HYDROCEPHALUS

Molecular hallmarks of hydrocephalus

Andrew T. Hale[1,2]*, Blake Zhou[3], Arjun Rajan[4], Phan Q. Duy[5], Mubeen Goolam[2], Seth L. Alper[6,7,8], Maria K. Lehtinen[8,9], Madeline A. Lancaster[10], Ryann M. Fame[3,11]*, Kristopher T. Kahle[8,12,13,14]*

Hydrocephalus (HC) is a failure of brain and cerebrospinal fluid (CSF) homeostasis often associated with dilation of the CSF-filled ventricles (ventriculomegaly). Hallmarks of HC include aberrant CSF dynamics, neural stem cell dysfunction resulting in impaired neurogenesis and corticogenesis, biomechanical instability at the brain-CSF interface, and disrupted synaptogenesis and neural circuitry. Pleiotropic mechanisms, including genetic and environmental insults to the brain, contribute to neurodevelopmental comorbidities. Hypothesis generation from genome-wide, single-cell multi-omic analyses coupled to experimental validation using induced pluripotent stem cell–derived cerebral organoids will refine molecular classification of HC subtypes and may lead to precision-based surgical and pharmacologic treatments.

INTRODUCTION

Hydrocephalus (HC) is the most common indication for childhood brain surgery (*1*). HC is a disorder of aberrant brain development and cerebrospinal fluid (CSF) homeostasis that is often associated with dilation of the cerebroventricular system (ventriculomegaly), increases in intracranial pressure, or both (*1*, *2*). HC includes primary (genetic or developmental) (*3*, *4*) and acquired forms (*5*) across the age spectrum (*6*–*8*). Systemic and intracranial infections are the leading causes of HC across the world. However, numerous molecular mechanisms and genetic factors underlie congenital HC (*6*, *7*). Current HC treatments are limited to surgical procedures such as ventriculoperitoneal (VP) shunting, endoscopic third ventriculostomy (ETV), or ETV with choroid plexus cauterization (CPC) (*2*, *9*). Although age, etiology, treatment history, and other factors may help to predict rates of surgical success (*10*), failure rates remain unacceptably high, with approximately 25% requiring reoperation within the first postoperative year (*11*). Some children undergo more than 100 neurosurgical procedures over the course of their abbreviated life spans (*11*, *12*). Rational design of pharmacologic treatments could substantially alleviate morbidity and mortality associated with HC, but these efforts have been impeded by an incomplete mechanistic understanding of the disease.

The path of CSF production and flow is classically represented as secretion by the choroid plexus (ChP), with unidirectional flow from the lateral ventricles through the foramina of Monro to the third ventricle, through the cerebral aqueduct of Sylvius into the fourth ventricle, followed by CSF reabsorption into the subarachnoid space through the foramina of Luschka and Magendie (Fig. 1). Although useful as a general framework for ventricular system anatomy, this model is widely challenged and neglects emerging contributors to CSF flow, including subventricular zone neural stem cells (NSCs), ependymal cilia, and the glia-lymphatic (glymphatic) system, including the CSF connection to the perineural space (*12*). For example, defects in ependymal cilia can cause HC in animal models through disruption of cilia organization, beat frequency, and alterations in CSF flow dynamics, with pleiotropic effects on brain development and function (*13*). However, the role of cilia as a primary pathophysiologic driver of human HC remains contested. In addition, the contribution of the glymphatic system (*14*) to HC is attributed to attenuated CSF waste clearance from germinal matrix hemorrhage and/or inflammation (*5*, *14*), but additional mechanistic studies are needed. Last, the nasopharyngeal lymphatic plexus and meningeal lymphatic vessels may represent alternative routes of CSF drainage in mice (*15*, *16*), but the role of these systems in human pathophysiology remains unexplored. These emerging mechanisms challenge dogmatic models of CSF flow and highlight our incomplete understanding of the relationships connecting CSF flow, brain development, and HC pathophysiology.

Molecular studies reveal HC as a phenotypically heterogenous disorder with an impact on both "drain and brain." Here, we outline molecular hallmarks of human HC, to serve as a framework for understanding human brain development and HC pathobiology and to identify remaining knowledge gaps. The hallmarks of HC include genetic predisposition, NSC dysfunction, alterations in corticogenesis, perturbation of brain volume and growth, biomechanical disruption of brain architecture, defects in synaptogenesis and neural circuitry, aberrant CSF dynamics and secretion, and augmentation of ChP developmental trajectories (Fig. 2). Much of our molecular understanding of HC is derived from in vivo and in vitro model systems. Although valuable, these approaches do not fully recapitulate the diverse genetic, ancestral, physiologic, or phenotypic characteristics of human HC. Recent advances in the generation and study of human induced pluripotent stem cell (iPSC)–derived cerebral organoids suggest that many mechanisms of HC will soon be directly testable in human systems. We will discuss in detail relevant considerations in leveraging this model system to advance our understanding of HC.

[1]Department of Neurosurgery, University of Alabama at Birmingham, Birmingham, AL 35233, USA. [2]Department of Human Biology and Neuroscience Institute, University of Cape Town, Cape Town 7925, South Africa. [3]Neurosciences Graduate Program, Stanford University, Stanford, CA 94305, USA. [4]Developmental Biology Graduate Program, Stanford University, Stanford, CA 94305, USA. [5]Department of Neurosurgery, University of Virginia School of Medicine, Charlottesville, VA 22903, USA. [6]Division of Nephrology and Vascular Biology Research Center, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA. [7]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA. [8]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA. [9]Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115, USA. [10]Medical Research Council, Laboratory for Molecular Biology, University of Cambridge, Cambridge CB2 0QH, UK. [11]Department of Neurosurgery, Stanford University School of Medicine, Palo Alto, CA 94304, USA. [12]Department of Neurosurgery, Harvard Medical School and Massachusetts General Hospital, Boston, MA 02114, USA. [13]Division of Genetics and Genomics and Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA 02115, USA. [14]Harvard PhD Program in Neuroscience (PiN), Harvard University, Cambridge, MA 02115, USA.
*Corresponding author. Email: andrewthale@uabmc.edu (A.T.H.); fame@stanford.edu (R.M.F.); kahle.kristopher@mgh.harvard.edu (K.T.K.)

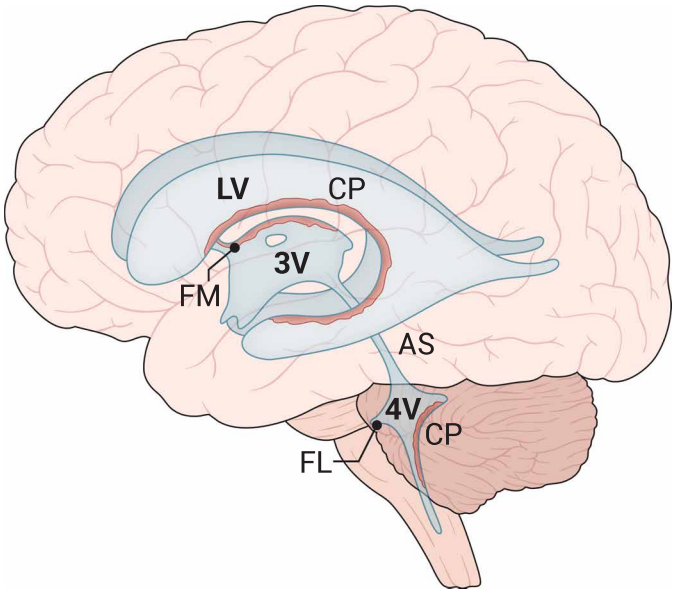


Fig. 1. CSF flow in the human cerebroventricular system. Illustration of the cerebroventricular system with the ChP (shown in red) and the ventricular system (shown in blue). CSF is secreted by the choroid plexus and is thought to flow from the lateral ventricles (LV) through the foramina of Monro (FM) to the third ventricle (3V), through the cerebral aqueduct of Sylvius (AS) into the fourth ventricle (4V), followed by CSF reabsorption into the subarachnoid space through the foramina of Luschka and Magendie (FL).

GENETIC PREDISPOSITION

The numerous challenges to understanding genetic contributions to HC include locus heterogeneity, the sporadic nature of HC, and phenotypic complexity among patients with HC. Moreover, HC is commonly accompanied by neurodevelopmental traits [for example, autism spectrum disorder (ASD), epilepsy, cognitive dysfunction, etc.] through pleiotropic mechanisms. Strategies to overcome these challenges include Mendelian genetic analysis using whole-exome sequencing (WES) of rigorously phenotyped human cohorts to identify inherited and de novo mutations, as well as copy number and other structural variations (SVs) across the genome. This approach enables precise identification of disease-causing mutations (at single–base pair resolution in select cases) and has been successful in identifying causative genes for HC (*3*, *17*), ASD (*18*), and epilepsy (*19*). WES is unbiased, systematic, and reproducible, enabling robust characterization of implicated loci. HC risk genes *TRIM71*, *SMARCC1*, *PTEN*, *FOXJ1*, and *PI3KCA* have been identified using this approach (*3*). As larger cohorts are sequenced and additional genetic mechanisms are explored, diagnostic refinement of congenital HC by genetic subtype and endophenotype might enable precision medicine–based treatment, given that WES was diagnostically informative in approximately 38% of cases (*20*).

Genome-wide and transcriptome-wide association studies (GWAS and TWAS, respectively) identify single-nucleotide polymorphisms (SNPs) and gene-based associations, respectively. GWAS requires a large sample size and best captures associations with common coding or noncoding variants (minor allele frequency > 0.1%), typically of low effect size. In addition, GWAS typically relies on array-based SNP detection and imputation methods based on reference population genetic architecture (*21*). In contrast, TWAS (which aggregates the effect of single variants across a gene locus) benefits from a higher *P* value threshold, reflecting the lower number of statistical tests (for thousands of genes versus millions of variants). TWAS also accounts for tissue-specific gene regulatory information [benefitting from reference tissue platforms such as the Genotype-Tissue Expression (GTEx) project (*22*)] and can infer molecular mechanism owing to the directionality of the gene-tissue pair association with the phenotype (*23*). Whereas GWAS identifies single SNPs, often in noncoding regions of often obscure functional impact, TWAS can suggest tissue- and gene-level directionality to guide hypotheses to elucidate underlying molecular mechanisms. Integration of these human genetic approaches with "omic" technologies such as neuroimaging linked to genetics and scRNA, epigenetic, and proteomic atlases of the developing brain can provide complementary and convergent means to define HC mechanisms. For example, a TWAS of HC identified maelstrom (*MAEL*) in the cortex as the gene-tissue pair reaching experiment-wide significant association

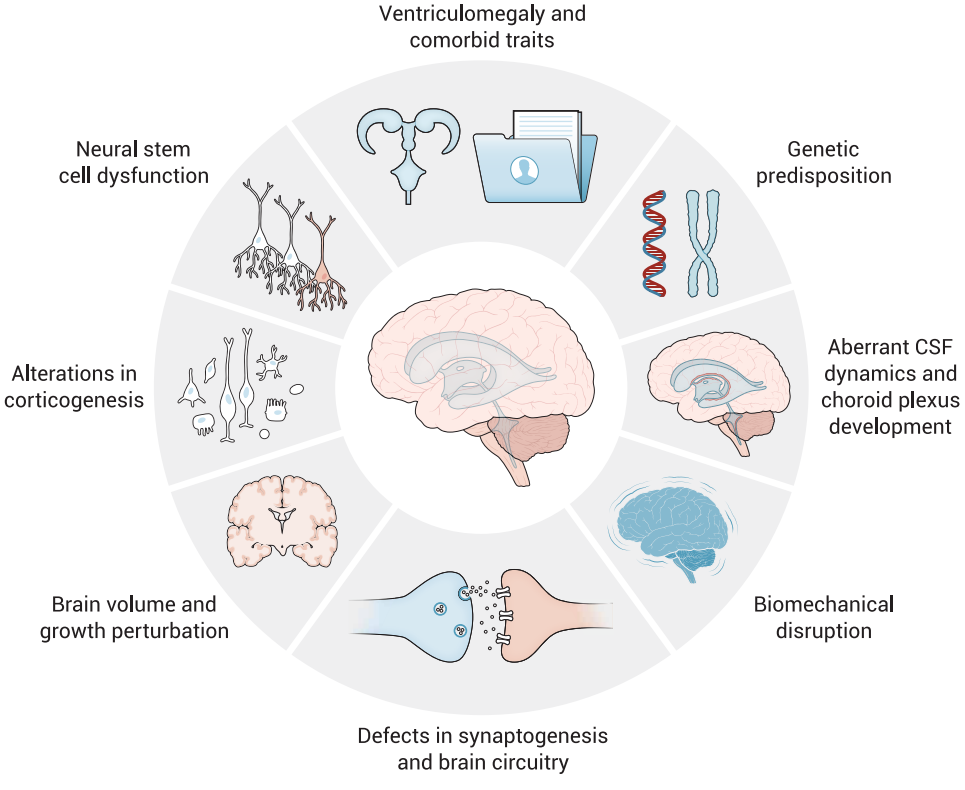


Fig. 2. The hallmarks of human hydrocephalus. Molecular hallmarks of HC include NSC dysfunction, alterations in corticogenesis, brain volume and growth perturbation, biomechanical disruption, defects in synaptogenesis and brain circuitry, aberrant cerebrospinal fluid dynamics and choroid plexus development, genetic predisposition, and ventriculomegaly and comorbid traits.

with HC. This finding was supported by rare variant analysis, integrative genomic analysis of neuroimaging phenotypes, ChP transcriptome analysis in a mouse model of HC, and CSF proteomics from patients with HC (*4*). In summary, use of multiple parallel approaches will continue to be paramount for the productive study of human cohorts.

