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Invited Mini Review

Neuronal function and dysfunction of CYFIP2: from actin dynamics to early infantile epileptic encephalopathy

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The cytoplasmic FMR1-interacting protein family (CYFIP1 and CYFIP2) are evolutionarily conserved proteins originally identified as binding partners of the fragile X mental retardation protein (FMRP), a messenger RNA (mRNA)-binding protein whose loss causes the fragile X syndrome. Moreover, CYFIP is a key component of the heteropentameric WAVE regulatory complex (WRC), a critical regulator of neuronal actin dynamics. Therefore, CYFIP may play key roles in regulating both mRNA translation and actin polymerization, which are critically involved in proper neuronal development and function. Nevertheless, compared to CYFIP1, neuronal function and dysfunction of CYFIP2 remain largely unknown, possibly due to the relatively less well established association between *CYFIP2* and brain disorders. Despite high amino acid sequence homology between CYFIP1 and CYFIP2, several *in vitro* and animal model studies have suggested that CYFIP2 has some unique neuronal functions distinct from those of CYFIP1. Furthermore, recent whole-exome sequencing studies identified *de novo* hot spot variants of *CYFIP2* in patients with early infantile epileptic encephalopathy (EIEE), clearly implicating CYFIP2 dysfunction in neurological disorders. In this review, we highlight these recent investigations into the neuronal function and dysfunction of CYFIP2, and also discuss several key questions remaining about this intriguing neuronal protein. [BMB Reports 2019; 52(5): 304-311]

INTRODUCTION

Actin cytoskeleton dynamics are critically involved in various aspects of neuronal development and function, including early migration, dendritic and axonal outgrowth and branching, and synapse formation and plasticity (1-3). Consistently, several mutations of genes encoding key regulators of the neuronal actin cytoskeleton have been associated with numerous neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorders (ASDs), intellectual disability (ID), schizophrenia (SCZ), and bipolar disorder (4-6). Among the key regulators, the WAVE regulatory complex (WRC) is a ∼400 kDa heteropentameric protein complex consisting of the Wiskott–Aldrich syndrome protein family verprolin-homologous protein (WAVE), cytoplasmic FMR1-interacting protein (CYFIP), Nck-associated protein (NAP), Abelson-interacting protein (ABI), and hematopoietic stem progenitor cell 300 (HSPC300) (7, 8). The WRC is basally inactive toward its downstream actin-nucleator, i.e., the actin-related protein 2/3 (Arp2/3) complex, because the activity-bearing C-terminal VCA (verprolin homology, central, and acidic regions) domain of WAVE is sequestered by an intermolecular interaction with CYFIP (8). However, diverse cellular signaling can release the VCA domain from the CYFIP, enabling it to bind to and activate the Arp2/3 complex. Specifically, inositol phospholipids and the active form of the small GTPase Rac1 (Rac1-GTP) can cooperatively recruit the WRC to the plasma membrane and induce its conformational changes (8). Considering that Rac1-GTP directly binds to the CYFIP during the activation process, CYFIP is involved in both basal inhibition and Rac1-induced activation of the WRC. Furthermore, phosphorylation of several residues of WAVE, CYFIP, and ABI can also modulate the WRC activity (9-11).

Two evolutionarily highly conserved members of the *CYFIP* gene family, namely *CYFIP1* and *CYFIP2* (also referred to as *SRA-1* and *PIR121*, respectively) exist (12). They are ∼145 kDa proteins, with a high homology in their amino acid sequences (88% identity and 95% similarity), suggesting similar functions at the molecular level. Originally, the CYFIP1/2 proteins were identified as binding partners of the fragile X mental retardation protein (FMRP) (12), whose loss causes the fragile X syndrome (FXS), which is characterized by ID, autistic behaviors, and dysmorphic features (13). Indeed, in addition to the WRC, CYFIP1 forms a complex with both the FMRP and the eukaryotic initiation factor 4E (eIF4E) to regulate the translational initiation of target messenger RNAs (mRNAs) (14). Notably, the brain-derived neurotrophic factor (BDNF)-induced activation of Rac1 has been shown to redistribute CYFIP1 from the FMRP-eIF4E complex to the WRC, thereby

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orchestrating both activity-dependent local protein synthesis and actin remodeling in neuronal synapses (15). Clinically, structural abnormalities (deletions and duplications) of the chromosomal region (15q11-q13) encompassing *CYFIP1* were identified in patients with ASDs and ID (16). *CYFIP1* has also been associated with SCZ (17). Furthermore, several lines of *Cyfip1* mutant mice expressed abnormal synaptic morphology and function, as well as either FXS- or ASD-like behaviors (18-20).

