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Kalirin Signaling: Implications for Synaptic Pathology

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Abstract Spine morphogenesis and plasticity are intimately linked to cognition, and there is strong evidence that aberrant regulation of spine plasticity is associated with physiological, behavioral, and pathological conditions. The neuronal guanine nucleotide exchange factor (GEF) kalirin is emerging as a key regulator of structural and functional plasticity at dendritic spines. Here, we review recent studies that have genetically and functionally linked kalirin signaling to a number of human disorders. Kalirin signaling may thus represent a disease mechanism and provide a novel therapeutic target.

Keywords Mental disorder · DISC1 · NRG1 · erbB4 · Glutamatergic · Postmortem · Genetic · Schizophrenia · Alzheimer's disease · ADHD · Addiction · Stroke

Introduction

Most excitatory synapses in the mammalian forebrain are located on dendritic spines. Actin dynamics can rapidly induce changes in spine morphology, which modulates synaptic properties and the potential for plasticity [1, 2]. During development, spine dynamics play a critical role in neural circuit formation. In mature neurons, synaptic activity drives spine dynamics, contributing to remodeling of neural circuits and experience-dependent plasticity [1, 2]. Several decades of research have documented changes in spine size and number associated with a number of physiological, behavioral, and pathological conditions. Importantly, circuit-level analysis indicates that relatively small changes in synapse strength or number may have a much greater effect on the overall function of circuits [3, 4].

Postmortem neuropathological studies using brain tissue from human patients have revealed that dendritic spine morphology and number are altered in a number of disorders of the central nervous system [5]. These alterations have been well characterized in intellectual disability, Down's syndrome [6], Rett syndrome [7], Fragile X syndrome [8], autism spectrum disorders (ASD) [9], schizophrenia [10, 11], and addiction [12–14]. Furthermore, synaptic pathology has been associated with neurodegenerative disorders including Alzheimer's [15], Parkinson's [16], and Huntington's disease [17].

Spine morphogenesis is driven by actin remodeling and membrane trafficking events, which are controlled by small GTPase signaling [18]. Small GTPases function as molecular switches: in the active, GTP-bound state they promote actin remodeling and trafficking, and they become inactive by hydrolyzing GTP to GDP. Abnormal small GTPase signaling has been associated with several human pathologies. Signaling pathways involving Rac and Ras are particularly important since a large proportion of genes causing intellectual disability encode proteins in these small GTPase pathways [19, 20]. Importantly, the only anatomical alteration reported in non-syndromic mental retardation is abnormal spine morphology, intimately connecting small GTPase signaling, spine morphogenesis, and human cognition [19, 20]. GTPases are modulated by guanine nucleotide exchange

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factors (GEFs) and GTPase activating proteins (GAPs). GEFs and GAPs are complex, multidomain signaling proteins that may integrate multiple signals and have tissue- and cell-specific functions [1]. Among these, the Rac/Rho-GEF kalirin has been associated with a range of psychiatric and neurodegenerative disorders.

Kalirin is highly is enriched in the forebrain and almost absent outside of the CNS; its most abundant isoform, kalirin-7, is localized to dendritic spines on cortical pyramidal neurons (Fig. 1a, b). Kalirin-7 plays a key role in structural and functional plasticity at excitatory synapses [21]. By activating Rac1 and its downstream effector, p21-activated kinase (PAK), kalirin facilitates actin remodeling [22, 23] such that overexpression of kalirin-7 leads to increased spine number, while knockdown leads to spine loss (Fig. 1c). Kalirin-7 has also been shown to mediate activity-dependent plasticity in dendritic spines. Xie and colleagues [24] found that NMDA receptor (NMDAR) activation-induced spine enlargement and increases in synaptic expression of AMPA receptors (AMPARs) were kalirin-7-dependent. Based on these well-characterized effects of kalirin on dendritic spines, it seems plausible that mutations or changes in expression of kalirin, or alterations in its upstream or downstream signaling partners, in human disorders would lead to aberrant dendritic spine number and morphology. Consistent with this, kalirin has been functionally and genetically implicated in the pathogenesis of several human disorders (see Table 1 for summary), most of which affect cognition and present with dendritic spine pathology. Here, we will discuss recent studies linking aberrant regulation of dendritic spine plasticity through altered kalirin signaling with several psychiatric and neurological disorders.