NSC DYSFUNCTION

The consequences of molecular errors within NSCs for the developing brain include HC (*24*, *25*). Human genetics studies of HC have identified de novo and inherited mutations, copy number variations, and other alterations in genes with roles in NSC maintenance, renewal, and development, such as *TRIM71*, *PTEN*, *PI3KCA*, *SMARCC1*, *FOXJ1*, and *MAEL* (*3*, *4*, *17*). Mutations in these genes can disrupt NSC function through divergent mechanisms, as illustrated by the examples below. HC-associated mutations in *TRIM71*, an RNA binding protein and E3 ubiquitin ligase, alter NSC differentiation and commitment to neural lineage through repression of β-catenin and lysine demethylase 1a (LSD1) translation (*26*, *27*). Long noncoding RNA (lnRNA) *Trinc1* (an inhibitor of *Trim71* gene expression) blocks tripartite motif containing 71 (TRIM71)–dependent fibroblast growth factor/extracellular signal-regulated kinase (FGF/ERK) signaling and NSC self-renewal (*28*). Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) phosphatase and PI3KCA kinase regulate the production and subcellular localization of phosphatidyl-inositol 3,4,5-triphosphate, thereby controlling NSC fate, proliferation, and identity. These enzymes also play pleiotropic roles in brain overgrowth phenotypes, among other neurodevelopmental pathologies (*29*, *30*). SWI/SNF related, matrix-associated, actin-dependent regulator of chromatin, subfamily C, member 1 (SMARCC1) is a key regulator of transcription through chromatin remodeling. Smarcc1 deficiency in mice results in embryonic lethality due to exencephaly (*31*), and SMARCC1 dysfunction in mouse telencephalon results in aberrant epigenetic regulation in NSCs, causing global alterations in transcription and chromatin states (*32*, *33*). Loss-of-function *Smarcc1* mutations in *Xenopus* also result in midbrain overgrowth and CSF obstruction, inducing ventriculomegaly without forebrain abnormalities (*33*). Transcription factor *FOXJ1* is required for cilia formation and differentiation of ChP epithelial and ependymal cells in the postnatal brain, but it is not expressed in radial glia cells (RGCs) (*34*). These data together suggest that convergent, disruptive pathways of NSC differentiation broadly underlie congenital HC.

A TWAS of human HC identified *MAEL* expression in the cortex as an experiment-wide predictor of HC across all gene-tissue pairs tested. Gene set enrichment analysis of statistically significant HC-associated genes identified NSC regulation as a shared mechanism (*4*). *MAEL* is an evolutionarily divergent gene that regulates Piwi-interacting RNA–mediated transposon silencing and may contribute to HC risk by regulating transcription through rearrangement of transposons (*35*, *36*), potentially inducing mutations to shape cell identity and augment genome size by altering the genomic loci of DNA sequences. Loss of *MAEL* can recruit RNA polymerase II to increase transcription and steady-state levels of transposon RNAs. The accompanying increase in heterochromatin spreading despite modest changes in histone-3 lysine-9 trimethylation (H3K9me3) patterns (*35*) suggests that MAEL may act independently or downstream of H3K9me3. Nevertheless, H3K9me3-dependent changes were among the most significantly enriched gene sets (gene ontology) and H3K9me3 methyltransferases among the genes most highly differentially expressed comparing HC cases and controls (*4*). However, the precise mechanisms by which H3K9me3-dependent alterations cause HC remain to be elucidated. These data collectively implicate NSC function, proliferation, differentiation, and renewal as key mechanistic drivers of HC.

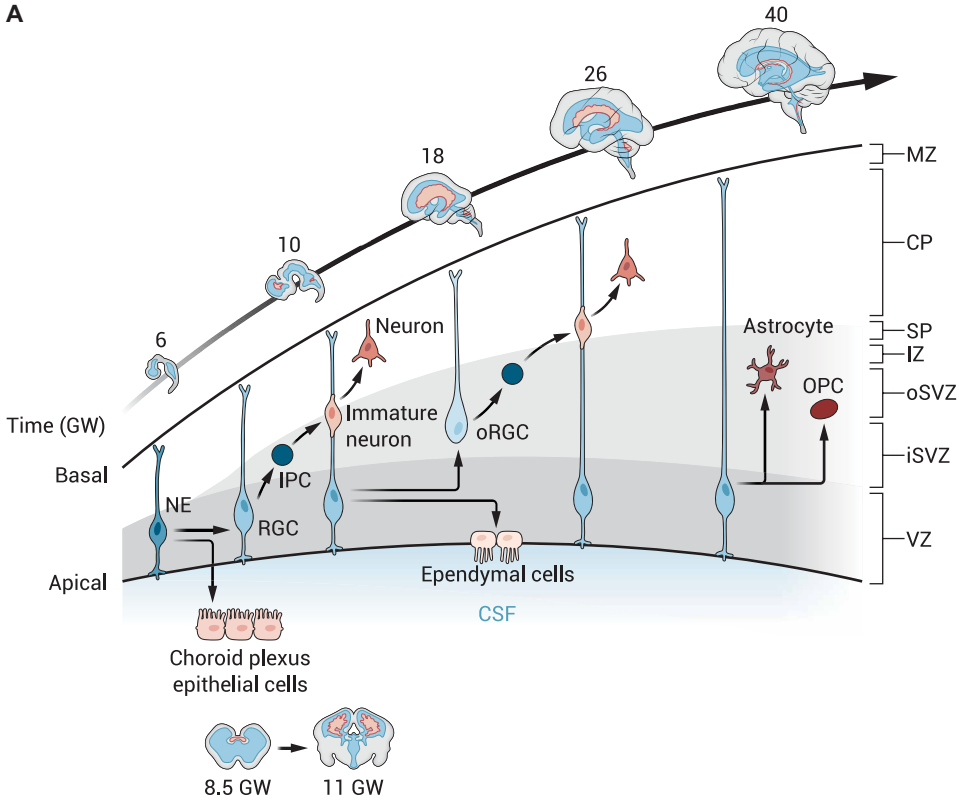
NORMAL DEVELOPMENT AT THE CSF-CORTICAL INTERFACE

Normal development of the cerebral cortex and ventricular system reflects a highly coordinated series of molecular, genetic, and cell-signaling events at the brain-CSF interface. The brain parenchyma and the CSF-producing ChP share a common tissue origin in the NSC at the dorsal midline and rhombic lip (*37*, *38*). Dorsal pallial NSCs give rise to neurons and glia of the cerebral cortex and the ChP of the lateral ventricle (*39*–*41*). Distinct NSC subsets can self-renew through symmetric cell division and then differentiate to generate de novo all neural and neuroglial cell lineages (*42*). Defects within the NSC compartment or its precursor lineages, including RGCs, outer RGCs (oRGCs), and intermediate progenitor cells (IPCs), can cause alterations in cortical patterning, folding, and cell-type identity (*43*), with global functional consequences in cognition and neurodevelopment (*44*). Epigenetic alterations within the NSC compartment may also affect corticogenesis and drive HC pathogenesis (*45*).

Human neuroepithelial cells arise from the neuroectoderm after neural tube closure (between days 28 and 32 postconception) and give rise to much of the brain parenchyma (Fig. 3A). Neuroepithelial cells generate multiple progenitor cell types, including RGCs beginning around gestational week (GW) 7 (*46*), ChP epithelial cells around GW8 (*47*, *48*), and ependymal cells around GW20 (*49*). RGCs rapidly proliferate to expand around GW10 to generate neurons and IPCs that, in turn, generate immature neurons (*50*). RGCs also produce more specialized RGC subtypes such as oRGCs by GW18 (*41*, *51*). Together, RGCs, oRGCs, and IPCs are the progenitors responsible for producing cortical neurons through the stereotyped inside-out process in which deep-layer neurons are born before the neurons that reside in more superficial layers (*52*). After generating neurons and neuronal progenitors, RGCs switch to a gliogenic role, producing astrocytes and oligodendrocyte precursor cells by the second trimester (*53*). Gliogenesis is a protracted process of differentiation that extends after neurogenesis through early childhood. Cerebrovascular and immune cell development in the human brain, though of possible relevance to HC, are beyond the scope of this review.

NSCs are the common precursor of neurons, neuroglia, ChP epithelial cells, and ependymal cells. Disruption of this lineage may impede CSF homeostasis by several mechanisms. For example, mutations that affect the earliest-stage NSCs could induce overgrowth and ventricular obstruction, alter CSF production and regulation, or perturb ependymal cells in the CSF-brain barrier. Forebrain expansion, neuroepithelial differentiation into oRGCs, and long gliogenic periods are highly species-specific, dynamic processes essential for cortical formation, maturation, and growth. For example, mutations in L1 cell adhesion molecule (L1CAM) have been hypothesized to cause X-linked HC through aberrant radial neuronal migration (*54*). In contrast, mutations that affect later-born, more highly specified progenitors (including RGCs, neuroblasts, or glioblasts) may regulate particular cell types implicated in disease.

A



B

Cell type	HC-associated risk genes with verified expression in cell type
Neuroepithelial cell (NE) Radial glial cell (RGC) Outer radial glial cell (oRGC)	PLOD2, PTCH1, PIK3CA, SMARCC1, PTEN, SGSM3, MAEL (unknown in NE), TRIM71, and SHH (low in NE)
Intermediate progenitor cell (IPC)	PTCH1, PIK3CA, SMARCC1, PTEN, SGSM3, and TRIM71
Immature neuron Neuron	PTCH1, PIK3CA, SMARCC1, PTEN, SGSM3, MAEL, and TRIM71
Astrocyte Oligodendrocyte precursor cell (OPC)	PLOD2 (OPC only), PIK3CA, SMARCC1, PTEN, and SHH
Choroid plexus epithelial cell Ependymal cell	SHH (choroid plexus only) and FOXJ1

Fig. 3. Overview of human neocortical development and cell type–specific expression of HC-associated genes. (**A**) Cellular differentiation during cortical development between GWs 4 and 40. NSCs successively expand, generate progenitors, and differentiate to form the principal components of the human cortex and ventricular system (*40*, *45*). (**B**) Summary of cell types and their HC-associated gene expression.

The timeline of human cortical development at the cellular level is central to HC. Identification of cell types expressing HC-associated genes during development may therefore help to clarify disease mechanisms. The expression of individual, congenital HC-associated genes, identified through unbiased human genetic studies (*3*, *4*, *17*), is enriched in divergent cell subpopulations in the brain neocortex (Fig. 3B and fig. S1), suggesting cell type–specific roles for HC-associated genes (*55*). We surmise that mapping expression patterns of human HC-associated genes (*3*, *4*, *17*) to the developing human brain may yield mechanistic insights. For example, *MAEL* expression is highest in the cortex [GTEx, Human Protein Atlas, and (*4*)], whereas other HC-associated genes (*PLOD2, PTCH1, PIK3CA, SMARCC1, PTEN,* and *SGSM3*) map onto specific subpopulations of cells in the human prenatal brain neocortex (*55*) (fig. S1). Although these human studies did not validate that NSCs express HC-associated genes, mouse studies suggest that many of the genes expressed by human RGC/oRGCs could also be expressed in NSCs (*56*). Other HC-associated genes (*SHH and FOXJ1*), although not strongly captured in the prenatal brain dataset (*55*), display cell type–specific expression. Sonic hedgehog (SHH) ligand is detectable in CSF, and source cells include epithelial cells, blood vessels, postmitotic neurons, Cajal-Retzius cells, and ChP (*57*). *FOXJ1* is expressed by multiciliated cells, including ChP epithelial cells and ependymal cells that line the brain ventricles (GTEx and Human Protein Atlas). Mapping HC risk genes to epigenetic atlases of the developing human brain can reveal additional mechanistic insights into how genetic errors cause HC and comorbidities (*4*, *58*–*62*). Moreover, to our knowledge, no whole-genome sequencing studies of HC have been performed to elucidate the disease contributions of noncoding, regulatory regions or SVs. The highly human-specific and elaborately orchestrated genetic and molecular events underlying NSC development, CSF regulation/dynamics, and corticogenesis will require human models to delineate HC mechanisms.

