Altered presynaptic function and number of mitochondria in the medial prefrontal cortex of adult Cyfip2 heterozygous mice

Gyu Hyun Kim1†, Yinhua Zhang2,3†, Hyae Rim Kang2,3†, Seung-Hyun Lee4†, Jiwon Shin1, Chan Hee Lee1, Hyojin Kang5, Ruiying Ma2,3, Chunmei Jin2,3, Yoonhee Kim2, Su Yeon Kim2,6, Seok-Kyu Kwon6, Se-Young Choi4*, Kea Joo Lee1,7* and Kihoon Han2,3*

Abstract

Variants of the cytoplasmic FMR1-interacting protein (CYFIP) gene family, CYFIP1 and CYFIP2, are associated with numerous neurodevelopmental and neuropsychiatric disorders. According to several studies, CYFIP1 regulates the development and function of both pre- and post-synapses in neurons. Furthermore, various studies have evaluated CYFIP2 functions in the postsynaptic compartment, such as regulating dendritic spine morphology; however, no study has evaluated whether and how CYFIP2 affects presynaptic functions. To address this issue, in this study, we have focused on the presynapses of layer 5 neurons of the medial prefrontal cortex (mPFC) in adult Cyfip2 heterozygous (Cyfip2+/−) mice. Electrophysiological analyses revealed an enhancement in the presynaptic short-term plasticity induced by high-frequency stimuli in Cyfip2+/− neurons compared with wild-type neurons. Since presynaptic mitochondria play an important role in buffering presynaptic Ca2+, which is directly associated with the short-term plasticity, we analyzed presynaptic mitochondria using electron microscopic images of the mPFC. Compared with wild-type mice, the number, but not the volume or cristae density, of mitochondria in both presynaptic boutons and axonal processes in the mPFC layer 5 of Cyfip2+/− mice was reduced. Consistent with an identification of mitochondrial proteins in a previously established CYFIP2 interactome, CYFIP2 was detected in a biochemically enriched mitochondrial fraction of the mouse mPFC. Collectively, these results suggest roles for CYFIP2 in regulating presynaptic functions, which may involve presynaptic mitochondrial changes.

Keywords: Presynapse, Mitochondria, Medial prefrontal cortex, Cytoplasmic FMR1-interacting protein 2

© The Author(s). 2020 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Main text

The two members of the cytoplasmic FMR1-interacting protein (CYFIP) family, CYFIP1 and CYFIP2, are evolutionarily highly conserved proteins involved in actin cytoskeleton dynamics and mRNA regulation in neurons [1]. Importantly, both CYFIP1 and CYFIP2 genes are associated with various types of brain disorders, including autism spectrum disorders, intellectual disability, schizophrenia, and epilepsy, suggesting critical roles of CYFIP1 and CYFIP2 in proper brain development and function [2, 3]. Specifically, in the synaptic compartment, CYFIP1 and CYFIP2 in proper brain development and function [2, 3].

Importantly, both CYFIP1 and CYFIP2 genes are associated with various types of brain disorders, including autism spectrum disorders, intellectual disability, schizophrenia, and epilepsy, suggesting critical roles of CYFIP1 and CYFIP2 in proper brain development and function [2, 3]. Specifically, in the synaptic compartment, CYFIP1 regulates presynaptic vesicle release [4], excitatory postsynaptic dendritic spine morphology [5], and inhibitory synaptic assembly and transmission [6]. The roles of CYFIP2 in regulating dendritic spine morphology and excitatory synaptic transmission have been investigated [7–9], but its roles in presynaptic functions are largely unknown.