Kalirin and Schizophrenia

Schizophrenia is a psychiatric disorder that affects cognition and perception of reality that impacts 0.5–1% of the population. Symptoms emerge during late adolescence or early adulthood. The causes of this disease are not known and there are no effective treatments for the negative and cognitive symptoms. One of the most consistent neuropathological findings in postmortem studies of schizophrenic patients is reduced spine density in forebrain regions. Spine loss in the dorsolateral prefrontal cortex (DLPFC) [10] and auditory cortex have been observed in postmortem studies in schizophrenic patients [11]. Other studies have also reported reduced spine density in the subiculum and CA3 [25, 26]. Genetic and anatomical studies have linked a number of classical regulators of spine plasticity to schizophrenia; conversely, a number of schizophrenia susceptibility molecules have been shown to modulate spine morphology [5]. Such findings might shed light on the molecular mechanisms underlying spine pathology in schizophrenia. Among these molecules, several lines of evidence link altered kalirin signaling with schizophrenia.

In a postmortem study, kalirin mRNA expression was found to be reduced in the DLPFC of schizophrenia patients, irrespective of antipsychotic treatment [27]. Interestingly, kalirin loss correlated with spine loss on layer 3 PFC neurons [27]. A recent genome-wide association study (GWAS) in a Japanese population [28] detected association signals with schizophrenia at the region of the KALRN gene. Although single locus analysis did not reach genome-wide significance, the study confirmed a shared polygenic risk of schizophrenia between the Japanese and

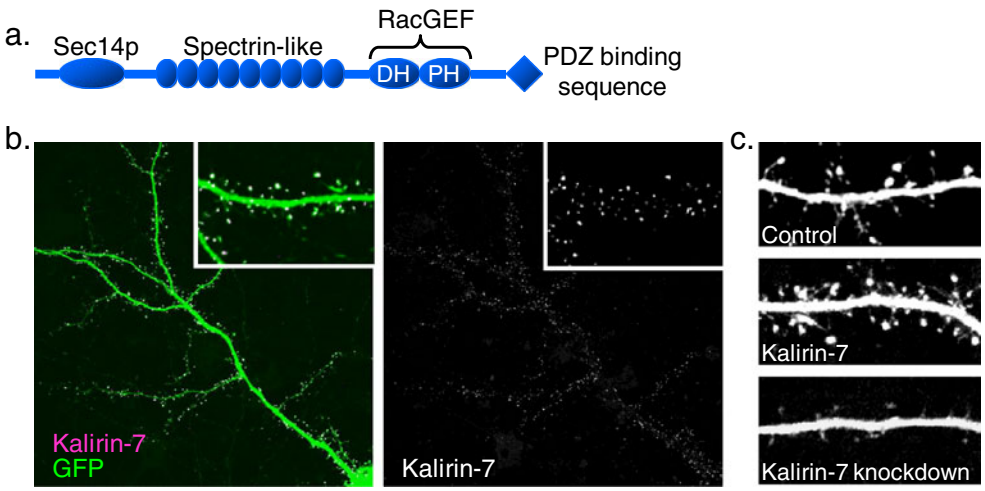

Fig. 1 Kalirin-7 is localized to dendritic spines and modulates dendritic spine morphogenesis. **a** This schematic of kalirin-7 domains shows the N-terminal Sec14p domain, followed by nine spectrin-like repeats. The Dbl homology (*DH*) and pleckstrin homology (*PH*) domains confer kalirin's GEF activity, and kalirin-7 has a C-terminal PDZ-binding domain. **b** Kalirin-7 is localized to dendritic spines of cortical pyramidal neurons. **c** Kalirin-7 regulates dendritic spine dynamics in cortical pyramidal neurons. Overexpression of kalirin-7 increases spine size and number, while RNAi-mediated knockdown reduces spine size and number