BRAIN VOLUME AND GROWTH PERTURBATION

The goal of treatment for pediatric patients with HC is to optimize brain growth and cognitive development, both primary outcomes in randomized controlled trials of surgical treatments for HC (*9*, *63*). Brain growth trajectories in HC correlate with neurodevelopmental outcomes in HC (*63*). However, determining "normal" brain growth patterns is challenging. Understanding how alterations in HC risk genes contribute to growth

of the brain, white matter, gray matter (*64*), and cortical patterning (*65*) is essential to inform precision treatments and to define successful outcomes. For example, patients with a genetic syndrome characterized by attenuated cortical development and mild ventriculomegaly in the setting of normal intracranial pressure may not require CSF diversion but, rather, referral to early intervention services for neurocognitive development. On the other hand, permanent CSF shunting may be indicated for patients with loss-of-function mutations in a key regulatory protein involved in CSF clearance. Precision medicine approaches for HC (*66*) have the potential to reduce morbidity and mortality associated with the disease and treatment complications. Indeed, integrative neuroimaging and genomics analyses have identified alterations in brain architecture as a key feature of neurodevelopmental diseases comorbid with HC (*64*, *65*, *67*, *68*). The top gene-level associations with HC include statistically significant enrichment for genes involved in total brain and white matter volumes, with *MAEL* among the topmost statistically significant genes (*4*). However, these population genetic models were developed from human genetic studies linked to neuroimaging phenotypes (*69*) lacking both birth-age data and adjustments for development. Brain growth charts coupled with multi-omics analysis across age, anthropometric, and population scales will further refine classification of HC subtypes.

HC rarely occurs in isolation, and the extent to which pleiotropic genetic mechanisms contribute to comorbid neurodevelopmental phenotypes is unknown. Detailed neuroimaging studies linked to genetic information may resolve these questions. Ventriculomegaly, but not necessarily HC, has been observed in patients with ASD, cognitive dysfunction, and neuropsychiatric disease (*70*–*73*). Normal ventricular size is highly subjective, and although brain charts across the age spectrum have been developed (with substantial effort) (*74*), these models have not been widely incorporated into clinical practice. For example, disruptive mutations in the phosphoinositide 3-kinase (PI3K)–Akt–mammalian target of rapamycin (mTOR) signaling pathway have been observed in patients with HC, macrocephaly, developmental delay, and ASD. Because dysregulation of this pathway can alter corticogenesis, ventriculomegaly in these cases is likely a harbinger of a primary structural brain disorder, rather than a primary driver of HC.

BIOMECHANICAL DISRUPTION OF BRAIN PARENCHYMA

Emerging data on perturbations in the development of brain architecture support direct roles for biomechanical disruption underlying subtypes of congenital HC. For example, HC-associated de novo mutations in *Trim71* and disruption of TRIM71 activity in the murine NSC compartment cause cortical hypoplasia and hypercompliant cortex with compensatory ventricular enlargement (*75*, *76*). Mice harboring *Trim71* mutations displayed impaired CSF outflow secondary to acquired aqueductal stenosis that could be ameliorated by CSF diversion. In contrast, the aqueduct in patients with L1CAM mutations remained stenotic even after CSF shunting (*75*). These data suggest a distinct mechanism by which altered cortical compliance and biomechanics may cause HC, such that the genetic etiology of HC determines, in large part, surgical treatment response. Independent mechanistic studies of *Trim71* further supporting the importance of defects in the NSC compartment have implicated impaired epigenetic mechanisms (*26*, *27*, *77*) as responsible for phenotypes of *Trim71*-deficient mice [and humans harboring *Trim71* loss-of-function mutations (*75*, *76*)]. These findings disrupt the classical paradigm of HC, in which defects of CSF production and absorption were the primary pathologic drivers of disease, and suggest primary disruption of brain parenchymal structural integrity as a causative mechanism for development of HC.

SYNAPTOGENESIS AND CIRCUIT DYSRUPTION

It remains unclear whether increased intracranial pressure and subsequent changes in CSF dynamics after CSF diversion are the primary causes of circuit disruptions associated with HC or, alternatively, whether HC-associated genetic errors result in pleiotropic effects leading to "passenger" phenotypes. For example, mutations in genes within the PI3K signaling pathway disrupt corticogenesis and are associated with the HC comorbidities of brain overgrowth and epilepsy (*78*, *79*). Genomic alterations within the PI3K pathway have also been identified by molecular phenotyping of patients with congenital HC (*3*, *17*), suggesting a pleiotropic basis for the wide phenotypic spectrum observed in humans with HC. Many independent mechanistic studies of the PI3K-PTEN signaling axis, a critical driver of synapse development (*80*), have identified defects in synaptogenesis and brain circuitry alterations caused by disruptions in this pathway. Disruptions in NSCs may delay gliogenesis or cause downstream defects in glial differentiation, impair processes required for synaptogenesis (astrocytes), and produce defects in circuit transduction through disrupted myelin production caused by oligodendrocyte dysfunction. Last, aberrant cortical neurogenesis leads to an imbalance between excitatory neurons and subpallial-generated inhibitory neurons or to defects in neuronal migration, both known mechanisms contributing to epilepsy (*81*).

Until recently, the mechanisms by which PI3K-PTEN alterations cause both HC and comorbid disorders such as ASD, epilepsy, and impaired cognition have remained elusive. Mice harboring de novo *Pten* mutations observed in patients with ASD and HC recapitulate the cerebral ventriculomegaly seen in humans (*82*, *83*). *Pten*-mutant mice developed aqueductal stenosis from proliferation of medial ganglionic eminence Nkx2.1 neural precursors and CSF hypersecretion (*82*, *83*). Unbiased brain network analysis identified similar neural networks in *Pten*-mutant mice with HC, reflecting diminished activity of Nkx2.1 NSC-derived inhibitory interneurons. These phenotypes were rescued by postnatal pharmacologic inhibition of mammalian target of rapamycin complex 1 (mTORC1) with Everolimus, which restored cortical architecture and decreased ventricular size (*82*, *83*). These data allow proposal of a mechanism by which primary alterations in brain circuitry and HC are caused by genetic disruption of PI3K-PTEN signaling. They demonstrate a possible pharmacological strategy to ameliorate both circuit-level and ventriculomegaly phenotypes through shared mechanisms. Refined molecular characterization of HC endophenotypes will enable clinical application of such precision-based approaches.

ABERRANT CSF DYNAMICS, SECRETION, AND CHOROID PLEXUS DEVELOPMENT

Precise regulation of CSF dynamics plays an important role in cerebral cortical development, and impairment of CSF homeostasis has been implicated in the pathophysiology of HC. The ChP epithelium is the primary source of CSF (*84*, *85*), based on observations from as far back as 1918, when neurosurgeon W. Dandy first described

surgical removal of ChP to decrease CSF production for HC treatment (*86*). Scarff (*87*) later described endoscopic approaches to treat HC through ChP cauterization. However, the complex mechanisms of ChP-mediated ion and water transport remain debated (*88*, *89*). The tightly regulated, concerted action of multiple integral membrane ion transport proteins such as Na^+/K^+-ATPase, $Na^+K^+Cl^-$ transporter NKCC1, and aquaporin water channels mediates transepithelial movement of solutes and water across the ChP epithelium (*84*, *88*). Other enzymes such as carbonic anhydrase secondarily contribute to mature ChP secretion without direct mediation of transmembrane transport. CSF secretion is achieved by the polarized localization of transport proteins in the ChP epithelium. For example, CSF $[Na^+]$ is regulated by the Na^+/K^+-ATPase and NKCC1 localized atypically on the ChP apical membrane, in contrast with their classically basolateral localization in other epithelia (*90*). Inhibitors of key ChP proteins such as ouabain (Na^+/K^+-ATPase), bumetanide and furosemide (NKCC1), and acetazolamide (carbonic anhydrase) decrease CSF secretion rates in vivo across model systems.

Increased cell mass or cell number due to ChP hyperplasia (*91*), ChP papilloma (*92*), and ChP carcinoma (*93*) can augment CSF production and obstruct CSF flow (*94*), leading to HC. Adult models of acquired HC secondary to hemorrhage (*5*), infection (*95*), and tumor (*96*) have demonstrated that increased CSF secretion in response to ChP inflammation is associated with up-regulated phosphorylation and activation of the SPS1-related proline/alanine-rich kinase (SPAK)–regulated NKCC1 cotransporter, which exists in a protein complex with the Na^+/K^+-ATPase on the apical ChP membrane. Genetic or pharmacologic inhibition of Toll-like receptor 4–associated ChP inflammation, as well as of the SPAK-NKCC1 complex, prevents development of ventriculomegaly in adult models of hemorrhagic and infectious HC (*95*, *97*–*99*). NKCC1 activity is required for CSF secretion by the adult ChP (*95*, *97*, *98*), consistent with the decreased epithelial secretion and "slit-ventricle" brain morphology indicative of decreased intraventricular CSF volumes in patients with loss-of-function NKCC1 mutations (*100*). However, the vector of net NaCl transport by NKCC1 (into or out of the ChP epithelial cell) reflects prevailing transepithelial ion gradients and its bidirectional and electroneutral transport mechanism (*101*), but remains debated. In developing mouse CSF in which ion concentrations have not yet achieved adult levels, NKCC1 may also play a role in CSF K^+ clearance (*102*, *103*). Interestingly, genetic overexpression of NKCC1 early in development through adeno-associated viral (AAV) transduction attenuates posthemorrhagic HC and obstructive HC in neonatal mice, which may be secondary to clearance of K^+ (*103*, *104*). Germline NKCC1 knockout animals display normal ventricular size (*105*), underscoring the complex, species-specific nature of CSF volume regulation. The lack of identified mutations in the NKCC1-SPAK driven pathway in human genetic studies of HC is not unusual, because the pathophysiologic and mechanistic bases of acquired and congenital HC are distinct (*6*). Importantly, the redundant pathways, molecular machinery, and mechanisms regulating ChP ion transport remain incompletely characterized. These data suggest that NKCC1 acts as a "rheostat" to mediate CSF homeostasis, albeit through incompletely understood mechanisms.

The molecular toolkit required to address fundamental questions in ChP biology has, until recently, been lacking. Large-scale developing brain atlases from multiple species offer increased resolution of NSCs and the ChP across neurodevelopment and enable unbiased insights into the function of these cell types (*106*–*108*). For example, *FOXJ1* mutations may cause HC through disruption of ependymal cell differentiation and ciliary biosynthesis in the absence of obstruction of CSF flow (*109*). Although primary disruption in ciliary function due to impaired ependymal cell development can disrupt CSF bulk flow and the transepithelial ion transport required for CSF production (*110*), primary ciliopathies are not classically associated with HC in humans. Moreover, *FOXJ1* in ChP is expressed predominantly at early developmental stages in nonmotile, multiciliated cells (*111*), suggesting that defects in FOXJ1-dependent pathways may also alter CSF production and homeostasis. Radial glial overproduction defects in SHH signaling (*112*) have also been shown to contribute to the development of the hindbrain ChP (*38*, *113*), as well as to HC (*114*) and to HC-related macrocephaly (*115*).

Single-cell and spatial sequencing atlases (*106*) have identified a shared progenitor cell for both ChP epithelial and neuronal cells in early ChP development, confirmed by Wnt1 lineage tracing (*106*) and consistent with previous Nestin1 lineage tracing (*116*). Cell-type composition, cell-cell interactions, and transition states shift considerably across the age spectrum. However, these analyses are currently restricted to the mouse brain, and the extent to which these paradigms apply to human ChP remains to be investigated. Further studies are needed to elucidate mechanisms of bidirectional CSF effects on neural development (*84*). Creation of a human model system could help resolve these mechanistic questions.

CEREBRAL ORGANOIDS AS A MODEL TO ELUCIDATE MECHANISMS UNDERLYING HC

Many in vitro, ex vivo, and in vivo [*Xenopus* (*33*, *117*, *118*), fish (*13*, *119*, *120*), rat (*97*, *121*), mouse (*122*, *123*), and pig (*124*)] models have been developed to elucidate fundamental mechanisms underlying hallmark features of HC, including alterations in ependymal barrier function (*125*), cortical development (*126*), and CSF dynamics (*127*). In view of the varied mechanisms and pathways implicated in HC and its comorbid phenotypes, a human model system at least partially recapitulating these complexities may enable a more detailed and translationally relevant mechanistic understanding of HC. Moreover, a human model system could serve as a screening platform for pharmacologic development. Human iPSC-derived cerebral organoids, in vitro three-dimensional structures resembling the developing human brain, have recently emerged as a promising experimental tool to answer fundamental questions about normal and abnormal brain development and disease (*128*). Cerebral organoids are self-organizing, recapitulate the spatial organization of the brain, and display transcriptional and epigenetic states that mirror brain development (*129*). These organoids have been critical tools to aid our mechanistic understanding of HC-related phenotypes, including microcephaly (*130*), macrocephaly (*131*), ASD (*132*), host-pathogen interactions (*133*), altered CSF production, and selectively modified transport of small molecules (*134*). Moreover, cerebral organoids form ventricular zones that resemble cerebral ventricles (*135*). Disruption of *ZEB2*, a gene crucial for neuroepithelial cell development, results in enlarged neuroepithelial buds resembling ventriculomegaly in cortical organoids (*135*). Leveraging this model system to study HC pathogenic features would be a logical next step to bridge the gap between mechanisms and patients.

