
		SCZ	Association	
SPTAN1 SPECTRIN, ALPHA, NONERYTROCYTIC, 1(SPECTRIN,ALPHAII) NM_0011304	9q34.11	West syndrome, profound ID, spastic quadriplegia	c.6619-6621del	p.E2207del
		West syndrome, profound ID, spastic quadriplegia	c.6923-6928dup	p.R2308_M2309dup
		Mild ID, IS	c.1697G>C	p.R566P
			c.6605-6607del	p.Q2202del
		West Syndrome, severely impaired psychomotor development, no visual attention	c.6908-6916 dup	p.D2303_L2305dup
		West syndrome	c.6910_6918dup	p.Q2304_G2306dup
		Infantile EE with IS and focal epilepsy,mild ID, ASD	c.5326C>T	p.R1776W
		Infantile EE with tonic spasms and FDS, profound DD, severe hypotonia, lack of visual attention; microcephaly	c.6184C>T	p.R2062W
		West syndrome,profound DD, minimal interaction, hypotonia, hypokinesia; microcephaly	c.6619_6621del	p.E2207del
		West syndrome,Profound DD, hypotonia, lack of visual attention; microcephaly	c.6619_6621del	p.E2207del
		West syndrome,Profound DD, severe hypotonia, lack of visual attention, thermic dysregulation; microcephaly	c.6622_6624del	p.N2208del
		West syndrome,Profound DD, hypotonia, multifocal myoclonus, dyskinetic movement disorder, lack of visual attention; microcephaly	c.6811G>A	p.E2271K
		Infantile EE with IS evolving to myoclonic seizures, severe DD, hypotonia, ataxic movement disorder	c.6850_6852del	p.D2284del
		West syndrome,Profound DD, hypotonia, ataxia, dyskinetic movement disorder, lack of visual attention; microcephaly	c.6908_6916dup	p.D2303_2305dup
		West syndrome (PEHO syndrome), profound DD, hypotonia, lack of visual attention; microcephaly	c.6908_6916dup	p.D2303_2305dup
		West syndrome, profound DD, hypotonia, lack of visual attention; microcephaly	c.6908_6916dup	p.D2303_2305dup
		West syndrome, profound DD, hypotonia, ataxia, dyskinetic movement disorder, lack of visual attention; microcephaly	c.6907_6915dup	p.D2303_2305dup
		West syndrome, profound DD, intermittent opisthotonus, hypotonia, lack of visual attention; microcephaly	c.6907_6915dup	p.D2303_2305dup
		West syndrome, mild ID, DD, delayed walking, mild attention deficit	c.6910_6918del	p.Q2304_G2306del
		West syndrome, profound DD, lack of visual attention; microcephaly	c.6923_6928dup	p.R2308_M2309dup
		Focal epilepsy	c.533G>A	p.G178D
		Epilepsy with myoclonic and atonic seizures ,moderate ID	c.917C>T	p.A306V
		No epilepsy, mild DD, ID, ASD; mild dysmorphic signs	c.3716A>G	p.H1239R
		Myoclonic epilepsy, mild–moderate DD, ID, ASD, hypotonia, mild spastic gait; Increased tone and deep tendon reflexes in lower limbs	c.4828C>T	p.R1610W
		FDS, moderate ID, ADHD	c.6908_6916del	p.D2303_L2305del
		Focal seizures, mild DD, ID, mild diffuse hypotonia, slowly progressive and severe cerebellar ataxia	arr[hg19] 9q34.11 (131,349,701–131,351,531)x1 exon 20–21 deletion	p.A927_L1002del
		Hereditary motor neuropathy	heterozygous c.415C4T	p.R139*
			heterozygous c.4615C4T	p.Q1539*
			heterozygous c.6385C4T	p.Q2149*
			heterozygous c.6781C4T	p.R2261*
SPTBN4 SPECTRIN, BETA, NONERYTHROCYTIC, 4 (SPECTRIN, BETAIV) NM_020971.2	19q13.2	Congenital myopathy,neuropathy, and central deafness	homozygous c.1597C>T	p.Q533*
		Global DD, hypotonia, dysphasia, recurrent respiratory infections, blue sclerae, hyporeflexia, failure to thrive	homozygous c.3394del	p.H1132Tfs*39
		Profound ID, congenital hypotonia, and motor axonal neuropathy	homozygous c.3820G>T	p.E1274*
			homozygous c.2709G>A	p.Trp903*
			c.1511G>A; c.7303C>T	p.R504Q; p.R2435C
			homozygous c.7453del	p.A2485Lfs*31
			c.1813C>T ; c.3829del	p.Q605*, p.Q1277Rfs*4
		Speech delay, ID, ataxia, seizures, cerebral atrophy	homozygous c.1665+2T>C	Intron
		Axonal neuropathy without ID	homozygous c.3949-1G>A	Intron