Table 1 Summary of evidence for kalirin association with disease

Disease	Genetic association	Postmortem evidence	Molecular association	Animal models	References
Schizophrenia	GWAS, rare missense mutations	↓ mRNA	DISC1, ErbB4, NRG1, 5-HT$_{2A}$, NMDAR, PSD-95	*KALRN* KO, Kal7 KO	[23, 27, 32, 34, 35, 41, 45, 50]
Alzheimer's disease	–	↓ mRNA ↓ protein	iNOS, PAK, EphB2	–	[59, 60, 66]
ADHD	GWAS	–	–	–	[68]
Addiction	–	–	–	Kal7 KO, addiction in mouse model	[75, 76]
Huntington's disease	–	–	HAP1	–	[73]
Stress	–	–	–	Chronic restraint stress in mice	[77]
Ischemic stroke	Case–control	–	–	Induced ischemia in mice	[80, 82]
Coronary artery disease	GWAS, association linkage	–	–	–	[78, 79]

the Caucasian samples. An independent study also supported this association [29]. Following up on these findings, Kushima and colleagues [30] re-sequenced all exons of the KALRN gene and identified several rare missense mutations enriched in patients with schizophrenia as compared to non-psychiatric controls. A number of these sequence alterations are predicted to have functional and damaging consequences. The authors detected a significant association of the P2255T mutation (OR=2.09) as well as combined association of all mutations (OR=2.07) with schizophrenia. Interestingly, several of these mutations are in exons encoding protein domains present in kalirin-9 or -12 but not kalirin-7. Thus, multiple rare missense mutations in *KALRN* may contribute to genetic risk in schizophrenia.

Kalirin-Interacting Proteins and Schizophrenia

Recent studies also demonstrate that kalirin-7 physically and functionally interacts with several proteins previously implicated in schizophrenia. Kalirin-7 directly interacts with DISC1 (disrupted in schizophrenia) [31], a protein product of a leading schizophrenia susceptibility gene. Hayashi-Takagi and colleagues [32] have shown that DISC1 functions as a scaffold for kalirin-7 and modulates the access of kalirin-7 to Rac1, controlling the duration and intensity of Rac1 activation in response to NMDAR activation. In this context, DISC1 functions as a scaffold that augments the kalirin-7/PSD-95 interaction; kalirin's release from DISC1 enhances its GEF activity leading to the modulation of spine structure. Knockdown of DISC1 in cultured neurons for extended periods of time causes a reduction in spine area [32]. If schizophrenia-associated mutations disrupt DISC1's scaffolding function, they would be expected to have deleterious consequences on spine morphogenesis though altered kalirin signaling (Fig. 2).

Kalirin also interacts with and is modulated by the 5-HT$_{2A}$ serotonin receptor (Fig. 2), a target of atypical antipsychotics which has also been genetically linked to schizophrenia [33]. Jones and colleagues [34] demonstrated that 5-HT$_{2A}$

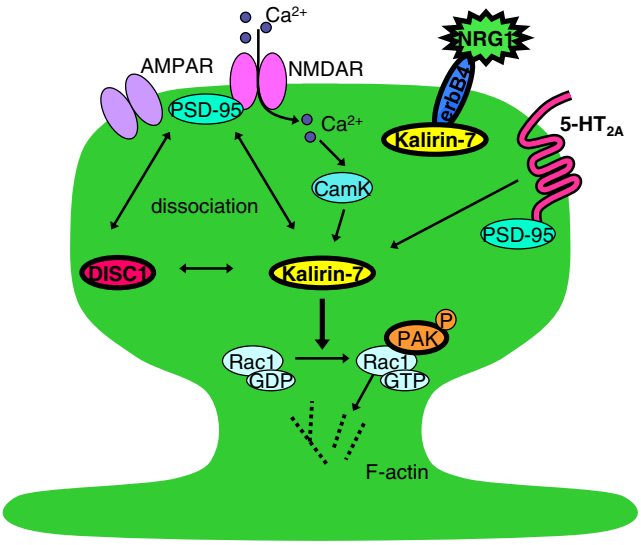

Fig. 2 Kalirin-7 interacts with schizophrenia-associated proteins. This model shows kalirin signaling and interactions under non-pathological conditions. Activation of postsynaptic NMDARs leads to dissociation of the PDS-95/DISC1/kalirin-7 complex, thus allowing kalirin-7 to activate its downstream target, Rac1. Activation of postsynaptic NMDARs also increases trafficking of AMPARs to the post-synaptic density. NRG1 activates post-synaptic erbB4 receptors which regulate kalirin-7. 5-HT$_{2A}$ receptor activation modulates dendritic spine density in a kalirin-dependent manner. Kalirin-7 activates Rac1 by exchanging GDP for GTP. Rac1 in turn activates PAK, which then initiates actin remodeling. Proteins outlined in bold have been genetically associated with schizophrenia (kalirin-7, DISC1, erbB4, NRG1, 5-HT$_{2A}$, PAK2, and PAK3)

