The autism and schizophrenia associated gene CYFIP1 is critical for the maintenance of dendritic complexity and the stabilization of mature spines

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INTRODUCTION

Copy number variation (CNV) at the 15q11.2 region has been identified as a significant risk locus for neurological and neuropsychiatric conditions such as schizophrenia (SCZ) and autism spectrum disorder (ASD). However, the individual roles for genes at this locus in nervous system development, function and connectivity remain poorly understood. Haploinsufficiency of one gene in this region, Cyfip1, may provide a model for 15q11.2 CNV-associated neuropsychiatric phenotypes. Here we show that altering CYFIP1 expression levels in neurons both in vitro and in vivo influences dendritic complexity, spine morphology, spine actin dynamics and synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor lateral diffusion. CYFIP1 is highly enriched at synapses and its overexpression in vitro leads to increased dendritic complexity. Neurons derived from Cyfip1 heterozygous animals on the other hand, possess reduced dendritic complexity, increased mobile F-actin and enhanced GluA2-containing AMPA receptor mobility at synapses. Interestingly, Cyfip1 overexpression or haploinsufficiency increased immature spine number, whereas activity-dependent changes in spine volume were occluded in Cyfip1 haploinsufficient neurons. In vivo, Cyfip1 heterozygous animals exhibited deficits in dendritic complexity as well as an altered ratio of immature-to-mature spines in hippocampal CA1 neurons. In summary, we provide evidence that dysregulation of CYFIP1 expression levels leads to pathological changes in CNS maturation and neuronal connectivity, both of which may contribute to the development of the neurological symptoms seen in ASD and SCZ.
involvement in the regulation of dendritic morphology, synaptic structural plasticity and neurotransmitter receptor mobility. Furthermore, how both deletions and duplications affecting Cyfip1 expression produce similar neuropsychiatric phenotypes has also not yet been addressed.\textsuperscript{34,35}

Here we show that both Cyfip1 and Cyfip2 are highly enriched at excitatory synapses in dendritic spines. In dissociated neurons, overexpression of Cyfip1 or Cyfip2 leads to increased dendritic complexity and altered spine morphology whereas conversely, reducing Cyfip1 levels causes decreased dendritic complexity and an increase in immature dendritic spines. Cyfip1 deficiency results in increased F-actin assembly within dendritic spines, occludes N-methyl-D-aspartate (NMDA) receptor-induced spine shrinkage and leads to alterations in the surface dynamics and synaptic mobility of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. \textit{In vivo}, Cyfip1 deletion in the mouse leads to delayed embryonic development and early embryonic death whereas Cyfip1 haploinsufficiency causes reduced dendritic complexity and an increase in immature spines in the hippocampus. Our results support a critical role for Cyfip1 in development, dendritic morphogenesis and synaptic connectivity and provide further support for the idea that dysregulated Cyfip1 mediates the molecular aetiology of 15q11.2 Copy number 2.\textsuperscript{2} Copy number variation–associated neuropsychiatric conditions such as ASD, SCZ and epilepsy.

MATERIALS AND METHODS

Cell culture and transfection

 Hippocampal cultures were obtained from E16 mouse embryos (produced via Cyfip1<sup>+/−</sup>xCyfip1<sup>−/−</sup> crosses) or E18 rat embryos as previously described and transfected using Lipofectamine-2000 (Life Technologies, Paisley, UK).\textsuperscript{36}

Microscopy, quantitative analysis of neuronal and dendritic spine morphology, and fluorescence recovery after photobleaching (FRAP)

 Confocal images were acquired on a Zeiss LSM700 upright confocal microscope using a 63X oil objective (NA: 1.4) unless otherwise stated (Carl Zeiss, Welwyn Garden City, UK). Image stacks for dendritic morphological and spine analysis had voxel dimensions of 0.39 μm x 0.39 μm x 0.57 μm and 0.19 μm x 0.19 μm x 0.57 μm, respectively. Neuronal arbours were reconstructed and subjected to Sholl analysis, using either Imaris (Bitplane AG, Zurich, Switzerland) or NeuronStudio and ImageJ (NIH, Bethesda, MD, USA).\textsuperscript{37} Spines were manually identified on 100–200 μm long secondary dendrites and analyzed for morphological classification or volume measurements in Imaris software (Bitplane). For spine classification custom parameters were used and spines were classified into stubby, mushroom, long and thin, and filopodia categories using a ratio of spine head and neck diameters to spine length. Classification was entirely automated until the final step where errors in classification were removed.

