Neurological diseases are the leading cause of disability and the second leading cause of death worldwide. Preventive strategies and interdisciplinary treatment regimens are improving outcomes of neurological conditions, but advances in treatment require accurate understanding of disease aetiology and progression. This knowledge can be acquired by studying the disease in patients and by studying in vitro and animal disease models.

Studying disease in patients — for example, through liquid biopsies, surgery or post-mortem tissue collection — mostly provides snapshots of disease development. However, disease initiation is rarely captured in such studies. Noninvasive methods, such as neuroimaging, enable prospective examination of disease traits and progression but also cannot track early pathological processes at the cellular level. For ethical reasons, randomized trials cannot be conducted to study disease initiation. Consequently, capturing early pathophysiology in patients requires time-consuming and expensive longitudinal investigations that rely on so-called experiments of nature. For example, an epidemiological study published in 2022 that provided strong evidence that the Epstein–Barr virus is the leading cause of multiple sclerosis (MS) required a sample size of >10 million people who were monitored over decades. However, even such studies cannot, in isolation, demonstrate causality.

Experiments in animal models can identify disease mechanisms and prove causality, as disease onset and progression can be controlled and monitored closely, enabling robust mechanistic research. This approach has been used to demonstrate causality in the example of Epstein–Barr virus in MS. However, translation of animal research depends on the conservation of disease processes between rodents and humans. As our understanding of the human brain and its development evolves, an increasing number of disparities between species are being uncovered that bring into question the utility of animal models. Therefore, human in vitro models could bridge the gap between animal research and clinical practice.

In this Review, we discuss advances in our understanding of human neurodevelopment, how these advances influence neurological disease modelling, and how organoids can improve such modelling. We describe different methodologies for producing organoids and how organoids can be used to model neurological disorders, including microcephaly, Zika virus infection, Alzheimer disease and other neurodegenerative disorders, and neurodevelopmental diseases, such as Timothy syndrome, Angelman syndrome and tuberous sclerosis. We also discuss the current limitations of organoid models and outline how organoids can be used to revolutionize research into the human brain and neurological diseases.
• Development of the human brain involves unique processes that are relevant to neurological disease but cannot be studied in animal models, so alternative model systems are required.

• Organoids are 3D human cell culture models that originate from pluripotent stem cells and recapitulate the hallmarks of human neurodevelopment, enabling studies of human brain development in vitro.

• Specific mutations can be introduced into organoids to study their effects on neurodevelopment; combined with high-throughput screening methods, this approach can determine the disease relevance of mutations in human tissue.

• To study specific diseases, brain organoids can be generated from induced pluripotent stem cells from individual patients, thereby preserving the specific genetic background of the individual and generating an insightful model.

• Through recapitulation of previously inaccessible periods of human brain development, brain organoids have enabled identification of novel mechanisms that underlie neurodevelopmental, neurodegenerative and infectious diseases.

• Combining organoids, patient research and animal models enables us to take full advantage of each of these systems and will provide unprecedented insights into neurodevelopment and neurological diseases.

The biology and development of oRGCs can be recapitulated in human cerebral organoids (hCOs)77. Studies of such organoids and primary tissues have revealed that the mTOR signalling pathway in oRGCs from humans...
Timing of studies

In later stages of primate development, aRGCs convert to truncated radial glia (tRGCs), which form a non-continuous scaffold that primarily depends on the basal processes of oRGCs15 (Fig. 2a). Thus, aRGCs in the subventricular zone are critical for establishing the vast neuronal population of the human cortex.

In addition to the transient progenitor zones, the developing human brain contains several transient neuronal compartments that differ from those in other species. The subplate undergoes particularly dramatic dynamic changes. This region contains various neurons and glia and is involved in various processes, such as migration, maturation, axon pathfinding and circuit organization (reviewed elsewhere10). The subplate in humans expands dramatically during development in comparison with that in other species — it becomes more than fourfold thicker than the cortical plate, which is twofold thicker than in non-human primates (Fig. 2a).

The subplate is important in the organization of circuits that connect the two hemispheres and the cortex to the thalamus11. To establish the topological organization, corticothalamic and thalamocortical projections require the formation of transient circuits within the subplate. Spontaneous activity patterns in the subplate shape the connectivity of the cortical plate, and dysregulation of these processes has been implicated in psychiatric disease (reviewed elsewhere11). Similarly, defects of the subplate can cause structural malformations, such as agenesis of the corpus callosum16. The subplate is also crucial for migration of newly generated neurons — synaptic interactions of immature neurons with the subplate leads to their transition from multipolar to bipolar, which initiates fast migration along the RGC scaffold43.