Genetic forms of HC are driven primarily by disruption of the NSC compartment and subsequent defects in cortical growth, patterning, and development. Extended culture protocols will aid our understanding of

the long-term consequences of genetically encoded early developmental dysfunction on downstream molecular events (*136*). Genetically tractable iPSCs can be used to understand the functional impact of patient-specific mutations using gene editing technologies. Such cell lines can also elucidate the consequences of genetic errors at the NSC stage on cortical development and the impact on neuronal synaptic function through organoid-wide electrophysiological recording. Modulation of tissue morphology has also been shown to influence brain organoid development, a feature that may mirror the effect of permanent CSF shunting on brain structural architecture (*137*). Important limitations of cerebral organoids include the absence of circulating immune cells, limited nutrients and oxygenation, formation of vascular networks, absent myelination, batch effects, and variation in iPSC lines necessitating stringent controls (*129*).

The mechanistically distinct acquired HC is driven by alterations in bulk CSF flow (production/absorption mismatch) and dysregulation of CSF dynamics. ChP-like cerebral organoids recapitulate the epithelial and stromal cell types observed in vivo. These organoids form a blood-CSF barrier–like tight junction interface, secrete CSF-like fluid, and produce a protein signature like human CSF, recommending organoids for the study of the effects of hemorrhage or infection on HC. Drawbacks to ChP organoids as an experimental model for acquired HC include the lack of a vascular network and circulating immune cells, the lack of the physiological contribution of adjacent brain structure to CSF composition, and the inability to analyze human ChP from surgical samples owing to safety concerns (for example, bleeding, stroke, etc.). Nonetheless, the composition of CSF-like fluid from ChP organoids appears to resemble that of human CSF, and transcriptomic studies of iPSC-derived ChP mirror human ChP datasets (*134*). Last, ChP organoids have been used to study infectious diseases like SARS-CoV-2 (*138*), highlighting their utility in understanding brain infections. Reconstitution experiments introducing genetically modified and/or additional cell types (such as microglia) into organoid cultures will provide a platform for well-controlled and hypothesis-driven experiments not feasible in other systems. Rigor and reproducibility of such experiments will require the broader scientific community to adhere to strict, clearly defined nomenclature and methods of organoid generation, culture, and assembly (*139*, *140*).

CONCLUDING REMARKS

We define the molecular hallmarks of human HC as a framework for future mechanistic studies, but many open questions remain. The most urgent unmet need is identification of therapeutic targets that reduce the requirement for and/or guide choice of neurosurgical interventions. We hypothesize that these targets will emerge from (i) careful study of cell-type and stage-specific roles of HC-associated genetic aberrations in human brain tissue; (ii) molecular characterization of implicated genetic mechanisms in improved human and animal models; and (iii) identification and validation of HC-associated genes in carefully phenotyped human cohorts. Such approaches will enable personalized characterization of genetic contributions to HC, will enhance validation of emerging pathogenic causes of HC in defined ancestral genomic backgrounds, and will provide resources for the broader HC research community.

Supplementary Materials

This PDF file includes:

Fig. S1

Reference (*141*)

REFERENCES AND NOTES

1. M. C. Dewan, A. Rattani, R. Mekary, L. J. Glancz, I. Yunusa, R. E. Baticulon, G. Fieggen, J. C. Wellons, K. B. Park, B. C. Warf, Global hydrocephalus epidemiology and incidence: Systematic review and meta-analysis. *J. Neurosurg.* **130**, 1065–1079 (2018).
2. K. T. Kahle, A. V. Kulkarni, D. D. Limbrick Jr., B. C. Warf, Hydrocephalus in children. *Lancet* **387**, 788–799 (2016).
3. S. C. Jin, W. Dong, A. J. Kundishora, S. Panchagnula, A. Moreno-De-Luca, C. G. Furey, A. A. Allocco, R. L. Walker, C. Nelson-Williams, H. Smith, A. Dunbar, S. Conine, Q. Lu, X. Zeng, M. C. Sierant, J. R. Knight, W. Sullivan, P. Q. Duy, T. De Spenza, B. C. Reeves, J. K. Karimy, A. Marlier, C. Castaldi, I. R. Tikhonova, B. Li, H. P. Peña, J. R. Broach, E. M. Kabachelor, P. Ssenyonga, C. Hehnly, L. Ge, B. Keren, A. T. Timberlake, J. Goto, F. T. Mangano, J. M. Johnston, W. E. Butler, B. C. Warf, E. R. Smith, S. J. Schiff, D. D. Limbrick Jr., G. Heuer, E. M. Jackson, B. J. Iskandar, S. Mane, S. Haider, B. Guclu, Y. Bayri, Y. Sahin, C. C. Duncan, M. L. J. Apuzzo, M. L. Di Luna, E. J. Hoffman, N. Sestan, L. R. Ment, S. L. Alper, K. Bilguvar, D. H. Geschwind, M. Günel, R. P. Lifton, K. T. Kahle, Exome sequencing implicates genetic disruption of prenatal neuro-gliogenesis in sporadic congenital hydrocephalus. *Nat. Med.* **26**, 1754–1765 (2020).
4. A. T. Hale, L. Bastarache, D. M. Morales, J. C. Wellons III, D. D. Limbrick Jr., E. R. Gamazon, Multi-omic analysis elucidates the genetic basis of hydrocephalus. *Cell Rep.* **35**, 109085 (2021).
5. J. K. Karimy, B. C. Reeves, E. Damisah, P. Q. Duy, P. Antwi, W. David, K. Wang, S. J. Schiff, D. D. Limbrick Jr., S. L. Alper, B. C. Warf, M. Nedergaard, J. M. Simard, K. T. Kahle, Inflammation in acquired hydrocephalus: Pathogenic mechanisms and therapeutic targets. *Nat. Rev. Neurol.* **16**, 285–296 (2020).
6. A. T. Hale, H. Boudreau, R. Devulapalli, P. Q. Duy, T. J. Atchley, M. C. Dewan, M. Goolam, G. Fieggen, H. L. Spader, A. A. Smith, J. P. Blount, J. M. Johnston, B. G. Rocque, C. J. Rozzelle, Z. Chong, J. M. Strahle, S. J. Schiff, K. T. Kahle, The genetic basis of hydrocephalus: Genes, pathways, mechanisms, and global impact. *Fluids Barriers CNS* **21**, 24 (2024).
7. K. T. Kahle, P. M. Klinge, J. E. Koschnitzky, A. V. Kulkarni, N. M. Aulay, S. Robinson, S. J. Schiff, J. M. Strahle, Paediatric hydrocephalus. *Nat. Rev. Dis. Primers.* **10**, 35 (2024).
8. J. Räsänen, S. Heikkinen, K. Mäklin, A. Lipponen, T. Kuulasmaa, J. Mehtonen, V. E. Korhonen, A. Junkkari, B. Grenier-Boley, C. Bellenguez, M. Oinas, C. Avellan, J. Frantzén, A. Kotkansalo, J. Rinne, A. Ronkainen, M. Kauppinen, M. V. U. Z. Fraunberg, K. Lönnrot, J. Satopää, M. Perola, A. M. Koivisto, V. Julkunen, A. M. Portaankorva, A. Mannermaa, H. Soininen, S. Helisalmi, J. E. Jääskeläinen, J.-C. Lambert, P. K. Eide, for FinnGen, A. Palotie, M. I. Kurki, M. Hiltunen, V. Leinonen, Risk variants associated with normal pressure hydrocephalus: Genome-wide association study in the FinnGen Cohort. *Neurology* **103**, e209694 (2024).
9. A. V. Kulkarni, S. J. Schiff, E. Mbabazi-Kabachelor, J. Mugamba, P. Ssenyonga, R. Donnelly, J. Levenbach, V. Monga, M. Peterson, M. MacDonald, V. Cherukuri, B. C. Warf, Endoscopic treatment versus shunting for infant hydrocephalus in Uganda. *N. Engl. J. Med.* **377**, 2456–2464 (2017).
10. A. V. Kulkarni, J. M. Drake, J. R. Kestle, C. L. Mallucci, S. Sgouros, S. Constantini, Canadian Pediatric Neurosurgery Study Group, Predicting who will benefit from endoscopic third ventriculostomy compared with shunt insertion in childhood hydrocephalus using the ETV Success Score. *J. Neurosurg. Pediatr.* **6**, 310–315 (2010).
11. G. K. Reddy, P. Bollam, G. Caldito, Long-term outcomes of ventriculoperitoneal shunt surgery in patients with hydrocephalus. *World Neurosurg.* **81**, 404–410 (2014).
12. A. P. Ligocki, A. V. Vinson, A. T. Yachnis, W. A. Dunn Jr., D. E. Smith, E. A. Scott, J. V. Alvarez-Castanon, D. E. Baez Montalvo, O. G. Frisone, G. A. J. Brown, J. E. Pessa, E. W. Scott, Cerebrospinal fluid flow extends to peripheral nerves further unifying the nervous system. *Sci. Adv.* **10**, eadn3259 (2024).
13. E. W. Olstad, C. Ringers, J. N. Hansen, A. Wens, C. Brandt, D. Wachten, E. Yaksi, N. Jurisch-Yaksi, Ciliary beating compartmentalizes cerebrospinal fluid flow in the brain and regulates ventricular development. *Curr. Biol.* **29**, 229–241.e6 (2019).
14. M. K. Rasmussen, H. Mestre, M. Nedergaard, The glymphatic pathway in neurological disorders. *Lancet Neurol.* **17**, 1016–1024 (2018).
15. D. Choi, E. Park, J. Choi, R. Lu, J. S. Yu, C. Kim, L. Zhao, J. Yu, B. Nakashima, S. Lee, D. Singhal, J. P. Scallan, B. Zhou, C. J. Koh, E. Lee, Y.-K. Hong, Piezo1 regulates meningeal lymphatic vessel drainage and alleviates excessive CSF accumulation. *Nat. Neurosci.* **27**, 913–926 (2024).
16. J.-H. Yoon, H. Jin, H. J. Kim, S. P. Hong, M. J. Yang, J. H. Ahn, Y.-C. Kim, J. Seo, Y. Lee, D. M. McDonald, M. J. Davis, G. Y. Koh, Nasopharyngeal lymphatic plexus is a hub for cerebrospinal fluid drainage. *Nature* **625**, 768–777 (2024).
17. C. G. Furey, J. Choi, S. C. Jin, X. Zeng, A. T. Timberlake, C. Nelson-Williams, M. S. Mansuri, Q. Lu, D. Duran, S. Panchagnula, A. Allocco, J. K. Karimy, A. Khanna, J. R. Gaillard, T. De Spenza, P. Antwi, E. Loring, W. E. Butler, E. R. Smith, B. C. Warf, J. M. Strahle, D. D. Limbrick, P. B. Storm, G. Heuer, E. M. Jackson, B. J. Iskandar, J. M. Johnston, I. Tikhonova, C. Castaldi, F. López-Giráldez, R. D. Bjornson, J. R. Knight, K. Bilguvar, S. Mane, S. L. Alper, S. Haider, B. Guclu, Y. Bayri, Y. Sahin, M. L. J. Apuzzo, C. C. Duncan,