 Single-plane confocal images were acquired for localization experiments \textit{in vitro}. To quantify Cyfip1 and Cyfip2 enrichment at synaptic sites, the fluorescence intensity of Cyfip1 or Cyfip2 was measured as the average intensity within the labelled synaptic puncta and normalized to the average intensity of the total process including synaptic puncta using Metamorph software (Molecular Devices, Sunnyvale, CA, USA).

 Dendritic morphology and spines in P5–60 mice were analysed using the Nikon Rapid Golgi Stain kit (FD NeuroTechnologies, Baltimore, MD, USA) and Neurolucida (MBF Bioscience, Williston, VT, USA). Golgi-imregnated brains were sliced at 150 μm using a vibratome (Leica Microsystems, Heerbrugg, Switzerland). Well-isolated hippocampal CA1 neurons were imaged at 60X using the Neurolucida software system and an upright light microscope with a motorized stage (MBF Bioscience). The entire extent (apical and basal) of the dendritic tree was traced and reconstructed. For spine analysis, two apical oblique and two basal dendrites were quantified per cell. Three males were analysed for each condition with a minimum of three cells traced per animal. When using animals from separate litters, each genotype was equally represented from each litter.

 Chemical long-term depression and fluorescence recovery after photobleaching (FRAP) on actin in dendritic spines was performed as described previously.\textsuperscript{38,39} FRAP movies were analysed in ImageJ (NIH) using a custom-built plugin, curves were fitted using \textit{Mathematica} (Wolfram Research, Champaign, IL, USA). For further details see Supplementary Methods.

Quantum dot (QD) imaging

 A total of 12–16DIV hippocampal cells transfected with GluA2<sup>2 GFP</sup> were live imaged under perfusion in ACSF at 37 ℃ and quantum dot (QD) movies were recorded at 8.5 Hz. Labelling of GluA2<sup>2 GFP</sup> with QDs was performed at RT using a mouse anti-GFP antibody (10 μg ml<sup>−1</sup>, Neuronab, N38) and an anti-mouse 605-nm QD (0.5 nm, Life Technologies). Analysis of QD receptor dynamics was performed as described (see Supplementary Methods).\textsuperscript{40}

RESULTS

Cyfip1 and Cyfip2 are enriched at excitatory synapses

 To explore the localization of Cyfip1 and Cyfip2 in neurons Cyfip1<sup>1 GFP</sup> or Cyfip2<sup>2 GFP</sup> (Supplementary Figure 1) were co-expressed in mature hippocampal neurons with DsRed as a marker of neuronal morphology and analysed using confocal microscopy. Compared with the localization of DsRed, which uniformly filled both dendritic processes and spines, Cyfip1<sup>1 GFP</sup> and Cyfip2<sup>2 GFP</sup> exhibited a nonuniform distribution appearing to be selectively targeted to punctate clusters in spines and along dendrites (Figures 1a and b). A similar enrichment in spines was observed for endogenous Cyfip1 using a Cyfip1-specific antibody and actin<sup>GFP</sup> to label neuronal morphology (Figure 1c; Supplementary Figure 1). In addition, Cyfip1 is detected within the post synaptic density fraction by western blotting (Supplementary Figure 4). Co-expression experiments with Cyfip1<sup>1 GFP</sup> and Cyfip2<sup>mCherry</sup> revealed that the majority of Cyfip1<sup>1 GFP</sup> co-localized with Cyfip2<sup>mCherry</sup> and overlapping puncta were found both in dendritic processes and spines (Supplementary Figure 2A).