modulates spine morphogenesis in a kalirin-dependent manner. 5-HT_{2A} receptors colocalize with PSD-95 and kalirin-7 in spines of mature cortical neurons. Treatment of cultured neurons with the 5-HT_{2A} receptor agonist DOI rapidly induced an increase in spine size and PAK phosphorylation, and both of these effects were dependent on kalirin-7 targeting to the PSD [34]. Thus, 5-HT_{2A} and kalirin-7 are functionally linked, and 5-HT signaling may modulate kalirin-7 activity and synapse size at mature cortical synapses. Based on these studies, 5-HT receptors may act as upstream regulators of kalirin-7 signaling and thus may contribute a neuromodulatory element to kalirin-7 signal integration in spines.

Kalirin-7 also participates in a common pathway with neuregulin 1 (NRG1) and erbB4, two prominent schizophrenia susceptibility molecules, to regulate the morphology of interneuronal dendrites [35]. The tyrosine kinase erbB4 is thought to be the principal receptor for NRG1. Polymorphisms in NRG1 have been identified in patients with schizophrenia [36]. A rare copy number variant (CNV) for erbB4 has also been associated with schizophrenia; this mutation results in a protein lacking most of its intracellular kinase domain that has the effect of a dominant negative protein [37]. ErbB4 is highly expressed in interneurons and, to a lesser extent, in cortical pyramidal cells and dendritic spines [38]. This suggested that NRG1/erbB4/kalirin signaling may play a role in interneurons. Indeed, while kalirin is robustly expressed in pyramidal neurons, it is also expressed in interneurons [39]. Interneuronal pathology is thought to play an important role in schizophrenia [40]. Ma and colleagues [41] demonstrated that endogenous kalirin-7 is localized to the postsynaptic side of excitatory synapses onto hippocampal interneurons. Consistent with this, Cahill and colleagues found that kalirin regulates interneuron dendritic growth downstream of NRG1 in mature cortical interneurons. Treatment of cortical interneurons with NRG1 increases dendritic length in a kalirin-dependent manner [35]. Conversely, cortical interneurons from erbB2/B4 conditional knockout mice have decreased dendritic length [35]. In cultured cortical interneurons, endogenous kalirin-7 colocalized with erbB4 and erbB4 co-immunoprecipitated with kalirin-7, indicating that kalirin-7 and erbB4 interact [35]. Kalirin may thus play a role in the normal development and function of inhibitory circuits, and its functional loss may contribute to the dysfunction of these circuits, in addition to excitatory circuits, in schizophrenia. NRG1 and erbB4 have been shown to affect spine morphology [38, 42], thus kalirin may also function downstream of these molecules in spines (Fig. 2).

P21-activated kinase (PAK) is a key regulator of actin remodeling, and a downstream effector of kalirin-7 and Rac1 [23]. Interestingly, missense mutations in PAK3 have been associated with psychotic disorders, primarily schizophrenia with premorbid mental retardation [43], and microdeletions that encompass PAK2 (3q29) have been found in schizophrenia patients [44]. These findings further implicate kalirin signaling in schizophrenia, as mutations in kalirin's downstream effectors are genetically associated with the disorder.

Taken together, these findings support a model whereby proteins associated with schizophrenia, NRG1, erbB4, DISC1, and 5-HT_{2A}, function in the same pathway with kalirin-7 and PAK to control spine morphogenesis (Fig. 2). These proteins might even form a "signalosome" [32], a multiprotein signaling complex in dendrites or spines. If the functional output of this pathway is the control of spine plasticity, alterations in any of the molecules in the pathway/complex might impair the output of the entire pathway, leading to spine pathology.