In our previous study, we observed a decrease in the number of presynaptic docked vesicles in layer 5 (L5) neurons in the medial prefrontal cortex (mPFC) of adult Cyfip2 heterozygous (Cyfip2+/−) mice compared with wild-type mice [8]. Therefore, in this study, we further investigated the presynaptic functional changes in the mPFC L5 neurons of Cyfip2+/− mice by measuring the short-term plasticity induced by trains of stimuli [see Additional File 1 for methods]. There was no significant difference between wild-type and Cyfip2+/− neurons in terms of the normalized evoked excitatory postsynaptic currents (eEPSCs) induced by a moderate-frequency (10 Hz) train of stimuli (Fig. 1a). Both neurons showed similar timings of depression during the train. However, with a high-frequency (20 Hz) train of stimuli, the normalized eEPSCs were significantly different between wild-type and Cyfip2+/− neurons (Fig. 1b). Specifically, Cyfip2+/− neurons showed an overall enhancement in normalized eEPSCs compared with wild-type neurons, suggesting altered short-term plasticity induced by high-frequency stimulation in Cyfip2+/− neurons.

Presynaptic Ca2+ is critically involved in short-term plasticity [10], and presynaptic mitochondria play an important role in buffering presynaptic Ca2+ [11, 12]. Notably, a recent study has revealed changes in mitochondrial activity and size in Drosophila Cyfip mutants [13]. Therefore, we also investigated the number and morphology of presynaptic mitochondria in the mPFC L5 neurons of Cyfip2+/− mice by re-analyzing electron microscopic image datasets previously acquired for dendritic spine analysis of the mPFC neurons [8] [see Additional File 1 for methods]. We found that mitochondria were observed only in a subpopulation of presynaptic boutons contacting dendritic spines of the mPFC L5 neurons, and that the ratio of presynaptic boutons containing mitochondria in Cyfip2+/− mice was significantly lower than that observed in wild-type mice (Fig. 1c). Considering normal densities of dendritic spines [8] and presynaptic boutons (Fig. 1c) of mPFC L5 neurons in Cyfip2+/− mice, these results indicate that Cyfip2+/− neurons have more mitochondria-free presynaptic boutons. Additionally, mitochondria number in axonal processes of the mPFC L5 was also significantly decreased in Cyfip2+/− mice compared with wild-type mice (Fig. 1d), suggesting that mitochondria transport along the axons can also be abnormal in Cyfip2+/− mice. In contrast, the mitochondrial volume in neither presynaptic boutons nor axonal processes was altered in Cyfip2+/− mice (Fig. 1c,d). As mitochondrial cristae density is highly correlated with its energy production and metabolic capacity [14], we next analyzed the mitochondrial cristae density in Cyfip2+/− and wild-type mice. Consistent with no difference in presynaptic mitochondrial volume (Fig. 1c), the cristae density of presynaptic mitochondria was comparable between genotypes (Fig. 1e). These results suggest that Cyfip2 haploinsufficiency affects the axonal and presynaptic localization, rather than the volume or cristae integrity, of mitochondria in the mPFC layer 5.

We have recently identified 140 proteins in the CYFIP2 interactome of mouse forebrain, which mainly included actin-regulatory proteins and RNA-binding proteins [15]. Notably, we found that 23 mitochondrial proteins were also detected in the CYFIP2 interactome (Fig. 1f). To further validate whether CYFIP2 was associated with mitochondria in the mPFC, we biochemically enriched the mitochondrial fraction from the mPFC homogenate of adult wild-type mice [see Additional File 1 for methods]. Western blot analysis showed that CYFIP2 was enriched in the mPFC mitochondrial fraction together with other mitochondrial proteins (Fig. 1g).

In this study, we showed changes in presynaptic short-term plasticity in the mPFC L5 neurons of adult Cyfip2+/− mice. The enhancement in the normalized eEPSCs in Cyfip2+/− neurons was induced by high-frequency, but not low-frequency, stimuli, suggesting that the decay of presynaptic Ca2+ signaling, such as Ca2+ clearance, rather than the action potential-induced Ca2+ increase, may be altered in Cyfip2+/− neurons. Presynaptic mitochondria positively regulate Ca2+ clearance in the axon terminal and thereby modulate neurotransmitter release [12]. Therefore, we speculate that the reduced number of presynaptic mitochondria in Cyfip2+/− neurons may decrease presynaptic Ca2+ clearance, which in turn elevates the residual Ca2+, and may trigger the short-term facilitating transmission. However, other possible mechanisms should also be considered. For example, mitochondria generate the majority of presynaptic ATP via oxidative phosphorylation, which is critical for synaptic vesicle recycling [11]. Therefore, changes in presynaptic ATP levels owing to the reduced
Fig. 1 (See legend on next page.)
mitochondria number may also contribute to the abnormal short-term plasticity in Cyfip2−/− neurons. In addition, mitochondria-independent mechanisms, such as changes in presynaptic actin dynamics, can be involved in the presynaptic functional changes of Cyfip2−/− neurons, as previously shown in Cyfip1+/− neurons [4]. Furthermore, in our previous study [8], we showed that the number of docked vesicles in a single presynaptic terminal was lower in Cyfip2−/− neurons compared with wild-type neurons, which is also in agreement with our current finding of an enhancement in the presynaptic short-term plasticity and an increase in the paired pulse ratio in Cyfip2−/− neurons.

