Mutations in the KIF21B kinesin gene cause neurodevelopmental disorders through imbalanced canonical motor activity

Laure Asselin1,2,3,4, José Rivera Alvarez1,2,3,4,20, Solveig Heide5,6,7,20, Camille S. Bonnet1,2,3,4,20, Peggy Tilly1,2,3,4, Hélène Vité8, Chantal Weber1,2,3,4, Carlos A. Bacino9,10, Kristin Baranaño11, Anna Chassevent11, Amy Dameron12, Laurence Faivre13,14, Neil A. Hanchard9, Sonal Mahida15, Kirsty McWalter12, Cyril Mignot5,6,7,16, Caroline Nava5,16, Agnès Rastetter16, Haley Streff9,10, Christel Thauvin-Robinet13,17, Marjan M. Weiss18, Frédéric Saudou8, Christel Depienne1,2,3,4,16,19, Christelle Golzio1,2,3,4, Delphine Héron5,6,7 & Juliette D. Godin1,2,3,4

KIF21B is a kinesin protein that promotes intracellular transport and controls microtubule dynamics. We report three missense variants and one duplication in KIF21B in individuals with neurodevelopmental disorders associated with brain malformations, including corpus callosum agenesis (ACC) and microcephaly. We demonstrate, in vivo, that the expression of KIF21B missense variants specifically recapitulates patients’ neurodevelopmental abnormalities, including microcephaly and reduced intra- and inter-hemispheric connectivity. We establish that missense KIF21B variants impede neuronal migration through attenuation of kinesin autoinhibition leading to aberrant KIF21B motility activity. We also show that the ACC-related KIF21B variant independently perturbs axonal growth and ipsilateral axon branching through two distinct mechanisms, both leading to deregulation of canonical kinesin motor activity. The duplication introduces a premature termination codon leading to nonsense-mediated mRNA decay. Although we demonstrate that Kif21b haploinsufficiency leads to an impaired neuronal positioning, the duplication variant might not be pathogenic. Altogether, our data indicate that impaired KIF21B autoregulation and function play a critical role in the pathogenicity of human neurodevelopmental disorder.
he development of the mammalian cerebral cortex depends on microtubule (MT)-related processes that coordinate birth, migration and differentiation of excitatory and inhibitory neurons. MT cytoskeleton acts in concert with microtubule-associated proteins (MAP) and motor proteins to promote the structural changes that underlie key developmental events such as neurogenesis, migration, neuritogenesis, axon pathfinding and synapse formation. Kinesin superfamily proteins (KIFs) are important molecular motors that control MT organization and dynamics in both axons and dendrites and mediate intracellular transport of various cargo, including vesicles, organelles, cellular proteins and mRNAs, along MTs. The importance of both the force-generating and MT-regulating functions of KIFs for brain development has become evident with loss of function studies demonstrating defects in mitosis, cytokinesis, polarity, migration, axonal growth and branching, survival and synaptogenesis. Further reflecting the key role of KIFs in neuronal development, variants in human KIF-encoding genes (KIF4A, KIF7, KIF11, KIF23, KIF24, KIF5C, KIF14, KIF3A, KIF4A, KIF26A) have been associated with various neurodevelopmental disorders, including malformation of cortical development (MCD), acrocallosal syndrome, ciliopathies, epilepsy and intellectual disability. Most KIF variants have been predicted to be highly pathogenic in silico but their direct implication in disease has been elicited for only a few of them.

The MT-plus-end directed kinesin-4 motor KIF21B is mainly expressed in spleen, testes and central nervous tissues and is particularly enriched in neurons. Within neurons, KIF21B is present in both axons and dendrites and especially abundant in growth cones. KIF21B has dual functions in neurons. First, it promotes intracellular transport through its N-terminal processive motor activity. However, except for BNDF-TrkB signaling endosomes, 2-subunit-containing GABAA receptor and neurobeachin recycling endosomes, our knowledge of KIF21B transported cargoes is very limited. Second, KIF21B influences MT dynamics through distinct MT binding domains. KIF21B positively regulates MTs dynamics in dendrites by favoring MT growth and catastrophes. In addition, in vitro studies demonstrate that KIF21B can also act as a MT pausing factor by accumulating at the MT-plus ends. Notably, KIF21B functions can be modulated by neuronal activity, which favors KIF21B trafficking activity at the expense of MT dynamics regulatory function as well as through an auto-inhibitory interaction between the N-terminal motor domain and an internal regulatory coiled-coil region.

KIF21B homozygous knockout (KO) mice display severe morphological abnormalities including microcephaly and partial loss of commissural fibers, cognitive deficits and altered synaptic transmission. Together with the reduced dendritic complexity and spines density observed in KIF21B−/− neurons in culture, these results highlight a critical role for KIF21B in brain development and function. Though, there are no clear KIF21B-related neurodevelopmental disorders, a duplication of the locus bearing KIF21B has been found in individuals with neurodevelopmental delay and intellectual disability (ID).

Here we provide the evidence of a causal relationship between variants in KIF21B and neurodevelopmental disorders. We report the identification of three missense variants and one truncating variant in patients with neurodevelopmental delay and brain malformations including corpus callosum (CC) agenesis (ACC) and microcephaly. By combining in vivo modeling tools, we show that KIF21B pathogenic variants impede neuronal migration and connectivity through at least two distinct mechanisms both leading to dysregulation of canonical kinesin motor activity.

Taken together our data suggest that KIF21B is a novel gene for ID associated with heterogeneous brain morphological anomalies.

### Results

#### Identification of human KIF21B variants

Using trio whole-exome sequencing, we identified a de novo variant (NM_001252100.1, c.2032A>C, p.Ile678Leu) in the KIF21B gene in a first patient (P1) presenting with developmental delay, learning and motor disabilities, associated with isolated complete agenesis of the corpus callosum (ACC) (Fig. 1a, e, Table 1, Supplementary Note 1). Through the GeneMatcher platform, variants in KIF21B were found in three additional patients. Patient 2 (P2) (NM_001252100.1, c.937C>A, p.Gln313Lys) presented with severe ID associated with microcephaly (Fig. 1b, f); patient 3 (P3) (NM_001252100.1, c.3001G>A, p.Ala1001Thr) presented with global developmental delay and mild to moderate ID (Fig. 1c) but normal brain structure at the MRI. This variant was inherited from the father, who presented with developmental delay and learning difficulties; and patient 4 (P4) (NM_001252100.1, c.2959_2962dup, p.Asn988Serfs*4-P4) presented with mild developmental delays and hypotonia, but no brain structural anomalies on brain MRI (Fig. 1d, g). The three identified KIF21B missense variants occur within highly conserved residues positioned in the motor domain (NM_001252100.1, c.937C>A, p.Gln313Lys-P2), the regulatory coiled-coil (rCC) region (NM_001252100.1, c.3001G>A, p.Ala1001Thr-P3), and the coiled-coil domain (NM_001252100.1, c.2032A>C, p.Ile678Leu-P1) (Fig. 1h, Supplementary Fig. 1a–c). The fourth variant is a duplication (NM_001252100.1, c.2959_2962dup, p.Asn988Serfs*4-P4) that leads to the introduction of a premature termination codon in exon 20. RT-qPCR analysis and sequencing of KIF21B transcripts isolated from P4’s blood revealed haploinsufficiency, likely due to the degradation of the mutant mRNA by nonsense-mediated decay (Supplementary Fig. 1d, e). All variants were predicted pathogenic by commonly used in silico software (Polyphen-2, Mutation Taster, SIFT and CADD; Supplementary Fig. 1f) and co-segregated with the phenotype in each pedigree (Fig. 1a–d). Of note, we found two other de novo variants of unknown significance in patients: one homozygous variant in ARHGA4 (chromosome X) in P1 that also segregated in his healthy brother and one de novo in UBR3 (NM_172070.3, c.5023G>C; p.Glu1675Gln) in P4, that showed a weak pathogenic score based on in silico predictions. None of the four KIF21B variants is reported in public databases, including dbSNP, 1000 Genomes and gnomAD. Overall, we identified variants in KIF21B gene in four patients presenting with mild to severe neurodevelopmental delay associated with heterogeneous brain malformations (Table 1, Supplementary Note 1).

### Kif21b expression is restricted to neurons

We first examined the expression pattern of Kif21b in the mouse developing cortex. Although levels of Kif21b mRNA transcripts are rather stable during development (Fig. 2a), protein expression tends to increase from embryonic day (E) 12.5 to postnatal day (P) 2 (Fig. 2b). Kif21b transcripts were mostly observed in the cortical plate (CP) and almost entirely excluded from the ventricular (VZ) and subventricular (SVZ) zones, where progenitors and newborn neurons reside (Supplementary Fig. 2a). As corticogenesis proceeds, mKif21b mRNAs accumulated in the intermediate zone, which is enriched in growing axons (Supplementary Fig. 2a). Immunolabeling of E12.5 to E18.5 embryo brain sections showed a restricted expression of mKif21b proteins to postmitotic compartments of the neuronal epithelium with a particularly intense signal in the axon-rich zone.
analyzed mKif21b protein in progenitor (YFP undoubtedly exclude any expression of Kif21b in progenitors, we stages of development (Fig. 2c, d and Supplementary Fig. 2b, c). To that Kif21b is exclusively expressed in post mitotic neurons at all axons from cell bodies and dendrites51 (Fig. 2e, f). Channels immunoblotting (Supplementary Fig. 2d). Although Kif21b was label neurons and intermediate progenitors respectively, con sections at birth (Supplementary Fig. 2e, f). Further immunostain- was validated by the absence of labeling in

(Fig. 2c and Supplementary Fig. 2b, c). mKif21b staining specificity was validated by the absence of labeling in Kif21b knockout brain sections at birth (Supplementary Fig. 2b, f). Further immunostainings with antibodies against mKif21b and βIII-Tubulin or Tbr2 that label neurons and intermediate progenitors respectively, confirmed that Kif21b is exclusively expressed in post mitotic neurons at all stages of development (Fig. 2c, d and Supplementary Fig. 2b, c). To undoubtedly exclude any expression of Kif21b in progenitors, we analyzed mKif21b protein in progenitor (YFP+; CD24+) and neuron populations (YFP+; CD24+) isolated from Rosa26-lox-STOP-YFP; NEXCRE/– mice. Nucleus staining using antibodies against mKif21b and Tau antibodies in these devices con...

**Fig. 1 Patients with KIF21B variants.** a-d Pedigrees of patients with identified KIF21B variants. e-g Sagittal brain section of patient’s MRI showing a complete agenesis of the corpus callosum in patient 1 (e, red arrow) and microcephaly in patient 2 (f). h Schematic representation of the human KIF21B (hKIF21B) protein indicating the different domains (motor domain, ATP binding site, coiled-coil domain (CC) 1 and 2, regulatory coiled-coil domain (rCC) and WD40 domain) and the position of the mutated amino acids for patient 1 (p.Ile678Leu), 2 (p.Gln313Lys), 3 (p.Ala1001Thr) and 4 (p.Asn988Serfs*4). T96N substitution that abolishes KIF21B mobility is also depicted.

**KIF21B variants impair migration of projection neurons.** To evaluate the pathogenic nature of KIF21B variants, we assessed the consequences of overexpressing hKIF21B variants on neuronal migration using in utero electroporation (IUE) in mice. Given that KIF21B is a post mitotic kinesin, we used plasmids allowing expression of human KIF21B under the control of the NeuroD promoter (NeuroD-hKIF21B). Transfection in N2A neuroblastoma cell line showed even expression of wild-type (WT) and all three missense hKIF21B variants by western blot, suggesting that p.Gln313Lys, p.Ile678Leu and p.Ala1001Thr missense variants are unlikely to affect the production, stability or turnover of the hKIF21B proteins (Supplementary Fig. 3a). Consistently, cycloheximide chase experiments in N2A cells revealed a similar half-life of WT and mutant hKIF21B proteins (Supplementary Fig. 3b).

To investigate the effects of the variants on neuronal positioning, we individually induced neuron-specific expression of the hKIF21B mutants using IUE of NeuroD-hKIF21B constructs together with a NeuroD-IRE-GFP reporter plasmid in mouse embryonic cortices at E14.5. Four days after IUE, whereas most of the GFP+ postmitotic neurons expressing full-length WT-hKIF21B reached the CP as in the control (Fig. 3a), neurons expressing missense variants accumulated in the intermediate zone, with a decrease of 27.7%, 60.3% and 23% of the cells reaching the upper CP in the p.Gln313Lys, p.Ile678Leu and p.Ala1001Thr conditions, respectively (Bonferroni adjusted P = 0.0001) (Fig. 3b). Noteworthy, hKIF21B missense variants likely disturbed neuronal migration in a cell-autonomous manner as their expression did not affect cell survival and glia scaffold integrity (Supplementary Fig. 3c). To assess the functional consequences of the p.Asn988Serfs*4 protein truncated variant (Supplementary Fig. 1d), we silenced mKif21b specifically in post mitotic neurons using IUE of CRE-dependent inducible shRNA vector together with a NeuroD-CRE-IRE-GFP construct at E14.5. Efficacy of the two shRNAs was confirmed by RT-qPCR (~61.4% for sh-Kif21b #1, ~45.1% for sh-Kif21b #2) (Supplementary Fig. 3d). Four days after IUE, Kif21b-silenced neurons displayed migration defects compared to control shRNA-electroporated cells with a reduction of 23.5% and 32.2% of cells distributed in the upper CP for sh-Kif21b #1 and sh-Kif21b #2, respectively (Supplementary Fig. 3e, f). To note, the migratory phenotype induced by sh-Kif21b #2 was fully recovered by co-electroporation of wild-type NeuroD-mKif21b construct (Supplementary Fig. 3e, f). Most of the cells overexpressing the p.Gln313Lys and p.Ala1001Thr mutants or silenced for Kif21b showed a correct positioning with nearly all cells found in the axial compartment (Supplementary Fig. 3f, g).
Table 1 Clinical summary of patients with *hKIF21B* variants.

| Patient | Patient 2 | Patient 3 | Patient 4 |
|---------|-----------|-----------|-----------|
| **Age at last evaluation** (y) | 10 | 12 | 9 | 3 y 8 m |
| **Sex** | Male | Male | Female | Male |
| **Genetics** | KIF21B | KIF21B | KIF21B | KIF21B |
| **NM_001252100.1** c.2032A>C | c.937C>A | c.3001G>A | c.2959_2962dupGCCAHGVS |  |
| **p.Ile678Leu** | p.Gln313Lys | p.Ala1001Thr | p.Asr988serfs*4 |  |
| **Inheritance** | De novo | De novo | Inherited from the affected father | De novo |
| **Pregnancy and delivery** | Normal | Oligohydramnios and IUGR | Normal | Small for gestational age |
| **Height (perc)/weight** | 51 cm (97th p), 3.750 kg (97th p), 32 cm (5th p) | 49 cm (49th p), 2.584 kg (7th p), 34 cm (25th p) | 3.480 kg (50th p) | 46.4 cm (7th p), 26.33 kg (5.87th p), 34 cm (25th p) |
| **Neonatal findings** | None | Nuchal cord at birth and was blue, mild respiratory distress, but discharged with mother | None | *Measurements done at age 6 days* |
| **Developmental stages** | | | | |
| **Age of sitting (months)** | 10 | Does not | 14 | 9 |
| **Age of walking (months)** | 18 | Does not | 24 | 15-16 |
| **Age of first words (m:months, y:years)** | 36 m | Yes | Yes | No |
| **Age of first sentences (m:months, y:years)** | NA | N/A | 36 m | 12 m |
| **Current language ability** | Short sentences, dysarthria | Non-verbal | Sentences | Short sentences |
| **Intellectual disability (ID)** | Borderline | Severe | Mild to moderate | Mild |
| **Estimated level of ID** (mild, moderate, severe) | | || |
| **Age at examination (y)** | 10 | 9 | 3 y 9 m | 3 y 8 m |
| **Total IQ** | 66-79 (WISC V) | 54-59 (WISC V) | || |
| **Clinical examination** | | | | |
| **Age at examination (years)** | 5 y | 12 y 1 m | 3 y 9 m | 3 y 8 m |
| **Height (SD)/weight** (SD)/head circumference (SD) | 118 cm (+1.5), 21.3 kg (+2), 52 cm (+0.5) | 139 cm (~3.3), 20 kg (~3.4), 48.5 cm (~3.9) | 99 cm (~1.3), 17.8 kg (~1.8), 49.6 cm (~0.2) | 90.7 cm (~1.67), 16.3 kg (~0.09), 50.7 cm (~0.72) |
| **Neurologic examination** | Poor visual fixation, constant tongue thrusting, poor gag, poor head control, bilateral ankle tightness, right wrist contracture | Large eyes, fleshy ears, hypertelorism | Mildly hypertonic legs | Hypotonia |
| **Dysmorphic features** | plagiocephaly | | | Upslanting palpebral fissures, prominent eyebrows, broad nose with bulbous tip, anteverted nares, Micrognathia, Right-sided Duane syndrome |
| **Brain imaging (MRI)** | Epicanthic folds, mild ptosis, tented upper lip | | | A few scattered punctate foci of T2 prolongation in subcortical white matter and periventricular white matter of bilateral cerebral hemispheres with no associated restricted diffusion or hemorrhage |
| **Age at examination (m:months, y:years)** | 3 | 6 m and 12 y | 2 y | 1y 9 m |
| **Brain anomalies (MRI)** | Complete agenesis of the corpus callosum | | | History of falling spells with normal EEG. Severe constipation, Central sleep apnea. History of feeding issues requiring G tube |
| **Other** | | | | |
the upper layer of the cortex after birth, indicating a delay in migration rather than a permanent arrest (Fig. 3c, d, Supplementary Fig. 3g, h). By contrast, the p.Ile678Leu variant induced a permanent migration defect as a large number of p.Ile678Leu-expressing neurons remained in the white matter and deep-layers at P2 (Fig. 3c, d). Remarkably, p.Ile678Leu-expressing projection neurons permanently arrested in the white matter were expressing the upper-layer marker Cux1, which supports a faulty migration rather than specification defects (Fig. 3e). Altogether, these results demonstrate that missense hKIF21B variants and Kif21b haploinsufficiency impede, to various extents, the radial migration of projection neurons.
KIF21B variants lead to aberrant KIF21B motility activity. To understand the molecular mechanisms by which variants in hKIF21B gene lead to defective radial migration, we tested for restoration of the hKIF21B variant-induced phenotype by increasing amount of wild-type protein. IUE of wild-type hKIF21B together with hKIF21B mutants at a 1:1 ratio failed to rescue the migration phenotype (Fig.3a, b). Strikingly, neurons overexpressing large amount of WT-hKIF21B (2 units of NeuroD-hKIF21B) failed to reach the upper CP 4 days after IUE (Fig. 4a, b). Collectively, these results raise the possibility that hKIF21B variants impair migration by enhancing KIF21B activity in a dominant manner. One possible mechanism by which hKIF21B mutants might exert this effect is by relieving autoinhibition imposed by the rCC to the motor domain as shown for CFEOM-causing variants in KIF21A46,53,54, a kinesin-4 family member that shares 61% identity with KIF21B40. Consistent with the hypothesis that KIF21B hyperactivation cause migration phenotypes, expression of a truncated mouse mKif21b protein that lacks the rCC domain (1 unit of NeuroD-mKif21bΔ) led to faulty migration (Fig.4c, d).

To test whether identified variants alter KIF21B autoinhibition, we next sought to explore the functions of KIF21B that were enhanced by KIF21B autoinhibition release in mutant conditions. Given the processive activity of KIF21B, we assessed the effect of the variants on KIF21B motility activity. Using immunofluorescence, we first compared the localization of the wild-type (WT) hKIF21B and variants. The localization of wild-type hKIF21B and variants was compared to the localization of other proteins such as Cux1. The percentage of electroporated GFP-cells in different regions showing effect of expressing hKIF21B variants was analyzed by two-way ANOVA (Bonferroni’s multiple comparisons test). Rescue experiments: n = 6 for each condition. Source data are provided in the Source Data file.
and the three missense mutant proteins in ST cells transfected with pcDNA-HA-hKIF21B cDNA constructs (Fig. 4e, Supplementary Fig. 4a). Although WT, p.Gln313Lys and p.Ala1001Thr proteins showed similar diffuse cytoplasmic localization, the p.Ile678Leu KIF21B protein tended to form aggregates localized mainly at the periphery of the cells, suggesting an enhanced motility toward the plus end of the microtubules (Fig. 4e). To note, WT and all mutant proteins showed similar distribution in soma and neurites when overexpressed in primary cortical neurons (Supplementary Fig. 4c). Interestingly, in 100% of the...
ST cells where the p.Ile678Leu variant is mislocalized, the cellular localization of the WT protein is altered, suggesting that the p.Ile678Leu protein might act as a dominant negative protein (Fig. 4f). In accordance, the p.Ile678Leu variant is competing with the WT protein to form KIF21B homodimer. Indeed, anti-Myc immunoprecipitation on extracts from HEK293T cells expressing myc-tagged WT, HA-tagged WT and WT or p.Ile678Leu GFP-tagged hKIF21B proteins revealed that the binding of the Myc and HA-tagged WT proteins was affected by the expression of the p.Ile678Leu missense variant (Supplementary Fig. 4d). We further analyzed the processivity of mutant hKIF21B in Cos7 cells transfected with GFP-tagged hKIF21B constructs (Supplementary Fig. 4b). Live-cell imaging revealed a shift of GFP-hKIF21B velocity toward high speed for all variant proteins compared to the WT protein (Fig. 4g). Notably the p.Ile678Leu variant showed a more drastic effect with an increased average velocity of 28% compared to the WT protein (Fig. 4h). We next assessed the effect of hKIF21B variants on the trafficking of BDNF vesicles and mitochondria, two potential cargoes of KIF21B (31, 44). Fast videomicroscopy experiments performed in Cos7 cells transfected with WT and mutant pcDNA-HA-hKIF21B cDNA (Supplementary Fig. 4b) and BDNF-mCherry or Mito-RFP constructs did not reveal any change in the dynamics of neither BDNF-mCherry-containing vesicles (Supplementary Fig. 4e–g) or mitochondria (Supplementary Fig. 4h, i), suggesting that hKIF21B variants might lead to excessive motility of other unidentified cargoes. Collectively, these data indicate that the variants enhanced KIF21B processive activity through lessening of the kinesin autoinhibition.

We finally tested the effect of expressing immotile hKIF21B mutant proteins on neuronal migration. We performed IUE of truncated WT and mutant hKIF21B that lacks the ATP binding domain (Fig. 1b) in wild-type E14.5 mouse cortices. Although a significant number of neurons expressing the p.Gln313Lys, p.Ile678Leu and p.Ala1001Thr hKIF21B variants were trapped in the IZ at E18.5, most of the cells expressing the immotile variants (NeuroD-p.Gln313Lys-ΔATPhKIF21B, NeuroD-p.Ile678Leu-ΔATPhKIF21B, NeuroD-p.Ala1001Thr-ΔATPhKIF21B) showed a correct distribution (Fig. 4i, j, Supplementary Fig. 3a), demonstrating that preventing the motility of the mutant proteins decreases the severity of the migration phenotype. These results confirmed that the mutant protein impairs radial migration at least by enhancing KIF21B motility activity through the release of the kinesin autoinhibition (Fig. 4k). Collectively, our data indicate that modulation of kinesin autoregulation is critical in KIF21B-associated cortical migration phenotypes.

hKIF21B p.Gln313Lys variant reduced head size in zebrafish. Considering the presence of microcephaly in the subject with p.Gln313Lys variant, we asked whether this mutant could induce head size defects in an appropriate animal model. We therefore turned toward the developing zebrafish embryo, a model that has been extensively used for microcephaly modeling, the measure of head size being a relevant proxy for brain size (55). drKIF21B protein is broadly distributed in zebrafish larval brain at 5 days post-fertilization (dpf), a stage characterized by strong upregulation of drKIF21B transcripts (Supplementary Fig. 5a, b). Larvae injected with p.Gln313Lys human mRNA showed a significant and physiologically relevant reduction of head size compared to control at 5 dpf (e–f, Welch two sample t-test P = 1.185 × 10−9), therefore exhibiting a phenotype analogous to the microcephaly observed in the human clinical condition (Fig. 5). By contrast, introduction of WT mRNAs or the two other missense variants (p.Ala1001Thr and p.Ile678Leu), that do not lead to head circumference defects in patients, barely affected zebrafish head size (Fig. 5, Supplementary Fig. 5c, d). These data suggest that this particular p.Gln313Lys variant in the motor domain of KIF21B likely drives the microcephaly phenotype observed in the individual carrier.

p.Gln313Lys KIF21B variant does not impair proliferation. We next sought to understand the mechanisms by which the p.Gln313Lys variant impairs brain size. Expression of hKIF21B missense variants in mouse cortical neurons did not induce cell death (Supplementary Fig. 3c), excluding the possibility that the microcephaly phenotype arises from a poor survival of neurons. Although KIF21B expression is restricted to neurons, we further tested whether expression of the p.Gln313Lys variant could non-cell autonomously affect the progenitors’ biology. We performed IUE of NeuroD-p.Gln313Lys hKIF21B at E14.5 in wild-type cortices and analyzed the progenitors located in the electroverted area at E16.5. The total number and the proliferative fraction (Ki67+) of both apical (Pax6+) and intermediate progenitors (Tbr2+) were indistinguishable from control condition (Supplementary Fig. 5e–j), suggesting that the brain size phenotype induced by the p.Gln313Lys hKIF21B variant was unlikely to have arisen from impaired neurogenesis. Altogether these results suggested that neither impaired birth nor poor survival of neurons or their progenitors contributed to the microcephaly phenotype observed in the subject with the p.Gln313Lys variant.

p.Ile678Leu hKIF21B variant expression impedes axonogenesis. The corpus callosum (CC), the major commissure connecting
Fig. 5 Expression of p.Gln313Lys hKIF21B decreases head size in zebrafish larvae. a Dorsal view of representative control zebrafish larvae (non-injected) or injected with 100 pg of wild-type (WT) or mutated hKIF21B mRNAs (p.Gln313Lys and p.Ala1001Thr) at 5 days post-fertilization (5 dpf). Double arrow indicates the distance between the forebrain and hindbrain, a measure used as a proxy for head size. b Dot plot of the head measurements (red double arrow) of control and injected larvae at 5 dpf. Red diamond corresponds to the mean of the batch measured. Number of embryos analyzed for this specific batch: control, n = 40; WT, n = 31; p.Gln313Lys, n = 38; p.Ala1001Thr, n = 31. Experiments were repeated six times for non-injected control embryos (n = 231), four times for WT-injected embryos (n = 127), three times for p.Gln313Lys-injected embryos (n = 108) and four times for p.Ala1001Thr-injected embryos (n = 158). ns, non-significant, *P < 0.05, **P < 0.005, ***P < 0.001. Significance was calculated by unpaired two-tailed Student’s t-test or a Welch’s two sample t-test between control and RNA-injected larvae. ns non-significant, *P < 0.05, **P < 0.005, ***P < 0.001. Source data are provided in the Source Data file.

the two cerebral hemispheres, is formed of hundreds of millions of axons projecting contralaterally from the callosal projection neurons. Callosal axons cross the midline around birth to reach, in the first postnatal week, the contralateral cortex where they branch extensively at layer II/III and V. Given the ACC in the patient carrying the p.Ile678Leu variant, we investigated how the missense variants in hKIF21B lead to aberrant inter-hemispheric connectivity by introducing WT or mutant cDNA (NeuroD-hKIF21B) together with a mScarlet-expressing vector (pCAG2-mScarlet) in wild-type callosal projection neuron via IUE of E15.5 mouse cortical progenitors. The p.Gln313Lys substitution variant was used as a negative control as we did not expect any commissural defects according to the patient clinical features (Table 1).

At P4, soon after the axons cross the midline and at P8, when axons start invading the contralateral CP, neither the expression of WT-hKIF21B nor of any of the variants perturbed midline crossing, as indicated by an equivalent scarlet intensity on each side of the CC (Fig. 6a–c, Supplementary Fig. 6a–c). In addition, at P22, when callosal axons achieve their adult-like arborization pattern, axons correctly invaded the homotopic contralateral cortex and successfully branched in layer II-III and V in all conditions (Supplementary Fig. 6g, h). Nonetheless, expression of the p.Ile678Leu, but not WT nor p.Gln313Lys mutant reduced by half the density of scarlet-positive axons in the white matter compared to the control both at P4 and P8 (– 49.7% at P8, P = 0.0043) (Fig. 6a, b, d, e, Supplementary Fig. 6a, b, d). These defects were unlikely due to delayed innervation, as the poor inter-hemispheric connections persisted at P22 (Supplementary Fig. 6e, f). Rerouting through alternate commissures was also excluded as no aberrant axonal projections were observed after electroporation of callosal neurons and as no other commissures were shown enlarged in the patient (P1). Altogether, these results indicate that the faulty CC innervation is due to an impaired axonal growth rather than to defective contralateral targeting. Accordingly, we measured the length of the longest neurites in primary cortical neurons transfected with pcDNA-HA-hKIF21B cDNA constructs. Although primary cortical neurons expressing WT-hKIF21B or p.Ala1001Thr and p.Gln313Lys hKIF21B variants showed normal axonal growth, expression of p.Ile678Leu mutant severely impaired axonogenesis at DIV2 and DIV5 (Fig. 6f, g, Supplementary Fig. 7a, b). To dig deeper into the pathogenic mechanism of the p.Ile678Leu hKIF21B variant, we performed a complementation experiment by co-electroporating p.Ile678Leu hKIF21B with increasing amounts of WT-hKIF21B at E15.5 and analyzed the percentage of projecting neurons at P8. Equivalent amounts of WT-hKIF21B failed to rescue the CC innervation phenotype (Fig. 6b, c). Further in vitro analysis of neurite length in primary cortical neurons expressing WT-hKIF21B together with p.Ile678Leu hKIF21B mutant at a 1:1 ratio confirmed the lack of rescue of axonal growth (Fig. 6f, g). We next reasoned that the p.Ile678Leu variant might exert its effect through attenuation of KIF21B autoinhibition. Accordingly, primary neurons transfected with two units of NeuroD-hKIF21B displayed shorter longest neurites in vitro (Fig. 6f, g), suggesting that enhanced KIF21B activity induces axonogenesis defects. To corroborate these findings in vivo, we assessed the ability of neurons electroporated with ΔATPlp.Ile678Leu variant (NeuroD-p.Ile678Leu-ΔATPhKIF21B) to project axons contralaterally at P8 (Fig. 6b, c, e). There was no significant difference between the control and the immotile p.Ile678Leu-ΔATPhKIF21B, suggesting that the p.Ile678Leu variant impedes CC innervation through aberrant motor activity. Consistent with a hyperactivation of KIF21B, neurons expressing NeuroD-mKif21bArCC failed to project axons at P8 (Fig. 6b, e). Altogether these results indicate that the loss of inter-hemispheric connectivity induced by the p.Ile678Leu hKIF21B variant, and the subsequent release of KIF21B autoinhibition, arises from impaired axonal growth rather than defective innervation and arborization in the contralateral cortex.
**hKIF21B p.Ile678Leu variant impairs ipsilateral connectivity.** Callosal neurons not only branch contraterally, but also send multiple ipsilateral axon collaterals within layer II-III and even more strongly to layer V. We further examined whether this hKIF21B missense variant also impacts the establishment of intracortical connections. We performed IUE of WT and mutant hKIF21B in wild-type embryos at E15.5 and analyzed ipsilateral cortical collaterals at P8. Although callosal neurons expressing...
WT or p.Gln313Lys hKIF21B displayed prominent ipsilateral branching, overexpression of the p.Ile678Leu variant greatly reduced the intracortical branching (−32%, Bonferroni adjusted P = 0.0016) (Fig. 7a–c). Co-electroporation of p.Ile678Leu hKIF21B variant with either half or equivalent dose of WT-hKIF21B gradually restored the intrahemispheric connectivity phenotype, suggesting that p.Ile678Leu hKIF21B possibly impairs formation of ipsilateral collaterals through a dominant negative mechanism (Fig. 7a, b, d). Notably, migration defects were not rescued in these experiments (Fig. 7e) demonstrating that the branching phenotype is not an indirect consequence of neuron mispositioning. Conversely, p.Ile678Leu-ΔATPhKIF21B
overexpressing neurons that migrated normally (Fig. 7c), failed to send axonal collaterals ipsilaterally in layer V (Fig. 7a, b, d). Also, those neurons (p.Ile678Leu-ΔATPhKIF21B) showed normal axonal growth (Fig. 6b, c, e), ruling out the possibility that defective intracortical branching arises from impaired axonogenesis (Fig. 7a, b, d). Accordingly, branching defects likely arose from impaired collateral growth as revealed by in vitro analysis of axon branching in primary neurons at DIV5. Expression of p.Ile678Leu-hKIF21B variant but not WT nor p.Gln313Lys or p.Ala1001Thr mutants led to a shift of branch length toward short branch classes that resulted in a large decrease of the mean length of axon collaterals (Fig. 7f, g, Supplementary Fig. 7a, c, d). Consistent with a dominant negative effect, intrahemispheric connectivity is not affected upon KIF21B hyperactivation with neurons expressing NeuroD-mKif21bΔrCC displaying normal ipsilateral collaterals (Fig. 7a, b, d). We finally investigated which function of WT-hKIF21B was negatively modulated by the mutant protein. We induced expression of p.Ile678Leu variant together with hKIF21B that either cannot bind (NeuroD-ΔATPhKIF21B; Fig. 1h) or hydrolyze ATP (T96N-hKIF21B; Fig. 1h) at a 1:1 ratio using IUE at E15.5. Both constructs failed to rescue the branching phenotype induced by p.Ile678Leu hKIF21B at P8, suggesting that p.Ile678Leu hKIF21B exerts its dominant negative effect on the processive activity of KIF21B (Fig. 7a, b, d, Supplementary Fig. 3a). Collectively, our results showed that the p.Ile678Leu variant alters the intrahemispheric connectivity beyond its effect on migration and axonal growth through a dominant negative effect on motility.

Discussion

Our findings highlight the critical role of KIF21B in the regulation of processes involved in cortical development and implicate variants in KIF21B in ID and brain malformation. We identified three missense variants and one duplication of four nucleotides. The duplication leads to a frameshift introducing a premature termination codon in exon 20. The resulting mutant mRNA is likely degraded by nonsense-mediated mRNA decay. Although we demonstrated in mice that Kif21b haploinsufficiency leads to an impaired neuronal positioning (Supplementary Fig. 5d-i), the p.Asn988Serfs+4 protein truncated variant is possibly not pathogenic. Indeed, hKIF21B gene might partially tolerate loss-of-function variants in the gnomAD (Genome Aggregation Database, v2.1.1 “non-neuro”) populations that is supposed to be depleted in severe pediatric conditions, 28 loss-of-function variants have been reported. Nonetheless, the ratio of the observed/expected loss-of-function variants in the gnomAD populations is low (0.32, confidence interval 0.23–0.43), still questioning the penetrance of loss-of-function variants in KIF21B.

Our study provides the molecular mechanisms by which the identified variants lead to an abnormal brain phenotype. We showed that all missense variants, to various extents, impaired neuronal migration by enhancing KIF21B motility activity through lessening of the kinesin autoinhibition (Fig. 4). First, WT-hKIF21B is unable to rescue the variant-induced migratory defects at equivalent dose (Fig. 3a, b). Second, the phenotype induced by overexpression of the variants is phenocopied by the expression of a constitutively active form of KIF21B (that is truncated for the rCC domain) (Fig. 4c, d). Third, loss of ATP binding is sufficient to abrogate the phenotype induced by the missense variants (ΔATP-hKIF21B; Figs. 4i, j and 7e). Fourth, mutant hKIF21B proteins showed enhanced microtubule-based motility compared to the WT protein (Fig. 4g, h). How do hKIF21B variants lead to autoinhibition release? KIF21B autoinhibition is mediated by a regulatory segment (rCC) within the second coiled-coil domain (CC2, Fig. 1h) that fastens the CC2 domain to the motor head.42,46 We hypothesize that the position of the missense variants within the motor (p.Gln313Lys), coiled-coil (p.Ile678Leu) and rCC (p.Ala1001Thr) domains (Fig. 1h) alters the protein conformation so that it varies the impact on the intramolecular interaction between the motor and the internal coiled-coil domains. This model raises the possibility that the level of disruption of these interactions correlates with the degree of autoinhibition release imposed by the different missense variants and therefore dictates the severity of the phenotype. In accordance, the increase in KIF21B processivity correlated with the extent of migration defects, the velocity of the p.Ile678Leu variant being the most drastically enhanced (Fig. 4g, h). We therefore propose a model in which a minimal level of autoinhibition is required to ensure proper function of KIF21B in the developing cortex. Below this threshold, the more KIF21B gets overactivated, the more severe and broad the phenotypes will present. In this model, the p.Gln313Lys and p.Ala1001Thr variants would partially relieve autoinhibition, whereas the p.Ile678Leu variant would completely loose autoinhibition. Consistently, p.Gln313Lys and p.Ala1001Thr KIF21B induce a delay of migration, whereas the p.Ile678Leu variant leads to a permanent arrest of migration and an additional connectivity phenotype (Figs. 3 and 6).

Beyond autoinhibition, maintaining a proper level of KIF21B activity seems to be crucial for its function during development. Indeed, KIF21B haploinsufficiency also leads to migratory defects. Whether those defects are caused by a loss of trafficking or MT...
regulator functions is not clear and should be further assessed. Overall, the model could be expanded to a threshold of activity, below (haptoinsufficiency) or above (identified missense variant) which KIF21B would not be properly functional leading to neurodevelopmental defects.

Convergent evidence suggests that the p.Ile678Leu variant alters axon branching through a dominant negative effect on KIF21B processivity (Fig. 7). First, co-expression of increasing amount of WT-hKIF21B together with the mutant protein gradually restored the intrahemispheric connectivity phenotype. Second, an immotile form of KIF21B failed to rescue the variant-induced branching defect. Third, expression of a constitutive active form (ΔCCR) of KIF21B does not affect the formation of axon branches. Fourth, p.Ile678Leu-ΔATPhKIF21B over-expressing neurons display abnormal ipsilateral collaterals. Fifth, consistent with a dominant negative effect, the WT KIF21B protein fails to form homodimer and is mislocalized when co-expressed with the p.Ile678Leu variant. Intriguingly, the p.Ile678Leu variant also perturbs axonal growth (Fig. 6) and migration (Figs. 3 and 4) through attenuation of autoinhibition, suggesting, as discussed above, a possible gain-of-function effect. To reconcile these seemingly conflicting findings, we propose that it could imply that KIF21B regulates the trafficking of different cargos in axon and branches. p.Ile678Leu-induced over-activation of KIF21B might therefore lead to excessive motility of specific cargos within axons. Conversely, the same variant could impede the transport of branch-specific cargos by interfering with the function of the wild-type protein. At this time, none of the few KIF21B cargoes identified is specific to axon or branches,44,45 so further work is needed to identify cargoes in the different cellular compartments and validate this hypothesis.

Expression of the KIF21B p.Gln313Lys variant recapitulates the microcephaly phenotype observed in the reported subject. Given that introduction of the p.Ala1001Thr variant that is expected to attenuate KIF21B autoinhibition at the same extent as the p.Gln313Lys substitution variant, but does not reduce brain size in zebrafish (Fig. 5), the hyperactivation of KIF21B is unlikely to be driving the phenotype of microcephaly. In a search for possible microcephaly-underlying mechanism, we exclude any non-cell autonomous effect on progenitors’ proliferation, or any impact on cell survival. Abnormal postnatal neuronal maturation may also contribute to the global microcephaly phenotype. Accordingly microcephaly may worsen with time: the patient carrying the KIF21B p.Gly313Lys variant was born with a head circumference below (haploinsufficiency) or above (identified missense variant) which KIF21B would not be properly functional leading to neurodevelopmental defects.

Methods
Whole-exome sequencing (WES). A parent–offspring trio approach was used for whole-exome sequencing (WES) in each family. Exomes were sequenced using DNA isolated from blood according to standard procedures. Informed consent was obtained from all participants in accordance with site-specific institutional review board approval. Patient 1: The SeeQCap EZ MedExome Enrichment Kit (Roche) was used for library preparation with 12 samples multiplexing, according to manufacturer’s instructions. This library was then loaded on a NextSeq 500 instrument at a 2 x 150 bp high output flowcell. The bioinformatic analyses was conducted by Polyweb using BWA 0.7.12, picard-tools-1.121, GenomeAnalysisTK-2014-3-17-g053013, SNPEff-4.2. Patient 2: The SeqCap EZ VCrome 2.0 (Roche) was used for library preparation. Exome libraries were sequenced on an Illumina HiSeq 2500 instrument using the following sites and pipelines to search for potential pathogenic variants and polymorphisms: the Human Gene Mutation Database (HGMD), the single Nucleotide Polymorphism database (dbSNP), 1000 genomes, HapMap data. Patient 3: Exome capture was done using the Nimblegen SeqCap EZ_Enome_v3 (Nimblegen). Exome libraries were sequenced on an Illumina HiSeq 2500. Phenotype information for patient 2 was provided by the Genetics Clinic at Children’s Hospital of Philadelphia.

Inclusion and genetic studies were approved by local ethics committee in France (CCP Ile de France, CPP No. 71-10/ 1ID RCB: 2010-A00802-37) and USA (Institutional Review Board at Baylor College of Medicine, protocol H-29697 and at the John Hopkins School of Medicine).

Cloning and plasmid constructs. Wild-type (WT) human KIF21B cDNA (NCBI Reference Sequence: NM_001252100.1) was obtained from Vector Builder by gene synthesis and subcloned by restriction-liquidation into the NeuroD-iresGFP61, the pDNA3.1+/-/N-HA and in the pcEGFP-N1 vectors. Myc-tagged hKIF21B construct was obtained by replacing the GFP sequence of the pcEGFP-N1 containing the KIF21B gene by a Myc by the following restriction enzymes: HindIII-KpnI (human KIF21B variants c.97C>A (p.Gln313Lys), c.2032A>C (p.Ile678Leu), c.3001G>A (p.Ala1001Thr) and the KIF21B with the KIF21B variants c.97C>A (p.Gln313Lys), c.2032A>C (p.Ile678Leu), c.3001G>A (p.Ala1001Thr) and the c.288C>T (196N) substitution that abolishes KIF21B mobility22 were created from WT CDS by Sequence and Ligation Independent Cloning (SLIC). hKIF21B ATP binding site (amino acids 87–94; UniProtKB O75037) was deleted by SLIC from NeuroD-WT-hKIF21B-iresGFP, NeuroD-hKIF21B-p.Gln313Lys-iresGFP, NeuroD-hKIF21B-p.Ile678Leu-iresGFP and NeuroD-hKIF21B-p.Ala1001Thr-iresGFP to generate p.Ile678Leu-ΔATPhKIF21B WT-ΔATPhKIF21B, p. Gl313Lys-ΔATPhKIF21B, p. Ile678Leu-ΔATPhKIF21B and p. Ala1001Thr-ΔATPhKIF21B constructs, respectively.

Wild-type mouse Kif21b CDS was isolated from E18.5 mouse mouse cortices by PCR and a new isoform has been amplified. This isoform is 4920 bp-long and has an insertion (c.4905_4906 ins C; NM_001039472.2) that leads to a frameshift and to the introduction of a premature stop codon in exon 35. This isoform has been subcloned by restriction-liquidation into the NeuroD-IRES-GFP plasmid. This new isoform has also been fused to eGFP in the N-terminal part (pEGFP-C1-WT-Mikif21b) via subcloning in pEGFP-C1 plasmid (NovoPro V12034). The amino acids 930-1010 corresponding to antioxidantinhibitory domain (rc)42 were deleted by site-directed mutagenesis to generate NeuroD-mKif21b-arC construct. Mouse Kif21b 3’UTR sequence (NCBI Reference Sequence: NM_001039472.2) were amplified by PCR and cloned by restriction-liquidation into the pEGFP-C1 plasmid and fused in the N-terminal part of pCR-BluntII-TOPO-ΔKIF21B 3’UTR used to synthesize RNA probes was generated by cloning part of the mKif21b 3’ UTR (255 bp, Geneprint template T36548) in pCR-BluntII-TOPO vector.

shRNAs against coding sequence 3390–3410 (NM_001252100.1) (sh-Kif21b #1) or the 3’ UTR (sh-Kif21b #2) were generated by annealing of sense and antisense oligos, the resulting duplex were doubled in pCALSmiR32 backbone vector digested with XhoI and EcoRI. The following oligos were used: sh-Kif21b #1: 5’-TCGAGaaggtatattgctgttgacagtgagcgGCCTTTAACAACCAGA 3’; antisense: 5’-AATTCgagggactgctcagcaagccacgACGATCCTAATGCTAATTgcagcatgtgtgcacctgtcactgctgG 3’; sh-Kif21b #2: 5’-TCGAGaaggtatattgctgttgacagtgagcgGCGCGATAG 3’; antisense: 5’-AATTCgagggactgctcagcaagccacgACGATCCTAATGCTAATTgcagcatgtgtgcacctgtcactgctgG 3’; Scrambled shRNA: Sense: 5’-TCGAGaaggtatattgctgttgacagtgagcgGCGCGATAG 3’; antisense: 5’-TCGAGaaggtatattgctgttgacagtgagcgGCGCGATAG 3’.

Nature Communications | (2020) 1:2441 | https://doi.org/10.1038/s41467-020-16294-6 | www.nature.com/naturecommunications

Nature Communications ISSN 2041-1391 (online) © 2020 Springer Nature Limited. All rights reserved.
For cryosections only, an antigen retrieval was performed by boiling sections in sodium citrate buffer (0.01 M, pH 6.0) during 15 min. Cryo- and vibratome sections were dissected, washed in PFA, and blocked for immunolabeling. Sections were maintained in PBS-azide 0.05% for short-term storage. Cryo- and vibratome sections as follows: after fixation, brains were dissection 2 and 4 days after surgery. For postnatal analysis, electroporated pups were further electroporated into the neuronal progenitors adjacent to the ventricle and thread. In situ hybridization was performed on E12.5 to E18.5 WT NMRI coronal embryonic brain cryosections as follow: sections were first rinsed in Phosphate buffered saline (PBS, HyClone) and dehydrated for 5 min in successive ethanol baths (70%, 95% and 100%) diluted in sterile milliQ water. The same amount of each antisense or sense Kif21b digoxigenin-labeled probes were diluted at 0.1 µg/µL in pre-warm Hybridization Buffer (4 M NaCl 0.2 M, 5 M EDTA pH 8, 10 mM Tris-HCl pH 7.5, 10 mM NaH₂PO₄·2H₂O, 10 mM Na₂HPO₄, 2 mg/mL Ficol, 2 mg/mL polyvinylpyrrolidone, 2 mg/mL bovine serum albumin, 10% Dextran Sulfate, 1 mg/mL Yeast tRNA (ThermoFisher Scientific), 50% deionized formamide and denatured salmon sperm DNA). Hybridizations were performed with digoxigenin-labeled probes at 50 °C in a water-bath O/N at 70 °C in a sealed humidiﬁed chamber. Slides were then washed twice with the pre-warmed 1x Sodium Citrate solution (SSC, 0.15 M NaCl, 15 mM Na Citrate) at 70 °C for 30 min and in the 0.2x SSC solution for one hour at 70 °C. Slides were then washed twice in PBS for 15 min, then slides were post-fixed in PFA4% diluted in PBS at 10 min for 4 °C. Slides were then washed 3 times in PBS for 10 min, air-dried and mounted in Pertex mounting medium (Leica Microsystems). Images were taken using a macroscope (Leica M420) connected to a Photomicrographics camera with the CoolSNAP software (v. 1.2).

### RNA in situ hybridization

**Mouse Kif21b sense and antisense probes were synthesized from pCR-BluntII-TOPO-mKif21b 3’UTR by either Barstar digestion followed by synthesis with T7 RNA polymerase (Roche) (sense probe) or EcoR digestion followed by synthesis with Sp6 RNA polymerase (Roche) (antisense probe).** Kif21b digoxigenin-labeled probes using the DIG RNA labelling Kit Sp6/T7 PCR Supermix protocol in an Antifreeze solution (10% Fetal Bovine Serum (FBS) medium (Polysciences Inc). All primary and secondary antibodies used for immunolabeling are described in Supplementary Table 1.

### In vitro electroporation (IUE)

Tied-pregnant mice were anesthetized with isoflurane (1.2 per min of oxygen, 4% isoflurane in the induction phase and 2% isoflurane during surgery operation; Tem Senga). The uterine horns were exposed, and a longitudinal incision of each embryo was injected using pulled glass capillaries (Harvard apparatus, 1.0 OD×0.58 ID×100 mm) with Fast Green (1 µg/µL; Sigma) combined with different amounts of DNA constructs using a micro injector (Eppendorf Femto Jet). We injected 1 µg/µL of WT or mutant human NeuroD-hKIF21B-ires-gfp constructs together with 0.5 µg/µL of empty NeuroD-ires-gfp vector into E14.5. 1.5 mg/µL of NeuroD-ires-gfp vector was used as control. We injected 1 µg/µL of NeuroD-Cre-gfp vector together with 3 µg/µL of either Cre inducible pCALSIL-miR30-shRNA-Kif21b #1 or #2 or pCALSIL-miR30-sh-scramble sequence and 1 µg/µL of NeuroD-ires-gfp or NeuroD-miR21b-ires-gfp (rescue experiment). For axonal pathfinding experiments, we injected 1 µg/µL of NeuroD-ires-gfp (empty or containing WT or mutated human Kif21b cDNA) together with 0.5 µg/µL of pCAG-Scarlet at E15.5. For rescue experiments, we co-injected 1 µg/µL of NeuroD-ires-gfp (WT or mutated human Kif21b cDNA) together with 0.25, 0.5 or 1 µg/µL of the indicated NeuroD-ires-gfp constructs. Plasmids were further electroporated into the neuronal progenitors adjacent to the ventricle by discharging five electric pulses (40 V) for 30 ms at 950 ms intervals using ultraviolet (VWR international). After electroporation, embryos were placed back in the abdominal cavity and the abdomen was sutured using surgical needle and thread. For E16.5 and E18.5 analysis, pregnant mice were killed by cervical dislocation 2 and 4 days after surgery. For postnatal analysis, electroporated pups were killed 2, 4, or 8 days after birth (P2, P4, P8) by head sectioning or 22 days after birth (P22) by terminal perfusion.

### Mouse brain fixation, cutting and immunolabeling

E12.5 to P8 animals were killed by head sectioning and brains were fixed in 4% paraformaldehyde (PFA, Electron Microscopy Sciences) in Phosphate buffered saline (PBS, HyClone) 2 h at room temperature (RT) or overnight (O/N) at 4 °C. P22 animals were killed by terminal perfusion of PBS then 4% PFA followed by overnight post-fixation at 4 °C in 4% PFA. For Kif21b expression pattern (Fig. 2 and Supplementary Fig. 2), immunolabeling was performed on cryosections as follows: after fixation, brains were rinsed and equilibrated in 20% sucrose in PBS overnight at 4 °C, embedded in Tissue-Tek O.C.T. (Sakura), frozen on dry ice and coronal sections were cut at the cryostat (12 to 18 µm thickness, Leica CM3050S) and processed for In situ hybridization or immunolabeling. Sections were maintained at ~80 °C. For IUE analysis (Figs. 3–7, Supplementary Figs. 3–7), immunolabeling was performed on cryosections as follows: after fixation, brains were rinsed and embedded in a solution of 4% low-melting agarose (Bio-Rad) and cut into coronal sections (60-µm-thick for E18.5 and P2 mice, 100-µm-thick for P4 to P22 mice) using a vibrating-blade microtome (Leica VT1000S, Leica Microsystems) and processed for immunolabeling. Immunolabeled sections were maintained in Antifreeze solution (30% Ethylene glycol, 20% Glycerol, 30% D.I.H₂O, 20% P.O₄ Buffer) for long-term storage.

Fluorescent-activated cell sorting (FACS). Cortices from 3 to 4 E16.5 mouse Rosasa-2loxSTOP-YFP, NEXCRe/+ embryos were dissected and dissociated as described above. After dissociation, cells were resuspended in 500 µL of staining solution (10% Fetal Bovine Serum (FBS) + 0.02% Sodium Azide in PBS) and stained for 20 min on ice in dark with CD24-APC Antibody (0.06 µg/100 µL final; clone 17-0242-82, BD Biosciences). Cells were then washed twice with HBSS (Gibco) and passed through a 40 µm filter (Falcon FACS). YFP-/CD24- population was sorted to enrich for progenitors and YFP+/CD24+ population was used to enrich for neurons using the BD Aria II flow cytometer with 488 and 633 lasers to excite YFP and APC respectively. Littermate YFP– embryos (Rosasa-2loxSTOP-YFP, NEXCRe–) were processed the same way, stained with the Rat IgG2b-APC isotype control antibody (clone eB149/10H5, Thermofisher Scientific [17-4031-82]) and used as controls to set the YFP and CD24 gates.
Microfluidic fabrication and neurons plating. Design of polydimethylsiloxane microfluidic device is based on the one described by Taylor et al.32 with modifications of the size of the microchannels (3-µm width, 3-µm height and 450-µm length to reduce the number of axons per microchannels)33. Briefly, microfluidic chambers were positioned and sealed on Iwaki boxes using plasma cleaner and then coated with poly-α-lysine (0.1 mg/mL) in the upper chamber, and with poly-α-lysine (0.1 mg/mL) and laminin (10 µg/mL) in the lower chamber. After overnight incubation at 4 °C, microfluidic devices were washed twice with Neurobasal medium and once with growing medium (Neurobasal medium supplemented with 2% B27, 2 mM Glutamax, and 1% penicillin/streptomycin). Microchambers were then placed in the incubator until neurons were plated. Primary cortical neurons were prepared as follows: E15.5 C57Bl/6J mouse embryos were collected and cortices were dissected, followed by papain and cytoeine digestion and trypsin inhibitor incubation. After mechanical dissociation, cortical neurons were resuspended in growing medium (5 × 10⁶ cells in 120 µL) and plated in the upper chamber with a final density of ~7000 cells/mm². Neurons were kept in the incubator for 1 h. Then, the two compartments were gently filled with growing medium.

Immunostaining in microchambers was performed from DIV5 culture34, after 30 min fixation in 4% PFA/Sucrose dissolved in PBS, all the compartments were blocked with a solution containing BSA 1%, normal goat serum 2%, Triton X-100 0.1%. For these two solutions, a bigger volume in the upper chamber was applied to create a pressure gradient. After 1 h incubation with blocking solution, neurons in both compartments were incubated overnight at 4 °C with primary antibody recognizing KIF21B and Tau. Secondary antibodies were added the following day for 4 h and microchambers were maintained in PBS for a few days in the dark at 4 °C (see Supplementary Table 1 for antibodies).

RNA extraction, cDNA synthesis and RT-qPCR. To assess Kif21b mRNA expression in mouse and zebrafish, total RNA was extracted from the cortices of WT NMR1 mouse embryos or from whole zebrafish (Danio rerio) embryos (AB strain) at different time points of development, with TRIzol reagent (ThermoFisher Scientific). We used mKif21b ex2-3 and hKIF21B ex33-34 primers to target mKif21b or zKif21b cDNA and mGAPDH or drEFlA (Elongation factor 1-alpha) as housekeeping genes normalizer (Table 2). shRNA-Kif21b knock-down efficiency was assessed by RT-qPCR. Total RNA was prepared from HEK293T cells overexpressing shKif21b, and once with growing medium (Neurobasal medium supplemented with 2% B27, 2 mM Glutamax, and 1% penicillin/streptomycin). Total RNA was prepared with TRIzol reagent (ThermoFisher Scientific) and submitted to DNAse I treatment (Turbo DNA free RNA Tube (Qiagen, Germantown, CA, USA) and total RNA extracted using PAXgene Blood miRNA kit (Qiagen, Germantown, CA, USA). As controls, RNA was prepared from HEK293T cells overexpressing sh-scrambled or shRNA-Kif21b, and once with growing medium (Neurobasal medium supplemented with 2% B27, 2 mM Glutamax, and 1% penicillin/streptomycin). 400 µg of proteins were then incubated with 15 µL of pre-washed magnetic Myc-coupled beads for 2 h at 4 °C under gentle shaking. Beads were collected using a magnetic stand and supernatants were discarded. Beads were washed twice with lysis buffer (10 mM HEPES, 150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with EDTA-free protease inhibitors (complete), Roche) and 0.01 M phosphatase inhibitor PMSF, for 30 min on ice. Cells debris were removed by high speed centrifugation at 4 °C for 5 min. After protein concentration measurement, samples were diluted to 1 µg/µL. Half of the protein was kept (Input) and diluted in 2× Laemmli Elution Buffer (Bio-Rad) containing 2% β-mercaptoethanol. 400 µg of proteins were then incubated with 15 µL of pre-washed magnetic Myc-coupled beads for 2 h at 4 °C under gentle shaking. Beads were collected using a magnetic stand and supernatants were discarded. Beads were washed twice with lysis buffer (10 mM HEPES, 150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with EDTA-free protease inhibitors (complete), Roche) and 0.01 M phosphatase inhibitor PMSF, for 30 min on ice. Cells debris were removed by high speed centrifugation at 4 °C for 5 min. Protein concentration was measured by spectrophotometry using Bio-Rad Bradford protein assay reagent. Samples were denatured at 95 °C for 10 min in Laemmli buffer (Biological) supplemented with 2% β-mercaptoethanol and then resolved by SDS–PAGE and transferred onto nitrocellulose membranes. Membranes were blocked in 5% milk in PBS buffer with 0.1% Tween (PBS-T) and incubated overnight at 4 °C with the appropriate primary antibody in blocking solution. Membranes were washed three times in PBS-T, incubated at room temperature for 1 h with HRP-coupled secondary antibodies (Invitrogen) at 1:10,000 dilution in PBS-T, followed by three times PBS-T washes. Visualization was performed by quantitative chemiluminescence using SuperSignal West Pico PLUS Chemiluminescent Substrate (Sigea). Signal intensity was quantified using ImageQuant LAS 600 (GE Healthcare). Primary and secondary coupled HRP antibodies used for western blot are described in Supplementary Table 1. Relative protein expression was quantified using Imagej software (Java 1.8.0_121).

Cycloheximide (CHX) treatment. To assess the protein half-life of WT- and mutated KIF21B proteins, treatments using the translational inhibitor cycloheximide (CHX) were performed. N2A cells were cultured on 6-well plates and transfected with the adequate NeoO-D KIF21B constructs using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. The day after, cells were treated with CHX (Sigma) diluted in media at 10 µg/mL for either 2, 4, 6, 8 or...
10 h. Cells were lysed as described above. For analysis, 10 ng of protein of each sample were loaded on a SDS-gel followed by western blotting analysis as described above. Experiments consisted of at least three independent replicates. Relative protein expression was quantified using ImageJ software (Java 1.8.0_112).

Zebrafish manipulation. Zebrafish (D. rerio) embryos (AB strain) were maintained as described elsewhere (1). Human WT and mutant full-length cDNA were cloned into the pHACE-Neo vector and expressed in Cos7 cells. For variant subcellular localization analysis (Fig. 4e, f), a z stack of 0.13 μm was acquired in 1024 × 1024 mode using a confocal microscope (Leica TCS SP5 equipped with a HCX PL APO ×63/1.40 oil objective) controlled by Leica LAS X software v3.7. To measure the axonal length (longest neurite) at DIV2 and DIV5, the longest Scarlet-positive labelled neurite was traced and the average length was measured using ImageJ software (Java 1.8.0_112). For DIV5 axonal length measurements, the same criterion was used with an HCX PL APO ×63/1.40 oil objective (Leica) controlled by Leica LAS X software v3.7. For each condition, 67 to 229 independent cells from at least three independent transfections were segregated according to cell morphology (diffuse localization versus impaired localization).

Live-cell imaging procedure and analysis. Cos7 cells were grown on 35-mm glass bottom microwell dishes No. 0 (MatTek, U.S.A.). Cells were transfected with the different pEGFP-N1-HA-KIF21B constructs or co-transfected with BDNF-mCherry (gift from Gary Baker, Oregon Health and Science University, Portland, USA) or Mito-RFP (gift from Hélène Puccio, IGBMC, Illkirch, France) and the different pDNA3.1-1×-HA-KIF21B constructs or empty vector with a DNA ratio of 1:2 as described above. Live-cell imaging was done 24 h after transfection. Live-cell videomicroscopy was performed on an inverted microscope Leica CSU W1 DMi8 (Leica) with an Adaptive Focus Control (AFC) controlled by Metamorph software v.7.6, using an HCX PL APO Lambda blue x63/1.40 objective or an HC PL APO ×63/1.40 oil objective and the different pDNA3.1-1×-HA-KIF21B constructs or empty vector with a DNA ratio of 1:2 as described above. Western blot experiments (Figs. 2c–e, f) were performed on n = 3 and n = 5 different brains per genotype, respectively, and gave similar results. Western blot expression profile of NeuroD-ires-GFP constructs (Supplementary Fig. 3a), HA-tagged, Myc-tagged (Supplementary Fig. 4a) and GFP-tagged constructs (Supplementary Fig. 4b) were performed once. For in vitro electroporation experiments, only brains with comparable electrophoretic regions and efficiencies were conserved for quantification.

FACS-sorting and western blot experiments (Figs. 2c–e, Supplementary Figs. 1d; 2d, f; 3a, b, d, f, h; 4a, b, d, f, g, h, i; 5a, d, g, j; 6c, d, h; 7b, c, d) were performed on n = 3 and n = 5 independent transfections or magnetofection (Figs. 7c–e, g, h, j; 6d, e, g, h; 5b; 6d, e, g, h; 4d, e, f, g, h, i). Data are reported in Supplementary Data 1. Statistical details (adjustments made for multiple comparisons, confidence intervals and exact P-values for Figs. 2a, b, c, d, e, f; Supplementary Figs. 1d, 3a, f; 4a, b, d, e, g, h, i, j; 5a, b, c, d, e, f, g, h, i; 6d, e, g, h, i; 7c, d) are reported in Supplementary Data 1. All statistical tests were calculated using Prism (GraphPad, version 6) and are represented as mean ± S.E.M. Graphs were generated using Prism and images were assembled with Adobe Photoshop 13.0.1 (Adobe Systems).

Statistics and reproducibility. Immunofluorescence, in situ hybridization, FACS-sorting and western blot experiments (Figs. 2c–e and 3e; Supplementary Fig. 4a) and GFP-tagged constructs (Supplementary Fig. 4b) were performed once. For in vitro electroporation experiments, only brains with comparable electrophoretic regions and efficiencies were conserved for quantification and statistical analyses. Analysis of IUE experiments were performed blinded. For one embryo or pup brain, cell counting and in vivo branching analyses were performed in three different slices. The exact numbers (n) of samples, animals, cells, particles, axons or branches used to derive statistics are mentioned in figure legends along with the respective data and are also reported in Supplementary Data 1. The number of times each experiment was repeated independently (i.e. the number of independent transfection or magnetofection) and statistical tests are mentioned in figure legends along with the respective data whenever possible, and are also reported in Supplementary Data 1. Statistical details (adjustments made for multiple comparisons, confidence intervals and exact P-values for Figs. 2a, b, c, d, e, f; Supplementary Figs. 1d, 3a, f; 4a, b, d, e, g, h, i, j; 5a, b, c, d, e, f, g, h, i; 6d, e, g, h, i; 7c, d) are reported in Supplementary Data 1. All statistical tests were calculated using Prism (GraphPad, version 6) and are represented as mean ± S.E.M. Graphs were generated using Prism and images were assembled with Adobe Photoshop 13.0.1 (Adobe Systems).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability The source data underlying Figs. 2a, b, 4d, e, 4d, e, f, 5a, b, 6c, d, e, f, 7c, d, e, g, h, i, j, k, l and Supplementary Figs. 1d, 6d, e, g, h, i, j, k, l, m, n and other Source Data file. All other data are available from the authors upon request. The following databases and in silico software were used in the study: Human Gene Mutation Databases (http://www.hgmd.cf.ac.uk/ac/ introduction.php?lang=en); the single Nucleotide Polymorphism database (http://ftp.ncbi.nih.gov/snp/), genome aggregation database (gnomAD, https://gnomad.broadinstitute.org/), 1000 Genomes Project (https://www.internationalgenome.org/); Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (http://www.mutationtaster.org/); Sorting Intolerant from Tolerant (SiFT, https://sift.biocore.de/v1/Plugin_KymoToolbox/releases). For each condition, 250–322 BDNF particles in 10–19 independent cells were counted from at least three independent transfections. For each condition, 173–256 Mito-RFP particles in 19–31 independent cells were counted from four independent transfections.
The three KIF21B missense variants have been deposited in LOVD (Leiden Open Variation Database) v3.0 (https://databases.lovd.nl/shared/genes/KIF21B) under the accession numbers 0000663938 (p.Ile678Leu), 0000663939 (p.Gln313Lys) and 0000663940 (p.Ala1001Thr).

Received: 17 June 2019; Accepted: 26 April 2020; Published online: 15 May 2020

References

1. Hirokawa, N. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279, 519–526 (1998).
2. Hirokawa, N. & Tanaka, Y. Kinesin superfamily proteins (KIFs): various functions and their relevance for important phenomena in life and diseases. Exp. Cell Res. 334, 16–25 (2015).
3. Carbalalona, A., Hu, D. J. & Vallee, R. B. KIF1A inhibition immobilizes brain stem cells but blocks BDNF-mediated neuronal migration. Nat. Neurosci. 19, 253–262 (2016).
4. Chen, J. L., Chang, C. H. & Tsai, J. W. Glis2 rescues delays in brain development induced by Kif3a dysfunction. Cereb Cortex 29, 751–764 (2018).
5. Foerster, P. et al. mTORC1 signaling and primary cilia are required for brain ventricle morphogenesis. Development 144, 201–210 (2017).
6. Sun, D. et al. Regulation of neural stem cell proliferation and differentiation by Kinesin family member 2a. PLoS ONE 12, e0179047 (2017).
7. Tsai, J. W., Lian, W. N., Kemal, S., Kriegstein, A. R. & Vallee, R. B. Kinesin 3 and cytoplasmic dynein mediate interkinetic nuclear migration in neural stem cells. Nat. Neurosci. 13, 1463–1471 (2010).
8. Wilson, S. L., Wilson, J. P., Wang, C., Wang, B. B. & McConnell, S. K. Primary cilia and Glis3 activity regulate cerebral cortical size. Dev. Neurobiol. 72, 1196–1212 (2012).
9. Geng, A. et al. KIF20A/MKLP2 regulates the division modes of neural progenitor cells during cortical development. Nat. Commun. 9, 2707 (2018).
10. Janisch, K. M. et al. The vertebrate-specific Kinesin-6, Kif20b, is required for normal cytokinesis of polarized cortical stem cells and cerebral cortex size. Development 140, 4672–4682 (2013).
11. Reilly, M. L. et al. Loss of function mutations in KIF14 cause severe microcephaly and kidney development defects in humans and zebrafish. Hum. Mol. Genet. 28, 778–795 (2018).
12. Falnikar, A., Takei, S. & Liu, M. Recurrent KIF2A mutations are responsible for classic infantile-onset epilepsy, absent language, and distinctive malformations of cortical development. Am. J. Med. Genet. A 173, 3127–3131 (2017).
13. Falnikar, A., Tole, S., Liu, M., Liu, J. S. & Baas, P. W. Polarity in migrating neurons is related to a mechanism analogous to cytokinesis. J. Cell Biol. 141, 763–773 (1998).
14. Midorikawa, R., Takei, Y. & Hirokawa, N. KIF4 motor regulates activity-dependent regulation of NMH dendritic kinesin sorting identified by quantitative cytoplasmic remodeling functions of the dendritic kinesin KIF21B. Cell Biol. 239, 1081–1091 (2007).
15. Peletti, D., Peris, L., Rosso, S., Quiroga, S. & Caceres, A. Evidence for the involvement of KIF4 in the anterograde transport of L1-containing vesicles. J. Cell Biol. 149, 141–152 (2000).
16. Xu, M. et al. Kinesin-12 influences axonal growth during zebrafish neural development. Cytoskeleton 71, 555–563 (2014).
17. Nakajima, K. et al. Molecular motor KIF5A is essential for GABA(A) receptor transport, and KIF5A deletion causes epilepsy. Neuron 76, 945–961 (2012).
18. Swarnkar, S., Avchalamov, Y., Raveendra, B. L., Grimm, E. & Puthanveettil, S. V. Kinesin family of proteins Kif11 and Kif21b act as inhibitory constraints of excitatory synaptic transmission through distinct mechanisms. Sci. Rep. 8, 17419 (2018).
19. Willemse, M. H. et al. Involvement of the kinesin family members KIF4A and KIF5C in intellectual disability and synaptic function. J. Med. Genet. 51, 487–494 (2014).
20. Najmabadi, H. et al. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature 478, 57–63 (2011).
21. Lai, W. Y., Wei, C. M. & Chen, F. Novel mutations cause familial Hoarseness and Acroosteolysis syndrome. Nat. Genet. 43, 601–606 (2011).
22. Wang, W. & Tsai, J. W. Microtubuleassociated motor KIF21B associates with congenital lymphedema and chorioretinopathy. Hum. Mutat. 35, 1–10 (2014).
23. Tole, S. et al. Mutations in KIF11 cause autosomal-dominant microcephaly and cerebellar atrophy. Brain Dev. 36, 532–538 (2014).
24. Girelli, A. E. et al. Activity-dependent regulation of distinct transport and cytoskeletal remodeling functions of the dendritic kinesin KIF21B. Mol. Biol. Cell 25, 2290–2298 (2014).
25. Makryangas, P. et al. Biallelic variants in KIF4 cause intellectual disability with microcephaly. Eur. J. Hum. Genet. 26, 330–339 (2018).
26. Boux, L. et al. Ciliogenesis and cell cycle alterations contribute to KIF2A-related malformations of cortical development. Hum. Mol. Genet. 27, 224–238 (2018).
27. Furtado, D., Thies, E. & Kneussel, M. The kinesin KIF21B participates in the cell surface delivery of gamma2 subunit-containing GABAA receptors. Eur. J. Cell Biol. 93, 338–346 (2014).
28. Marszałek, J. R., Weiner, J. A., Farlow, S. J., Chun, J. & Goldstein, L. S. Novel dendritic kinesin sorting identified by different process targeting of two related kinesins: KIF21A and KIF21B. J. Cell Biol. 145, 469–479 (1999).
29. Huang, C. F. & Ranker, G. The translocation selectivity of the kinesins that mediate neuronal organelle transport. Traffic 13, 549–564 (2012).
30. van Riel, W. E. et al. Kinesin-4 KIF21B is a potent microtubule pausing factor. Elife 6, e24746 (2017).
31. Gromova, K. V. et al. Neurodegeneration and the kinesin KIF21B are critical for endocytic recycling of NMH3A receptors and regulate social behavior. Cell Rep. 23, 2705–2717 (2018).
32. Shi, X. et al. Activity-dependent regulation of distinct transport and cytoskeletal remodeling functions of the dendritic kinesin KIF21B. Neuron 92, 557–572 (2016).
33. Labonte, D. et al. TRIM3 regulates the motility of the kinesin motor protein KIF21B. PLoS ONE 8, e75603 (2013).
34. Bianchi, S. et al. Structural basis for misregulation of kinesin KIF21A autophosphorylation by CFEOM1 disease mutations. Sci. Rep. 6, 30668 (2016).
35. Kannan, M. et al. WD-repeat repeat 47, a microtubule-associated protein, is essential for brain development and autophagy. Proc. Natl Acad. Sci. USA 114, E9308–E9317 (2017).
36. Morikawa, M., Tanaka, Y., Cho, H. S., Yoshihara, M. & Hirokawa, N. The molecular motor KIF21B mediates synaptic plasticity and fear extinction by terminating Rac1 activation. Cell Rep. 23, 3864–3877 (2018).
37. Olson, H. E. et al. Micro-duplications of 1q23.1 associated with neurodevelopmental delay. Eur. J. Med. Genet. 55, 145–150 (2012).
38. Sobrerio, N., Schiettecatte, F., Valle, D. & Hamosh, A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum. Mutat. 36, 928–930 (2015).
39. Taylor, A. M. et al. A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nat. Methods 2, 599–605 (2005).
40. Matsuda, T. & Cepko, C. L. Controlled expression of transgenes introduced by in vitro electroporation. Proc. Natl Acad. Sci. USA 104, 1027–1032 (2007).
41. Cheng, L. et al. Human CFEOM1 mutations attenuate KIF21A autophosphorylation and cause oculomotor axon stalling. Neuromuscul Disord. 22, 334–349 (2014).
42. van der Vaart, B. et al. CFEOM1-associated kinesin KIF21A is a cortical microtubule growth inhibitor. Dev. Cell 27, 145–160 (2013).
43. Mantino, C. et al. KCTD12 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number variant. Nature 543, 363–367 (2012).
60. Retterer, K. et al. Clinical application of whole-exome sequencing across
clinical indications. Genet Med 18, 696–704 (2016).

61. Hand, R. & Polleux, F. Neurogenin2 regulates the initial axon guidance of
cortical pyramidal neurons projecting medially to the corpus callosum. Neuronal
Dev. 6, 30 (2011).

62. Skarnes, W. C. et al. A conditional knockout resource for the genome-wide
study of mouse gene function. Nature 477, 337–342 (2011).

63. Zala, D. et al. Vesicular glycolysis provides on-board energy for fast axonal
transport. Cell 152, 479–491 (2013).

64. Virlogeux, A. et al. Reconstituting corticostriatal network on a-chip reveals
the contribution of the presynaptic compartment to Huntington’s disease. Cell
Rep. 22, 110–122 (2018).

65. Tretel, F. et al. Dominant phenotypes produced by the HD mutation in
SH3T4(11q11) striatal cells. Hum. Mol. Genet. 9, 2799–2809 (2000).

66. Courchet, J. et al. Terminal axon branching is regulated by the LKB1-NUAK1
kinase pathway via presynaptic mitochondrial capture. Cell 153, 1510–1525
(2013).

Acknowledgements

This work was funded by grants from INSERM (ATIP-Avenir program, J.D.G.), the Fyssen
foundation (J.D.G.), the French state funds through the Agence Nationale de la Recherche
under the project JCJC CREDO ANR-14-CE13-0008-01 (J.D.G.), CILAXCAL (C.D.) and
AXTON ANR-18-CE16-0009-01 (F.S.), and the program Investissements d’Avenir labeled
(ANR-10-IDEX-0002, ANR-10-LABX-0030-INRT, to J.D.G. and C.G.), the Fondation
pour la recherche sur le cerveau (F.S.), INSERM/CNRS and University of Strasbourg. N.A.H.
and G.Z. are supported by USDA Grant Number 3902-51000-057-04S. L.A. and J.R.A. are
funded through the IGBMC PhD program (ANR-10-IDEX-0002-02, ANR-10-LABX-0030-
INRT). L.A is currently supported by Fondation pour la recherche médicale
(FDF2018S0055184). F.T. and C.W. are, respectively, research assistant and research engi-
neer at the University of Strasbourg. J.D.G. and C.G. are INSERM investigators. F.S. is a
professor at Univ. Grenoble Alpes. H.V. is supported by a PhD fellowship from Association
Huntington France. We thank the Imaging Center of IGBMC (igbmc.fr), in particular,
Elvire Guzet and Erwan Grandgirard for their assistance in the imaging experiments. We are
grateful to the staff of the mouse facilities of the Institut Clinique de la souris (ICS) and
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), the staff of the
neuroscience facility of the IGBMC and, the molecular biology service (in particular Thierry
Leregone and Paola Rossolillo) for their involvement in the project. We thank Sandra Bour
and IGBMC communication service. We also thank Dr Courchet, Dr Banlcer, Dr Puccio and
member of Chelly lab for sharing reagents and for discussion. We are really grateful to Pr
Jamel Chelly and Dr Laurent Nguyen for their continuous support, discussion and time
reading this manuscript. We warmly thank Dr Sandrine Humbert and Dr Binaz Yalcin for
helpful comments and advice. We are also grateful to members of J.D.G. and Chelly
laboratories for discussion and technical assistance. In particular, we thank Dr Ehl Bayam for
cell sorting and advice in writing the manuscript. We thank Dr Gabrielle Rudolf for reading
the manuscript and for her suggestions. We finally thank Paula Hernandez for her help
collecting patient samples.

Author contributions

L.A. and J.R.A. conceived and designed the experiments, performed the experiments,
performed statistical analysis and analyzed the data related to cellular, and functional
studies in mice. P.T. provided technical assistance and performed in utero electropa-
tion. C.G. and C.S.B. conceived, designed and performed experiments in zebrafish. C.W.
provided technical assistance for zebrafish studies. S.H., K.B., C.A.B., A.C., A.D., S.M.,
L.F., N.A.H., K.M., C.M., H.S., C.T.R., M.M.W., P.J.G.Z., G.Z. and D.H. contributed
clinical and imaging data and follow-up of patients and families. S.H., A.R., C.N. and
C.D. contributed to the generation of whole-exome sequencing, bioinformatics tools and
analysis of sequencing data. H.V. and F.S. conceived and performed the expression
analysis in microdevices. J.D.G. conceived, coordinated and supervised the study,
designed experiments, analyzed data and wrote the manuscript.

Competing interests

A.D. and K.M. are employees of GeneDx, Inc. The other authors declare no competing
interest.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41467-
020-16294-6.

Correspondence and requests for materials should be addressed to J.D.G.

Peer review information Nature Communications thanks the anonymous reviewer(s) for
their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

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

© 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 license, and indicate if changes were made. The images or other third party
material in this article are included in the article’s Creative Commons license, unless
indicated otherwise in a credit line to the material. If material is not included in the
article’s Creative Commons license 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 license, visit http://creativecommons.org/
licenses/by/4.0/.

© The Author(s) 2020