In contrast to CYFIP1, the neuronal function and dysfunction of CYFIP2 remain largely unknown, possibly given the relatively less well established association between *CYFIP2* and brain disorders thus far (16). Nevertheless, despite the high sequence homology between CYFIP1 and CYFIP2, several lines of evidence suggest that they may have distinct functions *in vivo*. For example, both *Cyfip1* and *Cyfip2* null mice display lethality at different developmental time points (i.e., at early embryonic and perinatal stages, respectively) (20-23). Furthermore, recent whole-exome sequencing (WES) studies identified *de novo* hot spot mutations of *CYFIP2* in patients with early infantile epileptic encephalopathy (EIEE) (24, 25). In this review, we highlight recent *in vitro*, animal model, and human genetic investigations revealing the neuronal function and dysfunction of CYFIP2, and discuss the key remaining issues for a better understanding of this intriguing, yet largely uncharacterized, protein in the nervous system.

IN VITRO NEURONAL STUDIES

Through the overexpression of green fluorescent protein (GFP)-tagged CYFIP1 and CYFIP2, Pathania *et al.* investigated the subcellular localization of these proteins in cultured rat hippocampal neurons (26). Both CYFIP1-GFP and CYFIP2-GFP exhibited punctate clusters highly localized to the dendritic spines, which are small protrusions on the neuronal dendrites representing excitatory postsynaptic compartments in mature neurons (27). Consistently, both proteins co-localized with endogenous excitatory synaptic markers, namely the Homer protein homolog (Homer) and the vesicular glutamate transporter (VGLUT). Indeed, the excitatory synaptic localization of the CYFIP1/2 proteins was further supported by several biochemical studies. For example, the CYFIP1/2 proteins were identified as components of the postsynaptic proteome through mass-spectrometry analyses of both the purified human and rodent postsynaptic density (28, 29). Moreover, both proteins were recognized as key nodes of the mouse *in vivo* postsynaptic protein interactome (30), whereas CYFIP2 was identified as an *in vivo* postsynaptic scaffold SH3 and multiple ankyrin repeat domains 3 (Shank3) interactor (31). In addition to their postsynaptic localization, Pathania *et al.* also reported that overexpression of either CYFIP1 or CYFIP2 increased dendritic branching and outgrowth of cultured hippocampal neurons (26). In the same study, the effects of *Cyfip1*, but not of *Cyfip2*, haploinsufficiency (in *Cyfip1*$^{+/-}$ mice) on both hippocampal dendritic spine density and morphology were explored, and an increase in immature long and thin spines compared to wild-type (WT) mice was observed.

A large-scale *in vivo* phospho-proteomic analysis of nine mouse tissues (32) identified two brain-specific phosphorylation sites (S582 and T1067) of CYFIP2 (11). Furthermore, Lee *et al.* overexpressed WT, phospho-blocking (i.e., S582A and T1067A), and phospho-mimetic (i.e., S582D and T1067E) mutants of CYFIP2 in cultured hippocampal neurons to understand functional significance of such sites (11). With regard to dendritic spine regulation, overexpression of the T1067A mutant, but not that of either WT or other mutants, decreased the presence of stubby spines compared to control neurons, suggesting a dominant-negative effect of T1067A. In addition, while overexpression of WT CYFIP2 increased neurite outgrowth, the same was not true for T1067A. Intriguingly, when mapped on the crystal structure of the WRC (31), CYFIP2 T1067 was positioned at the interaction interface between the CYFIP and the NAP, and T1067 phosphorylation was predicted to weaken such an interaction (11). Lee *et al.* proposed a model based on these findings. It described that CYFIP2 T1067 phosphorylation, together with additional modifications of the WRC (such as bindings of inositol phospholipids and Rac1-GTP (8)), contributed to WRC activation through the disassembling of the self-inhibitory interactions among its components (11). In contrast to T1067 phosphorylation, the functional roles of CYFIP2 S582 phosphorylation, which is known to occur on the N-terminal outer surface of CYFIP2 in the WRC, are yet to be defined (11).