M. L. Di Luna, M. Günel, R. P. Lifton, K. T. Kahle, De novo mutation in genes regulating neural stem cell fate in human congenital hydrocephalus. *Neuron* **99**, 302–314.e4 (2018).
18. S. J. Sanders, M. T. Murtha, A. R. Gupta, J. D. Murdoch, M. J. Raubeson, A. J. Willsey, A. G. Ercan-Sencicek, N. M. Di Lullo, N. N. Parikshak, J. L. Stein, M. F. Walker, G. T. Ober, N. A. Teran, Y. Song, P. El-Fishawy, R. C. Murtha, M. Choi, J. D. Overton, R. D. Bjornson, N. J. Carriero, K. A. Meyer, K. Bilguvar, S. M. Mane, N. Sestan, R. P. Lifton, M. Günel, K. Roeder, D. H. Geschwind, B. Devlin, M. W. State, De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature* **485**, 237–241 (2012).
19. K. L. Helbig, K. D. F. Hagman, D. N. Shinde, C. Mroske, Z. Powis, S. Li, S. Tang, I. Helbig, Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genet. Med.* **18**, 898–905 (2016).
20. A. B. W. Greenberg, N. H. Mehta, G. Allington, S. C. Jin, A. Moreno-De-Luca, K. T. Kahle, Molecular diagnostic yield of exome sequencing in patients with congenital hydrocephalus: A systematic review and meta-analysis. *JAMA Netw. Open* **6**, e2343384 (2023).
21. R. M. Cantor, K. Lange, J. S. Sinsheimer, Prioritizing GWAS results: A review of statistical methods and recommendations for their application. *Am. J. Hum. Genet.* **86**, 6–22 (2010).
22. The GTex Consortium, The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans. *Science* **348**, 648–660 (2015).
23. M. Wainberg, N. Sinnott-Armstrong, N. Mancuso, A. N. Barbeira, D. A. Knowles, D. Golan, R. Ermel, A. Ruusalepp, T. Quertermous, K. Hao, J. L. M. Björkegren, H. K. Im, B. Pasaniuc, M. A. Rivas, A. Kundaje, Opportunities and challenges for transcriptome-wide association studies. *Nat. Genet.* **51**, 592–599 (2019).
24. M. M. Guerra, R. Henzi, A. Ortloff, N. Lichtin, K. Vío, A. J. Jiménez, M. D. Dominguez-Pinos, C. González, M. C. Jara, F. Hinostroza, S. Rodríguez, M. Jara, E. Ortega, F. Guerra, D. A. Sival, W. F. A. den Dunnen, J. M. Pérez-Fígares, J. P. McAllister, C. E. Johanson, E. M. Rodríguez, Cell junction pathology of neural stem cells is associated with ventricular zone disruption, hydrocephalus, and abnormal neurogenesis. *J. Neuropathol. Exp. Neurol.* **74**, 653–671 (2015).
25. E. M. Rodríguez, M. M. Guerra, K. Vío, C. González, A. Ortloff, L. F. Bátiz, S. Rodríguez, M. C. Jara, R. I. Muñoz, E. Ortega, J. Jaque, F. Guerra, D. A. Sival, W. F. A. den Dunnen, A. J. Jiménez, M. D. Domínguez-Pinos, J. M. Pérez-Fígares, J. P. McAllister, C. Johanson, A cell junction pathology of neural stem cells leads to abnormal neurogenesis and hydrocephalus. *Biol. Res.* **45**, 231–241 (2012).
26. Q. Liu, M. K. Novak, R. M. Pepin, K. R. Maschhoff, W. Hu, Different congenital hydrocephalus-associated mutations in Trim71 impair stem cell differentiation via distinct gain-of-function mechanisms. *PLoS Biol.* **21**, e3001947 (2023).
27. Q. Liu, M. K. Novak, R. M. Pepin, K. R. Maschhoff, K. Worner, X. Chen, S. Zhang, W. Hu, A congenital hydrocephalus-causing mutation in Trim71 induces stem cell defects via inhibiting Lsd1 mRNA translation. *EMBO Rep.* **24**, e55843 (2023).
28. Y.-P. Li, F.-F. Duan, Y.-T. Zhao, K.-L. Gu, L.-Q. Liao, H.-B. Su, J. Hao, K. Zhang, N. Yang, Y. Wang, A TRIM71 binding long noncoding RNA Trincr1 represses FGF/ERK signaling in embryonic stem cells. *Nat. Commun.* **10**, 1368 (2019).
29. M. Groszer, R. Erickson, D. D. Scripture-Adams, R. Lesche, A. Trumpp, J. A. Zack, H. I. Kornblum, X. Liu, H. Wu, Negative regulation of neural stem/progenitor cell proliferation by the *Pten* tumor suppressor gene in vivo. *Science* **294**, 2186–2189 (2001).
30. J.-B. Rivière, G. M. Mirzaa, B. J. O'Roak, M. Beddaoui, D. Alcantara, R. L. Conway, J. St-Onge, J. A. Schwartzentruber, K. W. Gripp, S. M. Nikkel, T. Worthylake, C. T. Sullivan, T. R. Ward, H. E. Butler, N. A. Kramer, B. Albrecht, C. M. Armour, L. Armstrong, O. Caluseriu, C. Cytrynbaum, B. A. Drolet, A. M. Innes, J. L. Lauzon, A. E. Lin, G. M. S. Mancini, W. S. Meschino, J. D. Reggin, A. K. Saggar, T. Lerman-Sagie, G. Uyanik, R. Weksberg, B. Zirn, C. L. Beaulieu, Finding of Rare Disease Genes (FORGE) Canada Consortium, J. Majewski, D. E. Bulman, M. O'Driscoll, J. Shendure, J. M. Graham Jr., K. M. Boycott, W. B. Dobyns, De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nat. Genet.* **44**, 934–940 (2012).
31. L. Harmacek, D. E. Watkins-Chow, J. Chen, K. L. Jones, W. J. Pavan, J. M. Salbaum, L. Niswander, A unique missense allele of BAF155, a core BAF chromatin remodeling complex protein, causes neural tube closure defects in mice. *Dev. Neurobiol.* **74**, 483–497 (2014).
32. R. Narayanan, M. Pirouz, C. Kerimoglu, L. Pham, R. J. Wagener, K. A. Kiszka, J. Rosenbusch, R. H. Seong, M. Kessel, A. Fischer, A. Stoykova, J. F. Staiger, T. Tuoc, Loss of BAF (mSWI/SNF) complexes causes global transcriptional and chromatin state changes in forebrain development. *Cell Rep.* **13**, 1842–1854 (2015).
33. A. K. Singh, G. Allington, S. Viviano, S. McGee, E. Kiziltug, S. Ma, S. Zhao, K. Y. Mekbib, J. P. Shohfi, P. Q. Duy, T. DeSpenza Jr., C. G. Furey, B. C. Reeves, H. Smith, A. M. M. Sousa, A. Cherskov, A. Allocco, C. Nelson-Williams, S. Haider, S. R. A. Rizvi, S. L. Alper, N. Sestan, H. Shimelis, L. K. Walsh, R. P. Lifton, A. Moreno-De-Luca, S. C. Jin, P. Kruszka, E. Deniz, K. T. Kahle, A novel SMARCC1 BAFopathy implicates neural progenitor epigenetic dysregulation in human hydrocephalus. *Brain* **147**, 1553–1570 (2024).
34. B. V. Jacquet, R. Salinas-Mondragon, H. Liang, B. Therit, J. D. Buie, M. Dykstra, K. Campbell, L. E. Ostrowski, S. L. Brody, H. T. Ghashghaei, FoxJ1-dependent gene expression is required for differentiation of radial glia into ependymal cells and a subset of astrocytes in the postnatal brain. *Development* **136**, 4021–4031 (2009).
35. G. Sienski, D. Dönertas, J. Brennecke, Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression. *Cell* **151**, 964–980 (2012).
36. S. F. C. Soper, G. W. van der Heijden, T. C. Hardiman, M. Goodheart, S. L. Martin, P. de Boer, A. Bortvin, Mouse maelstrom, a component of nuage, is essential for spermatogenesis and transposon repression in meiosis. *Dev. Cell* **15**, 285–297 (2008).
37. D. S. Currle, X. Cheng, C.-M. Hsu, E. S. Monuki, Direct and indirect roles of CNS dorsal midline cells in choroid plexus epithelia formation. *Development* **132**, 3549–3559 (2005).
38. N. L. Hunter, S. M. Dymecki, Molecularly and temporally separable lineages form the hindbrain roof plate and contribute differentially to the choroid plexus. *Development* **134**, 3449–3460 (2007).
39. A. Alvarez-Buylla, J. M. García-Verdugo, A. D. Tramontin, A unified hypothesis on the lineage of neural stem cells. *Nat. Rev. Neurosci.* **2**, 287–293 (2001).
40. A. Kriegstein, A. Alvarez-Buylla, The glial nature of embryonic and adult neural stem cells. *Annu. Rev. Neurosci.* **32**, 149–184 (2009).
41. D. V. Hansen, J. H. Lui, P. R. L. Parker, A. R. Kriegstein, Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* **464**, 554–561 (2010).
42. M. Uhlen, C. Zhang, S. Lee, E. Sjöstedt, L. Fagerberg, G. Bidkhori, R. Benfeitas, M. Arif, Z. Liu, F. Edfors, K. Sanli, K. von Feilitzen, P. Oksvold, E. Lundberg, S. Hober, P. Nilsson, J. Mattsson, J. M. Schwenk, H. Brunnström, B. Glimelius, T. Sjöblom, P.-H. Edqvist, D. Djureinovic, P. Micke, C. Lindskog, A. Mardinoglu, F. Ponten, A pathology atlas of the human cancer transcriptome. *Science* **357**, eaan2507 (2017).
43. N. Gaspard, T. Bouschet, R. Hourez, J. Dimidschstein, G. Naeije, J. van den Ameele, I. Espuny-Camacho, A. Herpoel, L. Passante, S. N. Schiffmann, A. Gaillard, P. Vanderhaeghen, An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature* **455**, 351–357 (2008).
44. P. Vanderhaeghen, F. Polleux, Developmental mechanisms underlying the evolution of human cortical circuits. *Nat. Rev. Neurosci.* **24**, 213–232 (2023).
45. C. MuhChyi, B. Juliandi, T. Matsuda, K. Nakashima, Epigenetic regulation of neural stem cell fate during corticogenesis. *Int. J. Dev. Neurosci.* **31**, 424–433 (2013).
46. B. M. Howard, Z. Mo, R. Filipovic, A. R. Moore, S. D. Antic, N. Zecevic, Radial glia cells in the developing human brain. *Neuroscientist* **14**, 459–473 (2008).
47. S. A. Bayer, J. Altman, *The Human Brain During the Early First Trimester* (CRC Press, 2008).
48. S. A. Liddelow, Development of the choroid plexus and blood-CSF barrier. *Front. Neurosci.* **9**, 32 (2015).
49. S. J. Gould, S. Howard, L. Papadaki, The development of ependyma in the human fetal brain: An immunohistological and electron microscopic study. *Brain Res. Dev. Brain Res.* **55**, 255–267 (1990).
50. M.-P. Pebworth, J. Ross, M. Andrews, A. Bhaduri, A. R. Kriegstein, Human intermediate progenitor diversity during cortical development. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2019415118 (2021).
51. S. A. Fietz, I. Kelava, J. Vogt, M. Wilsch-Bräuninger, D. Stenzel, J. L. Fish, D. Corbeil, A. Riehn, W. Distler, R. Nitsch, W. B. Huttner, OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nat. Neurosci.* **13**, 690–699 (2010).
52. P. Rakic, Specification of cerebral cortical areas. *Science* **241**, 170–176 (1988).
53. L. Yang, Z. Li, G. Liu, X. Li, Z. Yang, Developmental origins of human cortical oligodendrocytes and astrocytes. *Neurosci. Bull.* **38**, 47–68 (2022).
54. K. Itoh, S. Fushiki, The role of L1 cam in murine corticogenesis, and the pathogenesis of hydrocephalus. *Pathol. Int.* **65**, 58–66 (2015).
55. L. Wang, C. Wang, J. A. Moriano, S. Chen, G Zuo, A. Cebrián-Silla, S. Zhang, T. Mukhtar, S. Wang, M. Song, L. G. de Oliveira, Q. Bi, J. J. Augustin, X. Ge, M. F. Paredes, E. J. Huang, A. Alvarez-Buylla, X. Duan, J. Li, A. R. Kriegstein, Molecular and cellular dynamics of the developing human neocortex at single-cell resolution. bioRxiv 575956 [Preprint] (2024). https://doi.org/10.1101/2024.01.16.575956.
56. K. F. Chau, M. L. Shannon, R. M. Fame, E. Fonseca, H. Mullan, M. B. Johnson, A. K. Sendamarai, M. W. Springel, B. Laurent, M. K. Lehtinen, Downregulation of ribosome biogenesis during early forebrain development. *eLife* **7**, e36998 (2018).
57. O. R. Yabut, S. J. Pleasure, Sonic Hedgehog signaling rises to the surface: Emerging roles in neocortical development. *Brain Plast.* **3**, 119–128 (2018).
58. O. A. Bayraktar, T. Bartels, S. Holmqvist, V. Kleshchevnikov, A. Martirosyan, D. Polioudakis, L. B. Haim, A. M. H. Young, M. Y. Batiuk, K. Prakash, A. Brown, K. Roberts, M. F. Paredes, R. Kawaguchi, J. H. Stockley, K. Sabeur, S. M. Chang, E. Huang, P. Hutchinson, E. M. Ullian, M. Hemberg, G. Coppola, M. G. Holt, D. H. Geschwind, D. H. Rowitch, Astrocyte layers in the mammalian cerebral cortex revealed by a single-cell in situ transcriptomic map. *Nat. Neurosci.* **23**, 500–509 (2020).
59. H. J. Kang, Y. I. Kawasawa, F. Cheng, Y. Zhu, X. Xu, M. Li, A. M. M. Sousa, M. Pletikos, K. A. Meyer, G. Sedmak, T. Guennel, Y. Shin, M. B. Johnson, Z. Krsnik, S. Mayer, S. Fertuzinhos, S. Umlauf, S. N. Lisgo, A. Vortmeyer, D. R. Weinberger, S. Mane, T. M. Hyde, A. Huttner, M. Reimers, J. E. Kleinman, N. Sestan, Spatio-temporal transcriptome of the human brain. *Nature* **478**, 483–489 (2011).