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bipolar disorder (BD); schizophrenia (SCZ); developmental delay (DD); epileptic encephalopathy (EE); infantile spasms (IS); focal dyscognitive seizures (FDS); progressive encephalopathy (PEHO).

In general, the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) states that neurodevelopmental disorders (NDDs) include intellectual disability (ID), communication disorders, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), specific learning disorder, and motor disorders [80]. However, NDDs are complex conditions [81]; therefore, this review will broadly cover various classes of central nervous system (CNS) disorders that manifest during development, such as epilepsy, mood disorders, and schizophrenia.

Among the many AIS-localized proteins, as shown in Table 1, we focus on three genes: *ANK-3*, which encodes human ankyrin-3 protein (also known as ankyrin-G), *SPTAN1*, which encodes spectrin alpha chain, non-erythrocytic 1 protein, and *SPTBN4*, which encodes spectrin beta chain, non-erythrocytic 4 protein. Ion channel mutations that cause channelopathy are well-described in excellent reviews [82,83].

In patients with bipolar disorder (BD), several studies have shown that *ANK-3* has strong predisposition to BD in a genome-wide association study [84-86]. Exon variation has also been intensively investigated in patients with BD. Interestingly, exon variation at a BD-associated SNP correlates with a significant difference in cerebellar expression of a brain-specific *ANK-3* transcript and contributes to disease pathology [87,88]. *ANK-3* is also associated with schizophrenia [89-91].

Recently, whole-exome sequencing allowed the identification of a pathogenic mutation in the *ANK-3* gene of patients with ASD (Table1) [92]. In addition, another mutation has been identified within in a family of patients with ASD [93]. Iqbal et al. reported multiple types of *ANK-3* mutations in patients with NDDs [94]: one patient had borderline intelligence, ADHD, ASD, and cognitive problems caused by the balanced chromosomal translocation of *ANK-3*, and in a Pakistani family, moderate ID, and ADHD-like phenotype and behavioral problems were associated with a homozygous single base pair truncating frameshift mutation in *ANK-3* [94]. Subsequently, several *ANK-3* mutations were found in patients with ID [95-98].More recently, two patients with NDDs and *ANK-3* mutations were identified; each exhibited mild-to-borderline ID, ASD-like features, and speech delays. One patient also exhibited developmental delays, seizures, cognitive impairment, ataxia, retinal dystrophy, and small stature [99].

The first pathogenic variant was found in *SPTAN1* in patients with West syndrome, a constellation of symptoms primarily characterized by epileptic/infantile spasms, abnormal brain electroencephalography (EEG) patterns called hypsarrhythmia, and ID (Table1); two patients possessed a heterozygous in-frame 3bp deletion or 6bp duplication in *SPTAN1* [100]. The patients exhibited intractable seizures at 3 months of age and showed ID, poor visual attention, lack of speech development, and spastic quadriplegia; in addition, brain magnetic resonance imaging (MRI) showed diffuse hypomyelination and extensive brain atrophy. Subsequently, several studies have also identified a mutation in *SPTAN1* in patients with a wide spectrum of neurodevelopmental phenotypes ranging from mild to severe (Table1) [101-104].

In addition, four heterozygous mutations in *SPTAN1* were recently identified in four different families with juvenile-onset hereditary motor neuropathy, a particularly rare subgroup of inherited peripheral neuropathies [105,106]. These patients typically exhibited a length-dependent axonal degeneration of lower motor neurons without apparent involvement of sensory neurons [105,106].

Recently, the first pathogenic variant of *SPTBN4* was reported [107]. Other studies have also show mutations in *SPTBN4* [108-112]. These congenital diseases are defined as SPTBN4 disorder or, alternatively, neurodevelopmental disorder with hypotonia, neuropathy, and deafness (NEDHND) [113].

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These results indicate that mutations in these AIS regulatory proteins are important for the development of the CNS.