Using immunofluorescence and confocal microscopy, both Cyfip1<sup>1 GFP</sup> and Cyfip2<sup>2 GFP</sup> levels were found to be ~90% enriched at Homer and VGLUT-positive excitatory synaptic sites compared with total dendritic processes (Cyfip1, 91.4 ± 9.8% increase, P < 0.001; Cyfip2, 85.9 ± 12.6% increase, P < 0.001; Figures 1d and e, Supplementary Figure 2B). In addition, endogenous Cyfip1 was also highly enriched at excitatory synapses and co-localized with the excitatory marker PSD-95 (Figure 1f). Thus endogenous Cyfip1, along with Cyfip1<sup>1 GFP</sup> and Cyfip2<sup>2 GFP</sup>, are found at excitatory synapses and exhibit high levels of expression at sites of F-actin accumulation, like dendritic spines.

Overexpression of Cyfip1 and Cyfip2 enhances dendritic complexity and alters dendritic spine morphology

 Microduplication of the Cyfip1-encoding region at 15q11.2 is associated with increased neuropsychiatric disease burden, and increased expression of Cyfip1 has specifically been linked to ASD.\textsuperscript{1,3,10} Overexpression of Cyfip1<sup>1 GFP</sup> or Cyfip2<sup>2 GFP</sup> resulted in a significant increase in dendritic complexity as a function of distance from the soma compared with control neurons expressing GFP, measured using either number of intersections or number of branch points as indicators of morphological complexity (Figures 2a–c). In addition, total dendritic length and the total number of branch points per cell were also increased in the Cyfip1 and Cyfip2 overexpressors (dendritic length: Cyfip1, 37% increase, 2876 ± 174 μm, P < 0.01; Cyfip2, 33% increase, 2787 ± 152 μm, P < 0.01; GFP, 2099 ± 163 μm; branch points: Cyfip1, 48% increase, 66.5 ± 7.0, P < 0.05; Cyfip2, 101% increase, 90.1 ± 5.6, P < 0.001; GFP 44.8 ± 7.7; Figures 2d and e).

To examine whether changes in dendritic complexity were accompanied by changes in excitatory spine number or shape, we measured spines in Cyfip1<sup>mCherry</sup> and actin<sup>GFP</sup> expressing neurons at 21DIV. Compared with DsRed+actin<sup>GFP</sup> transfected neurons,
Figure 1. CYFIP1 and CYFIP2 are localized at dendritic spines and enriched at excitatory synapses. In 17–20DIV rat hippocampal neurons, CYFIP1<sub>GFP</sub> (a), CYFIP2<sub>GFP</sub> (b) or endogenous CYFIP1 (c) localize to dendritic spines. DsRed (a, b) or actin<sub>GFP</sub> (c) were used to label processes. A line-scan through the dendritic shaft and spine head (right graphs) shows the fluorescence intensity of the green and red channels depicting the enrichment of CYFIP1<sub>GFP</sub>, CYFIP2<sub>GFP</sub> or endogenous CYFIP1 in the spine compared with the dendrite. CYFIP1<sub>GFP</sub> and CYFIP2<sub>GFP</sub> clusters co-localize with the excitatory synaptic marker Homer (d) (arrowheads). Quantification shows that there is an enrichment of CYFIP1<sub>GFP</sub> and CYFIP2<sub>GFP</sub> at excitatory synapses compared with the total process (excitatory: CYFIP1<sub>n</sub> = 61, CYFIP2<sub>n</sub> = 54, ***<i>P</i> < 0.001) (e). Endogenous CYFIP1 also co-localizes with the excitatory synaptic marker PSD-95 (arrowheads) in actin<sub>GFP</sub>-expressing cells (f). Scale bars, 2 μm.
CYFIP1mCherry+actinGFP-expressing cells displayed no significant difference in spine density (data not shown). However, CYFIP1 overexpressors had significantly fewer stubby spines (30% decrease in stubby spines, DsRed+actinGFP: 0.32 ± 0.01 spines μm⁻¹, P < 0.01) and increased numbers of long, thin spines and filopodia (24% increase in long, thin spines, DsRed+actinGFP: 0.14 ± 0.01 spines μm⁻¹, P < 0.05; 145% increase in filopodia, DsRed+actinGFP: 0.006 ± 0.001 spines μm⁻¹, CYFIP1mCherry+actinGFP: 0.016 ± 0.003 spines μm⁻¹, P < 0.01; Figures 2f and g). Furthermore, spines were significantly longer in CYFIP1 overexpressors regardless of subtype classification (DsRed+actinGFP: 1.13 ± 0.005 μm, CYFIP1mCherry+actinGFP: 1.29 ± 0.005 μm, P = 0.0001, Figure 2h). CYFIP1 overexpression also significantly decreased spine diameter, whereas spine volume showed an overall increase due to the dominating effect of the increase in spine length (Supplementary Figures 6C and D). These results demonstrate that increasing CYFIP1 expression levels above baseline enhances dendritic complexity and alters the ratio of mature versus immature spines in neurons, thereby changing network connectivity.