Kalirin Knockout Mice Have Disease-Relevant Phenotypes

Alterations in kalirin signaling may thus contribute to behavioral alterations relevant for schizophrenia. To test this, Cahill and colleagues generated a mouse line carrying a full knockout of KALRN [45]. Analysis of these mice revealed robustly reduced spine density in the cortex. Interestingly, KALRN KO mice have impaired working memory but do not show deficits in reference memory, indicating that kalirin may play a specific role in modulating working memory. Importantly, working memory deficits are a core endophenotype of schizophrenia. Recurrent excitation in prefrontal cortical microcircuits has been hypothesized to underlie working memory [46]. Reduced spine density on pyramidal neurons in such circuits, as it occurs in KALRN KO mice or human subjects with schizophrenia, may lead to gradual signal loss and diminished output of these circuits (Fig. 3). In addition to working memory deficits, KALRN KO mice exhibit other schizophrenia-related behavioral phenotypes such as impaired prepulse inhibition, decreased social interaction, and locomotor hyperactivity, which is reversed by the antipsychotic clozapine in KALRN heterozygote but not KO mice [45].

One unique feature of these mice is that the morphological and behavioral phenotypes emerge during adolescence, paralleling the onset of schizophrenia in humans, which also occurs during adolescence or young adulthood [47]. This particular feature might be useful in understanding the age dependence of schizophrenia emergence in humans. The molecular and cellular mechanisms underlying this delayed phenotype emergence are not known, and warrant more investigation. One contributor could be "phenotype unmasking", whereby natural changes in expression levels of similar proteins might uncover the effects of a genetic defect later in life. While multiple synaptic Rac1-GEFs (kalirin-7, Tiam1,

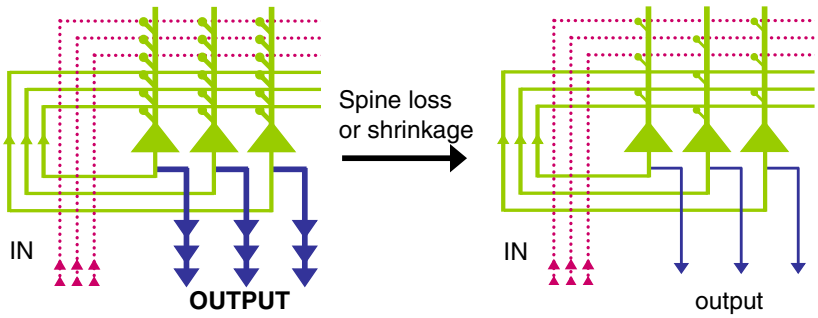

Fig. 3 The effects of spine loss on recurrent excitation in neocortical circuits. Reduced spine numbers may diminish signal output in recurrent cortical networks, and thus impair working memory

βPIX) are present in both the hippocampus and cortex in young animals, the expression levels of Tiam1 and βPIX decrease significantly in the adult cortex, but not hippocampus [48]. Thus, by adulthood, kalirin-7 is the predominant synaptic Rac1-GEF in the cortex. Kalirin loss in the KALRN KO mouse, or reduced levels in humans, may thus lead to enhanced spine elimination or impaired activity-dependent stabilization in the cortex. Interestingly, prenatal knockdown of DISC1 also results in delayed onset circuit dysfunction [49], suggesting that the kalirin/DISC1 interaction may play a role in peri-adolescent spine stabilization. An important future direction for these studies is to characterize the development of spine morphological and behavioral phenotypes that correspond to presymptomatic and symptomatic stages of the disease.

Ma and colleagues generated another kalirin mutant mouse line, Kal7 KO, in which the exon unique to kalirin-7 has been deleted [50]. While kalirin-7 and Δkalirin-7, a truncated isoform of kalirin-7 that is also expressed in the brain, are absent in the brains of Kal7 KO mice, kalirin-9 and kalirin-12 are upregulated by approximately 50% [50]. Contrary to the KALRN KO mice, Kal7 KO mice have significantly reduced spine size and density in the hippocampus [50]. As loss of the entire KALRN gene does not effect hippocampal spine density [45], upregulation of other kalirin isoforms may account for this, as both kalirin-9 and -12 (but not kalirin-7) contain RhoA GEF domains [51], which have been shown to play a role in spine elimination [52]. This suggests that compensatory upregulation of kalirin-9 or -12 in response to reduced kalirin-7 signaling might be a pathological mechanism in schizophrenia worth further investigation.