8. Abnormal AIS characteristics in neurodevelopmental disorders

Studies on human mutations have shown that molecules that regulate the AIS are highly related to brain development. Finally, we discuss the changes in the properties of the AIS in animal models and patients with NDDs, as well as the potential use of homeostatic regulation for the treatment of NDDs.

The first report by Kaphzan et al. showed a significant increase in the AIS length of hippocampal pyramidal neurons in vivo in Angelman syndrome (AS) mouse model [114]. AS is an NDD caused by a loss of function of the maternally inherited *UBE3A* gene and is associated with symptoms of ID, epilepsy, cerebellar ataxia, sleep disorders; AS also shows high comorbidity with ASD. Changes were also found in the amplitude of the resting membrane potential, threshold potential, and action potential [114]. Interestingly, CA1 and CA3 pyramidal neurons have significantly longer AIS, whereas cortical neurons in the somatosensory area do not show alterations [114]. This is the first in vivo evidence of changes in AIS in an animal model of NDDs [114].

As mentioned earlier, a mutation in the *ANK-3* gene, which encodes the 480 kDa ankyrin-G isomer, induces NDDs in humans [99]. In addition, disruption of exon 37 induces specific giant ankyrin-G disruption, and exon 37 knockout mice showed reduced cortical gamma oscillations, which are associated with higher cognitive processes [99]. Further behavioral investigations of exon 37 knockout mouse may strongly support the use of the mouse as an ideal model animal for NDDs.

More recently, in a mouse model of fragile X syndrome, Booker et al. showed that the length of the AIS in CA1 neurons in the $Fmr1^{-/y}$ mouse hippocampus elongated with increasing cellular excitability. This change in length was not due to a decrease in AIS plasticity but instead to reduced input from the entorhinal cortex. These results indicate that the length of the AIS and cellular intrinsic hyperexcitability may reflect a decrease in synaptic input to CA1 neurons in a non-cell-autonomous fashion by homeostatic mechanisms [115].

We also found that ASD model mice exhibit abnormal AIS length at the forebrain, and it is likely that we could detect the abnormality in a circuit-specific fashion (unpublished results).

In humans, Hong et al. showed that the α2-$GABA_AR$ protein is reduced in the AIS of pyramidal cells in the prefrontal cortex in patients with ASD [116]. They first showed a significant decrease in the number of ChCs in the prefrontal cortex of patients with ASD [117], as well as that the reduction in α2-$GABA_AR$ in the pyramidal cell AIS is localized to layer III in ASD patients, presumably due to ChCs reduction [116]. These results support the idea that ChCs regulate cortical excitability of pyramidal neurons in the AIS domain.

Another study found that, in mouse models of ASD, mice with mutations in the transcription factor PAX6 exhibited changes of AIS that were significantly further from the cell body and exhibited longer AnkG staining of prethalamic neurons [118].

Taken together, these results suggest that the AIS is an indicator of structural changes as well as abnormal and homeostatic plastic changes in response to dysfunction of neural circuits.

9. Conclusions

As discussed above, a growing body of evidence suggests the importance of the pathophysiological role of abnormal AISes in NDDs. This then prompts the question: can we take advantage of these concepts to develop future treatments or diagnostic tools based on AIS changes? One of the most promising uses of AIS is as a biomarker for aberrant neural circuits in neurological and psychiatric disorders. In a variety of neurological and psychiatric disease models, abnormal AIS length has been widely observed [68-

74,114,115]. To determine cell-specific neural circuit abnormalities, researchers can use viral or classical retrograde or anterograde tracing methods in disease models. Then, chemogenetic or optogenetic techniques can be applied to modulate circuit activity and prove the aberrant circuits responsible for disease.

On the other hand, we may also ask: are there any potential therapies for NDDs? According to an excellent 2021 review, we now have the opportunity to treat NDDs therapeutically at the protein, neural circuit, and individual levels [119].

In particular, according to the results of a human study on ChCs in ASD [116], it may be useful to transplant human ChCs that have been differentiated from pluripotent cells into patients with ASD [119].

Recent advances in ultra-sensitive optogenetics may allow the use of implant-free deep brain optogenetics that could be used for human neural circuit-specific therapy [120,121]. If abnormal neural circuits were determined in disease models, human neural circuits could be modulated by the viral vector with optogenetic method, even deep in the brain.

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