Our observations of reduced number, but normal volume, of presynaptic mitochondria in Cyfip2−/− neurons contradict the results of a recent report showing increased mitochondrial activity and size in Drosophila Cyfip mutants [13]. Only one Cyfip gene exists in Drosophila, but there are two genes, Cyfip1 and Cyfip2, in mice. Therefore, one possibility can be that CYFIP1 and CYFIP2 have different roles in regulating presynaptic mitochondrial activity in the mouse brain. Our recent interactome analysis suggested that CYFIP1 and CYFIP2 have distinct pools of binding partners [15], supporting their differential molecular functions in vivo.

Detailed mechanisms underlying the reduced number of mitochondria in presynaptic boutons and axonal processes of Cyfip2−/− mPFC require further investigation. Presynaptic mitochondrial transport and localization can be regulated by several mechanisms, including the Ca2+-dependent detachment of the mitochondria from the molecular motor and its immobilization by mitochondrial docking proteins [16]. Whether CYFIP2 is involved in mitochondrial capture at presynaptic sites via interactions with mitochondrial proteins will be an interesting topic of future studies.

Notably, there are several intra-mitochondrial and mitochondrial inner membrane proteins in the CYFIP2 interactome (Fig. 1f). However, we could not find CYFIP2 in the publicly available mitochondrial protein databases (MitoCarta [17] and MitoProteome [18]). Furthermore, intra-mitochondrial localization was not predicted for CYFIP2 by a prediction tool of protein sub-mitochondrial localization (DeepMito [19]). These results suggest that CYFIP2 is not an intra-mitochondrial protein, and that those interactions between CYFIP2 and intra-mitochondrial proteins can be indirect via mitochondrial outer membrane proteins. Further investigations are necessary to identify key mitochondrial proteins mediating CYFIP2-mitochondria interaction and its potential regulation.

In conclusion, our results provide in vivo evidence that CYFIP2 regulates presynaptic functions, which may involve presynaptic mitochondrial changes. These results may be potentially implicated for CYFIP2-associated brain disorders, given that presynaptic mitochondrial dysfunction contributes to the pathogenesis of various brain disorders [11].

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13041-020-09658-4.

Additional file 1.

Abbreviations

CYFIP: Cytoplasmic FMR1-interacting protein; eEPSC: Evoked excitatory postsynaptic current; HET: Heterozygous; LS: Layer 5; MFN2: Mitofusin 2; mPFC: Medial prefrontal cortex; OPAL: Optic atrophy 1; SB-SEM: Serial block-face scanning electron microscopy; TOMM40: Translocase of outer mitochondrial membrane 40; WT: Wild-type

Acknowledgements
We thank the Laboratory Animal Research Center at Korea University College of Medicine for their animal care and support, and the Brain Research Core Facilities at Korea Brain Research Institute for electron microscopic support.
The Cyfip2+/− mice were bred and maintained in a C57BL/6J background according to the Korea University College of Medicine Research Requirements. All the experimental procedures were approved by the Committees on Animal Research at the Korea University College of Medicine (KOREA-2018-0174). The experiments. HK, S-KK, S-YC, KJL, and KH designed and performed the data. S-YC, KJL, and KH wrote the paper. All authors have read and approved the manuscript.

Availability of data and materials
The datasets used and analyzed in the current study are available from the corresponding authors on reasonable request.