Kal7 KO mice have impaired contextual fear learning [50]. This learning deficit seems specific to fear associations, as Kal7 KO mice performed well in radial arm maze and novel object recognition tasks [50]. Consistent with this, in KALRN KO mice both context and cue-dependent fear conditioning are impaired, suggesting an amygdala deficit [53]. Both of these mouse models have deficits in contextual learning; however, the relevance of these deficits for schizophrenia is unclear. It seems plausible that behaviors in mouse models do not closely mimic behaviors observed in human disease because kalirin loss is global in the mouse, whereas it is likely cell population or region specific in humans. Both the KALRN KO and the Kal7 KO mice exhibit disease-relevant phenotypes and thus will be powerful tools for future investigation of mechanisms underlying synaptic plasticity and pathology.

Taken together, several lines of evidence support a role for kalirin in spine pathology in schizophrenia. Animal model studies suggest that these spine abnormalities might underlie specific behavioral alterations. Further studies in human subjects and model systems are needed to clarify whether kalirin alterations are a cause or consequence of spine pathology in schizophrenia, whether kalirin-mediated spine pathology is preferentially associated with specific disease diagnostic domains or phenotypes, and whether kalirin dysfunction is associated with specific subtypes of schizophrenia. In any case, therapeutically manipulating kalirin activity might provide an efficient way to reverse spine plasticity deficits in schizophrenia.

Kalirin and Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by loss of memory and other cognitive functions. AD affects 13% of Americans over the age of 65. Synapse and spine loss in the hippocampus and cortex are consistent postmortem findings in AD patients [54, 55]. Remarkably, synapse and dendrite loss are more robustly correlated with cognitive decline than neurofibrillary tangles or neuronal loss [54]. Postmortem analysis has also indicated that synapse loss occurs in mild cognitive impairment and worsens in AD, suggesting that synapse loss precedes the onset of AD [56]. Compensatory synaptic changes may counteract the effects of synapse loss in AD [57]. A better understanding of the early stages of the disease and the compensatory mechanisms that are acting at this stage would profoundly impact the direction of future investigation of therapeutic interventions in AD.

Several recent studies implicate kalirin, along with its upstream regulators and downstream targets, in the pathology of AD (Fig. 4) [58]. Postmortem studies determined that kalirin mRNA and kalirin-7 protein are specifically and significantly reduced in the hippocampus of AD patients compared to controls [59, 60]. It is unknown whether kalirin loss plays a causal role in the pathogenesis of AD, but it is possible that these effects are secondary and occur in response to the presence of Aβ. Furthermore, it remains to be determined if kalirin loss leads to spine loss in AD, or if spine loss precedes kalirin loss.

EphB2 receptors are upstream regulators of kalirin signaling [23] that have been implicated in synaptic degeneration induced by Aβ. Aβ oligomers accumulate in the brain of AD patients and have been shown to cause synapse dysfunction and degeneration, as well as impairments in glutamatergic function [61]. Postmortem studies of AD patient brains and studies using hAPP transgenic mice have found that EphB2 receptors are reduced in the hippocampus in AD [62]. Furthermore, treating cultured hippocampal neurons with Aβ oligomers leads to decreased surface expression of NMDARs and EphB2 receptors [63]. In addition, EphB2 and Aβ oligomers may interact directly as they can be co-immunoprecipitated in a cell-free system as well as from primary neuron homogenates [64]. A critical downstream effector of EphB2 and kalirin-7 signaling, PAK, has also been associated with AD. Postmortem analysis of AD patient brains found that PAK protein and activation are markedly reduced and that phosphoPAK was mislocalized in the hippocampus of AD patients as well as in AD transgenic mice [65].