ANIMAL MODEL STUDIES

The *in vivo* neuronal functions of CYFIP were initially characterized in the *Drosophila*, which expresses only one homolog of CYFIP (*dCYFIP*) (16). In fact, Schenck *et al.* demonstrated that CYFIP is specifically expressed in the central nervous system (CNS) of *Drosophila*, and is localized at synaptic terminals in the neuromuscular junctions (NMJs) (33). The length of the synaptic terminal was reduced in the NMJs of *dCYFIP* mutant larvae, compared to WT larvae, while the synaptic boutons had immature supernumerary buds (33). Notably, the reduced synaptic terminal length of *dCYFIP* mutants was the opposite phenotype of that observed in the *Drosophila FMR1* (*dFMR1*) null NMJs (34). Indeed, Abekhoukh *et al.* determined that the NMJs of *dCYFIP;dFMR1* double homozygous mutants were similar to WT NMJs, and not to those present in either of the single mutants. This suggests a genetic interaction between *dCYFIP* and *dFMR1* associated with the regulation of NMJ synaptic structures (35). Furthermore, Zhao *et al.* identified further detailed structural and functional changes in *dCYFIP* mutant NMJs (36). Specifically, enlarged synaptic vesicles and additional cisternae in the synaptic boutons were found through electron microscopic analysis of *dCYFIP* mutants. Moreover, given that *dCYFIP* mutants also showed functional defects related to the release of neurotransmitters under high-frequency stimulation, Zhao *et al.* suggested that dCYFIP regulates synaptic endocytosis by inhibiting F-actin assembly (36).

The *nevermind* (*nev*) mutant zebrafish was isolated from a large-scale forward genetic screening to identify the genes involved in retinotectal axon pathfinding (37, 38). Specifically, the dorsonasal axons of retinal ganglion cells (RGCs) are missorted with ventral axons in *nev* mutant zebrafish, and are abnormally projected through the dorsal tectum (even though they still targeted to the ventral tectum). Pittman *et al.* identified the *nev* gene to encode zebrafish *Cyfip2* (39). In fact, while the zebrafish expresses both CYFIP1 and CYFIP2 (86% identical), its CYFIP2 is 98% identical to that in humans (12). Pittman *et al.* also reported a broad *Cyfip2* expression in the zebrafish CNS, even though the other axonal tracts of *nev* mutants, including those of both the primary motor neurons and Mauthner neurons, were normal (39). Furthermore, Pittman *et al.* found that retinal lamination was disrupted in *nev* mutants (39). Additionally, Cioni and colleagues identified more detailed molecular and cellular mechanisms behind the optic tract missorting in *Cyfip2* mutant zebrafish (40). By combining the molecular replacement approach and time-lapse imaging of axonal growth cones, they showed the involvement of CYFIP2 in both homotypic axonal fasciculation and heterotypic axonal repulsion. Specifically, CYFIP2 was translocated to the growth cone in response to axon-axon contact, and regulated the filopodial dynamics to induce either axonal fasciculation or repulsion. Unlike *Cyfip2*, CRISPR/Cas9-mediated deletion of *Cyfip1* caused axonal growth defects in the optic tract, suggesting that CYFIP1 and CYFIP2 are specifically involved in axonal growth and sorting, respectively (40). In addition to the visual system, the CYFIP2 function in the auditory system of the zebrafish was also characterized. Marsden *et al.* performed an n-ethyl-n-nitrosourea (ENU)-based forward genetic screening to identify zebrafish mutants with reduced auditory startle thresholds (i.e., enhanced startle response) and isolated the *Cyfip2* mutant as one of five lines having such a phenotype (41). The zebrafish startle circuit is composed of auditory afferents (VIII), excitatory spiral fiber (SF) interneurons, and Mauthner cells. Marsden *et al.* showed that the activity of this circuit was enhanced in *Cyfip2* mutant zebrafish, as a result of either the increased excitability of SF interneurons or the enhanced excitatory synaptic input to SF interneurons (41). Although the detailed molecular mechanism underlying such a phenomenon is yet to be defined, the FMRP was not shown to be involved in this auditory phenotype of *Cyfip2* mutant, as the *fmr1* mutant zebrafish presented a normal innate startle threshold.