60. D. Polioudakis, L. de la Torre-Ubieta, J. Langerman, A. G. Elkins, X. Shi, J. L. Stein, C. K. Vuong, S. Nichterwitz, M. Gevorgian, C. K. Opland, D. Lu, W. Connell, E. K. Ruzzo, J. K. Lowe, T. Hadzic, F. I. Hinz, S. Sabri, W. E. Lowry, M. B. Gerstein, K. Plath, D. H. Geschwind, A single-cell transcriptomic atlas of human neocortical development during mid-gestation. *Neuron* **103**, 785–801.e8 (2019).
61. J. Seidlitz, F. Váša, M. Shinn, R. Romero-Garcia, K. J. Whitaker, P. E. Vértes, K. Wagstyl, P. K. Reardon, L. Clasen, S. Liu, A. Messinger, D. A. Leopold, P. Fonagy, R. J. Dolan, P. B. Jones, I. M. Goodyer, NSPN Consortium, A. Raznahan, E. T. Bullmore, Morphometric similarity networks detect microscale cortical organization and predict inter-individual cognitive variation. *Neuron* **97**, 231–247.e7 (2018).
62. R. S. Ziffra, C. N. Kim, J. M. Ross, A. Wilfert, T. N. Turner, M. Haeussler, A. M. Casella, P. F. Przytycki, K. C. Keough, D. Shin, D. Bogdanoff, A. Kreimer, K. S. Pollard, S. A. Ament, E. E. Eichler, N. Ahituv, T. J. Nowakowski, Single-cell epigenomics reveals mechanisms of human cortical development. *Nature* **598**, 205–213 (2021).
63. J. G. Mandell, A. V. Kulkarni, B. C. Warf, S. J. Schiff, Volumetric brain analysis in neurosurgery: Part 2. Brain and CSF volumes discriminate neurocognitive outcomes in hydrocephalus. *J. Neurosurg. Pediatr.* **15**, 125–132 (2015).
64. G. Ball, J. Seidlitz, R. Beare, M. L. Seal, Cortical remodelling in childhood is associated with genes enriched for neurodevelopmental disorders. *Neuroimage* **215**, 116803 (2020).
65. J. Seidlitz, A. Nadig, S. Liu, R. A. I. Bethlehem, P. E. Vértes, S. E. Morgan, F. Váša, R. Romero-Garcia, F. M. Lalonde, L. S. Clasen, J. D. Blumenthal, C. Paquola, B. Bernhardt, K. Wagstyl, D. Polioudakis, L. de la Torre-Ubieta, D. H. Geschwind, J. C. Han, N. R. Lee, D. G. Murphy, E. T. Bullmore, A. Raznahan, Transcriptomic and cellular decoding of regional brain vulnerability to neurogenetic disorders. *Nat. Commun.* **11**, 3358 (2020).
66. G. Allington, P. Q. Duy, J. Ryou, A. Singh, E. Kiziltug, S. M. Robert, A. J. Kundishora, S. King, S. Haider, K. T. Kahle, S. C. Jin, Genomic approaches to improve the clinical diagnosis and management of patients with congenital hydrocephalus. *J. Neurosurg. Pediatr.* **29**, 168–177 (2022).
67. A. F. Alexander-Bloch, A. Raznahan, S. N. Vandekar, J. Seidlitz, Z. Lu, S. R. Mathias, E. Knowles, J. Mollon, A. Rodrigue, J. E. Curran, H. H. H. Görring, T. D. Satterthwaite, R. E. Gur, D. S. Bassett, G. D. Hoftman, G. Pearlson, R. T. Shinohara, S. Liu, P. T. Fox, L. Almasy, J. Blangero, D. C. Glahn, Imaging local genetic influences on cortical folding. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 7430–7436 (2020).
68. V. Warrier, E.-M. Stauffer, Q. Q. Huang, E. M. Wigdor, E. A. W. Slob, J. Seidlitz, L. Ronan, S. L. Valk, T. T. Mallard, A. D. Grotzinger, R. Romero-Garcia, S. Baron-Cohen, D. H. Geschwind, M. A. Lancaster, G. K. Murray, M. J. Gandal, A. Alexander-Bloch, H. Won, H. C. Martin, E. T. Bullmore, R. A. I. Bethlehem, Genetic insights into human cortical organization and development through genome-wide analyses of 2,347 neuroimaging phenotypes. *Nat. Genet.* **55**, 1483–1493 (2023).
69. C. Bycroft, C. Freeman, D. Petkova, G. Band, L. T. Elliott, K. Sharp, A. Motyer, D. Vukcevic, O. Delaneau, J. O'Connell, A. Cortes, S. Welsh, A. Young, M. Effingham, G. M. Vean, S. Leslie, N. Allen, P. Donnelly, J. Marchini, The UK Biobank resource with deep phenotyping and genomic data. *Nature* **562**, 203–209 (2018).
70. Y.-J. Ge, B.-S. Wu, Y. Zhang, S.-D. Chen, Y.-R. Zhang, J.-J. Kang, Y.-T. Deng, Y.-N. Ou, X.-Y. He, Y.-L. Zhao, K. Kuo, Q. Ma, T. Banaschewski, G. J. Barker, A. L. W. Bokde, S. Desrivières, H. Flor, A. Grigis, H. Garavan, P. Gowland, A. Heinz, R. Brühl, J.-L. Martinot, M.-L. P. Martinot, E. Artiges, F. Nees, D. P. Orfanos, H. Lemaitre, T. Paus, L. Poustka, S. Hohmann, S. Millenet, J. H. Fröhner, M. N. Smolka, N. Vaidya, H. Walter, R. Whelan, IMAGEN Consortium, J.-F. Feng, L. Tan, Q. Dong, G. Schumann, W. Cheng, J.-T. Yu, Genetic architectures of cerebral ventricles and their overlap with neuropsychiatric traits. *Nat. Hum. Behav.* **8**, 164–180 (2024).
71. V. Kyriakopoulou, A. Davidson, A. Chew, N. Gupta, T. Arichi, C. Nosarti, M. A. Rutherford, Characterisation of ASD traits among a cohort of children with isolated fetal ventriculomegaly. *Nat. Commun.* **14**, 1550 (2023).
72. D. Vojinovic, H. H. Adams, X. Jian, Q. Yang, A. V. Smith, J. C. Bis, A. Teumer, M. Scholz, N. J. Armstrong, E. Hofer, Y. Saba, M. Luciano, M. Bernard, S. Trompet, J. Yang, N. A. Gillespie, S. J. van der Lee, A. Neumann, S. Ahmad, O. A. Andreassen, D. Ames, N. Amin, K. Arfanakis, M. E. Bastin, D. M. Becker, A. S. Beiser, F. Beyer, H. Brodaty, R. N. Bryan, R. Bülow, A. M. Dale, P. L. De Jager, I. J. Deary, C. De Carli, D. A. Fleischman, R. F. Gottesman, J. van der Grond, V. Gudnason, T. B. Harris, G. Homuth, D. S. Knopman, J. B. Kwok, C. E. Lewis, S. Li, M. Loeffler, O. L. Lopez, P. Maillard, H. E. Marroun, K. A. Mather, T. H. Mosley, R. L. Muetzel, M. Nauck, P. A. Nyquist, M. S. Panizzon, Z. Pausova, B. M. Psaty, K. Rice, J. I. Rotter, N. Royle, C. L. Satizabal, R. Schmidt, P. R. Schofield, P. J. Schreiner, S. Sidney, D. J. Stott, A. Thalamuthu, A. G. Uitterlinden, M. C. Valdés Hernández, M. W. Vernooij, W. Wen, T. White, A. V. Witte, K. Wittfeld, M. J. Wright, L. R. Yanek, H. Tiemeier, W. S. Kremen, D. A. Bennett, J. W. Jukema, T. Paus, J. M. Wardlaw, H. Schmidt, P. S. Sachdev, A. Villringer, H. J. Grabe, W. T. Longstreth, C. M. van Duijn, L. J. Launer, S. Seshadri, M. A. Ikram, M. Fornage, Genome-wide association study of 23,500 individuals identifies 7 loci associated with brain ventricular volume. *Nat. Commun.* **9**, 3945 (2018).
73. B. Zhao, T. Luo, T. Li, Y. Li, J. Zhang, Y. Shan, X. Wang, L. Yang, F. Zhou, Z. Zhu, Alzheimer's Disease Neuroimaging Initiative, Pediatric Imaging, Neurocognition and Genetics, H. Zhu, Genome-wide association analysis of 19,629 individuals identifies variants influencing regional brain volumes and refines their genetic co-architecture with cognitive and mental health traits. *Nat. Genet.* **51**, 1637–1644 (2019).
74. R. A. I. Bethlehem, J. Seidlitz, S. R. White, J. W. Vogel, K. M. Anderson, C. Adamson, S. Adler, G. S. Alexopoulos, E. Anagnostou, A. Areces-Gonzalez, D. E. Astle, B. Auyeung, M. Ayub, J. Bae, G. Ball, S. Baron-Cohen, R. Beare, S. A. Bedford, V. Benegal, F. Beyer, J. Blangero, M. B. Cábez, J. P. Boardman, M. Borzage, J. F. Bosch-Bayard, N. Bourke, V. D. Calhoun, M. M. Chakravarty, C. Chen, C. Chertavian, G. Chetelat, Y. S. Chong, J. H. Cole, A. Corvin, M. Costantino, E. Courchesne, F. Crivello, V. L. Cropley, J. Crosbie, N. Crossley, M. Delarue, R. Delorme, S. Desrivieres, G. A. Devenyi, M. A. Di Biase, R. Dolan, K. A. Donald, G. Donohoe, K. Dunlop, A. D. Edwards, J. T. Elison, C. T. Ellis, J. A. Elman, L. Eyler, D. A. Fair, E. Feczko, P. C. Fletcher, P. Fonagy, C. E. Franz, L. Galan-Garcia, A. Gholipour, J. Giedd, J. H. Gilmore, D. C. Glahn, I. M. Goodyer, P. E. Grant, N. A. Groenewold, F. M. Gunning, R. E. Gur, R. C. Gur, C. F. Hammill, O. Hansson, T. Hedden, A. Heinz, R. N. Henson, K. Heuer, J. Hoare, B. Holla, A. J. Holmes, R. Holt, H. Huang, K. Im, J. Ipser, C. R. Jack Jr., A. P. Jackowski, T. Jia, K. A. Johnson, P. B. Jones, D. T. Jones, R. S. Kahn, H. Karlsson, L. Karlsson, R. Kawashima, E. A. Kelley, S. Kern, K. W. Kim, M. G. Kitzbichler, W. S. Kremen, F. Lalonde, B. Landeau, S. Lee, J. Lerch, J. D. Lewis, J. Li, W. Liao, C. Liston, M. V. Lombardo, J. Lv, C. Lynch, T. T. Mallard, M. Marcelis, R. D. Markello, S. R. Mathias, B. Mazoyer, P. McGuire, M. J. Meaney, A. Mechelli, N. Medic, B. Misic, S. E. Morgan, D. Mothersill, J. Nigg, M. Q. W. Ong, C. Ortinau, R. Ossenkoppele, M. Ouyang, L. Palaniyappan, L. Paly, P. M. Pan, C. Pantelis, M. M. Park, T. Paus, Z. Pausova, D. Paz-Linares, A. P. Binette, K. Pierce, X. Qian, J. Qiu, A. Qiu, A. Raznahan, T. Rittman, A. Rodrigue, C. K. Rollins, R. Romero-Garcia, L. Ronan, M. D. Rosenberg, D. H. Rowitch, G. A. Salum, T. D. Satterthwaite, H. L. Schaare, R. J. Schachar, A. P. Schultz, G. Schumann, M. Schöll, D. Sharp, R. T. Shinohara, I. Skoog, C. D. Smyser, R. A. Sperling, D. J. Stein, A. Stolicyn, J. Suckling, G. Sullivan, Y. Taki, B. Thyreau, R. Toro, N. Traut, K. A. Tsvetanov, N. B. Turk-Browne, J. J. Tuulari, C. Tzourio, É. Vachon-Presseau, M. J. Valdes-Sosa, P. A. Valdes-Sosa, S. L. Valk, T. van Amelsvoort, S. N. Vandekar, L. Vasung, L. W. Victoria, S. Villeneuve, A. Villringer, P. E. Vértes, K. Wagstyl, Y. S. Wang, S. K. Warfield, V. Warrier, E. Westman, M. L. Westwater, H. C. Whalley, A. V. Witte, N. Yang, B. Yeo, H. Yun, A. Zalesky, H. J. Zar, A. Zettergren, J. H. Zhou, H. Ziauddeen, A. Zugman, X. N. Zuo, 3R-BRAIN, AIBL, Alzheimer's Disease Neuroimaging Initiative, Alzheimer's Disease Repository Without Borders Investigators, CALM Team, Cam-CAN, CCNP, COBRE, cVEDA, ENIGMA Developmental Brain Age Working Group, Developing Human Connectome Project, FinnBrain, Harvard Aging Brain Study, IMAGEN, KNE96, Mayo Clinic Study of Aging, NSPN, POND, PREVENT-AD Research Group, VETSA, E. T. Bullmore, A. F. Alexander-Bloch, Brain charts for the human lifespan. *Nature* **604**, 525–533 (2022).
75. P. Q. Duy, S. C. Weise, C. Marini, X.-J. Li, D. Liang, P. J. Dahl, S. Ma, A. Spajic, W. Dong, J. Juusola, E. Kiziltug, A. J. Kundishora, S. Koundal, M. Z. Pedram, L. A. Torres-Fernández, K. Händler, E. De Domenico, M. Becker, T. Ulas, S. A. Juranek, E. Cuevas, L. T. Hao, B. Jux, A. M. M. Sousa, F. Liu, S.-K. Kim, M. Li, Y. Yang, Y. Takeo, A. Duque, C. Nelson-Williams, Y. Ha, K. Selvaganesan, S. M. Robert, A. K. Singh, G. Allington, C. G. Furey, A. T. Timberlake, B. C. Reeves, H. Smith, A. Dunbar, T. De Spenza Jr., J. Goto, A. Marlier, A. Moreno-De-Luca, X. Yu, W. E. Butler, B. S. Carter, E. M. R. Lake, R. T. Constable, P. Rakic, H. Lin, E. Deniz, H. Benveniste, N. S. Malvankar, J. I. Estrada-Veras, C. A. Walsh, S. L. Alper, J. L. Schultze, K. Paeschke, A. Doetzlhofer, F. G. Wulczyn, S. C. Jin, R. P. Lifton, N. Sestan, W. Kolanus, K. T. Kahle, Impaired neurogenesis alters brain biomechanics in a neuroprogenitor-based genetic subtype of congenital hydrocephalus. *Nat. Neurosci.* **25**, 458–473 (2022).
76. P. Q. Duy, B. Jux, S. Zhao, K. Y. Mekbib, E. Dennis, W. Dong, C. Nelson-Williams, N. H. Mehta, J. P. Shohfi, J. Juusola, G. Allington, H. Smith, S. Marlin, K. Belhous, B. Monteleone, G. B. Schaefer, M. D. Pisarska, J. Vásquez, J. I. Estrada-Veras, B. Keren, C. Mignot, L. A. Flore, I. V. Palafoll, S. L. Alper, R. P. Lifton, S. Haider, A. Moreno-De-Luca, S. C. Jin, W. Kolanus, K. T. Kahle, TRIM71 mutations cause a neurodevelopmental syndrome featuring ventriculomegaly and hydrocephalus. *Brain* **147**, 4292–4305 (2024).
77. T. Welte, A. C. Tuck, P. Papasaikas, S. H. Carl, M. Flemr, P. Knuckles, A. Rankova, M. Bühler, H. Großhans, The RNA hairpin binder TRIM71 modulates alternative splicing by repressing MBNL1. *Genes Dev.* **33**, 1221–1235 (2019).
78. L. A. Jansen, G. M. Mirzaa, G. E. Ishak, B. J. O'Roak, J. B. Hiatt, W. H. Roden, S. A. Gunter, S. L. Christian, S. Collins, C. Adams, J.-B. Rivière, J. St-Onge, J. G. Ojemann, J. Shendure, R. F. Hevner, W. B. Dobyns, PI3K/AKT pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia. *Brain* **138**, 1613–1628 (2015).
79. J. H. Lee, M. Huynh, J. L. Silhavy, S. Kim, T. Dixon-Salazar, A. Heiberg, E. Scott, V. Bafna, K. J. Hill, A. Collazo, V. Funari, C. Russ, S. B. Gabriel, G. W. Mathern, J. G. Gleeson, De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nat. Genet.* **44**, 941–945 (2012).
80. L. Wang, K. Pang, L. Zhou, A. Cebrián-Silla, S. González-Granero, S. Wang, Q. Bi, M. L. White, B. Ho, J. Li, T. Li, Y. Perez, E. J. Huang, E. A. Winkler, M. F. Paredes, R. Kovner, N. Sestan, A. A. Pollen, P. Liu, J. Li, X. Piao, J. M. García-Verdugo, A. Alvarez-Buylla, Z. Liu, A. R. Kriegstein, A cross-species proteomic map reveals neoteny of human synapse development. *Nature* **622**, 112–119 (2023).