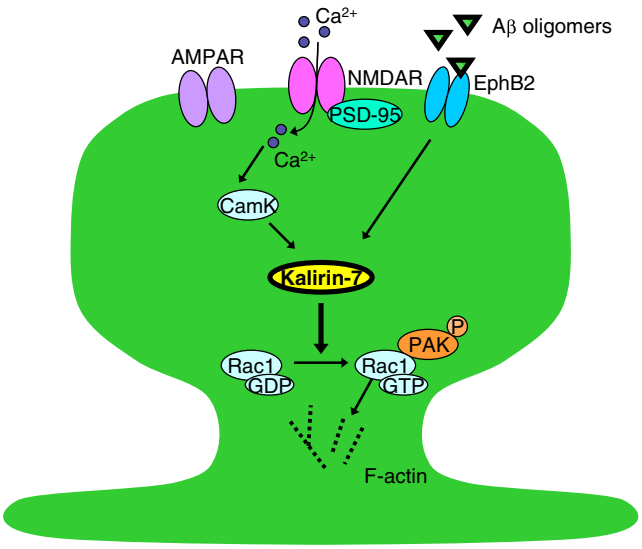

Fig. 4 Kalirin-7 interacts with Alzheimer's disease-associated proteins. Amyloid β oligomers cause a decrease in surface expression of NMDARs and EphB2 receptors in AD, which likely leads to decreased kalirin and PAK signaling, leading to synapse loss and cognitive impairment. Proteins associated with AD are outlined in *bold* (Aβ oligomers and kalirin-7)

Interestingly, kalirin-7 interacts with inducible nitric oxide synthase (iNOS) [66], which has been shown to be neurotoxic *in vitro* [67]. Kalirin inhibits iNOS activity by preventing formation of active homodimers in a heterologous system [66]. Postmortem studies found that iNOS activity was significantly increased in AD hippocampus [59]. Co-immunoprecipitation from human brain lysates confirmed that kalirin-7 and iNOS interact in the human brain [59]. Furthermore, overexpression of kalirin-7 in cultured cells reduces iNOS activity [59]. Thus, it has been hypothesized that reduced kalirin-7 expression in the hippocampus of AD patients could account for increased iNOS activity and possibly cell death [59].

Taken together, postmortem neuropathological and functional data suggest that the interactions of EphB and iNOS with kalirin-7 and its downstream effector PAK may be altered in AD, possibly due to the presence of Aβ oligomers at the synapse. Although there is no published genetic evidence implicating kalirin or any of the above proteins in the etiology of AD, current data support a potential role for these proteins in AD pathophysiology. Reduced function or loss of these proteins in spines could precipitate spine collapse, leading to synapse loss and cognitive decline (Fig. 4). Further studies in human subjects as well as in animal and cellular models are required to substantiate this hypothesis. If this proves to be the case, pharmacological enhancement of this pathway could provide a modality to delay or reverse synaptic pathology in AD, by fortifying synapses.

Kalirin and Other Disorders

Kalirin has been genetically and functionally associated with several other human disorders (Table 1). The KALRN gene has been genetically linked to adult ADHD. In a recent study, Lesch and colleagues [68] identified 15 SNPs in the *KALRN* open reading frame that associated with ADHD. In the same study, SNPs associated with ADHD were also identified in *CDH13*, the gene encoding cadherin 13 and *CTNNA2*, the gene encoding αN-catenin [68]. Cadherins have been shown to regulate spine morphology in a kalirin-dependent manner, thus aberrant function of one or both of these proteins may have detrimental effects on spine remodeling [69].

Huntington's disease (HD) is a genetic neurodegenerative disorder that is caused by expanded trinucleotide repeats in the widely expressed huntingtin protein [70, 71]. Huntingtin-associated protein 1 (HAP1) is one of only a few known proteins that interact with huntingtin in a repeat length-dependent manner [72]. Kalirin has been shown to

interact with HAP1 [73]. While this interaction requires further examination, it is of interest as it may provide insight into the functional significance of the interaction between huntingtin and HAP1, and also suggests a potential role for Rac signaling in HD.