The initial genetic evidence supporting the neuronal functions of CYFIP2 in mice derived from quantitative trait locus (QTL) analyses aimed at identification of the genetic loci contributing to the behavioral differences between the C57BL/6J and C57BL/6N mouse substrains (21, 42). Specifically, the C57BL/6N substrain was branched out from original C57BL/6J substrain in the 1950s, and is currently the second major C57BL/6 mouse substrain. However, whole genome sequencing identified only approximately 10,000 single nucleotide polymorphisms (SNPs) between the two substrains (43). Therefore, QTL analysis can be useful for detecting the causal genetic factors underlying certain phenotypic differences between such substrains. Kumar *et al.* described the C57BL/6N substrain as having a lower behavioral response (as measured by locomotor activity) to psychostimulants (such as cocaine and methamphetamine), compared to C57BL/6J (21). Through the performance of a QTL analysis, Kumar *et al.* identified that a nonsynonymous SNP (G to A at the 46,036,117 base pair of chromosome 11, mm9) producing a serine-to-phenylalanine missense mutation (S968F) in CYFIP2 was responsible for the difference in the psychostimulant responses (21). Successively, Kirkpatrick *et al.* used a similar QTL analysis to show that such a *Cyfip2* variant was also causally associated with the enhanced binge eating-like behavior of the C57BL/6N substrain, compared to the C57BL/6J substrain (42). With regard to the molecular mechanism behind such a phenomenon, Kumar *et al.* reported the S968F CYFIP2 proteins to be less stable than WT CYFIP2 when overexpressed in HEK293T cells (21). However, recent structural analysis of a WRC-Rac1 complex by cryo-electron microscopy suggested that the S968F CYFIP2 could cause excessive activation of the WRC by Rac1 (44). Therefore, further molecular and cellular studies are required to understand the process by which a single CYFIP2 variant changes the behavioral phenotypes in mice.

Both *Cyfip1* and *Cyfip2* null mice show lethality at different developmental time points. Specifically, while the *Cyfip1* null embryos die around embryonic day 9.5 (E9.5) (20, 26), the *Cyfip2* null (*Cyfip2*$^{-/-}$) mice die perinatally (21, 22). Recently, Zhang *et al.* revealed that, although *Cyfip2*$^{-/-}$ mice survived until E18.5, with an expected Mendelian inheritance ratio they would all die at postnatal day 0 (P0) (23). Therefore, it is conceivable that *Cyfip2*$^{-/-}$ mice could die very soon after birth (21). Although the detailed cause of lethality remains unknown, Zhang *et al.* also reported that *Cyfip2*$^{-/-}$ mice were smaller than either WT or heterozygous (*Cyfip2*$^{+/-}$) littermates at E18.5 (23). Specifically, the crown-rump length was shorter in *Cyfip2*$^{-/-}$ mice, suggesting defects in their body curvature. Nevertheless, both the brain size and overall cytoarchitecture were comparable between WT, *Cyfip2*$^{+/-}$ and *Cyfip2*$^{-/-}$ mice at E18.5. Subsequent to an RNA-sequencing analysis, however, Zhang *et al.* found the extracellular matrix (ECM)-related genes to be significantly altered in the *Cyfip2*$^{-/-}$ embryonic cortex, compared with the WT cortex (23). Whether the abnormal expression of ECM-related genes can affect the neuronal development and function in *Cyfip2*$^{-/-}$ embryos, and whether such changes can be associated with their early postnatal lethality remains to be investigated.