81. K. Staley, Molecular mechanisms of epilepsy. *Nat. Neurosci.* **18**, 367–372 (2015).
82. T. DeSpenza Jr., E. Kiziltug, G. Allington, D. Barson, D. O'Connor, S. M. Robert, K. Y. Mekbib, P. Nanda, A. Greenberg, A. Singh, Dual impact of PTEN mutation on CSF dynamics and cortical networks via the dysregulation of neural precursors and their interneuron descendants. bioRxiv 533275 [Preprint] (2023). https://doi.org/10.1101/2023.03.18.533275.
83. T. De Spenza Jr., E. Kiziltug, G. Allington, D. G. Barson, S. M. Gee, D. O'Connor, S. M. Robert, K. Y. Mekbib, P. Nanda, A. B. W. Greenberg, A. Singh, P. Q. Duy, F. Mandino, S. Zhao, A. Lynn, B. C. Reeves, A. Marlier, S. A. Getz, C. Nelson-Williams, H. Shimelis, L. K. Walsh, J. Zhang, W. Wang, M. L. Prina, A. O. Yang, A. F. Abdulkareem, H. Smith, J. Shohfi, N. H. Mehta, E. Dennis, L. R. Reduron, J. Hong, W. Butler, B. S. Carter, E. Deniz, E. M. R. Lake, R. T. Constable, M. Sahin, S. Srivastava, K. Winden, E. J. Hoffman, M. Carlson, M. Gunel, R. P. Lifton, S. L. Alper, S. C. Jin, M. C. Crair, A. Moreno-De-Luca, B. W. Luikart, K. T. Kahle, PTEN mutations impair CSF dynamics and cortical networks by dysregulating periventricular neural progenitors. *Nat. Neurosci.* **28**, 536–557 (2025).
84. M. P. Lun, E. S. Monuki, M. K. Lehtinen, Development and functions of the choroid plexus–cerebrospinal fluid system. *Nat. Rev. Neurosci.* **16**, 445–457 (2015).
85. H. H. Damkier, P. D. Brown, J. Praetorius, Cerebrospinal fluid secretion by the choroid plexus. *Physiol. Rev.* **93**, 1847–1892 (2013).
86. W. E. Dandy, Extirpation of the choroid plexus of the lateral ventricles in communicating hydrocephalus. *Ann. Surg.* **68**, 569–579 (1918).
87. J. E. Scarff, The treatment of nonobstructive (communicating) hydrocephalus by endoscopic cauterization of the choroid plexuses. *J. Neurosurg.* **33**, 1–18 (1970).
88. J. Praetorius, H. H. Damkier, Transport across the choroid plexus epithelium. *Am. J. Physiol. Cell Physiol.* **312**, C673–C686 (2017).
89. B. K. Owler, T. Pitham, D. Wang, Aquaporins: Relevance to cerebrospinal fluid physiology and therapeutic potential in hydrocephalus. *Cerebrospinal Fluid Res.* **7**, 15 (2010).
90. P. Quinton, E. M. Wright, J. M. Tormey, Localization of sodium pumps in the choroid plexus epithelium. *J. Cell Biol.* **58**, 724–730 (1973).
91. E. A. Robson, L. Dixon, L. Causon, W. Dawes, M. Benenati, M. Fassad, R. A. Hirst, P. Kenia, E. F. Moya, M. Patel, D. Peckham, A. Rutman, H. M. Mitchison, K. Mankad, C. O'Callaghan, Hydrocephalus and diffuse choroid plexus hyperplasia in primary ciliary dyskinesia-related MCIDAS mutation. *Neurol. Genet.* **6**, e482 (2020).
92. M. Fujimura, T. Onuma, M. Kameyama, O. Motohashi, H. Kon, K. Yamamoto, K. Ishii, T. Tominaga, Hydrocephalus due to cerebrospinal fluid overproduction by bilateral choroid plexus papillomas. *Childs Nerv. Syst.* **20**, 485–488 (2004).
93. P. Pencalet, C. Sainte-Rose, A. Lellouch-Tubiana, C. Kalifa, F. Brunelle, S. Sgouros, P. Meyer, G. Cinalli, M. Zerah, A. Pierre-Kahn, D. Renier, Papillomas and carcinomas of the choroid plexus in children. *J. Neurosurg.* **88**, 521–528 (1998).
94. J. K. Karimy, D. Duran, J. K. Hu, C. Gavankar, J. R. Gaillard, Y. Bayri, H. Rice, M. L. Di Luna, V. Gerzanich, J. M. Simard, K. T. Kahle, Cerebrospinal fluid hypersecretion in pediatric hydrocephalus. *Neurosurg. Focus* **41**, E10 (2016).
95. S. M. Robert, B. C. Reeves, E. Kiziltug, P. Q. Duy, J. K. Karimy, M. S. Mansuri, A. Marlier, G. Allington, A. B. W. Greenberg, T. De Spenza Jr., A. K. Singh, X. Zeng, K. Y. Mekbib, A. J. Kundishora, C. Nelson-Williams, L. T. Hao, J. Zhang, T. K. T. Lam, R. Wilson, W. E. Butler, M. L. Diluna, P. Feinberg, D. P. Schafer, K. Movahedi, A. Tannenbaum, S. Koundal, X. Chen, H. Benveniste, D. D. Limbrick Jr., S. J. Schiff, B. S. Carter, M. Gunel, J. M. Simard, R. P. Lifton, S. L. Alper, E. Delpire, K. T. Kahle, The choroid plexus links innate immunity to CSF dysregulation in hydrocephalus. *Cell* **186**, 764–785.e21 (2023).
96. Y. Li, C. Di, S. Song, Y. Zhang, Y. Lu, J. Liao, B. Lei, J. Zhong, K. Guo, N. Zhang, S. Su, Choroid plexus mast cells drive tumor-associated hydrocephalus. *Cell* **186**, 5719–5738.e28 (2023).
97. J. K. Karimy, J. Zhang, D. B. Kurland, B. C. Theriault, D. Duran, J. A. Stokum, C. G. Furey, X. Zhou, M. S. Mansuri, J. Montejo, A. Vera, M. L. Di Luna, E. Delpire, S. L. Alper, M. Gunel, V. Gerzanich, R. Medzhitov, J. M. Simard, K. T. Kahle, Inflammation-dependent cerebrospinal fluid hypersecretion by the choroid plexus epithelium in posthemorrhagic hydrocephalus. *Nat. Med.* **23**, 997–1003 (2017).
98. J. Zhang, M. I. H. Bhuiyan, T. Zhang, J. K. Karimy, Z. Wu, V. M. Fiesler, J. Zhang, H. Huang, M. N. Hasan, A. E. Skrzypiec, M. Mucha, D. Duran, W. Huang, R. Pawlak, L. M. Foley, T. K. Hitchens, M. B. Minnigh, S. M. Poloyac, S. L. Alper, B. J. Molyneaux, A. J. Trevelyan, K. T. Kahle, D. Sun, X. Deng, Modulation of brain cation-Cl^- cotransport via the SPAK kinase inhibitor ZT-1a. *Nat. Commun.* **11**, 78 (2020).
99. L. Ø. Johnsen, K. A. Friis, H. H. Damkier, In vitro investigation of the effect of proinflammatory cytokines on mouse choroid plexus membrane transporters Ncbe and NKCC1. *Fluids Barriers CNS* **20**, 71 (2023).
100. T. Stödberg, M. Magnusson, N. Lesko, A. Wredenberg, D. M. Munoz, H. Stranneheim, A. Wedell, *SLC12A2* mutations cause NKCC1 deficiency with encephalopathy and impaired secretory epithelia. *Neurol Genet.* **6**, e478 (2020).
101. J. M. C. Gregoriades, A. Madaris, F. J. Alvarez, F. J. Alvarez-Leefmans, Genetic and pharmacological inactivation of apical Na^+-K^+-2Cl^- cotransporter 1 in choroid plexus epithelial cells reveals the physiological function of the cotransporter. *Am. J. Physiol. Cell Physiol.* **316**, C525–C544 (2019).
102. R. M. Fame, H. Xu, A. Pragana, M. Lehtinen, Age-appropriate potassium clearance from perinatal cerebrospinal fluid depends on choroid plexus NKCC1. *Fluids Barriers CNS* **20**, 45 (2023).
103. H. Xu, R. M. Fame, C. Sadegh, J. Sutin, C. Naranjo, D. Syau, J. Cui, F. B. Shipley, A. Vernon, F. Gao, Y. Zhang, M. J. Holtzman, M. Heiman, B. C. Warf, P.-Y. Lin, M. K. Lehtinen, Choroid plexus NKCC1 mediates cerebrospinal fluid clearance during mouse early postnatal development. *Nat. Commun.* **12**, 447 (2021).
104. C. Sadegh, H. Xu, J. Sutin, B. Fatou, S. Gupta, A. Pragana, M. Taylor, P. N. Kalugin, M. E. Zawadzki, O. Alturkistani, F. B. Shipley, N. Dani, R. M. Fame, Z. Wurie, P. Talati, R. L. Schleicher, E. M. Klein, Y. Zhang, M. J. Holtzman, C. I. Moore, P.-Y. Lin, A. B. Patel, B. C. Warf, W. T. Kimberly, H. Steen, M. L. Andermann, M. K. Lehtinen, Choroid plexus-targeted NKCC1 overexpression to treat post-hemorrhagic hydrocephalus. *Neuron* **111**, 1591–608.e4 (2023).
105. V. I. Dzhala, D. M. Talos, D. A. Sdrulla, A. C. Brumback, G. C. Mathews, T. A. Benke, E. Delpire, F. E. Jensen, K. J. Staley, NKCC1 transporter facilitates seizures in the developing brain. *Nat. Med.* **11**, 1205–1213 (2005).
106. N. Dani, R. H. Herbst, C. M. Cabe, G. S. Green, K. Kaiser, J. P. Head, J. Cui, F. B. Shipley, A. Jang, D. Dionne, L. Nguyen, C. Rodman, S. J. Riesenfeld, J. Prochazka, M. Prochazkova, R. Sedlacek, F. Zhang, V. Bryja, O. Rozenblatt-Rosen, N. Habib, A. Regev, M. K. Lehtinen, A cellular and spatial map of the choroid plexus across brain ventricles and ages. *Cell* **184**, 3056–3074.e21 (2021).
107. U. C. Eze, A. Bhaduri, M. Haeussler, T. J. Nowakowski, A. R. Kriegstein, Single-cell atlas of early human brain development highlights heterogeneity of human neuroepithelial cells and early radial glia. *Nat. Neurosci.* **24**, 584–594 (2021).
108. M. P. Lun, M. B. Johnson, K. G. Broadbelt, M. Watanabe, Y.-J. Kang, K. F. Chau, M. W. Springel, A. Malesz, A. M. M. Sousa, M. Pletikos, T. Adelita, M. L. Calicchio, Y. Zhang, M. J. Holtzman, H. G. W. Lidov, N. Sestan, H. Steen, E. S. Monuki, M. K. Lehtinen, Spatially heterogeneous choroid plexus transcriptomes encode positional identity and contribute to regional CSF production. *J. Neurosci.* **35**, 4903–4916 (2015).
109. C. C. Hou, D. Li, B. C. Berry, S. Zheng, R. S. Carroll, M. D. Johnson, H. W. Yang, Heterozygous FOXJ1 mutations cause incomplete ependymal cell differentiation and communicating hydrocephalus. *Cell. Mol. Neurobiol.* **43**, 4103–4116 (2023).
110. B. Banizs, M. M. Pike, C. L. Millican, W. B. Ferguson, P. Komlosi, J. Sheetz, P. D. Bell, E. M. Schwiebert, B. K. Yoder, Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development* **132**, 5329–5339 (2005).
111. J. L. Stubbs, I. Oishi, J. C. Izpisúa Belmonte, C. Kintner, The forkhead protein Foxj1 specifies node-like cilia in *Xenopus* and zebrafish embryos. *Nat. Genet.* **40**, 1454–1460 (2008).
112. R. F. Hevner, Brain overgrowth in disorders of RTK-PI3K-AKT signaling: A mosaic of malformations. *Semin. Perinatol.* **39**, 36–43 (2015).
113. X. Huang, T. Ketova, J. T. Fleming, H. Wang, S. K. Dey, Y. Litingtung, C. Chiang, Sonic hedgehog signaling regulates a novel epithelial progenitor domain of the hindbrain choroid plexus. *Development* **136**, 2535–2543 (2009).
114. I. S. Shimada, B. N. Somatilaka, S.-H. Hwang, A. G. Anderson, J. M. Shelton, V. Rajaram, G. Konopka, S. Mukhopadhyay, Derepression of sonic hedgehog signaling upon Gpr161 deletion unravels forebrain and ventricular abnormalities. *Dev. Biol.* **450**, 47–62 (2019).
115. S. D. Klein, D. C. Nguyen, V. Bhakta, D. Wong, V. Y. Chang, T. B. Davidson, J. A. Martinez-Agosto, Mutations in the sonic hedgehog pathway cause macrocephaly-associated conditions due to crosstalk to the PI3K/AKT/mTOR pathway. *Am. J. Med. Genet. A* **179**, 2517–2531 (2019).
116. M. L. Shannon, R. M. Fame, K. F. Chau, N. Dani, M. L. Calicchio, G. S. Géléoc, H. G. W. Lidov, S. Alexandrescu, M. K. Lehtinen, Mice expressing Myc in neural precursors develop choroid plexus and ciliary body tumors. *Am. J. Pathol.* **188**, 1334–1344 (2018).
117. P. Date, P. Ackermann, C. Furey, I. B. Fink, S. Jonas, M. K. Khokha, K. T. Kahle, E. Deniz, Visualizing flow in an intact CSF network using optical coherence tomography: Implications for human congenital hydrocephalus. *Sci. Rep.* **9**, 6196 (2019).
118. A. H. Dur, T. Tang, S. Viviano, A. Sekuri, H. R. Willsey, H. D. Tagare, K. T. Kahle, E. Deniz, In *Xenopus* ependymal cilia drive embryonic CSF circulation and brain development independently of cardiac pulsatile forces. *Fluids Barriers CNS* **17**, 72 (2020).
119. S. Yang, A. Emelyanov, M.-S. You, M. Sin, V. Korzh, Camel regulates development of the brain ventricular system. *Cell Tissue Res.* **383**, 835–852 (2021).
120. R. M. Fame, J. T. Chang, A. Hong, N. A. Aponte-Santiago, H. Sive, Directional cerebrospinal fluid movement between brain ventricles in larval zebrafish. *Fluids Barriers CNS* **13**, 11 (2016).
121. K. R. Lodhia, P. Shakui, R. F. Keep, Hydrocephalus in a rat model of intraventricular hemorrhage. *Acta Neurochir. Suppl.* **96**, 207–211 (2006).
122. P. Vogel, R. W. Read, G. M. Hansen, B. J. Payne, D. Small, A. T. Sands, B. P. Zambrowicz, Congenital hydrocephalus in genetically engineered mice. *Vet. Pathol.* **49**, 166–181 (2012).
123. L. Crews, T. Wyss-Coray, E. Masliah, Insights into the pathogenesis of hydrocephalus from transgenic and experimental animal models. *Brain Pathol.* **14**, 312–316 (2004).