Recent studies have also identified kalirin as a key factor in a mouse model of addiction. It has been suggested that drug addiction is the result of aberrant learning mechanisms, which could be explained by aberrant regulation of plasticity at dendritic spines [74]. Since kalirin has a key role in regulation of structural and functional plasticity at dendritic spines, it is possible that kalirin may play a role in addiction. Kiraly and colleagues used Kal7 KO mice to demonstrate that kalirin-7 is required for normal morphological and behavioral responses to chronic cocaine administration [75]. In wild-type mice, chronic cocaine treatment induced an increase in kalirin-7 protein and mRNA in the nucleus accumbens (NAc) as well as an increase in dendritic spine density and size in the NAc. Interestingly, chronic cocaine treatment in Kal7 KO mice did not alter spine density and induced a decrease in spine size. Moreover, Kal7 KO mice showed increased locomotor sensitization to cocaine following chronic treatment and maintained this higher sensitization for at least 1 week following the last dose of cocaine. In addition, Kal7 KO mice showed significantly diminished conditioned place preference for cocaine. Importantly, Kal7 KO mice showed no difference from wild-type mice in place preference for food, indicating a cocaine-specific deficit in place conditioning. Furthermore, chronic treatment with cocaine has been shown to alter the expression profile of specific kalirin isoforms by altering usage of isoform-specific promoters. Chronic cocaine exposure increases the usage of the promoter and the 3′ exon that are specific to kalirin-7 and Δ-kalirin-7, while usage of the kalirin-9 specific 3′ exon was reduced [76].

Kalirin signaling may also be involved in depression and chronic stress. Chronic restraint stress (CRS) causes a depression-like behavioral and physiological response in mice, including decreased time to immobility on a forced-swim test and reduced body weight [77]. Interestingly, recent studies have demonstrated that kalirin-7 expression is significantly reduced in the mouse hippocampus following CRS [77]. Furthermore, administration of estrogen during CRS attenuated the behavioral and physiological responses and increased kalirin-7 expression [77].

Genetic studies have identified KALRN as a risk factor for coronary artery disease [78, 79]. Based on this genetic evidence and the fact that kalirin has been implicated in nitric oxide (NOS) signaling, recent genetic studies have sought to determine if KALRN is genetically linked to ischemic stroke. In a case–control study, Krug and colleagues identified two SNPs in the 5′ region of *KALRN* and one in the *ROPN1–KALRN* intergenic region that is associated with increased risk for ischemic stroke [80]. However, the link between SNPs in the KALRN gene and risk for ischemic stroke requires further validation, as another study was unable to replicate the linkage of all three SNPs in two of the three sample populations used [81]. In addition, a recent study has characterized changes in kalirin-7 protein expression after ischemic stroke in the gerbil hippocampus. Beresewicz and colleagues [82] found that under control conditions, kalirin-7 levels were significantly higher in the ischemia-resistant CA2/3 and DG regions than in the ischemia-vulnerable CA1 region of the hippocampus. Interestingly, after 5 min of ischemia and 1 h of reperfusion, kalirin-7 was significantly reduced in CA2/3 and DG and was significantly elevated in CA1. In another study, Jourdain and colleagues [83] observed cytoskeletal rearrangements in organotypic hippocampal cultures following oxygen and glucose deprivation, these were found to be Ca^{2+}- and NMDAR-dependent. Thus, changes in kalirin-7 levels may act downstream of NMDARs in ischemia to induce cytoskeletal rearrangements.

In sum, accumulating evidence implicates kalirin dysfunction in a broad range of neurological and psychiatric disorders, in addition to schizophrenia and AD. As all of these disorders are associated with abnormal spine plasticity and neuronal connectivity, kalirin may be part of the disease pathway and may provide a therapeutic target for reversing these deficits.

Conclusions and Future Directions

Genetic, morphological, and functional studies suggest that kalirin might be a common mediator of synaptic pathology in several psychiatric and neurological disorders. The strongest evidence to date links kalirin to schizophrenia, but there is mounting evidence that also suggests possible roles in AD, addiction, chronic stress, stroke, and Huntington's. This is consistent with the fact that kalirin functions in common pathways with molecules previously associated with these disorders to regulate normal spine formation, maintenance, and plasticity. Kalirin-mediated spine pathology might thus be a cellular phenotype of interest in these disorders. It may be possible to rescue abnormal spine plasticity, and thus the associated behavioral deficits, by altering kalirin activity or function. Further studies, preferably in human subjects, are needed to strengthen these conclusions.

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