WD40-repeat 47, a microtubule-associated protein, is essential for brain development and autophagy

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Edited by Stephen T. Warren, Emory University School of Medicine, Atlanta, GA, and approved September 7, 2017 (received for review August 11, 2017)

The family of WD40-repeat (WDR) proteins is one of the largest in eukaryotes, but little is known about their function in brain development. Among 26 WDR genes assessed, we found 7 displaying a major impact in neuronal morphology when inactivated in mice. Remarkably, all seven genes showed corpus callosum defects, including thinner (Atg16l1, Coro1c, Dmxl2, and Herc1) thinner (Kif21b and Wdr89), or absent corpus callosum (Wdr47), revealing a common role for WDR genes in brain connectivity. We focused on the poorly studied WDR47 protein sharing structural homology with LIS1, which causes lissencephaly. In a dosage-sensitive manner, mice lacking Wdr47 showed lethality, extensive fiber defects, microcephaly, thinner cortices, and sensory motor gating abnormalities. We showed that WDR47 shares functional characteristics with LIS1 and participates in key microtubule-mediated processes, including neural stem cell proliferation, radial migration, and growth cone dynamics. In absence of WDR47, the exhaustion of late cortical progenitors and the consequent decrease of neurogenesis together with the impaired survival of late-born neurons are likely yielding to the worsening of the microcephaly phenotype postnatally. Interestingly, the WDR47-specific C-terminal to Lish (CTLH) domain was associated with functions in autophagy described in mammals. Silencing WDR47 in hypothalamic GT1-7 neuronal cells and yeast models independently recapitulated these findings, showing conserved mechanisms. Finally, our data identified superior cervical ganglion-10 (SCG10) as an interacting partner of WDR47. Taken together, these results provide a starting point for studying the implications of WDR proteins in neuronal regulation of microtubules and autophagy.

Significance

We present an identification of the relevance of WD40-repeat (WDR) genes in brain connectivity, highlighting the power of unbiased mouse studies in the field of neuroscience. We focus on the poorly studied WDR47 protein sharing structural homology with LIS1, which causes lissencephaly. WDR47 plays a role in progenitor proliferation, neuronal migration, and fiber tract projections in a similar fashion to LIS1 but with the distinctive particularity that WDR47 inhibits autophagic flux. This provides a functional link between autophagy biology and the C-terminal to Lish domain in mammals. Importantly, WDR47 uncovers an aspect of corpus callosum biology pointing toward a link between the regulation of microtubule dynamics and autophagic flux for axonal outgrowth and guidance.


The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1713625114/-/DCSupplemental.
identified 20 y ago to regulate dynein activity and neuronal migration (6) is linked with lissencephaly type 1, a severe malformation where the brain develops without convolutions (Online Mendelian Inheritance in Man 607432), and the corpus callosum is thinner (7). Mutations in WDR62 cause autosomal recessive primary microcephaly and hypoplasia of the corpus callosum (8), and WDR73 is implicated in Galloway-Mowat syndrome characterized by microcephaly and thin corpus callosum (9). Understanding the underlying pathophysiological mechanisms of callosal disorders is critical for patient stratification and therapy development.

Made of ~190 million axonal projections, the human corpus callosum is the largest interhemispheric white matter tract in the brain, with neurons located mainly in neocortical layers II/III, giving rise to callosal axons (10). The genetics of corpus callosum biology is, however, highly heterogeneous, and despite technological advances in next generation sequencing, 75% of callosal disorders have no identified genetic cause (11). Recent studies have suggested that a smaller corpus callosum is associated with a higher risk for autisms (12), bipolar disorder (13), and schizophrenia (14). Corpus callosum abnormalities are often seen in conjunction with other defects, such as smaller or larger brain size and malformations of cortical development (15). The formation of the corpus callosum is a process relying on axonal guidance cues, such as Netrin/DCC, ROBO, and Slit (16). This developmental process also relies on microtubule polymers that localize to the tip of the axon, known as the growth cone (17). However, much less is known about microtubules at the growth cone, but they are the primary effectors of axonal movement and guidance (18).

Less than 3% of WDR proteins have been functionally defined in the CNS, while for the remaining 97%, the function remains completely unknown (Dataset S1). Interestingly, several WDR proteins have been linked to microtubules in KO mouse studies (for example, LIS1 (19) and WDR62 (20). Microtubules are critical components of the cytoskeleton, and their dynamics refers to the continuous remodeling between assembly (rescue) and disassembly (catastrophe) at their tip (21). Proper regulation of this dynamic is essential and achieved through microtubule-associated proteins.

In this study, we ask whether microtubule-mediated processes might be affected by mutations of WDR genes that result in corpus callosum anomalies, what the underlying cellular and molecular mechanisms are, and ultimately, how these underlie corpus callosum biology.