124. J. P. McAllister II, M. R. Talcott, A. M. Isaacs, S. H. Zwick, M. Garcia-Bonilla, L. Castaneyra-Ruiz, A. L. Hartman, R. N. Dilger, S. A. Fleming, R. K. Golden, D. M. Morales, C. A. Harris, D. D. Limbrick Jr., A novel model of acquired hydrocephalus for evaluation of neurosurgical treatments. *Fluids Barriers CNS* **18**, 49 (2021).
125. M. R. Del Bigio, The ependyma: A protective barrier between brain and cerebrospinal fluid. *Glia* **14**, 1–13 (1995).
126. J. Mariani, M. V. Simonini, D. Palejev, L. Tomasini, G. Coppola, A. M. Szekely, T. L. Horvath, F. M. Vaccarino, Modeling human cortical development in vitro using induced pluripotent stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 12770–12775 (2012).
127. A. Benninghaus, O. Balédent, A. Lokossou, C. Castelar, S. Leonhardt, K. Radermacher, Enhanced in vitro model of the CSF dynamics. *Fluids Barriers CNS* **16**, 11 (2019).
128. O. L. Eichmüller, J. A. Knoblich, Human cerebral organoids—A new tool for clinical neurology research. *Nat. Rev. Neurol.* **18**, 661–680 (2022).
129. I. Chiaradia, M. A. Lancaster, Brain organoids for the study of human neurobiology at the interface of in vitro and in vivo. *Nat. Neurosci.* **23**, 1496–1508 (2020).
130. M. A. Lancaster, M. Renner, C.-A. Martin, D. Wenzel, L. S. Bicknell, M. E. Hurles, T. Homfray, J. M. Penninger, A. P. Jackson, J. A. Knoblich, Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379 (2013).
131. W. Zhang, L. Ma, M. Yang, Q. Shao, J. Xu, Z. Lu, Z. Zhao, R. Chen, Y. Chai, J.-F. Chen, Cerebral organoid and mouse models reveal a RAB39b–PI3K–mTOR pathway-dependent dysregulation of cortical development leading to macrocephaly/autism phenotypes. *Genes Dev.* **34**, 580–597 (2020).
132. P. Wang, R. Mokhtari, E. Pedrosa, M. Kirschenbaum, C. Bayrak, D. Zheng, H. M. Lachman, CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in cerebral organoids derived from iPS cells. *Mol. Autism.* **8**, 11 (2017).
133. D. Dutta, H. Clevers, Organoid culture systems to study host–pathogen interactions. *Curr. Opin. Immunol.* **48**, 15–22 (2017).
134. L. Pellegrini, C. Bonfio, J. Chadwick, F. Begum, M. Skehel, M. A. Lancaster, Human CNS barrier-forming organoids with cerebrospinal fluid production. *Science* **369**, eaaz5626 (2020).
135. S. Benito-Kwiecinski, S. L. Giandomenico, M. Sutcliffe, E. S. Riis, P. Freire-Pritchett, I. Kelava, S. Wunderlich, U. Martin, G. A. Wray, K. M. Dole, M. A. Lancaster, An early cell shape transition drives evolutionary expansion of the human forebrain. *Cell* **184**, 2084–2102.e19 (2021).
136. S. L. Giandomenico, M. Sutcliffe, M. A. Lancaster, Generation and long-term culture of advanced cerebral organoids for studying later stages of neural development. *Nat. Protoc.* **16**, 579–602 (2021).
137. I. Chiaradia, I. Imaz-Rosshandler, B. S. Nilges, J. Boulanger, L. Pellegrini, R. Das, N. D. Kashikar, M. A. Lancaster, Tissue morphology influences the temporal program of human brain organoid development. *Cell Stem Cell* **30**, 1351–1367.e10 (2023).
138. L. Pellegrini, A. Albecka, D. L. Mallery, M. J. Kellner, D. Paul, A. P. Carter, L. C. James, M. A. Lancaster, SARS-CoV-2 infects the brain choroid plexus and disrupts the blood-CSF barrier in human brain organoids. *Cell Stem Cell* **27**, 951–961.e5 (2020).
139. S. P. Paşca, P. Arlotta, H. S. Bateup, J. G. Camp, S. Cappello, F. H. Gage, J. A. Knoblich, A. R. Kriegstein, M. A. Lancaster, G.-L. Ming, A. R. Muotri, I.-H. Park, O. Reiner, H. Song, L. Studer, S. Temple, G. Testa, B. Treutlein, F. M. Vaccarino, A nomenclature consensus for nervous system organoids and assembloids. *Nature* **609**, 907–910 (2022).
140. S. O. Sandoval, G. Cappuccio, K. Kruth, S. Osenberg, S. M. Khalil, N. M. Méndez-Albelo, K. Padmanabhan, D. Wang, M. J. Niciu, A. Bhattacharyya, J. L. Stein, A. M. M. Sousa, E. A. Waxman, E. D. Buttermore, D. Whye, C. L. Sirois, Cross-IDDRC Human Stem Cell Consortium, A. Williams, M. Maletic-Savatic, X. Zhao, Rigor and reproducibility in human brain organoid research: Where we are and where we need to go. *Stem Cell Rep.* **19**, 796–816 (2024).
141. F. A. Wolf, P. Angerer, F. J. Theis, SCANPY: Large-scale single-cell gene expression data analysis. *Genome Biol.* **19**, 15 (2018).

Acknowledgments: We apologize to colleagues whose studies we could not include because of space limitations. **Funding:** This work was supported by the National Institutes of Health (NIH) R21NS135321, Neurosurgery Research and Education Foundation (NREF) and American Academy of Neurological Surgery Grant, UAB Civitan Scholar Award, Thrasher Early Career Award, Mission Brain Foundation Scholarship, NIH Fogarty Global Health Fellowship (Harvard University and University of Cape Town), and Oppenheimer Memorial Trust (A.T.H.); National Science Foundation (NSF) Graduate Research Fellowship Program (GRFP) and ARC Institute PhD Fellowship (B.Z.); NIH 5-T32 GM141828-02 (A.R.); the Gabriel Foundation, South African Medical Research Council, and the University of Cape Town Neuroscience Institute (M.G.); Hydrocephalus Association, Pediatric Hydrocephalus Foundation, and NIH R01NS129823 (M.K.L.); Medical Research Council Core Funding and European Research Council Starting Grant (M.A.L.); Hydrocephalus Association, Brain Research Foundation Seed Grant, McCormick and Gabilan Faculty Award, Shurl and Kay Curci Foundation, and NIH R01MH136258 (R.M.F.); and NIH R01NS111029, R01NS109358, and R01NS117609 (K.T.K.). **Competing interests:** M.A.L. is an inventor on a patent application (WO2020152272A1) submitted by United Kingdom Research and Innovation that covers choroid plexus organoids. All other authors declare that they have no competing interests.

Submitted 30 April 2024
Resubmitted 13 September 2024
Accepted 12 May 2025
Published 4 June 2025
10.1126/scitranslmed.adq1810