Given the early postnatal lethality of *Cyfip2*$^{-/-}$ mice, *Cyfip2*$^{+/-}$ mice were used to characterize the functions of CYFIP2 in adult brains. Han *et al.* hypothesized that CYFIP2 could be one of the key actin-regulatory proteins mediating the dendritic spine abnormalities observed in fragile X mental retardation 1 (*Fmr1*)-null (*Fmr1*$^{-/y}$) mice, an animal model of FXS (22). In addition to the protein-protein interaction between CYFIP2 and FMRP (12), the *Cyfip2* mRNA has been ranked as the ninth FMRP target among 842 FMRP-associated brain polyribosomal mRNAs (45). Therefore, it is plausible that the basal and/or activity-induced translation of *Cyfip2* mRNA can be altered in *Fmr1*$^{-/y}$ mice, possibly contributing to the abnormal actin-rich dendritic spines found in these mice. Moreover, Han and colleagues found that, similar to *Fmr1*$^{-/y}$ mice, adult *Cyfip2*$^{+/-}$ mice exhibited some FXS-like behaviors, including hyper locomotor activity, reduced acoustic startle response, and enhanced pre-pulse inhibition (22). Notably, such behavioral phenotypes were aggravated in the *Fmr1*$^{-/y}$;*Cyfip2*$^{+/-}$ double mutant mice. In concordance, the dendritic spine abnormality seen in the *Fmr1*$^{-/y}$ cortex (i.e., the increase in immature thin spines) was aggravated in double mutant mice (22). The basal levels of both the CYFIP2 and WAVE1 proteins were normal in the hippocampus and cortex of *Fmr1*$^{-/y}$ mice. Han *et al.* showed the treatment of cultured cortical neurons with a metabotropic glutamate receptor (mGluR) agonist 3,5-dihydroxyphenylglycine (DHPG) to induce the expression of CYFIP2 proteins, which was blocked by the translational inhibitor cycloheximide (22). Notably, both the DHPG-induced expression of CYFIP2 and the DHPG-induced dendritic spine remodeling were impaired in both *Fmr1*$^{-/y}$ and *Cyfip2*$^{+/-}$ cortical neurons. Therefore, *Cyfip2* mRNA can be translated in an mGluR-dependent manner, as a target of the FMRP-associated polyribosome, and newly expressed CYFIP2 proteins may be involved in dendritic spine remodeling (22). Nevertheless, the causal relationship between such molecular and cellular changes in *Cyfip2*$^{+/-}$ neurons and the behavioral phenotypes of *Cyfip2*$^{+/-}$ mice remains to be further validated.

CYFIP2 MUTATIONS IN EARLY INFANTILE EPILEPTIC ENCEPHALOPATHY

In contrast to the well-established associations between *CYFIP1* and either ASDs, ID, or SCZ, *CYFIP2* has not been clearly associated with any brain disorders until recently (16). Although interstitial deletions of the chromosomal region 5q33.3-q35.1 harboring *CYFIP2* have been identified in individuals with developmental delay, mild to severe ID, and seizures (46, 47), those deletions included tens of additional genes, preventing the identification of the causal gene(s). Furthermore, reduced protein expression of CYFIP2 in postmortem brains of patients with SCZ (48) and Alzheimer's disease (49) was reported, even though its causal relationship with the disease pathophysiology was not clearly established. Notably, two recent WES studies similarly identified *de novo* hot spot (Arg87) variants in *CYFIP2* from patients with EIEE (24, 25). EIEE (OMIM 308350, also referred to as the Ohtahara syndrome) is a neurological disorder characterized by the occurrence of seizures, which begin within a year of birth (50). This disorder often progresses to West syndrome, which is characterized by epileptic spasms with hypsarrhythmia on the electroencephalogram (EEG) and severe psychomotor developmental delay (51).