**Results**

**Mouse WDR Proteins Are Implicated in Corpus Callosum Biology.**

Twenty-six WDR domain-containing mouse mutants were randomly selected among a manually curated list of 286 family members (Dataset S1) and studied at 16 wk of age. We first carried out gene ontology enrichment analysis in both 286- and 26-gene sets and found the three most significant terms (P < 0.001): protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone protein complex binding, actin filament binding, and histone

Using a quantification approach of 66 morphological and 115 cellular measurements across 19 different brain regions in two histological sections at Bregma +0.98 mm and −1.34 mm (Datasets S2 and S3), we found that mutations of seven WDR genes (Atg16l1<sup>−/−</sup>, Coro1c<sup>−/−</sup>, Dmxd2<sup>−/−</sup>, Herc1<sup>−/−</sup>, Kif21b<sup>−/−</sup>, Wdr47<sup>−/−</sup>, and Wdr89<sup>−/−</sup>) were associated with neuroanatomical phenotypes (Fig. 1; Datasets S4 and S5) show P values and percentage changes. At the morphological level, Dmxd2<sup>−/−</sup> and Herc1<sup>−/−</sup> displayed macrocephaly, with increased sizes of 37% (P = 0.0005) and 20% (P = 0.002), respectively. By contrast, Wdr47<sup>−/−</sup> and Kif21b<sup>−/−</sup> revealed microcephaly, with decreases in size of 25% (P = 0.03) and 20% (P = 0.05), respectively. Atg16l1<sup>−/−</sup> was associated with increased height (+11%) of the motor cortex (P = 0.003) and increased height (+38%) of the radiatum layer of the hippocampus (P = 0.004), whereas Wdr89<sup>−/−</sup> and Coro1c<sup>−/−</sup> were associated with ventricular atrophy (~13%) of the dorsal third ventricle (P = 0.0009).
and an enlargement (+13%) of the lateral ventricles ($P = 0.0002$), respectively. At the cellular level, $Herc1^{−/−}$ displayed a 37% increase in cell numbers in the mammillothalamic tract ($P = 0.003$) and a 20% increase in the granular cortex ($P = 0.001$), while $Kif21b^{−/−}$ showed a decreased number of cells (−17%) in the cingulate cortex ($P = 0.03$).

All seven WDR mutants displayed corpus callosum anomalies (Fig. 1A and Datasets S4 and S5). Developmental mechanisms regulating the dorsoven-tral axes of the corpus callosum being distinctive, with pioneering axons projecting from the cingulate cortex crossing the dorsal region and neurons from the neocortex regulating formation of the ventral region (22), we quantified several regions of the corpus callosum (the genu, soma, and splenium). $DmnaL2^{−/−}$ and $Herc1^{−/−}$ strongly impacted the genu, while $Atgl61^{+/+}$, $Corolc^{+/+}$, $Kif21b^{−/−}$, and $Wdr89^{−/+}$ affected the soma only. In addition, $Atgl61^{+/+}$, $Corolc^{+/+}$, $Wdr37^{−/−}$, and $Wdr89^{−/+}$ exhibited cell count defects in the corpus callosum, the directionals of which were in line with those of the morphological phenotypes. $Wdr47^{−/−}$ stood out as the most severely affected gene, with agenesis of the corpus callosum (Fig. 1B), defined as a failure to develop the large bundle of fibers that connect the cerebral hemispheres (11).

**Wdr47 Is Highly Expressed in the Adult Brain and Is Essential for Survival in Mice.** We chose to focus on the poorly studied WDR47 gene (23) given the severity of the associated neuroanatomical phenotypes and developed two mouse models (tm1a and tm1b) (Materials and Methods and Fig. S1B). We validated both models using qRT-PCR and determined that tm1a is a hypomorph allele in a series of tissues, suggesting that Wdr47 is skipping over the LacZ cassette restoring gene expression, while tm1b is a complete loss-of-function (LoF) of Wdr47 (Fig. 2A). Based on average relative expression to the WT, tm1a heterozygous (het) mice, hereafter referred to as $Wdr47^{+/tm1a}$, expressed 70%, and tm1b het $Wdr47^{tm1b}$ expressed 50% (Dataset S6). In homozygous (hom) animals, tm1a ($Wdr47^{tm1a/tm1a}$) and tm1b ($Wdr47^{tm1b/tm1b}$) expressed 30% and 0%, respectively (Dataset S6), offering the opportunity to study the impact of gene dosage (70, 50, and 30%) and complete gene LoF. WDR47 protein analysis confirmed minimal expression in $Wdr47^{tm1a/tm1a}$ (Fig. 2B). LacZ spatial expression throughout the brain and in peripheral tissues revealed Wdr47 expression mainly in layers II/III of the cortex, pyramidal cells of the hippocampus, spinal cord, ventromedial hypothalamus, and arcuate nucleus (Fig. 2C). Wdr47 was less expressed in peripheral tissues (Fig. 2A and Fig. S1C).

Adult mouse survival was assessed from 1,085 successfully genotyped mice derived from a heterozygous-by-heterozygous (“het-by-het”) breeding scheme (Dataset S7); 5.7% hom, 54.2% het, and 40.1% WT were obtained in $Wdr47^{tm1a}$, and 0% hom, 55% het, and 45% WT were obtained in $Wdr47^{tm1b}$, indicating lethality in both het and hom mice. Wdr47 expression levels and lethality (expressed as percentages) exhibited a high negative correlation (Fig. 2D), with males and females being equally affected. To determine the window of death, we then tested mouse viability from embryonic d 9.5 (E9.5) to 153 d of age (P153) (Fig. 2E). Death rate was unaffected during embryogenesis, and no abnormality in number of somites, limb morphology, and heartbeat was observed in E9.5 embryos ($n = 34$) and E18.5 $Wdr47^{tm1a/tm1a}$ embryos ($n = 132$). However, the percentage of $Wdr47^{tm1a/tm1a}$ decreased exponentially from birth to P55, with a reduction of 36% by P16 and a further reduction of 64% by P55. Mice that survived until P55 survived until adulthood. The cause of lethality remains unknown; however, histological assessment at E18.5 excluded lung defects.

It has been recently reported that a lipid-enriched diet rescues lethality in a mouse model of amyotrophic lateral sclerosis (24). We thus maintained a separate colony of mice on a fortified diet (Mouse Breeder Diet 5021) with extra lipids (10.8% as opposed to 3% in a normal diet) and folic acid (3 vs. 0.7 mg) using a het-by-het breeding scheme and examined its effects on 591 mice in both $Wdr47^{tm1a}$ and $Wdr47^{tm1b}$ (Fig. 2F). Remarkably, we found an almost complete transgenerational rescue of the lethal phenotype at the second generation in $Wdr47^{tm1a}$ mice. These results indicate that diet enrichment counterbalances the lethality effect, possibly by altering nutrient levels necessary in key processes for survival. There was no rescue in $Wdr47^{tm1b}$, suggesting that residual Wdr47 expression is necessary for diet-induced survival reversal.

**Wdr47 Deficiency Results in Severe Microcephaly and Fiber Tract Hypoplasia in Adult Male and Female Mice.** Sexual dimorphism was assessed through a newly designed sagittal analysis of 95 variables for 22 unique brain regions across three selected sections (lateral 0.72, 1.32, and 2.52 mm) (Fig. S2). This analysis had...
The advantage of adding new brain regions (such as the substantia nigra) while maintaining existing ones in 16-wk-old Wdr47+/tm1a and Wdr47+/tm1a mice (Datasets S8 and S9). Consistently, male and female Wdr47+/tm1a mice showed a similar set of neuroanatomical anomalies (for example, at lateral 0.72 mm), a reduction in the total brain area of 27.5% for female (P = 0.014) and 25.1% (P = 0.00057) in both groups (Fig. 3B). In Wdr47+/tm1a mice, the area of the corpus callosum was smaller by 55.6% (P = 0.007), and the corpus callosum area was smaller by 55.6% (P = 0.007) compared with WT. (Fig. 4A). While the total brain area was not significantly affected across the three coronal planes, it showed a clear tendency toward reductions of size of 10.3% (P = 0.26), 10% (P = 0.06), and 7.1% (P = 0.31) at Bregma 2.19, 3.51, and 6.75 mm, respectively (Dataset S9). Similar phenotypes emerged in Wdr47+/tm1b, with fewer regions affected and smaller percentage changes compared with Wdr47+/tm1a (Fig. 4B and Dataset S9). To further investigate the reduction of the cortical thickness, we measured individual layers and found a reduction originating specifically from the cortical plate and subventricular zones at Bregma 2.19 mm (Fig. 4B).

Additionally, we measured 63 parameters in Wdr47+/tm1b and Wdr47+/tm1b at P8 and found a similar set of regions being affected compared with in 16-wk-old mice (Fig. S6A and Dataset S9); in particular, the total brain area was reduced by 21.6% (P = 0.046), and the corpus callosum area was smaller by 55.6% (P = 0.033). We also analyzed mice at 56 wk of age and found that the brain size phenotypes did not worsen (Fig. S6B and C). In summary, Wdr47+/tm1b mice exhibited reductions of the total brain size of 9, 22, 26, and 29% at E18.5, P8, 16 wk of age, and 56 wk of age, respectively, pointing toward primary microcephaly that worsens postnatally.

Wdr47 Regulates Progenitor Proliferation and Survival of Neurons in Late Corticogenesis. Wdr47 cortical expression, assessed using Western blot analysis, gradually increased from E12.5 to P2, reaching a peak at E18.5 (Fig. 4C). Accordingly, LacZ spatiotemporal expression revealed an enriched Wdr47 expression in layers II/III as corticogenesis progresses (Fig. 4D), suggesting a role of WDR47 in late corticogenesis. The reduction of the thickness of the cortical plate at E18.5 (Fig. 4B) could arise from a poor survival of progenitors or neurons. In agreement with this hypothesis, Wdr47+/tm1b/tm1b showed an increased level of apoptosis, mainly in upper-layer neurons (Fig. 4E), indicating that WDR47 is not required for the survival of the
Using immunolabeling, we analyzed cortical progenitors in Wdr47tm1b/tm1b embryos and WT E18.5 embryos and found a reduced number of Ki67+ cycling progenitors (−33.8%, P = 0.0016) (Fig. 4F) and a decrease in the absolute number of both Pax6+ apical (−39.7%, P = 0.0064) (Fig. 4G) and Tbr2+ intermediate (−37.2%, P = 0.0042) (Fig. 4F) progenitors. Noteworthy, the proliferative potential of both progenitor types remained unchanged (Fig. 4I and J).

To understand the loss of progenitor cells at E18.5, we repeated our experiments at an earlier stage. Wdr47tm1b/tm1b E16.5 Pax6+ apical and Tbr2+ intermediate progenitors behaved as WT, with no observable phenotype in their absolute number (Fig. S7 C and D) or proliferative potential (Fig. S7 E and F). However, we observed a milder decrease in the number of Ki67+ cycling progenitors by 17% (P = 0.018) (Fig. S7B) compared with −33.8% at E18.5 (Fig. 4F). In addition, we assessed cell cycle exit and fate of newborn cells by injecting 5-ethyl-2-deoxyuridine (EdU) at E15.5 and studying corresponding Wdr47tm1b/tm1b embryos 24 h later at E16.5. We found no differences in the fate of newborn Pax6+ and Tbr2+ progenitors (Fig. S7 G and H) but a slight increase in the number of progenitors that exited the cell cycle (+10.7%, P = 0.027) (Fig. S7I) together with decreased proliferation (Fig. S7B), suggesting that the reduction of progenitors self-renewal starts from E16.5 onward and progressively increases until E18.5.

### Specific Deletion of Wdr47 in Postmitotic Neurons Impairs Radial Migration

Given that WDR47 is involved in the neurogenic program and has been identified as a microtubule-associated protein (23), we tested whether, in addition to neurogenesis, WDR47 could also regulate neuronal migration. We performed acute deletion of WDR47 in projection neurons by in utero electroporation of plasmids, allowing the expression of the CRE recombinase and the GFP under the control of the NeuroD promoter (NeuroD:CRE-GFP) at E14.5 (Fig. 4K). Four days after in utero electroporation, while most of the GFP+ postmitotic neurons reached the cortical plate in the control (Fig. 4L), neurons depleted for WDR47 (NeuroD:CRE-GFP in Wdr47tm1b/tm1b embryos) accumulated in the intermediate zone, with a decrease of 20% of the cells reaching the cortical plate (Bonferroni adjusted P = 0.0001) (Fig. 4G). The role of WDR47 in radial migration of projection neurons was confirmed in KO mice, as we observed similar positioning defects of EdU-labeled cells 4 d after a single EdU injection in E14.5 Wdr47tm1b/tm1b embryos compared with WT littermates (reduction of 51.3% in the cortical plate; Bonferroni adjusted P < 0.0001) (Fig. S7J). WDR47 is, therefore, required for proper radial migration of projection neurons.

**Wdr47 Depletion Impairs Growth Cone Morphology and Microtubule Stability.
**Next, we turned to the investigation of fiber projections during neurodevelopment considering the extreme hypoplasia of fiber tracts in 16-wk-old mice (Fig. 3). Consistently, MRI corroborated these findings throughout the brain, showing the corpus callosum as the most affected region (Fig. 5A). Using the axonal (SMI-312K) and LIMAC markers to visualize neurofilaments and axonal projections, respectively, we found fewer axonal processes both at E14.5 and E16.5 Wdr47tm1b/tm1b embryos, with thalamocortical projections unable to cross the diencephalon–telencephalon boundary (Fig. 5B).

Because endogenous WDR47 is expressed throughout the cytoplasm, neurites, and in the growth cone in neurons (Fig. S8A), we thought to assess neuronal architecture when Wdr47 is deleted using cortical and hippocampal primary neuronal cultures at E17.5. We visualized the axon and growth cone using a marker for neurofilaments and microtubule-associated protein 2 (MAP2), a microtubule-associated protein enriched in the dendrites of neurons,

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Fig. 4. Wdr47 is a key regulator in multiple steps of the neurogenic program. (A) Heat map of neuroanatomical defects in Wdr47tm1a/tm1a at E18.5 (n = 4 Wdr47tm1a/tm1a; n = 5 Wdr47tm1b/tm1b; n = 5 WT) (Dataset S9) and images illustrating neuroanatomical anomalies. (Magnification: 20×C (B, Upper) Zoom in of boxed area in A showing height of neocortical layers in sections stained with cresyl violet from WT (n = 5) and Wdr47tm1b/tm1b (n = 4) embryos at E18.5. (B, Lower) Quantification of individual cortical layers. ***P < 0.01 (Student’s t test, two-tailed). (C) Western blot of WDR47 expression in WT cortical tissues from E14.5 to E18.5 (wt) and images illustrating neuroanatomical anomalies (Magnification: 10×). L, Caudal; VZ, ventricular zone; SVZ, subventricular zone; IL, lateral ventricle; LV, lateral ventricle; M, motor cortex; MZ, marginal zone; OR, olfactory region; RS, retrosplenial granular cortex; SP, subplate; SVZ, subventricular zone; TIL, total pyramidal cell layer; VZ, ventricular zone. (Scale bars: E, F, and I, 100 μm; G–I, 50 μm.) ***P < 0.0001 (two-way ANOVA followed by Bonferroni correction).
and measured the area of the cell body (n = 215), axonal length (n = 519), and area of the growth cone (n = 775). Primary neuronal cultures derived from Wdr47+tm1a allele displayed a severe reduction in growth cone areas by 41% for the hippocampus (P = 2.69E−10) and 42% for hippocampus (P = 1.88E−10) (Fig. 5C), resembling the physiological collapse or catastrophe state in growth cone behavior (21). Neuronal cultures derived from Wdr47+tm1b allele also showed smaller growth cone areas of 17% for the hippocampus (P = 1.08E−10) and 37% for the cortex (P = 8.6E−10), showing that this phenotype is also sensitive to Wdr47 dosage (Fig. 5C). Furthermore, these structures displayed a blunt tip and reduced filopodia protrusions, while the cell area and length of axon did not differ (Fig. 5C). These observations were confirmed in rat hypothalamic neurons treated with WDR47-specific siRNA using scanning EM (Fig. S8B and C). In addition, time-lapse recordings from live neurons over 24 h showed that mutant growth cones are much less dynamic compared with WT (Movies S1 and S2).

The analysis of the microtubule distribution network at the growth cone of neurons derived from Wdr47+tm1a allele using acetylated tubulin as a marker of stable microtubules revealed unusual shapes, with a ring-like structure at the soma (Fig. S5D). Similar abnormalities were seen in rat hypothalamic neurons using super-resolution structured illumination microscopy; however, tubulin protein levels were not significantly altered (Fig. S5D). Super-resolution single-molecule localization microscopy also showed these unusual shapes and further established that tubulin molecules were widely dispersed in mutant as opposed to uniform and denser distribution in WT cells (Fig. 5E). Tau protein level, a microtubule-associated protein known to modulate the stability of axonal microtubules, was increased by about twofold in Wdr47+tm1a cortical tissue samples compared with WT (Fig. 5F). Given the role of Tau in microtubule dynamics, we hypothesized that WDR47 might participate in microtubule stabilization. We tested this by treating hippocampal primary neuronal cultures derived from Wdr47+tm1a and characterized by a reduction of growth cone areas of ~75% (n = 162, P = 1.89E−10) (Fig. 5G), with a microtubule stabilizer compound [Epothilone D (EpoD)] at two concentrations (10 and 100 nM) for 1.5 h as recommended elsewhere (26). Remarkably, EpoD was able to dose-dependently rescue growth cone size up to +69.7% relative to vehicular control (DMSO) cells (Fig. 5G). Treatment with 10 nM EpoD increased the size of growth cones by 2.1 times (n = 102, P = 1.2E−10), and treatment with 100 nM EpoD increased the size of growth cones by 3.3 times (n = 143, P = 1.9E−10). No significant changes were observed between EpoD-treated and nontreated groups in the WT. To determine whether these anomalies might be causing cell motility defects, we used a previously tested assay in neurite outgrowth (27), which relies on creating a scratch in cell culture dishes and quantifying the time required for the cells to close it (28). At 24 h postscratch introduction, the migration distance and velocity (Fig. S5E) as well as the percentage wound closing (Fig. S5F) were reduced with WDR47 siRNA treatment. All together, these results show that Wdr47 plays a role in stabilizing microtubules, facilitating tubulin network dynamics in both genetic mutant cells and siRNA-treated cells.

**Superior Cervical Ganglion-10 Is an Interacting Partner of WDR47.** To understand the molecular mechanisms by which WDR47 might regulate microtubule stability, we next searched for interacting partners by screening a human fetal cDNA library using a yeast two-hybrid system.

Using the N terminus of WDR47 as bait, the superior cervical ganglion-10 (SCG10) protein was identified as a putative WDR47-interacting partner (Dataset S10). SCG10 is a well-established microtubule-stabilizing protein (29) regulated by JNK1, a protein kinase of the MAPK family known to phosphorylate SCG10, rendering it inactive (30). To gain insight into the mechanistic basis of this interaction, we first studied localization of WDR47, SCG10, and JNK1 in primary cortical neurons. WDR47 colocalized with SCG10 in the cytoplasm but not in the growth cone, whereas JNK1 showed colocalization with SCG10 in the cytoplasm as well as neurites (Fig. S9A). SCG10 relative mRNA expression levels showed no difference between Wdr47+tm1a and WT mice preparations derived from the cortex, spinal cord, thalamus, and liver (Fig. S9B). Colocalization of WDR47 and SCG10 was confirmed to occur in the cytoplasm of hypothalamic cells (Fig. S9C). Western blot analysis of endogenous SCG10 normally gives rise to four bands that range from 20 to 25 kDa, representing distinct structures.
To establish if the neuroanatomical defects in Wdr47 KO mice lead to specific behavioral phenotypes, we assessed forelimb and memory were tested using the Y maze (short-term memory), Morris water maze (spatial memory), and novel object recognition with retention time of 24 h (long-term memory). (C) Learning and Memory paradigms reveal hyperactivity. (D) Grip strength for both forelimb and hind limb. (E) Motor coordination assessed using the MoRaG. (F) Sensation Hot plate latency to react (s). All plots are represented as mean ± SEM. ***P < 0.001. **P < 0.01; *P < 0.05.

Wdr47 Mice Are Hyperactive and Display Sensory Motor Gating Abnormalities. To establish if the neuroanatomical defects in Wdr47 KO mice lead to specific behavioral phenotypes, we assessed a broad range of paradigms in both male and female mice (Dataset S11 shows a list of P values).

The circadian activity test revealed an increase in traveled distance (P = 0.02) during the active phase (Fig. 6A). The open-field test was used to examine basic locomotor activity as well as anxiogenic behavior and showed increased total traveled distance in the arena and total number of rears (P = 0.0001) (Fig. 6A) but did not show an increase in the time spent in the center, suggesting no traits of anxiety (Fig. S10A). The elevated plus maze test confirmed the absence of anxiogenic behavior in mice (Fig. S10B). Since Wdr47 is highly expressed in the hippocampus implicated in the consolidation of information, we next looked for deficits in memory using the Y-maze test to evaluate working memory, the Morris water maze for spatial memory performance, and the novel object recognition for long-term memory as well as social recognition test. Mice did not show any difficulties in learning each given task or in memorizing various objects and cues; however, they displayed, again, hyperactivity in the Y-maze (P = 0.04) and novel object recognition paradigms (P = 0.0004) (Fig. 6B). Because of prominent corpus callosum abnormalities, we studied forelimb laterality and dexterity using the Mouse Reaching and Grasping (MorRaG) test (32) and found reduced reaching (P = 0.02 for male and P = 0.005 for female) abilities, while forelimb laterality was unaffected (Fig. 6C). Accordingly, forepaws strength was decreased as studied in a grip strength test used to assess muscular strength (Fig. 6D). Hind paws motor coordination was affected in the notched bar (P = 0.01) and gait test (Fig. 6E) but not ataxia (Fig. S10D). We also tested somatosensation using the hot plate-adhesive removal (33), and shock tests. Mice showed very significant decreased latency to react to nociceptive heat (P = 0.0001), indicative of increased sensitivity (Fig. 6F). This, however, was specific to heat stimuli, since touching sensitivity and electric shock showed no difference (Fig. S10F). Phenotypes were replicated in a validation cohort of het male mice but were milder, suggesting that the dosage sensitivity of Wdr47 is also reflected at the behavioral level (Dataset S11). Together, these results support that the neuroanatomical defects associated with Wdr47 KO result in hyperactivity and sensory motor gating abnormalities both in male and female mice.

To test whether behavioral phenotypes worsen with age, we generated a 56-wk-old cohort but found no difference between phenotypes detected from this cohort and at 6 wk age, suggesting that Wdr47 is unlikely to be implicated in neurodegeneration (Fig. S10F). In addition, we tested the impact of enriched diet on behavioral performances but saw no phenotypic improvement or rescue compared with mice on chow diet (Dataset S11).

WDR47 Plays a Role in Cell Homeostasis and Autophagy. Several important WDR proteins implicated in autophagy [Atg18 (34), PIK3R4 (35)] have been identified in yeast before transposition to a mammalian system. WDR47 is not normally expressed in yeast; however, this unicellular model allows us to rapidly test for a wider spectrum of biological processes. We took advantage of this to study the WDR47 C-terminal to LisH (CTLH) domain, a predicted alpha-helical sequence with function that remains completely unknown in mammals (36) (Fig. 7A), by overexpression of human WDR47 and LIS1 in Saccharomyces cerevisiae (Fig. 7B). While WDR47 is less expressed than LIS1 (Fig. 7B), delayed growth was recorded exclusively in WDR47-GFP-transformed cells (Fig. 7C), suggesting that WDR47 is hijacking important cellular functions. After staining of the vacuolar membrane with the lipophilic dye FM4-64 (Fig. S11A), we observed that LIS1-GFP was mainly cytosolic; however, overexpression, it associated with a large punctate structure adjacent to the vacuole, whereas WDR47-GFP was associated with smaller structures in the cytoplasm (Fig. S11A) but not with endosomes or Golgi complex (Fig. S11B). These results show that, when WDR47 and LIS1 are overexpressed in yeast cells, they have different intracellular localization, and only WDR47 overexpression impairs growth.

Autophagy being essential for cell viability on nutrient starvation in yeast (37) and autophagosomes being formed at a single site next to the vacuolar membrane (38), we asked whether WDR47 or LIS1 could be localized to autophagy sites. We first used expression of WDR47 or LIS1 with mCherry-Atg8, a homolog of mammalian LC3 (39). This revealed that WDR47 impaired yeast autophagy, whereas LIS1 did not. Indeed, mCherry-Atg8 did not reach the lumen of the vacuole and accumulated into the cytoplasm (Fig. 7D). Coexpression of WDR47- or LIS1-GFP with mCherry-Atg8 also impaired yeast autophagy and further showed that WDR47 was associated with punctate structures that did not colocalize with Atg8 (Fig. 7E). These results suggest that WDR47 might interact with some yeast autophagy effector, thereby inhibiting this cellular process.

To investigate this hypothesis in mammalian cells, we tested autophagy in GT1-7 WDR47 siRNA-treated cells and quantified phosphorylation states (31). We assessed this in WDR47 siRNA-treated cells and quantified 22-Kda (unphosphorylated) and 25-Kda (phosphorylated) bands, but we only detected a trend for decreased 22-Kda SCG10 and increased 25-Kda SCG10 (Fig. S9D). Together, these results suggest that WDR47 physically interacts with the microtubule-destabilizing protein SCG10.
Discussion

WDR proteins have recently emerged in the field of neuroscience, but their function and ultimately, their participation in shaping the mammalian brain remain to be addressed. Here, we report the analysis of 26 WDR proteins in brain anatomy and focus on the functional characterization at the whole-organism level using a combination of cellular, invertebrate, and vertebrate model systems (siRNA, yeast, and KO mice) of a poorly characterized member, WDR47. WDR47 is also known as Neunitin (23), a protein sharing structural homology with LIS1, a WDR protein identified 20 years ago and associated with lissencephaly (7). Our findings highlight four important points.

First, 27% of assessed WDR genes gave rise to severe brain anomalies when inactivated in mice, showing the functional importance of WDR genes in brain connectivity, particularly in the genesis of the corpus callosum, a commissure that provides higher-order neurological advantages in placental mammals. This is in line with the emerging role of WDR genes in human brain pathologies associated with corpus callosum anomalies. Examples include WDR73, WDR81, ERC28, and HERC1 (40). Herc1/−/− was coincidently processed in our study and showed macrocephaly and enlarged corpus callosum, reminiscent of the radiographic features of human patients with HERC1 mutations, showing the pertinence of rodent screens for translating neuroanatomical disorders in humans.

Second, while WDR47 has previously been shown to associate with microtubules (23), we report here its implication in brain development and corpus callosum genesis. Our working model is shown in Fig. 8. In the absence of WDR47, we show that both callosal and corticofugal neurons have severe fiber tract defects and abnormally shaped growth cones in conjunction with microcephaly linked to the exhaustion of late cortical progenitors and the concomitant decrease of neuronal populations in the cerebral cortex, yielding to abnormal genesis of glial cells. Together, these could provide a molecular model underlying altered motor coordination, skilled movements, and pain sensitivity. Our work also provides insights showing that WDR47 plays a microtubule stabilizer role in the growth cone. This is further supported by the interaction between the N terminus of WDR47 and SCG10 [a very well-known microtubule destabilizer promoting catastrophe at the growth cone (41)], which led us to think that WDR47 might also be a regulator of SCG10 activity in the JNK1 pathway. Interestingly, KO mouse studies of Jnk1 (42) show high behavioral similarities (for example, motor coordination defects) compared with Wdr47.

Third, altered levels of LC3-II and p62 proteins, concomitantly with increased autophagy flux, suggest that WDR47, unlike LIS1, is involved in the regulation of key effectors of autophagy in the brain. Autophagy is a protein clearance process of aberrant or obsolete cellular structures and organelles crucial in cell homeostasis. Microtubules seem an important player in the autophagy process in mammals (43); however, their specific implication in autophagy is unclear (44). A potential molecular mechanism by which WDR47 regulates autophagy might be through its interaction with the light chain of MAP8 (also known as MAPIS) (23). In a similar way to MAP8 (45), WDR47 might bridge the autophagy machinery, in particular, LC3-II–bound autophagosomes, with microtubules. Unlike other cells of the mammalian system, postmitotic neurons are more vulnerable to damage from cellular debris and thus, would require a well-regulated degradation pathway.

levels of LC3 and p62 (or SQSTM1), two key proteins involved in autophagy. We found that LC3-II levels, but not LC3-I, were reduced (Fig. 7F). To assess whether this is caused by increased autophagic flux or decreased autophagosomal synthesis, we treated both WDR47 siRNA-treated and siRNA control groups with bafilomycin A1, a potent inhibitor of the H+ ATPase that reduces lysosomes functionality, and found that both LC3-II and p62 levels were increased (Fig. 7F), indicating enhanced autophagy flux. We also tested the expression of p62 and mTOR (an upstream regulator of autophagy) in cortices from adult Wdr47−/− mice, showing a clear specificity of WDR47 function in p62-mediated autophagy (Fig. 7G).

In addition, transmission EM revealed abnormal autophagosomes in the cytoplasm of primary neuronal cultures (Fig. 7H). Together, these data show an additional role of WDR47 in cell homeostasis and protein clearance by modulating autophagic activity.

Fig. 7. WDR47 is a key effector of autophagy. (A) WDR47 and LIS1 structures. (B) Western blot on yeast protein extracts with anti-GFP antibodies. (C) Drop test growth assays done on WT yeast cells (BY4742) transformed with pAG413 [low-copy number centromere (CEN) plasmid; expression] or pAG423 (2 microns; over-expression) plasmids bearing LIS1 or WDR47. Midlog phase cultures of the indicated yeast cells serially diluted to the indicated OD600 and spotted onto synthetic medium without histidine (SC-H9). Growth evaluated after 2 d of incubation at 30 °C. (D) WT BY4742 (control) or Wdr47−/− (negative control) yeast cells transformed with mCherry-Atg8 plasmid and WT BY4742 cells cotransformed with expression plasmid (pAG413) bearing LIS1 or WDR47 cDNA observed by fluorescence microscopy after incubation for 4 h in nitrogen starvation medium (SD-N) to induce autophagy. (Scale bars: 5 μm) (E) Living WT yeast cells (BY4742) expressing human LIS1-GFP or WDR47-GFP and mCherry-Atg8 observed by fluorescence microscopy after induction of autophagy by incubation in SD-N medium. (F) Western blot quantification of LC3 and p62 relative protein levels in the presence and absence of Bafilomycin A1 (Baf) control treatment in response to WDR47 siRNA treatment. GAPDH was used as a loading control. (G) Western blot images of p62, mTOR, and phospho-mTOR in the cortex of WT and Wdr47−/− embryos. Quantification of relative protein expression normalized against β-actin is plotted as mean ± SEM (n = 6 Wdr47−/− and n = 5 WT, male and female), *P < 0.05; **P < 0.001. (H) Transmission EM of cortical neurons from Wdr47−/− embryos (n = 3) at E18.5 compared with WT (n = 3). (Scale bars: Left, 2 μm; Right, 1 μm.)
WDR47 involvement in the survival of postmitotic neurons in late corticogenesis from upper cortical layers, where WDR47 is the most expressed, could be mediated through the proper regulation of autophagy, reinforcing the molecular cross-talk between autophagy and apoptosis (46). Furthermore, the possible association of WDR47-related autophagy to brain wiring is supported by a recent report showing that the WDR autophagy scaffold protein (ALFY) is required for neuronal connectivity in the mouse brain (47). Strikingly, the lack of ALFY in mice led to perinatal lethality, microcephaly, absence of the corpus callosum, and hypoplasia of the intermediate zone, reminiscent of Wdr47 phenotypes, suggesting a similar mode of action. It may, therefore, be that WDR47 regulates the cell ability to detect environmental cues via its microtubule stabilization role, which in turn, regulates the protein degradation pathway necessary for neuronal shape and motility.

Fourth, the final significant finding is the essential role of WDR47 for survival. The scarcity of patients harboring mutations in WDR47 supports this notion. Indeed, we found no truncating mutations in WDR47, despite sharing data at numerous genetic meetings, and only three stop-gained mutations are reported in the ExAC database (48), suggesting a selection bias against LoF mutations in WDR47. Extending our analysis to three specific cohorts of unknown genetic cause made up of patients with lissencephaly, intellectual disability, and nonsyndromic agenesis of the corpus callosum, we identified three missense variants (Datasets S12 and S13); however, pathogenicity and transmission mode were not compatible with WDR47 gene causality. The inability of S. cerevisiae to grow, possibly because of the inhibition of autophagy on overexpression of WDR47, further supports the essentiality of WDR47 for survival but also suggests that mirrored protein levels (too little or too much) have identical effects on pathogenicity and underlying biological processes. This is a characteristic of scaffold proteins, indicating that WDR47 might be serving as a support for the interaction of other proteins, such as SCG10. Although we were unable to identify the precise reason of death, cardiac and breathing failure were excluded. Autophagy being dramatically up-regulated in the neonatal stage to overcome the starvation period (49), mice could be dying of lack of nutrients, since an enriched lipid diet rescued lethality.

In conclusion, this study presents identification of the relevance of WDR genes in brain connectivity, highlighting the power of unbiased and high-throughput mouse LoF studies in the field of neuroscience. These mouse models could explain some of the missing genetics in corpus callosum biology (11) and help pave the way for a better stratification system of complex neurodevelopmental steps. WDR47 plays a role in the regulation of microtubule dynamics, progenitor proliferation, neuronal migration, and fiber tract projections in a similar fashion to LIS1 (50, 51) but with the distinctive particularity that WDR47 inhibits autophagic flux. This provides a functional link between autophagy biology and the CTLH domain (52) in mammals (this association was previously made with the vacuole import and degradation pathways in yeast (53)), while strengthening the emerging link between autophagy and microtubules assembly. Although a definite association of WDR47 with human brain disorders has not been made yet, WDR47 should be considered as a candidate gene for corpus callosum abnormalities and motor coordination deficiencies, possibly through compound heterozygosity or somatic mosaicism. Considering that the microtubule stabilizer drug restored growth cone defects, it will be interesting to test whether the behavioral anomalies, as already shown in an Alzheimer mouse model (54), could also be restored. This might open up therapeutic perspectives in the clinic with the aim of relieving symptoms in patients suffering from this class of diseases that we refer to as “WDRopathies.”

**Materials and Methods**

Mutant mice were obtained through collaboration with the Sanger Mouse Genetics Project. Wdr47-targetted mice were generated using the International Mouse Phenotyping Consortium targeting mutation strategy (55). This relies on the identification of an exon common to all transcript variants, upstream of which an LacZ cassette was inserted to make a KO (tm1a), whereas tm1b creates a frameshift mutation on deletion of the selected exon. Animal procedures were approved by the local ethics committee.
We thank Jonathan Flint for helpful comments on the manuscript, Christel Deplenie, and Albert Weixlbaumer for scientific discussions. We thank Nadia Messadeq for the development of the genotyping strategy. We thank Mustapha Oulad-Abdelghani for producing WDR47 antibody. We also thank animal care workers at the MCI and at the Electron Microscopy Facility for their superb images. We thank Hugues Jacobs at the MCI for advice on histology and all of the members of the imaging facility at Institut de Génétique et de Biologie Moléculaire et Cellulaire. We thank Fulvio Reggio (Heidelberg University) for the rh1 (McHerry-Aktig plasmid and Gabriele Grennell) (École Polytechnique Fédérale de Lausanne) for SCG10 antibodies. J.D.G. is funded by the Action Thématique et Incitative sur Programme (ATIP-Avenir) joint CNRS-INSERM program and the Fysen foundation. This study was funded by CNRS (S.F.), INSERM (E.B. and S.F.), Strasbourg University (S.F.), initiative Stratilab (IDEX 2015-2019), grants ANR-10-LBX-0030-INRT, a French State fund managed by the Agence Nationale de la Recherche under the frame program Investissements d'Avenir ANR-10-IDEX-0002-02 (to B.Y. and J.D.G.). C.K. is supported by funding from the South African Medical Research Council. B.Y. is supported by the Jérôme Lejeune Foundation, the French National Research Agency (ANR-11-PDOC-0029), and the Gutenberg Circle.

ACKNOWLEDGMENTS. We thank Jonathan Flint for helpful comments on the manuscript, Christel Deplenie, and Albert Weixlbaumer for scientific discussions. We thank Nadia Messadeq for the development of the genotyping strategy. We thank Mustapha Oulad-Abdelghani for producing WDR47 antibody. We also thank animal care workers at the MCI and at the Electron Microscopy Facility for their superb images. We thank Hugues Jacobs at the MCI for advice on histology and all of the members of the imaging facility at Institut de Génétique et de Biologie Moléculaire et Cellulaire. We thank Fulvio Reggio (Heidelberg University) for the rh1 (McHerry-Aktig plasmid and Gabriele Grennell) (École Polytechnique Fédérale de Lausanne) for SCG10 antibodies. J.D.G. is funded by the Action Thématique et Incitative sur Programme (ATIP-Avenir) joint CNRS-INSERM program and the Fysen foundation. This study was funded by CNRS (S.F.), INSERM (E.B. and S.F.), Strasbourg University (S.F.), initiative Stratilab (IDEX 2015-2019), grants ANR-10-LBX-0030-INRT, a French State fund managed by the Agence Nationale de la Recherche under the frame program Investissements d'Avenir ANR-10-IDEX-0002-02 (to B.Y. and J.D.G.). C.K. is supported by funding from the South African Medical Research Council. B.Y. is supported by the Jérôme Lejeune Foundation, the French National Research Agency (ANR-11-PDOC-0029), and the Gutenberg Circle.