CYFIP1 is essential for embryonic development and its loss leads to marked developmental delay. To study the effects of decreased Cyfip1 levels in vivo, we characterized a Cyfip1 KO mouse41 and found that deletion was embryonically lethal. However, fertilized KO oocytes from Cyfip1⁺/⁻ × Cyfip1⁺/⁻ crosses did persist up to the blastocyst stage in vitro (not shown), and in pregnant dams, they were detectable until embryonic day 8.5 (E8.5) postcoitum (Supplementary Table 1). At this stage, Cyfip1 KO embryos were...
significantly reduced in size and displayed delayed development compared with their heterozygous (Cyfip1+/−) or wild-type (WT) counterparts (Supplementary Figures 3A–C). Further examination revealed complete developmental failure resulting in reduced body elongation and patterning. Cyfip1−/−/ heterozygous animals on the other hand, were viable until adulthood, were fertile and did not show any gross morphological abnormalities in brain structure when compared with WT (Supplementary Figure 3H). These data suggest that adequate levels of CYFIP1 are indispensable for proper development through early stages of embryogenesis.

**Cyfip1 haploinsufficiency results in decreased dendritic complexity and increased immature spine number**

Microdeletion at 15q11.2 renders people haploinsufficient for Cyfip1 and is associated with ASD and SCZ.4,5,7 We therefore investigated the effects of decreased CYFIP1 levels by measuring dendritic and spine morphologies in Cyfip1−/− hippocampal neurons (Supplementary Figures 3F and G). Sholl analysis of 14DIV, actinGFP-labelled neurons revealed that reduced CYFIP1 levels had the opposite effect on dendritic complexity to that of CYFIP1 overexpression, leading to a marked decrease in dendritic complexity between 30 and 110 μm away from the soma (Figures 3a–c). Furthermore, both total dendritic length and the total number of branch points were reduced in Cyfip1−/− neurons compared with control (15% decrease in total dendritic length in Cyfip1−/− neurons, WT: 2704 ± 96.8 μm, Cyfip1−/−: 2298 ± 80.2 μm, P < 0.01; 20% decrease in number of branch points in Cyfip1−/− neurons, WT: 76.7 ± 4.1, Cyfip1−/−: 61.4 ± 3.6, P < 0.01; Figures 3d and e). Spine density was unchanged in Cyfip1−/− neurons compared with WT controls; however, if spines were further classified into the different morphological subtypes, long and thin spines and filopodia were significantly increased (30% increase in long thin spines, WT: 0.15 ± 0.01 per μm−1, Cyfip1−/−: 0.20 ± 0.01 spines μm−1, P < 0.05; 125% increase in filopodia, WT: 0.007 ± 0.001 spines μm−1, Cyfip1−/−: 0.016 ± 0.003 spines μm−1, P < 0.05, respectively; Figures 3f–h). In addition, spines were significantly longer in the Cyfip1−/− neurons irrespective of subtype classification (WT: 1.14 ± 0.003 μm, Cyfip1−/−: 1.27 ± 0.003 μm, P < 0.0001, Figure 3i). However, no change in spine diameter or volume was observed in the Cyfip1 haploinsufficient neurons (Supplementary Figures 6A and B). These experiments demonstrate that Cyfip1 haploinsufficiency results in decreased dendritic complexity and produces alterations in spine morphology.

**CYFIP1 regulates actin assembly at dendritic spines**

CYFIP1 negatively regulates local actin filament assembly, suggesting that actin dynamics may be altered in Cyfip1−/− dendritic spines. FRAP of actinGFP in spines of Cyfip1−/− neurons revealed a significant difference in amount of fluorescence recovery compared with WT controls. Fluorescence intensity within the spine head of WT neurons returned to only 64.1 ± 5.2% of the pre-bleached fluorescence by 40 s compared with 80.0 ± 4.1% in the Cyfip1−/− neurons (P < 0.05; Figures 4a and b). Moreover, the fluorescence recovery of Cyfip1−/− neurons plateaued at a greater intensity than WT neurons, which was confirmed by a significant increase in the total mobile fraction (i.e. the proportion of final recovered fluorescence compared with the total bleached fluorescence, 16% increase, WT: 68.4 ± 5.46%, Cyfip1−/−: 84.4 ± 3.97%, P < 0.05; Figure 4c). However, there was no change in the recovery rate constant between Cyfip1−/− and WT neurons. Similar results were also obtained using LifeactGFP, an F-actin-specific fluorescent probe suitable for FRAP experiments.42 Photobleaching spines expressing LifeactGFP produced a significantly greater amount of recovered fluorescence in Cyfip1−/− neurons compared with WT again by 40 s (WT: 67.9 ± 4.8%, Cyfip1−/−: 85.5 ± 7.0%, P < 0.05; Figure 4d) resulting in a significant increase in the total mobile fraction (9.8% increase, WT: 70.1 ± 3.03%, Cyfip1−/−: 79.3 ± 3.12%, P < 0.05; Figure 4e).42,43 Taken together, the increased recovery of actinGFP and LifeactGFP suggests an increase in F-actin assembly in the Cyfip1−/− spines.

**Cyfip1 haploinsufficiency occludes NMDA receptor-dependent structural plasticity**

As Cyfip1 haploinsufficiency changes actin availability in spines and alters the ratio of mature versus immature spines at steady-state, we hypothesised that stimulus-dependent structural plasticity would also be altered in Cyfip1−/− spines. To study the role that CYFIP1 has in spines during structural plasticity, we utilized an established chemical long-term depression protocol.38 Brief bath application of 20 μM NMDA plus 20 μM glycine induces a 15–20% spine shrinkage within a timeframe of 40 min, which is measured as a decrease in spine volume without a concomitant change in either spine diameter or length (Figures 4f and g; Supplementary Figures 6E and F). Using this stimulus, we were able to induce spine shrinkage in WT neurons in response to NMDA treatment (Figures 4f and g). Cumulative frequency curves of spine volume show a leftward shift following NMDA application in WT neurons, indicating a reduction of spine volume across the population (Figure 4h). Cyfip1−/− spines showed no change in spine volume following NMDA application and spine volume remained unchanged from its basally low levels (16% decrease in WT spine volume following NMDA treatment, no change in Cyfip1−/− spine volume following NMDA treatment, WT saline: 0.30 ± 0.003 μm3, WT NMDA: 0.25 ± 0.001 μm3, Cyfip1−/− saline: 0.26 ± 0.002 μm3, Cyfip1−/− NMDA: 0.26 ± 0.002 μm3, P < 0.0001; Figures 4f–h). These data reveal a role for CYFIP1 in mediating structural reorganization during synaptic plasticity, possibly through dynamic regulation of the local actin cytoskeleton in spines.

Altered surface AMPA receptor mobility due to Cyfip1 haploinsufficiency

AMPA receptors mediate fast excitatory synaptic transmission within the CNS.44,45 Actin dynamics in spines have a major role in coordinating the delivery and removal of AMPA receptors and the strength of synapses.45 To address whether CYFIP1-mediated alterations in spine morphology and actin dynamics also impinge on the stability or mobility of AMPA receptors within synapses, we investigated whether surface GluA2-containing receptor dynamics were different from control in Cyfip1−/− neurons. Initial observations suggested that total protein levels of the AMPA receptor subunit GluA2 are unchanged in WT and Cyfip1−/− neurons (Supplementary Figures 5A and B). Therefore, we expressed pH-sensitive superrecluptic pHluorin (SEP)-tagged GluA2 in neurons to visualize GluA2-containing synaptic clusters in dendrites. Single GluA2SEP-containing receptors were labelled with 605 nm QDs to follow the diffusion of individual receptors within synaptic clusters.40–42 QD-labelled GluA2SEP receptors were less mobile when spines were bleached within clusters (Dout = 0.023 μm2 s−1, Din = 0.057 μm2 s−1, Dout = 0.034 μm2 s−1, Din = 0.023 μm2 s−1, Dout = 0.016 μm2 s−1, Din = 0.034 μm2 s−1) where D is the median diffusion coefficient, P < 0.0001, Supplementary Figures 5C and D), indicating confined motion at synapses, as previously shown.46 In Cyfip1−/− neurons, however, GluA2SEP-containing receptors exhibited an increased synaptic mobility within clusters when compared with WT neurons, (WT: Dout = 0.023 μm2 s−1, Cyfip1−/−: Dout = 0.034 μm2 s−1, P < 0.01; Figures 4i and j) as well as a reduced confinement at synapses (Supplementary Figure 5E). GluA2SEP receptor mobility outside synapses remained unchanged between WT and Cyfip1−/− neurons (WT: Din = 0.057 μm2 s−1, Cyfip1−/−: Din = 0.062 μm2 s−1, Figure 4k). These observations suggest that the restricted mobility of GluA2 receptors at synapses is significantly diminished in Cyfip1−/− spines.
CYFIP1 regulates neuronal development and connectivity in vivo.

To confirm that Cyfip1 haploinsufficiency produces similar effects on dendritic spines in the intact animal, we investigated dendritic complexity and spine morphology in the hippocampi of WT versus Cyfip1<sup>+/−</sup> mice using Golgi staining. Cyfip1 haploinsufficiency in postnatal day 55–60 (P55–60) animals was confirmed by western blotting (46.8% CYFIP1 protein decrease, WT control 100 ± 15.4%, Cyfip1<sup>+/−</sup> 53.2 ± 7.8%, P < 0.05; Supplementary Figures 3D and E). Compared with WT male littermate controls, CA1 neurons in Cyfip1<sup>+/−</sup> animals possessed a significantly decreased dendritic complexity within 100 μm from the soma (Figures 5a and b). Furthermore, spine density was unchanged, but long and thin spine morphology was increased (Figures 5f–h).

**Figure 3.** Cyfip1 haploinsufficient neurons display decreased dendritic complexity and increased immature spines. Cyfip1<sup>+/−</sup> hippocampal neurons had reduced dendritic complexity compared with WT controls at 14DIV (a, b). Scale bar, 20 μm. Dendritic morphology was examined using Sholl analysis. Cyfip1<sup>+/−</sup> neurons show significantly less complex morphology as a function of distance from the soma when compared with WT neurons (n = 18 cells per condition, *P < 0.05, **P < 0.01) (c). Total dendritic length (d) and total number of branch points (e) (n = 18 cells per condition, **P < 0.01) are also reduced in Cyfip1<sup>+/−</sup> neurons compared with WT. Spine morphology was analysed at 21DIV in Cyfip1<sup>+/−</sup> neurons and compared with WT cells (f, g) (upper panel: representative image; lower panel: 3-dimensional reconstruction). Scale bar, 5 μm. Colour key for spine type in 3-dimensional reconstruction: green = mushroom, red = stubby, blue = long and thin, pink = filopodia. Cyfip1<sup>+/−</sup> neurons possessed increased long, thin spines and filopodia (h) (n = 27–31 cells per condition, *P < 0.05). Spine length is increased across the entire population regardless of subtype classification in Cyfip1<sup>+/−</sup> neurons compared with control neurons (i) (n = ~39 000 spines per condition, ***P < 0.001).
Figure 4. Cyfip1 deficiency affects F-actin dynamics in spines, occludes structural responses to cLTD and increases surface AMPA receptor mobility. Cyfip1+/− and WT hippocampal neurons were transfected with actinGFP or LifeactGFP at 17–20DIV and subjected to FRAP live imaging 2 days later. Spines were imaged for 100 s and bleaching occurred after the first 20 s. (a) Representative images over time in seconds of actinGFP fluorescence recovery in WT and Cyfip1+/− spines. The red circles highlight the bleached spine. Scale bar, 2 μm. Quantification of GFP fluorescence intensity within the spine head region of WT compared with Cyfip1+/− neurons transfected with actinGFP (b) or LifeactGFP (d) shows Cyfip1+/− spines recover to a greater extent than WT (data points represent an average of 25–33 movies, *P < 0.05). Data are fitted with single exponentials (coloured lines). The mobile fraction, quantified as the final amount of recovered fluorescence presented as a percentage of the total bleached fluorescence, is significantly increased in Cyfip1+/− neurons for both actinGFP (c) (n = 29–33, *P < 0.05) and LifeactGFP (e) (n = 24–29, *P < 0.05). 21DIV WT and Cyfip1+/− hippocampal neurons were transfected with actinGFP and treated with either 20 μM NMDA+20 μM glycine (NMDA) or saline (SAL). (f) Representative images of spines following each treatment. Scale bar, 5 μm. Spine volume is significantly decreased in WT neurons following NMDA treatment compared with saline (n = 8000–13 000 spines per condition, ****P < 0.0001). However, Cyfip1+/− spines have basally low volumes and fail to remodel in response to NMDA (n = 12 000–14 000 spines per condition, ****P < 0.0001) (g). Spine volume is significantly decreased in WT NMDA-treated neurons compared with saline treatment whereas Cyfip1+/− saline- and NMDA-treated neurons show no leftward shift (n = 8000–14 000 spines, ****P < 0.0001, one-way analysis of variance (ANOVA)) (h). GluA2SEP-containing receptors were labelled with quantum dots (QDs) and live imaged. (i) Representative QD trajectories are shown for WT and Cyfip1+/− neurons, arrowheads indicate GluA2 SEP-containing synapses and green dotted line indicates synapse area. Scale bar, 2 μm. Quantification of receptor diffusion inside (j) and outside (k) synaptic clusters revealed that GluA2 SEP-containing receptors are more mobile in synaptic clusters of Cyfip1+/− neurons compared with WT (n = 140–160 QD tracks, **P < 0.01). cLTD, chemical long-term depression.
spines were increased in the apical oblique dendrites in Cyfip1+/− animals (40% increase, WT: 0.56 ± 0.066 spines μm−1, Cyfip1+/−: 0.79 ± 0.078 spines μm−1, P < 0.05; Figures 5c and d). These results indicate that genetic dysregulation of Cyfip1 produces penetrant effects on pyramidal neuron dendritic complexity and spine morphology in vivo.

DISCUSSION

Altered levels of CYFIP1 are associated with several neurological disorders including SCZ and ASD.4,5,10 However, it remains unclear what role CYFIP1 has in the establishment or maintenance of neuronal connectivity in the mammalian CNS, and how changes in its expression contribute to neurological dysfunction. Here we report that CYFIP1 regulates the maturation of neuronal dendrites and dendritic spines, controls the availability of F-actin within spines and impacts both spine structural plasticity and mobility of AMPA receptors at synapses. Furthermore, modelling SCZ- and ASD-associated Cyfip1 haploinsufficiency in mice produces deficits in dendritic complexity and spine morphology in hippocampal pyramidal neurons in vivo.