Funding
This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea Government Ministry of Science and ICT (NRF-2018R1C1B601235, NRF-2018M3C7A1024603, NRF-2017M3C7A1048086, and NRF-2020R1A2C3011464) and the KBRI Basic Research Programs (20-BR-01-08 and 20-BR-04-01).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Neural Circuits Research Group, Korea Brain Research Institute, 61, Cheoerdan-ro, Dong-gu, Daegu 41062, South Korea. 2Department of Neuroscience, College of Medicine, Korea University, 73, Goryeodae-ro, Seongbuk-gu, Seoul 02841, South Korea. 3Department of Biomedical Sciences, College of Medicine, Korea University, Seoul, South Korea. 4Division of National Supercomputing, Korea Institute of Science and Technology Information, Daejeon, South Korea. 5Korea Institute of Science and Technology, Center for Functional Connectomics, Brain Science Institute, Seoul 02792, South Korea. 6Department of Brain and Cognitive Sciences, DGIST, Daegu, South Korea.

Received: 28 July 2020 Accepted: 8 September 2020

Published online: 11 September 2020

References
1. Zhang Y, Lee Y, Han K. Neuronal function and dysfunction of CYFIP2: from action dynamics to early infantile epileptic encephalopathy. BMF Rep. 2019; 52(5):304–11.
2. Abelekhough S, Bardoni B. CYFIP family proteins between autism and intellectual disability: links with fragile X syndrome. Front Cell Neurosci. 2014;8:81. https://doi.org/10.3389/fncel.2014.00081.
3. Nakashima M, Kato M, Aoto K, Shiina M, Belal H, Mukaida S, et al. De novo hotspot variants in CYFIP2 cause early-onset epileptic encephalopathy. Ann Neurol. 2018;83(4):794–806. https://doi.org/10.1002/ana.25208.
4. Hisao K, Harony-Nicolás H, Bushbaum JD, Bozdagi-Gunal O, Benson DL. CyFip1 regulates presynaptic activity during development. J Neurosci. 2016; 36(5):1564–76. https://doi.org/10.1523/JNEUROSCI.0511-15.2016.
5. De Rubens S, Pasciuto E, Li Kw, Fernandez E, Di Marino D, Buzzi A, et al. CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation. Neuron. 2013;79(6):1169–79. https://doi.org/10.1016/j.neuron.2013.06.039.
6. Davenport EC, Sulic BR, Drew J, Taylor J, Morgan T, Higgs NF, et al. Autism and schizophrenia-associated CYFIP1 regulates the balance of synaptic excitation and inhibition. Cell Rep. 2019;26(8):2037–51 e6. https://doi.org/10.1016/j.celrep.2019.01.092.
7. Han K, Chen H, Gennarino VA, Richman R, Lu HC, Zoghbi HY. Fragile X-like behaviors and abnormal cortical dendritic spines in cytoplasmic FMRI-2-mutant mice. Hum Mol Genet. 2015;24(7):1813–23. https://doi.org/10.1093/hmg/ddu595.
8. Lee SH, Zhang Y, Park J, Kim B, Kim Y, Lee SH, et al. Haploinsufficiency of Cyfip2 causes lithium-responsive prefrontal dysfunction. Ann Neurol. 2020. https://doi.org/10.1002/ana.25827.
9. Lee Y, Kim D, Ryu J, Zhang Y, Kim S, Kim Y, et al. Phosphorylation of CYFIP P2, a component of the WAVE-regulatory complex, regulates dendritic spine density and neurite outgrowth in cultured hippocampal neurons potentially by affecting the complex assembly. Neuroreport. 2017;28(12):749–54. https://doi.org/10.1097/WNR.0000000000001838.
10. Roehr WG. Short-term presynaptic plasticity. Cold Spring Harb Perspect Biol. 2012;4(7):a005702. https://doi.org/10.1101/cshperspect.a005702.
11. Devine MJ, Kittler J. Mitochondria at the neuronal presynapse in health and disease. Nat Rev Neurosci. 2018;19(2):63–80. https://doi.org/10.1038/s41584-017-0170.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions