Schizophrenia-related microdeletion causes defective ciliary motility and brain ventricle enlargement via microRNA-dependent mechanisms in mice

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Progressive ventricular enlargement, a key feature of several neurologic and psychiatric diseases, is mediated by unknown mechanisms. Here, using murine models of 22q11-deletion syndrome (22q11DS), which is associated with schizophrenia in humans, we found progressive enlargement of lateral and third ventricles and deceleration of ciliary beating on ependymal cells lining the ventricular walls. The cilia-beating deficit observed in brain slices and in vivo is caused by elevated levels of dopamine receptors (Drd1), which are expressed in motile cilia. Haploinsufficiency of the microRNA-processing gene Dgcr8 results in Drd1 elevation, which is brought about by a reduction in Drd1-targeting microRNAs miR-382-3p and miR-674-3p. Replenishing either microRNA in 22q11DS mice normalizes ciliary beating and ventricular size. Knocking down the microRNAs or deleting their seed sites on Drd1 mimicked the cilia-beating and ventricular deficits. These results suggest that the Dgcr8-miR-382-3p/miR-674-3p-Drd1 mechanism contributes to deceleration of ciliary motility and age-dependent ventricular enlargement in 22q11DS.
The volume of cerebral ventricles often increases in various neurologic and psychiatric disorders, such as Parkinson disease, Alzheimer disease, vascular dementia, bipolar disorder, copy number variation disorders, as well as during normal aging. In individuals with schizophrenia (SCZ), cerebral ventricular enlargement is one of the most common and replicable findings; it has been observed in more than 80% of SCZ cases. The progressive increase in the total or regional ventricular volumes usually becomes evident at the first episode of disease. Despite this strong linkage, the mechanisms of ventricular enlargement in psychiatric disorders are mostly unknown.

Many neurodevelopmental disorders, such as SCZ, are polygenic diseases that are difficult to reproduce in animal models. Substantial progress has been made in studying such pathologies by modeling well-defined genomic lesions that increase the risk of neurodevelopmental diseases. For instance, 22q11.2 deletion syndrome (22q11DS) is a copy number variation disorder that is associated with multiple physical and neuropsychiatric morbidities, such as autism spectrum disorders, attention deficit hyperactivity disorder, anxiety disorder, intellectual disability, Parkinson disease, and others. Despite this strong linkage, the mechanisms of ventricular enlargement in psychiatric disorders are mostly unknown.

Dilated LVs have been found in 22q11DS patients, and ventricular enlargement is a common neuroanatomical finding in individuals who have a 22q11.2 chromosomal microdeletion. The most frequent changes are seen in the lateral ventricles (LVs) and third ventricle (TV). The 22q11DS is associated with abnormally dilated LVs, and SCZ. The 1.5-Mb deletion is thought to be the minimal region containing the genes that account for the increased risk of SCZ among individuals with 22q11DS. Ventricular enlargement is a common neuroanatomical finding in individuals who have a 22q11.2 chromosomal microdeletion. The most frequent changes are seen in the lateral ventricles (LVs) and third ventricle (TV) in 22q11DS patients. The 22q11DS is associated with abnormally dilated LVs, and SCZ. The 1.5-Mb deletion is thought to be the minimal region containing the genes that account for the increased risk of SCZ among individuals with 22q11DS. Ventricular enlargement is a common neuroanatomical finding in individuals who have a 22q11.2 chromosomal microdeletion. The most frequent changes are seen in the lateral ventricles (LVs) and third ventricle (TV) in 22q11DS patients. The 22q11DS is associated with abnormally dilated LVs, and SCZ. The 1.5-Mb deletion is thought to be the minimal region containing the genes that account for the increased risk of SCZ among individuals with 22q11DS. Ventricular enlargement is a common neuroanatomical finding in individuals who have a 22q11.2 chromosomal microdeletion. The most frequent changes are seen in the lateral ventricles (LVs) and third ventricle (TV) in 22q11DS patients. The 22q11DS is associated with abnormally dilated LVs, and SCZ. The 1.5-Mb deletion is thought to be the minimal region containing the genes that account for the increased risk of SCZ among individuals with 22q11DS. Ventricular enlargement is a common neuroanatomical finding in individuals who have a 22q11.2 chromosomal microdeletion. The most frequent changes are seen in the lateral ventricles (LVs) and third ventricle (TV) in 22q11DS patients. The 22q11DS is associated with abnormally dilated LVs, and SCZ. The 1.5-Mb deletion is thought to be the minimal region containing the genes that account for the increased risk of SCZ among individuals with 22q11DS. Ventricular enlargement is a common neuroanatomical finding in individuals who have a 22q11.2 chromosomal microdeletion. The most frequent changes are seen in the lateral ventricles (LVs) and third ventricle (TV) in 22q11DS patients. The 22q11DS is associated with abnormally dilated LVs, and SCZ. The 1.5-Mb deletion is thought to be the minimal region containing the genes that account for the increased risk of SCZ among individuals with 22q11DS. Ventricular enlargement is a common neuroanatomical finding in individuals who have a 22q11.2 chromosomal microdeletion.

Results

Haploinsufficiency of the 22q11DS gene Dgcr8 causes age-dependent ventricular enlargement. We performed magnetic resonance imaging (MRI) analysis in Dgcr8 heterozygous (Dgcr8+/−) mice and murine models of 22q11DS (ref. 53; Fig. 1a). The onset of clinical manifestations of psychosis in patients with 22q11DS or SCZ typically occurs during late adolescence or early adulthood (16–30 years). This age is developmentally equivalent to 3–4 months in mice. Thus, we examined the neuroanatomical features in mice that were 4 months or older. We detected a considerable increase in the total ventricular volume in 8-month-old but not 4-month-old Dgcr8+/− mice compared to wild-type (WT) littermates (Fig. 1b, c). At the regional level, we observed a significant enlargement of the LVs and TV but no difference in the size of the fourth ventricle or the aqueduct in 8-month-old Dgcr8+/− mice compared to age-matched controls (Supplementary Table 1). Similar results were achieved by measuring ventricle-to-brain ratios (VBRs), where ventricular volumes were normalized to the total brain volume (Supplementary Table 1). Despite the ventricular enlargement, the volumetric characteristics of the total brain, cortex, and hippocampus of Dgcr8+/− mice were comparable to those of WT littermates (Supplementary Fig. 1a–e).

Dgcr8+/− mice showed age-dependent volumetric abnormalities that were similar to those of Dgcr8−/− mice. Haploinsufficiency of Dgcr8 resulted in a significant increase in the ventricular volume in 8-month-old but not 4-month-old mice (Fig. 1d, e). The volume of the LVs and TV increased, but that of the fourth ventricle and aqueduct did not (Supplementary Table 1, 1). As in Dgcr8−/− mice, the volumes of the whole brain, cortex, and hippocampus of Dgcr8+/− mice did not differ from those of WT littermates at both ages (Supplementary Fig. 1f). Although the increase in the ventricular volumes in Dgcr8+/− mice was similar to that in Dgcr8−/− mice, it did not replicate it (Supplementary Table 1, 1). Specifically, the increases in the total ventricular volume and LV volume were not statistically different between 8-month-old Dgcr8+/− and 8-month-old Dgcr8−/− mice; however, the increase in the TV was significantly larger in 8-month-old Dgcr8+/− mice than in 8-month-old Dgcr8−/− mice (Supplementary Table 1, 1). This suggests that Dgcr8 haploinsufficiency is a major (but not the sole) contributor to ventricular enlargement in Dgcr8−/− mice.

To test if deletion of Dgcr8 limited to the subventricular zone (SVZ), especially in ependymal cells, replicates ventricular enlargement, we generated Dgcr8 conditional–knockout mice by crossing mice carrying a Dgcr8 floxed allele with Foxj1Cre mice that express Cre recombinase in ependymal cells. The Dgcr8 transcript level in the LV walls was reduced in Dgcr8+/− mice compared to WT mice (Supplementary Fig. 2b). It was also reduced in Dgcr8 conditional–knockout mice (Supplementary Fig. 2b). However, only Foxj1Cre;Dgcr8fl/fl mice but not Foxj1Cre;Dgcr8+/− mice showed lower Dgcr8 transcript levels in the LV walls compared to that in control littermates (Supplementary Fig. 2b). This suggests that conditional heterozygous deletion of Dgcr8 in ependymal cells does not accurately replicate Dgcr8 dosage achieved in Dgcr8−/− mice. Nonetheless, homozygous deletion of Dgcr8 in ependymal cells has a drastic effect on the ventricular size (Supplementary Fig. 2a, c). The total ventricular volume and volumes of the LVs, TV, and fourth ventricle substantially increased, whereas the aqueduct remained normal in Foxj1Cre;Dgcr8−/− mice (Supplementary Table 2, 2). Furthermore, the volumes of the whole brain, cortex, and hippocampus were reduced in Foxj1Cre;Dgcr8−/− mice but not in Foxj1Cre;Dgcr8+/− mice compared to the control littermates (Supplementary Fig. 2d–h). In addition, SVZ neurogenesis was disrupted in the Foxj1Cre;Dgcr8−/− mice but remained normal in Foxj1Cre;Dgcr8+/− mice (Supplementary Fig. 3). Neural progenitor proliferation (measured by the presence of proliferation markers Ki67 and BrdU in the SVZ) (Supplementary Fig. 3a–d), apoptotic cell death (measured by cell positivity for cleaved caspase-3 in the SVZ) (Supplementary Fig. 3e, f), and SVZ neuronal migration (measured by the presence of the neuroblast marker doublecortin and BrdU+ cells in the SVZ neuronal migratory stream)
from 8-month-old WT (Supplementary Fig. 1k) or abnormal characteristics of neural progenitors in the SVZ (Supplementary Fig. 4). Neural progenitor proliferation (Supplementary Fig. 4a–g), apoptotic cell death (Supplementary Fig. 4h–j), and SVZ neuronal migration (Supplementary Fig. 4k, l) were normal in Dgcr8/fl mice compared to WT littermates. These observations indicate that although Dgcr8 haploinsufficiency may cause ventricular enlargement, the mechanism for such a result does not involve changes in CSF composition or SVZ neurogenesis.

Slower ependymal flow and ciliary beating in the lateral ventricles of Dgcr8/fl mice. To test whether Dgcr8 deficiency affects ependymal function, we first examined ependymal flow that is directed by motile ciliary beating. We compared individual fluorescent microbead movements at the apical surface of ependymal cells in acute LV whole-mounts from 8-month-old WT and Dgcr8/fl mice. Although the overall direction of ependymal flow was comparable between Dgcr8/fl and WT mice, the average bead velocity was slower in the mutants (Fig. 2a, b, Supplementary Movies 1 and 2). Using this approach, we observed slower beating of motile cilia in Dgcr8/fl mice (Fig. 2a, b, Supplementary Movies 1 and 2).

Volumetric changes in Dgcr8/fl mice were not associated with changes in cerebral spinal fluid (CSF) osmolality (Supplementary Fig. 1k) or abnormal characteristics of neural progenitors in the SVZ (Supplementary Fig. 4). Neural progenitor proliferation (Supplementary Fig. 4a–g), apoptotic cell death (Supplementary Fig. 4h–j), and SVZ neuronal migration (Supplementary Fig. 4k, l) were normal in Dgcr8/fl mice compared to WT littermates. These observations indicate that although Dgcr8 haploinsufficiency may cause ventricular enlargement, the mechanism for such a result does not involve changes in CSF composition or SVZ neurogenesis.
that in addition to being slower, motile cilia movements were less stereotypical (more asynchronous) in acute brain slices (Supplementary Movies 3 and 4) and whole-mounts (Supplementary Movies 5 and 6) from older (>4 months) Dgcr8+/− mice compared to WT littermates.

To show ciliary abnormality in vivo, we measured ependymal CBF in anesthetized mice by using two-photon imaging (Fig. 2i). This approach enabled us to detect quickly beating individual cilia within the cilia tuft at the surface of the LV wall (Fig. 2j, Supplementary Movie 7). Similar to results from the ex vivo whole-mount experiments, the ependymal CBF in vivo was slower in 8-month-old Dgcr8+/−;Arl13beGFP mice than in Dgcr8+/+;Arl13beGFP littermates (Fig. 2k). Together, these results show a progressive reduction in ependymal cilia beating frequency in Dgcr8+/− mice.

Dgcr8 haploinsufficiency does not affect ependymal cell structure or planar polarity. The increase in ventricular volume in Dgcr8+/− mice was not associated with structural changes in ependymal cilia or planar cell polarity. Transmission electron microscopy (TEM) revealed normal microtubule configuration in the 9 + 2 axoneme and basal body docking to the apical membrane of ependymal cells in Dgcr8+/− mice (Fig. 3a–d). Scanning electron microscopy also showed no abnormalities in morphology, length, or the number of motile cilia on the wall of the LVs in
Dysregulation of the dopamine receptor Drd1 in ependymal cells of Dgcr8+/− mice. Previous reports indicate that Dgcr8 regulates the expression of dopamine receptors through specific miRNAs48. Dopamine receptors are present not only on neurons but also on ciliated ependymal cells67,68, where catecholamine projections from the subependymal layer may form synapses with them67,69. First, we determined whether Drd1 is present in the motile cilia. Immunostaining of the LV wall for motile cilia markers (i.e., acetylated tubulin or Arl13b) and dopamine receptors revealed that Drd1s localize to the motile cilia (Fig. 4a, Supplementary Fig. 5a, b) in both WT and Dgcr8+/− mice (Fig. 4a). Immunolabelling and super-resolution imaging confirmed the presence of Drd1s in the motile cilia (Fig. 4b) and revealed that immunolabeling of the motile cilia with antibodies against Drd1 was specific (Supplementary Fig. 5c). To further confirm the subcellular localization of Drd1 in ependymal cells, we performed immunogold EM experiments in sections containing ependymal cells in WT mice. The gold particles were detected in the cytoplasm of ependymal cells and cilia, including

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**Fig. 3 Normal cilia structure and planar polarity of ependymal cells in Dgcr8+/− mice.** a–d Representative TEM images of motile cilia in ependymal cells of WT and Dgcr8+/− mice. Cilia are transected at the levels of the axoneme (a, b) or basal body (c, d). e–h Representative SEM images of motile cilia in ependymal cells of WT and Dgcr8+/− mice. i–l Confocal images of basal body patch position as an anatomical indicator of ependymal planar polarity in the anterior dorsal (i, j) and anterior ventral (k, l) LV walls of WT and Dgcr8+/− mice. Whole-mount brains were stained with antibodies against β-catenin (green, intercellular junctions) and γ-tubulin (red, basal bodies). m–o Mean ciliary length (m), ciliary number in a bundle (n), and distance of the basal body (BB) displacement from the center of an ependymal cell (o) in WT and Dgcr8+/− mice. In m, 105 cilia in 3 mice of each genotype were analyzed using the Mann–Whitney rank-sum test (U = 5156, p = 0.42). In n, 72 cilia in three mice of each genotype were analyzed in the same manner (U = 2379, p = 0.39). In o, 309 cells in 3 WT mice and 305 cells in 3 Dgcr8+/− mice were analyzed using the Shapiro–Wilk normality test (U = 44,160, p = 0.18). p Distribution of the angles of the individual vectors in each imaged section were normalized to 0°, and distributions of the angles were compared in WT (235 cells, three mice) and Dgcr8+/− mice (Fig. 3i, j). Planar polarity of ependymal cells was not altered in Dgcr8+/− mice compared to WT littermates (Fig. 3e–h, m, n). Furthermore, planar polarity of ependymal cells was not altered in Dgcr8+/− mice (Fig. 3i–l). Planar polarity within the ependymal cells is determined by the location of the microtubule-based basal bodies, which give rise to the motile cilia60. Co-labeling of the cell adherens junction and basal body with antibodies against β-catenin and γ-tubulin in the LV wall showed preserved cell polarity in both genotypes. Displacement of the basal body patch from the cell center, an indicator of translational polarity, was similar between the genotypes (Fig. 3o). Furthermore, the alignment of rotational orientation of basal bodies, which is thought to determine the direction of motile cilia beating61–66, was not altered in Dgcr8+/− mice (Fig. 3p).
**Fig. 4** *Dgcr8* haploinsufficiency increases the expression of dopamine receptor *Drd1* in the LV wall.  

**a** Confoal images of *Drd1* expression in the ependymal cells of whole-mount LV walls from WT or *Dgcr8*+/− mice. Acetyl-tubulin antibody is used as a marker of motile cilia and DAPI stains the nuclei.  

**b** Super-resolution microscopic images of coronal brain sections from the WT (*Foxj1Cre;Drd1*+/+) mice show expression of *Drd1* in motile cilia. Acetyl-tubulin antibody is used as a marker of motile cilia.  

**c** Immunogold EM images show localization of immunogold-tagged *Drd1* in the cytoplasm of ependymal cells and near the microtubules of cilia, including the axoneme, basal body, and distal appendages of the basal body (arrows).  

**d-e** Representative immunoblotting of *Drd1* and *Drd2* in the LV wall of WT and *Dgcr8*+/− mice.  

**f-g** Quantification of *Drd1* (**f**) and *Drd2* (**g**) protein levels and the cAMP level (**g**) in the LV wall of WT and *Dgcr8*+/− mice. In **f**, the data represent eight mice per group and were analyzed using the two-tailed Student’s *t*-test (*t*_14 = 4.80, *p* < 0.001). In **g**, eight mice per group were included, and the data were analyzed in the same manner (*t*_14 = 0.89, *p* = 0.39). In **h**, 4 WT and 3 *Dgcr8*+/− mice were included in each experiment, which were run in triplicate and analyzed using the two-tailed Student’s *t*-test (*t*_5 = 3.72, *p* < 0.05).  

**h** Average CBF in ex vivo brain slices from 6-month-old WT and *Dgcr8*+/− mice in the absence (85 ciliary bundles in 3 WT mice and 88 ciliary bundles in 3 *Dgcr8*+/− mice) or presence of *Drd1* antagonist SCH23390 (93 ciliary bundles in 3 WT mice and 85 ciliary bundles in 3 *Dgcr8*+/− mice) (**h**). Data were analyzed using one-way ANOVA (*F*3,24 = 24.99, *p* < 0.001) (**h**) and in slices from 4- to 5-month-old WT mice in the presence of vehicle (DMSO) (67 ciliary bundles in 3 mice) or the *Drd1* agonist SKF81297 (60 ciliary bundles in 3 mice) (**i**). Data were analyzed using the Mann-Whitney rank-sum test (*U* = 1043, *p* < 0.001) (**i**).  

Source data are provided as a Source Data file.
in the axoneme, near the outer and central microtubules, and at the basal body and its distal appendages (Fig. 4c). In contrast, Drd2s were not detected in the motile cilia (Supplementary Fig. 5d). Together, these experiments determined that Drd1 receptors are present in the motile cilia.

To determine if Dgcr8 haploinsufficiency affects dopamine receptor expression, we performed Western blotting for Drd1 and Drd2 proteins in extracts from the LV walls. (Note, the LV wall contains several cell types, including ependymal cells.) The expression level of Drd1 but not Drd2 was significantly elevated in Dgcr8+/− mice compared to WT controls (Fig. 4d–f). Consistent with this finding, the levels of intracellular cAMP, the downstream effector of Drd1 signaling, was also significantly increased in the LV wall of Dgcr8+/− mice (Fig. 4g). Furthermore, blocking Drd1 activity with SCH23390, a Drd1-specific antagonist, rescued abnormal ependymal function in Dgcr8+/− mice (Fig. 4h). SCH23390 significantly accelerated ependymal CBF (p < 0.001), compared to vehicle in slices from Dgcr8+/− mice, making their CBF comparable to that of WT mice. Conversely, activation of Drd1 by SKF81297, a Drd1-specific agonist, mimicked the Dgcr8+/− ciliary beating phenotype in WT mice (Fig. 4i). Application of SKF81297 slowed ependymal CBF compared to vehicle treatment in slices from WT mice. Together, these results suggest that Dgcr8 haploinsufficiency upregulates Drd1 in the LV walls, and this reduces ependymal CBF. Because a major function of Dgcr8 is production of miRNAs, we next examined whether a Dgcr8–miRNA–Drd1 mechanism contributes to the deceleration of ependymal CBF in 22q11DS mice.

Depleting miR-382-3p or miR-674-3p or deleting their seed sites on Drd1 3’UTR decelerates ependymal ciliary beating and mediates ventricular enlargement. To identify the miRNAs involved in the Dgcr8–miRNA–Drd1 mechanism, we performed miRNA microarray analysis in the LV wall of 8-month-old Dgcr8+/− and WT littermates. Among the miRNAs that potentially target the 3’ untranslated region (UTR) of the Drd1 transcript (on the basis of the miRWalk and TargetScan miRNA-target-prediction algorithms), only three miRNAs (miR-153-5p, miR-382-3p, and miR-674-3p) were significantly decreased in Dgcr8+/− mice (Fig. 5a, Supplementary Table 3). The RT-qPCR analysis verified that the levels of all three Drd1-targeting miRNAs were significantly reduced in Dgcr8+/− mice compared to WT mice (Fig. 5b). All three miRNAs were substantially enriched in the brain, including the LV wall, compared to non-brain areas such as kidney or liver (Fig. 5c). Of the three identified miRNAs, overexpression of two, miR-382-3p (miR-382-3p OE) and miR-674-3p (miR-674-3p OE), decreased the levels of Drd1 transcripts in vitro (Supplementary Fig. 6). Therefore, in further experiments we focused on these two miRNAs as the mediators of the Dgcr8–miRNA–Drd1 mechanism. The miR-382-3p is conserved across species, including humans and mice, but miR-674-3p does not have a human homolog. Interestingly, the predicted miRNA-target sites of miR-382-3p and miR-674-3p overlapped on the Drd1 3’UTR (Fig. 5d). The 13-bp sequence containing seed sequences from both miRNAs is unique throughout all 3’UTRs of reference-sequence genes in the mouse genome (GRCm38/mm10 assembly). The miR-674-3p has an additional 7-bp binding site in the Drd1 3’UTR (Fig. 5d). To test whether the miR-382-3p– or miR-674-3p–binding sites are involved in ventricular enlargement, we generated mice with deletions of the 13-bp seed site (Drd1Δ13bp) and the 7-bp seed site (Drd1Δ7bp), respectively, in the Drd1 3’UTR by using the CRISPR/Cas9 approach (Fig. 5e). Both Drd1Δ7bp+/− and Drd1Δ13bp+/− mice developed normally and had no detectible gross abnormalities, compared to their respective WT controls (data not shown). Drd1Δ13bp+/− mice showed age-dependent and region-specific ventricular enlargement (Fig. 5f, g). Specifically, 8-month-old but not younger Drd1Δ13bp+/− mice had significantly enlarged total and LV volumes compared to WT littermates (Fig. 5f, g, Supplementary Table 4, 4’). In contrast, we did not detect any differences in ventricular volumes in Drd1Δ7bp+/− mice compared to control mice (Fig. 5h, i). This suggests that the unique 13-bp miR-382-3p/miR-674-3p Drd1 seed site but not the 7-bp miR-674-3p Drd1 seed site mediates the effect of these miRNAs in the ventricular-enlargement phenotype.

To test if depletion of miR-382-3p or miR-674-3p mimics the cilia deceleration and ventricular enlargement in WT mice in vivo, we generated sponges specific for miR-382-3p or miR-674-3p. The miR-382-3p or miR-674-3p effectively inhibited reporters containing the sponge sequence in vitro, as measured by the luciferase assay (Supplementary Fig. 7). To deliver these sponges in vivo, we generated the recombinant adeno-associated virus serotype 1 (AAV1) encoding GFP under the ubiquitous CAG promoter (AAV1–CAG–GFP). We injected AAV1–CAG–GFP intracerebroventricularly in Foxj1+-/Ai14–/− mice, which express tdTomato in ependymal cells. Approximately 3 weeks after injection, we detected robust co-labeling of GFP with tdTomato, indicating that the AAV1 efficiently and specifically infected the ependymal cells (Fig. 6a and Supplementary Fig. 8). The GFP signal was co-localized with tdTomato+ and S100β+ ependymal cells but not with neural progenitor markers GFAP and Ki67 (Supplementary Fig. 8b), indicating that ependymal cells have a strong affinity for AAV1, as previously shown.70 In contrast, AAV5 viruses were less specific and infected ependymal cells and other cells, including neurons in other brain areas (data not shown).

To use this AAV1 tropism for specific molecular manipulations in ependymal cells, we constructed an AAV1 containing miR-382-3p, miR-674-3p sponges, or scramble control (AAV1–CAG–GFP–miR-382-3p sponge, AAV1–CAG–GFP–miR-674-3p sponge, and AAV1–CAG–GFP–scramble). The infection with AAV1 encoding the miR-382-3p or miR-674-3p sponge significantly reduced the levels of respective miRNAs and increased the expression of the Drd1 transcript in the LV wall of WT mice (Fig. 6b–d). The miR-674-3p sponge expression in ependymal cells of WT mice resulted in ventricular enlargement, including increased volume of the TV (Fig. 6e–g, Supplementary Table 5, 5’). The miR-382-3p sponge increased the volume of the TV but did not affect the total ventricular volume (Fig. 6g, Supplementary Table 5, 5’). These significant differences in ventricle size were not accompanied by other aberrant neuroanatomical features or changes in ependymal planar polarity (Supplementary Fig. 9). Furthermore, the AAV1 expressing the miR-674-3p sponge significantly reduced the ependymal CBF compared to control virus in WT mice (Fig. 6h). Ependymal CBF also appeared to be reduced when ependymal cells were infected with AAV1 expressing the miR-382-3p sponge, but that reduction was not significant. Together, these results suggest that depletion of miR-382-3p or miR-674-3p, which occurs in Dgcr8+/− mice, slows ependymal CBF and induces ventricular enlargement.

Replenishing miR-382-3p or miR-674-3p rescues the cilia-beating deficit and ventricular enlargement in 22q11DS mice. Given that depletion of miR-382-3p or miR-674-3p leads to ventricular enlargement in 22q11DS mice, we sought to rescue this phenotype by overexpressing these miRNAs in the ependymal cells. To this end, we constructed AAV1 expressing miR-382-3p or miR-674-3p with GFP (AAV1–miR-382-3p OE and AAV1–miR-674-3p OE). After we injected AAV1–miR-382-3p OE or AAV1–miR-674-3p OE...
Fig. 5 Drd1-targeting miRNAs are depleted in Dgrc8−/− mice, and deletion of miRNA seed site on Drd1 3' UTR causes ventricular enlargement. a A miRNA microarray volcano plot depicting changes in miRNA expression in the LV wall of male Dgrc8−/− mice compared to that in male WT mice. b Normalized relative levels of miR-153-5p, miR-382-3p, and miR-674-3p measured by qPCR in the LV wall in WT (8 mice) and Dgrc8−/− littermates (8 mice). The data were analyzed using Mann–Whitney rank-sum test (U = 12, *p < 0.001 for miR-153-5p; U = 133, *p < 0.001 for miR-382-3p; U = 0, *p < 0.001 for miR-674-3p). c Normalized relative expression of miR-153-5p (8 mice), miR-382-3p (8 mice), and miR-674-3p (8 mice) in cerebellum, cortex, hippocampus, LV wall, striatum, kidney, and liver, as measured by qPCR. Data were analyzed using Kruskal–Wallis one-way analysis (H = 133.96, p < 0.001 for miR-153-5p; H6 = 127.59, p < 0.001 for miR-382-3p; and H6 = 149.63, p < 0.001 for miR-674-3p). d The predicted miRNA target sites of miR-382-3p (1053–1059) mmu-miR-382-3p (1059–1065) mmu-miR-674–3p (1246–1252) and miR-674-3p (1246–1252) miR-153-5p miR-382-3p miR-674-3p.

d mmu-miR-382-3p (1053–1059)

3′-UUUUUCAACAGGACACUAU-5′

Drd1 3′ UTR 5′-UGCAUGAGUGUAAGCACUGUAGACUAGAA...GGUAAACUAACUGCUAGACAGCC...3′

3′-AACAGACUCUACCUGCAGAC-5′

mmu-miR-382–3p (1059–1065)

mmu-miR-674-3p (1246–1252)

13 bp

7 bp

f

g

h

i

20

15

10

5

0

2-m 4-m 8-m

20

15

10

5

0

2-m 4-m 8-m

20

15

10

5

0

2-m 4-m 8-m

Total ventricular volume (mm3)

Total ventricular volume (mm3)

Total ventricular volume (mm3)

20

15

10

5

0

2-m 4-m 8-m

2-m 4-m 8-m

2-m 4-m 8-m

Source data are provided as a Source Data file.
intracerebroventricularly, the expression of each miRNA was increased, compared to the levels in mice that received injections with a control virus (Supplementary Fig. 10a, b). This coincided with reduced Drd1 mRNA levels in the LV wall of Df(16)1/+ mice (Supplementary Fig. 10c). Neither AAV1–miR-382-3p OE nor AAV1–miR-674-3p OE affected Drd1 mRNA levels in WT mice. However, overexpression of AAV1–miR-674-3p significantly reduced Drd1 mRNA levels in Df(16)1/+ mice (p < 0.001), which became indistinguishable from WT. Drd1 mRNA levels in Df(16)1/+ mice were also significantly reduced (p < 0.001) after injection of AAV1–miR-382-3p OE but still remained higher than in WT mice (Supplementary Fig. 10c).

AAV1–miR-382-3p OE and AAV1–miR-674-3p OE but not a control AAV1 injected intracerebroventricularly into 2- to 3-month-old Df(16)1/+ mice prevented ventricular enlargement measured at 8 months of age (Fig. 7a, b, Supplementary Table 6, 6). The total ventricular volume was substantially larger in Df(16)1/+ mutants than in WT mice when both genotypes were injected with the control virus. However, this ventricular enlargement was eliminated when WT and Df(16)1/+ mice were injected with AAV1–miR-382-3p OE or AAV1–miR-674-3p OE (Fig. 7b). AAV1–miR-382-3p OE and AAV1–miR-674-3p OE did not affect ventricular volumes in WT mice, but ventricular volume was significantly smaller in Df(16)1/+ mutants injected with AAV1–miR-674-3p OE than those injected with control AAV1 (p = 0.006). A trend of decreased total ventricular volume was also observed in Df(16)1/+ mice injected with AAV1–miR-382-3p OE, but that decrease was not significant (p = 0.055) (Supplementary Table 6, 6).

The normalization of the ventricular size in Df(16)1/+ mice injected with AAV1–miR-674-3p OE or AAV1–miR-382-3p OE was accompanied by the rescue of ciliary beating (Fig. 7c). Ependymal CBF was significantly lower in Df(16)1/+ mice than WT littersmates when both genotypes were injected with the control virus. However, this difference was eliminated when mice were injected with either AAV1–miR-674-3p OE or AAV1–miR-382-3p OE. Similar to the ventricular volume, ependymal CBF in WT mice was not affected by AAV1–miR-674-3p OE or AAV1–miR-382-3p OE. However, AAV1–miR-674-3p OE significantly increased ependymal CBF in Df(16)1/+ mice (p = 0.04). The CBF in Df(16)1/+ mice injected with AAV1–miR-382-3p OE also appeared to be faster than that in mice injected with control virus, but the increase was not significant (p = 0.08).

The normalization of the ventricular volume and CBF following intracerebroventricular injections of AAV1–miR-674-3p OE or AAV1–miR-382-3p OE was also observed in Dgcr88/- mice (Fig. 7d–i). Dgcr88/- mice injected with the control virus had significantly enlarged ventricles and decreased CBF. However, injection of AAV1–miR-674-3p OE or AAV1–miR-382-3p OE eliminated these differences between WT and Dgcr88/- littersmates. In both measures, neither virus affected WT mice but rescued the defects in Dgcr88/- mice. AAV1–miR-674-3p OE significantly decreased the ventricular volume (p = 0.04) and increased ependymal CBF (p < 0.01) in Dgcr88/- mice. AAV1–miR-382-3p OE appeared to decrease...
ventricular volume, but the change was not significant ($p = 0.08$); however, it significantly increased CBF ($p < 0.001$) in Dgcr8+/− mice (Fig. 7e, f).

Together, these results indicate that Dgcr8 haploinsufficiency is a genetic factor significantly contributing to progressive enlargement of the ventricles in 22q11DS mice. Both ventricular enlargement and the deficit in ciliary beating are mediated by the Dgcr8-miR-382-3p/miR-674-3p–Drd1 mechanism.

**Discussion**

Here we identified a novel mechanism of age-dependent ventricular enlargement in mouse models of 22q11DS. We have shown that haploinsufficiency of the 22q11DS gene Dgcr8, which is involved in miRNA biosynthesis, leads to depletion of miR-382-3p and miR-674-3p and elevation of Drd1 expression in the LV wall. We also determined that in ependymal cells Drd1 is expressed on the motile cilia. The increased expression of Drd1
Fig. 7 Overexpression of Drd1-targeting miRNAs in the ependymal cells rescues 22q11DS-mediated ventricular enlargement. a Representative MRIs of the brains of WT or Df(16)-/- mice injected with an AAVV expressing control, miR-382-3p (miR-382 OE), or miR-674-3p (miR-674 OE). b Mean total ventricular volume of 8-month-old WT and Df(16)-/- mice injected with an AAVV expressing scramble control (control: 9 WT mice, 9 Df(16)-/- mice; two-tailed Student’s t-test; t9 = 2.39, p < 0.05), miR-382-3p (miR-382 OE: 21 WT mice, 17 Df(16)-/- mice; two-tailed Student’s t-test; t9 = 1.14, p = 0.26), or miR-674-3p (miR-674 OE: 20 WT mice, 16 Df(16)-/- mice; two-tailed Student’s t-test; t14 = 0.66, p = 0.52) into the intracerebroventricular space at 2–3 months of age. c CBF measured in ex vivo brain slices from 8-month-old WT mice injected with an AAVV expressing scramble control (control: 111 ciliary bundles in 5 WT mice, 90 ciliary bundles in 5 Df(16)-/- mice; Mann-Whitney rank-sum test; U = 3497, p < 0.0001), miR-382-3p (miR-382 OE: 139 ciliary bundles in 5 WT mice, 156 ciliary bundles in 5 Df(16)-/- mice; Mann-Whitney rank-sum test; U = 9962, p = 0.23), or miR-674-3p (miR-674 OE: 151 ciliary bundles in 5 WT mice, 148 ciliary bundles in 5 Df(16)-/- mice; Mann-Whitney rank-sum test; U = 10,622, p = 0.48. d Representative MRIs of the brains of WT or Dgcr8+/- mice injected with an AAVV expressing scramble (Control), miR-382-3p (miR-382 OE), or miR-674-3p (miR-674 OE). e Mean total ventricular volume of 8-month-old WT or Dgcr8+/- mice injected with AAVV expressing scramble control (Control: 10 WT mice, 9 Dgcr8+/- mice; two-tailed Student’s t-test; t9 = 3.20, p < 0.001), miR-382-3p (miR-382 OE: 22 WT mice, 24 Dgcr8+/- mice; two-tailed Student’s t-test; t9 = 1.17, p = 0.25), or miR-674-3p (miR-674 OE: 17 WT mice, 16 Dgcr8+/- mice; two-tailed Student’s t-test; t9 = 0.58, p = 0.57). f CBF measured in ex vivo brain slices from 8-month-old WT mice injected with AAVV expressing scramble control (Control: 153 ciliary bundles in 5 WT mice, 128 ciliary bundles in 5 Dgcr8+/- mice; Mann-Whitney rank-sum test; U = 6736.5, p < 0.001), miR-382-3p (miR-382 OE: 134 ciliary bundles in 5 WT mice, 131 ciliary bundles in 4 Dgcr8+/- mice; Mann-Whitney rank-sum test; U = 7640.5, p = 0.07), or miR-674-3p (miR-674 OE: 162 ciliary bundles in 6 WT mice, 168 ciliary bundles in 6 Dgcr8+/- mice; Mann-Whitney rank-sum test; U = 12,165, p = 0.10). Scale bars, 8 mm. Source data are provided as a Source Data file.

reduces ependymal CBF, which is associated with age-dependent ventricular enlargement in 22q11DS mice. Overall, these experiments suggest that Dgcr8 haploinsufficiency operating via the Dgcr8–miR-382-3p/miR-674-3p–Drd1 mechanism is a major contributing factor to ventricular enlargement in 22q11DS mice. However, our data do not rule out that other genes within the microdeletion may also contribute to age-dependent ventricular enlargement in 22q11DS.

Multiple factors may contribute to ventricular enlargement, including stenosis of the aqueduct of Sylvius, impaired CSF homeostasis, aging, certain diseases, and cilia dysfunction. Aqueduct stenosis and abnormal production or adsorption of CSF can cause the most drastic changes in the ventricular volume, leading to hydrocephalus, especially during early development. There was no stenosis of the aqueduct in 22q11DS mouse brains, indicating that this enlargement of ventricles was nonobstructive. CSF osmolality was also not changed in the mutant brains. The slow developmental trajectory of ventricular enlargement in 22q11DS mice starkly contrasts with that of rapidly developing congenital hydrocephalus, a severe neurological disorder with high mortality. It also contrasts with occasional sporadic hydrocephalus in mice, which is much more severe and evident at birth. Hydrocephalic animals develop severe lesions and die at 20–40 days of age, but there is no abnormal mortality in 22q11DS mice at this age. In humans and mice, ventricular volumes progressively increase during normal aging. This enlargement also has been often observed in neurological disorders, such as Alzheimer disease, vascular dementia, bipolar disorder, copy number variation disorders, and Parkinson disease. However, evidence of ventricular enlargement in SCZ is overwhelming and reported in more than 80% of SCZ studies. The slowly progressing ventricular enlargement described here resembled that which occurs in SCZ patients. The late onset of ventricular enlargement in 22q11DS mice also resembles idiopathic normal-pressure hydrocephalus. This normally occurs in older patients and is frequently misdiagnosed with comorbidities (i.e., Parkinson disease), and can be improved by CSF drainage. Similar clinical management could be potentially used in 22q11DS patients with advanced ventricular enlargement.

Structural neuroimaging studies of children with 22q11DS have reported multiple volumetric brain abnormalities. A recent study indicated the existence of three subgroups within 22q11DS based on severity of changes in midline structures. The changes we observed in 22q11DS mouse models and Dgcr8+/- mice were relatively mild and correspond to subgroup 1 or 2 of this classification. Indeed, we observed no difference in total brain size, total (brain–CSF) volumes, cortical thickness, or hippocampal volume in these mutant mice compared to WT controls. The only consistent change we observed in 22q11DS and Dgcr8+/- mice was an age-dependent enlargement of the LV and TV volumes.

The cumulative data show a strong association between aberrant ciliogenesis or ciliary beating and ventricular enlargement. In the present study, we detected a defect in cilia beating that was associated with ventricular enlargement in 22q11DS mice. Slower motile cilia beating in 22q11DS mice may arise from structural or functional abnormalities. EM and light imaging revealed that 22q11DS mice have no structural defects in the motile cilium. Planar polarity in ependymal cells was also normal in Dgcr8+/- mice. The unidirectional flow of CSF is generated by the well-coordinated stroke of motile cilia and is thought to depend on the planar polarity of the cell. Furthermore, we detected no abnormalities in SVZ neurogenesis (i.e., cell proliferation, apoptosis, or neuronal migration into the olfactory bulb) in mice with heterozygous deletion of Dgcr8. This is consistent with the notion that heterozygous Dgcr8 deletion has a negligible effect on SVZ neurogenesis, whereas homozygous Dgcr8 deletion substantially affects SVZ neurogenesis. One report indicates that heterozygous deletion of Dgcr8 does not affect proliferation of adult neural stem cells in the SVZ (but reduces cell proliferation and neurogenesis in the adult hippocampus). Another report indicates that mitotic frequency of basal progenitors in the late (E16.5) but not early (E13.5) stage of embryonic SVZ development is slightly reduced in the Dgcr8+/- mice. When corticogenesis was investigated in mice with conditional deletion of Dgcr8 using Emx1Cre, there was a substantial defect in homozygous mice but not in heterozygous mice, in terms of the cortical or SVZ thickness at E13.5. The homozygous ablation of Dgcr8 and, to a much less extent, heterozygous ablation of Dgcr8 affected the maintenance and differentiation of apical and basal progenitors in the cortical VZ/SVZ and reduced the expression of Ctip2 and Sox5 in newborn neurons during early corticogenesis. Our data show that homozygous deletion of Dgcr8 in Foxj1+ ependymal cells leads to ventricular enlargement but through a severe defect in SVZ neurogenesis. Our attempts to heterozygously delete Dgcr8 in ependymal cells failed to reduce the Dgcr8 transcript levels; therefore, the results of those experiments were inconclusive.
One example of functional defects of motile cilia is primary ciliary dyskinesia (PCD)\(^6\)\(^7\). PCD is associated with defects in ciliary ultrastructure and/or function of the components of the ciliary axoneme. The clinical features of PCD are present during the neonatal period and characterized by respiratory tract infections, chronic otitis media, situs inversus, male infertility, and in most severe cases, hydropsphalus\(^8\)\(^9\). Although hydropsphalus is not a consistent feature in PCD, several studies show a link between PCD and hydropsphalus in humans\(^9\)\(^2\)\(^-\)\(^4\) and mouse models\(^9\)\(^4\)\(^5\)\(^6\). Despite these numerous studies, it is still not clear whether PCD-related abnormalities in ciliary function cause hydropsphalus. Similarly, we cannot unequivocally claim that slower ciliary beating causes enlarged ventricles in 22q11DS. Nonetheless, our rescue experiments restored the normal levels of both ciliary beating and the ventricular size in 22q11DS mice, and our mimicking experiments resulted in both deceleration of ciliary beating and enlarged LVs in WT mice, suggesting a link between ciliary function and ventricular size. However, the formal causal link between defects in ciliary beating and ventricular enlargement is not yet established. In 22q11DS mice, LV enlargement is a common finding. These data suggest that miR-382-3p/miR-674-3p–Drd1 mechanism in ependymal cells regulates ciliary beating and the stability of ventricular volumes. Our data show that miR-382-3p and miR-674-3p are highly expressed in the brain, including the LV wall. It remains to be elucidated how the Dgcr8–miR-382-3p/miR-674-3p–Drd1 pathway affects other brain regions in 22q11DS mice. It is interesting to speculate that these miRNAs might have a differential role in Drd1 signaling in the 22q11DS brain.

The behavioral consequences of ventricular enlargement in 22q11DS or SCZ are unclear. Brain structures adjacent to the ventricles, especially subcortical regions adjacent to the LVs and TV, could be affected by ventricular enlargement. Although ventricular enlargement in SCZ does not correlate with volume diminution in the adjacent brain structures, it could affect neuronal function in those structures. Ventricular enlargement may also affect neuronal functions in subcortical structures (e.g., thalamus, hippocampus, amygdala, and striatum) and potentially explain the diverse symptoms of SCZ (i.e., positive, negative, and cognitive symptoms). Given that ventricular enlargement in 22q11DS mice does not appear until later ages, it could, in theory, contribute to the last onset of at least some symptoms\(^3\)\(^2\)\(^4\)\(^,\)\(^5\)\(^4\). Furthermore, several behavioral abnormalities of 22q11DS mice appear only after 4 months of age, coinciding with the developmental trajectory of ventricular enlargement. For instance, old (>4 months) but not young (<3 months) Df(16)1/+ mice or similar 22q11DS mouse models perform worse in paired-pulse inhibition, Morris water maze, cued fear conditioning, social memory, and active avoidance tests\(^4\)\(^8\)\(^–\)\(^5\)\(^0\). Moreover, the neural circuits associated with these deficits are affected in older but not younger mice\(^4\)\(^8\)\(^–\)\(^5\)\(^0\). For instance, thalamic projections to the auditory cortex or the amygdala become disrupted after 4 months in 22q11DS mice, but 2-month-old mutants have normal synaptic transmission in these circuits\(^4\)\(^7\)\(^,\)\(^4\)\(^8\)\(^,\)\(^5\)\(^0\). In this study, we did not investigate the connection between neuroanatomical changes and behavioral deficits in 22q11DS mouse models. Future studies are needed to elucidate those relations.

In summary, our data implicate a Dgcr8–miR-382-3p/miR-674-3p–Drd1 pathogenic mechanism of slower ciliary beating and ventricular enlargement in 22q11DS, a rare disease that substantially increases the risk of several neuropsychiatric disorders including SCZ. Our data indicate that replenishing miR-382-3p or miR-674-3p or inhibiting Drd1 in ependymal cells would be a potential therapeutic avenue for preventing progressive enlargement of brain ventricles, a robust and replicable neuroanatomical feature associated with neuropsychiatric disease.

Methods

Animals. Both male and female mice (2–9 months old) were used for all experiments. The generation of Df(16)1/+, Dgcr8−/−, Arl13bGFP, Foxj1Cre, and Ai14 mouse lines has been reported previously\(^4\)\(^9\)\(^,\)\(^5\)\(^0\)\(^–\)\(^5\)\(^2\). Df(16)1/+ and Dgcr8−/− mouse strains were back-crossed onto the C57BL/6j genetic background for at least 10 generations. The care and use of animals were reviewed and approved by the St. Jude Children’s Research Hospital Institutional Animal Care and Use Committee.

Magnetic resonance imaging. The animal MRI study was performed using a 7T Bruker ClinScan system (Bruker BioSpin MRI GmbH, Germany) equipped with a 12 S gradient coil. A mouse head volume coil (Bruker BioSpin) was used.

Transmission and scanning electron microscopy. For TEM, 8-month-old WT and Dgcr8−/− mice were perfused in 4% paraformaldehyde and postfixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (Tousimis Research Corp, Rockville, MD or Electron Microscopy Sciences, Hatfield, PA). The samples were postfixed in 2% osmium tetroxide and dehydrated via a graded series of alcohol baths, cleared in propylene oxide, embedded in epon araldite, and polymerized overnight at 70°C. Sections (70-nm thick) were cut on a Leica Ultracut E. The sections were stained with uranyl acetate and lead citrate for TEM. For TEM, the samples were cut on a Leica Ultracut E. The samples were stained with uranyl acetate and lead citrate for TEM. The images were acquired using a Zeiss Libra 120 microscope.
unstained sections were imaged on a JEOL 1200 EX transmission electron microscope with an AMT 2K digital camera.

For scanning electron microscopy, the brains were perfused with super-reagent perfusion wash and super-reagent perfusion fixation (Electron Microscopy Sciences, Hatfield, PA). Freshly collected brains were dissected and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.35 (Tousimis Research Corp, Rockville, MD) and 2% osmium tetroxide, pH 7.35, in 0.1 M sodium cacodylate buffer (Electron Microscopy Sciences). More dehydration in an ethanol series and critically point dried (Tousimis Sandai 790, Tousimis Research Corp). Samples were mounted, coated with gold/palladium, and imaged using a JEOL 7000 field emission gun scanning electron microscope. Cilia length and numbers were measured in a blinded manner (i.e., without prior knowledge of the mouse genotype).

Immunogold labeling. Mice were perfused with 1 M phosphate buffer (pH 7.3) containing 4% paraformaldehyde and 0.1% glutaraldehyde; their brains were then removed and immersed in the same fixative. Brain slices prepared using a vibra-tome were contrasted with tannic acid and uranyl acetate in 0.1 M sodium acetate, dehydrated in series of solutions with increasing concentrations of ethanol to 100%, infiltrated with resin/ethanol mixtures, and embedded into a modified formulation of Spurr’s resin[108]. The embedded brain slices were sectioned at 80-nm thickness and placed on nickel grids for immunolabeling. The brain slices were sequentially treated with 50 mM glycine in 10 mM PBS (pH 7.4); a blocking solution composed of 3% bovine serum albumin (BSA), 3% normal serum, and 0.1% cold water fish skin gelatin in PBS; and 0.1% BSA-c (Aurion) in PBS. The sections were incubated overnight at 4 °C with anti-DRD1 antibody (Sigma, D2944, 1:100) diluted in 0.1% BSA-c in PBS. After washing with 0.1% BSA-c in PBS, the brain sections were incubated with 12-nm Colloidal Gold AffiniPure donkey anti-rabbit IgG (Jackson Immuno Research, 711-205-152, 1:20) diluted in 0.1% BSA-c in PBS for 2 h at room temperature and washed sequentially with 0.1% BSA-c in PBS, PBS, and water, then allowed to air dry. Samples were examined at 80 kV on an FEI transmission electron microscope (Thermo Fisher Scientific) equipped with an AMT camera system.

Microbeads-based epifluorescent flow assay. The LV walls were dissected and pinned in a dish with L-15 Leibovitz media (Thermo Fisher Scientific) at room temperature. Latex beads (1-μm diameter, 5 mL; Sigma-Aldrich, L2778) were released over the dorsal surface of the lateral wall by using a stereotactic injector. Bead movements were recorded using a Rolera-XR high-speed digital camera and an Olympus U-CMAD3 fluorescence microscope using Q-Capture Pro7 imaging software (QImaging, Surrey, BC, Canada), with an acquisition rate of 20 frames/second and a time-lapse processing interval of 0.2 seconds. Three to five rounds of bead release and imaging were performed for each whole-mount to obtain optimal sensitivity threshold is applied to remove weak detections. Next, a nonminima suppression technique discards weak, overlapping detections[105]. Then remaining跟踪过程: (1) an additional threshold is related to the radius of the bead by σ = r/√2. In practice, we commonly use filter radii between 3 and 5 pixels. Local optima are extracted from filter-response maps, and a sensitivity threshold is applied to remove weak detections. Next, a nonminima suppression technique discards weak, overlapping detections[105]. Then remaining tracking features are further pruned in a two-step refinement process: (1) an additional threshold on local maxima from a Gaussian heatmap generated using the spatial location of each detection combines nearby beads into single detections; (2) agglomerative clustering using the complete-linkage criterion over Chebychev distance removes beads that are stationary for too many frames in the image sequence[106]. All thresholds are determined interactively by the user. After detection and refinement, the remaining candidate beads are tracked through time using a Kalman filter with a constant-velocity model[107]. We assume the initial variance of the detections is small (i.e., the Log filtering/refinement process yields strong detection/Bayesian approach to particle motion). The Kalman filter (i.e., the measurement process). The Kalman filter is more likely to have an error than is the detection filter due to irregular bead movement and time between image captures). There are additional thresholds on the expected time that a bead is visible in the image (on average); this helps to further prune tracks that are biologically unlikely.

Ex vivo ciliary beating frequency measurements. For ex vivo measurement of CBF, coronal brain sections (300-μm) were collected in L-15 Leibovitz media (Thermo Fisher Scientific) and placed in 37°C, 95%/5% O2/CO2 chamber for 15 minutes for incubation. Image sequences of CBF were acquired by line scan analysis using an LD C-Apochromat ×80 objective water-immersion lens (1.1 NA) in LSM780 confocal microscope (Zeiss Axios Observer). Slices were scanned through a line perpendicular to the axis of the cilia in the XTM mode along this line with an acquisition rate of 529 frames/second (512 × 1 frame size, 1.89-ms scan time, 5000 cycles). Imaging was performed within 1 h after the end of the incubation period.

Transmitted DIC and fluorescence emitted by GFP or Alexa Fluor 488 were collected with a 488- to 568-nm bandpass filter. Fluorescence excitation and transmission cilia beating frequency measurements were performed on a 488-nm diode laser (G7735-01, 10 mm). Time-lapse images were captured using a DX70 microslide stage (Tokai Hit, Tokyo, Japan). The filter set comprised a 510 nm (635 nm) barrier filter and a 510 nm long-pass filter. Each image was acquired in a 0.5 × 0.5 μm2 field of view with an IX71 IX71 microscope (Olympus, 1.05 NA). GFP+ cilia lining the left LV were imaged with 950-nm laser excitation. With a resis...
Recombinant AAVs were generated at the St. Jude Vector Development and Production Core as described previously49. Generation of miRNA sponge constructs. The miR-382-3p and miR-674-3p sponges were generated as described previously60. Six copies of the following sequences were inserted for the miR-382-3p sponge (TGTTCCTGAGATGGAGCCGCTGTTG), the miR-674-3p sponge (TCATTCGAAAGACACACATTTT), and the scrambled control (GAGCTAGCTAATGCCTCATCGTGGAAGTATACACTTCTGTTGTTGGTGGAGGGAATAGAAGAACCCCTTCCC), mDrd1 were injected with a metal cannula (33 gauge; Plastics One, Roanoke, VA). An incision was made in the scalp, and a small hole was drilled for the craniotomy. The injection was performed using a syringe attached to the cannula. The plasmid was transfected into HEK 293 cells (ATCC, CCL-3216) with control NLS. For Drd1, Drd1Δ13bp mice, Drd1Δ13bp and Drd1Δ13bp mice were generated using the CRISPR/Cas9 approach. Briefly, C57BL/6 J fertilized zygotes (Jackson Laboratories, Bar Harbor, ME) were cytoplasmically co-injected with 30 ng/µl sgRNA (Synthego) and brains were collected either the next day or 2 days post injection. For cell culture, mirVana RNA isolation kit (Ambion, Austin, TX) was used to isolate total RNA from the LV wall of 8-month-old WT and Drd1Δ13bp mice. Total RNA was isolated from the LV wall of 8-month-old WT and Drd1Δ13bp male mice by using the mirVana RNA isolation kit (Life Technologies). Total RNAs (100 ng) were labeled using the miRNA Complete Labeling and Hyb Kit (Agilent), followed by hybridizing to the Mouse miRNA v21 Microarray (Agilent-075055) that contains 4415 unique probes targeting 1881 mature miRNAs, according to the mouse miRBase version 21.0 (http://www.mirbase.org; June 2014). Microarrays were scanned using an Agilent array scanner (G2565CA) at 3-µm resolution. Microarray data were extracted by Agilent Feature Extraction software (v10.5.1.1) with the miRNA_107_Sep09 protocol. The data process was performed using Partek software (St. Louis, MO). The miRNA microarray data analysis was performed as described previously64. In brief, the signal intensities for each miRNA were summarized after quantile normalization among arrays; the Student’s t-test was then performed to determine statistical significance between sets of biological replicates from different experimental groups; and the significant differentially expressed miRNAs were further filtered using the p-value and fold-change cutoff for the group comparison. The miRNAs targeted by differentially expressed miRNAs were predicted using bioinformatics tools miRWalk and TargetScan. The microarray data have been deposited in the NCBI GEO database under accession number GSE123560. Luciferase assay. To test the effect of each miRNA on Drd1 expression, the 3’UTR of the Drd1 gene was cloned into the 3’ end of the Renilla luciferase gene contained within the psCHECK-2 vector (Promega, Madison, WI). The plasmids were transfected into HEK 293 cells (ATCC, CCL-3126) with control hsa-miR-30a, mI-153-5p, or the miR-647-3p OE plasmid. After 2 days in culture, Renilla and Firefly activities were measured using the dual-luciferase reporter assay (Promega) according to the manufacturer’s instructions. The Renilla luciferase activity was normalized to Firefly luciferase activity. Western blotting. Tissue from the LV wall (200- to 300-µm slice) was acutely dissected from the mouse brain and lysed in ice-cold RIPA buffer (50 mM Tris-HCl (pH 7.4), 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, and 1 mM EDTA) that included protease-inhibitor cocktail tablets (Roche, Indianapolis, IN). A total of 25 µg protein was loaded per lane. Sodium dodecyl sulfate/polyacylamide gel electrophoresis, protein transfer to polyvinylidene difluoride membranes, and Western blotting were performed using standard methods. The following antibodies were used: rabbit anti-Drd1 (Abcam; ab5367, 1:500); mouse anti-β-actin (Sigma-Aldrich, St. Louis, MO; A5316, 1:10,000). The following secondary antibodies were used: anti-rabbit (LI-COR Biosciences, Lincoln, NE; 926-68021, 1:15,000, and anti-mouse (LI-COR Biosciences, 926-32212, 1:15,000) and anti-bodies conjugated to 125 I (PerkinElmer, Waltham, MA). Blots were imaged and quantified using the Odyssey CLx infrared imaging system (LI-COR Biosciences). Histological analysis and immunohistochemistry. Mice were deeply anesthetized and intracardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and brains were fixed overnight. Each brain was sliced (50 µm) coronally with a vibratome (Leica), and the sections including the LV wall were immunolabeled as previously described60. The following primary antibodies were used: DRD1 (Sigma-Aldrich, D2944, 1:200), DRD2 (Millipore, Burlington, MA; AB5084p, 1:200), acetylated tubulin (Sigma-Aldrich, T7697, 1:1000), γ-tubulin (Sigma-Aldrich, T3192, 1:1000), β-catenin (BD Transduction Lab, San Jose, CA; 610133, 1:500), GFP (Abcam, ab13970, 1:1000), cleaved caspase-3 (Cell Signaling, Danvers, MA; 9661S, 1:250), Ki67 (Abcam, ab15850, 1:500), Brdu (Abcam, ab63262, 1:200), doublecortin (Abcam, ab18723, 1:1000). Appropriate Alexa dye–conjugated secondary antibodies (Thermo Fisher Scientific, Waltham, MA; 1:1000) were used to detect primary antibody binding. DAPI (Invitrogen, Carlsbad, CA) was used as the nuclear counterstain. For cell proliferation, in the SVZ or tracking neuronal migration from the SVZ to the olfactory bulb, BrdU (100 mg/kg) was injected twice at 24 hours intervals. Brains were collected either the next day for SVZ analysis or 21 days later for neuronal migration analysis. Measurements of basal body displacement were performed in a blind manner, without prior knowledge of the mouse genotype. Data availability. The raw data are available in the NCBI GEO database under accession number GSE123560. Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article. Statistical analyses. All statistics were computed using the Sigma Plot software (Systat Software, Inc., Point Richmond, CA). Differences in mean data were determined by the Student’s t-test, Mann-Whitney rank-sum test, Kruskal-Wallis one-way analysis, Watson’s U2 two-sample test, Shapiro-Wilk normality test, or a one-way ANOVA followed by Student–Newman–Keuls post hoc test and were considered significant if the p-value of the test result was <0.05.
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Author contributions
S.S.Z. and T.-Y.E. designed the study. T.-Y.E. performed the immunohistochemical, molecular, and imaging experiments. J.K. performed the MRI examinations. S.B.H. and J.I. performed the molecular cloning and qRT-PCR experiments. J.A.B. performed the in vivo CBF measurements. Y.-D.W. performed the miRNA microarray analysis. K.A. and D.B.K. assisted with the immunohistochemistry analysis and virus injections. M.E. and D.C.R. developed the bead-tracing software. S.M.S. and S.M.P.-M. designed and performed the gene-editing experiments. C.G.R., L.H. and B.W. performed the immunolabelling and electron microscopy experiments. S.S.Z. provided the reagents and equipment. S.S.Z. and T.-Y.E. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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