In the first study, Nakashima *et al.* identified three *de novo CYFIP2* variants at the Arg87 residue (Arg87Leu, Arg87Cys, and Arg87Pro) in four unrelated patients diagnosed with West syndrome (24). All four individuals presented their first seizure event prior to the age of 6 months and reported additional common features, including hypotonia, microcephaly, developmental delay, and ID. Using the available crystal structure of the WRC (8), Nakashima and colleagues descried the Arg87 residue of CYFIP1 (the solved WRC structure contains CYFIP1, not CYFIP2) to be at an interface between CYFIP1 and WAVE1 (24). Specifically, the side chain of Arg87 formed hydrogen bonds with both the main chain of Tyr151 in WAVE1 and the side chains of Glu625 and Glu690 in CYFIP1. Importantly, the three Arg87 variants were predicted to disrupt these hydrogen bonds, thereby destabilizing the interaction between CYFIP1 and WAVE1. Indeed, all three CYFIP2 variants showed weaker interaction with the purified VCA domain of the WAVE, compared to WT CYFIP2 (24). Furthermore, transfection of CYFIP2 mutants, but not WT, induced an aberrant F-actin accumulation in cultured mouse melanoma cells (24); an expected finding, given that the mutants released the VCA domain of the WAVE, leading to activation of the downstream Arp2/3 complex. In a subsequent investigation, Peng *et al.* performed a WES analysis of 56 Chinese families with West syndrome and identified 112 variants in 89 genes, which included a *de novo* Arg87Cys variant of *CYFIP2* (25). Intriguingly, an in-frame deletion variant of *CYFIP1* was also described. Overall, these studies suggest that *de novo* hot spot (Arg87) variants of *CYFIP2* can be causally associated with West syndrome, potentially through the induction of aberrant WRC activation (i.e., gain-of-function) and F-actin polymerization.

PERSPECTIVES AND CONCLUSIONS

As highlighted in the current review, although a growing amount of evidence supports distinct *in vivo* functions of CYFIP1 and CYFIP2 (35, 40), the detailed mechanisms underlying such a phenomenon are largely unknown. Distinct spatiotemporal expression patterns of CYFIP1 and CYFIP2 may contribute to this (40, 52). For example, while a preliminary analysis found both the *Cyfip2* mRNA and protein to be enriched in the cortex, compared with other brain regions of mice, those of *Cyfip1* were observed to be evenly expressed in different brain regions (unpublished data). This may, at least partly, explain the cortical, but not hippocampal, dendritic spine changes seen in *Cyfip2*$^{+/-}$ mice (22). Moreover, a different expression of CYFIP1 and CYFIP2 in brain non-neuronal cells cannot be excluded. Indeed, a mouse brain cell-type-specific RNA-sequencing database (http://www.brainrnaseq.org/) (53) highly detected the presence of *Cyfip1* mRNA in microglia/macrophage, whereas *Cyfip2* mRNA was abundant in newly-formed oligodendrocytes and neurons. In addition to their basal expression, neuronal activity-induced expression of *Cyfip1* and *Cyfip2* can be differentially regulated. For example, while *Cyfip2* mRNA was identified as a high ranked FMRP target (45), the same was not true for *Cyfip1*, indicating that only the dendritic transport, stability, and local translation of *Cyfip2* mRNA can be regulated by FMRP in an activity-dependent manner (22, 54). In accord, *Cyfip2* mRNA was detected in neuronal dendrites (55). It is also possible that, despite their high sequence homology, CYFIP1 and CYFIP2 may have distinct groups of protein interactors. For example, in the original study which identified both CYFIP1/2 as direct binding partners of FMRP (12), CYFIP2, but not CYFIP1, was also shown to interact with FMRP-related proteins (FXR1P/FXR2P). Several independent investigations, using different biochemical approaches, recently identified the protein interactors specific to both CYFIP1 and CYFIP2 (15, 21, 30, 40). A side-by-side comprehensive analysis of these protein lists may provide another important insight into understanding the diverse functions of CYFIP1 and CYFIP2 *in vivo*.

Detailed molecular and cellular mechanisms underlying the processes by which the Arg87 variants of *CYFIP2* lead to the neurological symptoms of West syndrome remain to be further investigated (Fig. 1). Nakashima *et al*. proposed that an aberrant increase in WRC activity and downstream actin polymerization may change the neuronal excitatory synaptic function, thereby disrupting the excitatory/inhibitory (E/I) synaptic balance (24) (Fig. 1A), which is considered one of the key mechanisms behind epilepsy, and other neurodevelopmental and neuropsychiatric disorders (56, 57). Not only postsynaptic functions, but also excitatory (and inhibitory) presynaptic functions may be regulated by CYFIP2 (Fig. 1B), since CYFIP1 regulates presynaptic terminal size and vesicle release probability in the developing hippocampus (58). Another possibility is that the change in neuronal excitability (neuronal propensity to generate action potential firing) by the *CYFIP2* variants, is caused by affecting the surface expression and/or functional properties of certain ion channels. Notably, Chen *et al*. indicated that the WRC can potentially interact

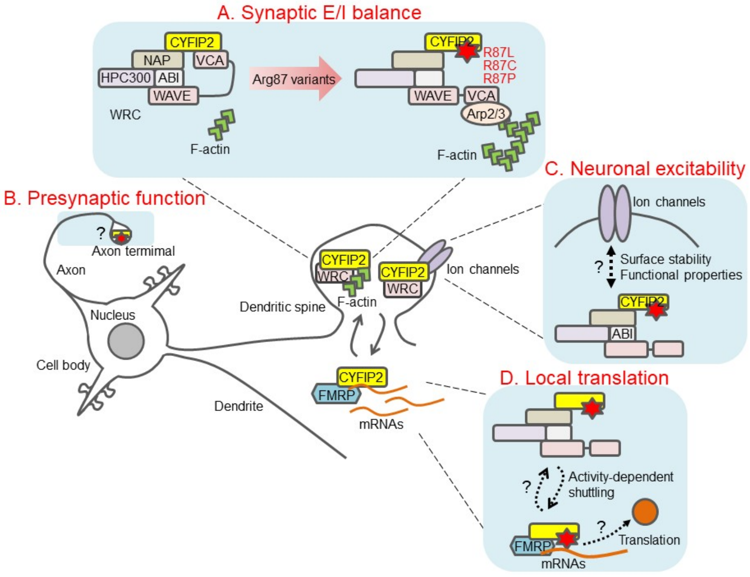

Fig. 1. Neuronal functions of CYFIP2 and potential effects of the Arg87 variants identified in the West syndrome on those functions. (A) The Arg87 variants weaken the inhibitory CYFIP2-VCA interaction, thereby leading to aberrant activation of WRC and the downstream actin polymerization. This may result in dendritic spine remodeling and change excitatory/inhibitory synaptic balance, in favor of more excitation. (B) Similar to CYFIP1, CYFIP2 may also regulate neurotransmitter release in the presynaptic compartment, and the Arg87 variants may affect this function of CYFIP2. (C) The WRC interacts with certain ion channels potentially involved in regulating neuronal excitability. Given that CYFIP and ABI of the WRC form an interaction surface toward those membrane proteins, the Arg87 variants may indirectly affect such interactions, thereby changing surface stability or other functional properties of ion channels. (D) In addition to the WRC, CYFIP2 also interacts with some mRNA-binding proteins, such as FMRP, to regulate target mRNA transport and local translation. The Arg87 variants may affect formation of these mRNA-regulatory protein complexes, or their function in regulating target mRNAs.

with ∼102 diverse membrane proteins, including protocadherins, G protein-coupled receptors (GPCRs), and ion channels (59). Importantly, a detailed structural analysis revealed that both the CYFIP and ABI of the WRC formed an interaction surface toward those membrane proteins. Therefore, the *CYFIP2* variants found in West syndrome may indirectly affect the interactions between the WRC and certain ion channels, possibly leading in turn to changes in both the ion channel properties and neuronal excitability (Fig. 1C). Furthermore, the FMRP-mediated regulation of expression of the proteins involved in modulating the synaptic E/I balance or the neuronal excitability may also play a role (60). Similarly to CYFIP1, CYFIP2 can form a complex with the FMRP and other RNA-binding proteins to regulate mRNA transport and local translation (40), whereas the Arg87 variants may affect this process (Fig. 1D). An additional comprehensive analysis of the expression, localization, interaction, and activity-dependent regulation of CYFIP2 using various *in vitro* and *in vivo* approaches will elucidate its unique functions, which can have implications for both the proper neuronal development and the pathophysiology of several brain disorders.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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