The activity of Rho GTPases (including RhoA, Rac1 and Cdc42) is associated with increased dendritic branch dynamics and extension.20,21,47,48 Activation of Rac1 releases the inhibitory action of CYFIP1 on WAVE, potentiating Arp2/3-mediated actin polymerization suggesting that changes in CYFIP1 levels could alter signalling events downstream of Rac1.31 Here we demonstrate that CYFIP1 expression levels are important for dendritic arborization and neuronal morphological complexity. Overexpression of CYFIP1 in hippocampal neurons in vitro leads to an increase in dendritic length and complexity whereas conversely, neurons from Cyfip1+/− animals have smaller, less complex dendrites both in vitro and in vivo. Interestingly, in addition to the effects on dendritic morphogenesis that we report here, both WAVE1 and CYFIP1 have previously been implicated in axonal outgrowth.49,50

A strict spatiotemporal balance between actin polymerization and turnover, mediated by Rho GTPases, is also important for spine formation, maturation and stability.20,21 We report that CYFIP1 is highly enriched in dendritic spines where it co-localizes with actin. Thus, CYFIP1 is critically placed, as a key Rac1 effector, to locally regulate WAVE in spines, and variations in its expression levels are likely to impact on Arp2/3-dependent spine actin dynamics downstream of Rac1. In agreement with this, we find that downregulating CYFIP1 expression levels in vitro or in vivo drives an increase in the ratio of immature-to-mature spines suggesting that CYFIP1 is important for actin-dependent regulation of spine maturation. Furthermore, we show that dendritic spines from Cyfip1+/− haploinsufficient neurons display enhanced F-actin availability suggesting that spatiotemporal control over actin polymerization is altered at Cyfip1+/− postsynaptic sites. A role for CYFIP1 in the regulation of Rac1-dependent F-actin assembly in Drosophila NMJ presynaptic terminals was also recently demonstrated, where it was shown to be important for synaptogenesis and presynaptic quantal content.51,52 In addition to previous functions described for CYFIP1 in the regulation of mRNA translation in dendrites and mGluR-long-term depression, our results suggest that CYFIP1 also has a vital role in the local regulation of spine actin dynamics via WAVE and Arp2/3.32,33 This is in agreement with a recent study showing that CYFIP1...
translocates between the FMRP-containing protein synthesis machinery and the actin-regulatory complex, and that disrupting CYFIP1’s interaction with members of either complex negatively impacted spine maturation in neurons.3,4

The surface mobility of neurotransmitter receptors is dynamically regulated to control synaptic activity and is emerging as another key component underlying plasticity at nerve terminals.40,53,54 The trapping of mobile AMPA receptors within the postsynaptic domain is thought to be critical for maintaining and strengthening synaptic communication, and a pathological impairment in this process could have significant implications for neurotransmission.3,55 The enrichment of CYFIP1 at excitatory synapses implies that CYF1 can locally regulate actin cytoskeletal dynamics in the postsynaptic domain. The fine-tuning of actin turnover has previously been demonstrated to be an important regulator of synaptic glutamate receptor mobility. Indeed, we find that the disruption of spine maturation due to decreased CYFIP1 expression levels also correlates with an increase in AMPA receptor mobility within the spine postsynaptic density. Consequently, CYFIP1 expression levels appear to be critical not just for the maturation of dendritic spines but may also be important for regulating the postsynaptic cytoskeletal architecture essential for the stabilization of postsynaptic AMPA receptors.

Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 In vitro, we report here that complete CYFIP1 deletion in mice leads to a decrease in spine volume upon CYFIP1 overexpression or decrease in immature spines upon CYFIP1 overexpression or depletion.63 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56 Dynamic regulation of F-actin assembly is repeatedly implicated in mechanisms underlying spine structural plasticity.21,56

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embryonic lethality observed in the CYFIP1 knockout suggests that CYFIP2 cannot compensate for all CYFIP1 functions. Intriguingly, we also observed CYFIP2 to be enriched in dendritic spines and its overexpression could drive increased dendritogenesis. This suggests that it will be interesting to further explore whether CYFIP2 is a candidate susceptibility gene for major mental illness. Our results provide important new insights into the role CYFIP1 has in the development of the nervous system and how Cyfp1 dysregulation can alter CNS function to contribute to the development of neuropsychiatric illness. Changes in the structure and function of excitatory synapses, and alterations in the local versus long-range dendritic connectivity of neurons, have been associated with the development of neuropsychiatric conditions involving imbalances of excitation and inhibition, such as ASD and SCZ.73,74 The roles for CYFIP1 described here may help inform how altered levels of this molecule gives rise to network-level dysfunction that defines these pathologies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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