At later developmental stages, after guiding the axonal projections of the cortical plate, the subplate reduces in size and the expanded white matter that is characteristic of the human brain is formed. Thus, the subplate orchestrates the emergence and connectivity of diverse cell types in the human brain — its greater size than in other animals is necessary to support these processes.

The outer subventricular zone becomes densely populated around mid-gestation and continues to produce excitatory neurons. Consequently, the prominent outer subventricular zone in gyrencephalic species results in greater expansion of the upper cortical layers than in rodents17,44 (Fig. 2b). These neurons form intralaminar connections and have been linked with human-specific cognitive abilities13,46. Introduction of the human-specific gene ARHGAP11B into non-human primate fetuses increases the size of the outer subventricular zone and the numbers of neurons in the upper cortical layers while deep layers are unchanged15,32, thereby making the developmental process more similar to that in humans.

Overall, excitatory neurogenesis in the human brain involves various human-specific processes in progenitor proliferation, transient developmental structures and neuronal subtype distribution that lead to the complex connectivity of the human brain. Given that these developmental processes cannot be studied in rodents, human-derived organoids can be used to model these processes and their specialized cell types for investigation of their physiological roles and contributions to human pathology.

Generation of inhibitory neurons. In addition to differences in excitatory neuron development, the development of interneurons in the human brain differs from that in other animals (Fig. 2c,d). The protracted development

![Organoids can bridge patient and animal studies to advance our understanding of neurological disease](Fig. 1)

Studies in patients (left), such as sequencing, neuropathology or patient-derived xenograft models, provide a snapshot of disease at a given time point. Furthermore, these studies are usually not started until symptoms become apparent, meaning that the earliest pathogenic processes are not captured. Noninvasive and longitudinal studies to capture these early processes require large sample sizes and a lot of time. In animal studies (right), disease initiation can be controlled, so disease initiation, pathogenesis and progression can be studied throughout the disease course. Transfer of knowledge from animal studies to humans and vice versa relies on the assumption that disease mechanisms are conserved between humans and animal models, which is not always true. 3D human model systems such as organoids could be useful for bridging this gap, as they enable studies of early disease stages in human-derived tissue.
of the human brain has a strong effect on the timeline of interneuron generation (reviewed elsewhere). In mice, neurogenesis in the ventral GE regions occurs from around embryonic day 10.5 (E10.5) to E16 (REFS 48,49), and the timing differs slightly between the MGE, LGE and CGE. By contrast, ventral neurogenesis in humans peaks around mid-gestation and continues until the end of pregnancy — proliferation occurs in the MGE earlier than in the CGE (Fig. 2c). Differences in tissue architecture in the MGE are also important.

In humans, this region contains doublecortin-positive cell-enriched nests, which are densely packed islands...
Fig. 2 | Innovations of human neurodevelopment. The human brain develops over a protracted period of time (centre), resulting in its complex structure. This development involves several processes that are unique to humans (parts a–e). a | Radial glial cell development. Apical radial glia cells (aRGCs, blue) are the neural stem cells that give rise to the human brain. aRGCs reside in the ventricular zone (V1) and are connected to the ventricular surface via apical processes. At gestational week (GW) 14 (left), they pass through the cortical plate (CP) and connect to the pial surface via basal processes. Outer aRGCs (oRGCs, brown) emerge in the subventricular zone (SVZ) and connect only to the pial surface via their basal process. Subsequently (at GW 18, centre), the basal processes of aRGCs detach from the pial surface, and these cells become truncated radial glial cells (tRGCs). At this stage, the progenitor zone (right) is organized into a VZ that contains tRGCs, an inner SVZ (iSVZ) that contains intermediate progenitor cells (IPCs, dark yellow) and an outer SVZ (oSVZ) that contains oRGCs. Newly generated neurons (red) ascend along the basal processes of the radial glial cells towards the CP. b | Expansion of cortical layers II and III. Excitatory neurons are generated in an inside–out manner. Neurons migrate through the intermediate zone (IZ) towards the CP, which is delineated by the marginal zone (MZ) towards the pial surface. The first neurons to be generated are subplate neurons (dark blue), which form the subplate (SP). The deep SP and upper SP are formed sequentially. In humans, the SP expands greatly during development (compare GW 13.5 with GW 26–29) — during mid-gestation, the SP becomes larger than the CP and cortical layers 1 to 6 combined. At later stages, an increased contribution of oRGCs to neurogenesis results in expansion of cortical layers II and III in the human brain (purple; compare GW 26–29 with newborn). The SP also reduces in size and the prominent white matter (WM) emerges. c | Interneuron generation in the ventral forebrain within the ganglionic eminences. The human medial ganglionic eminence (MGE, left) contains doublecortin-positive cell-enriched nests (yellow) that contribute to neuronal production during the later stages of development. The MGE, lateral ganglionic eminence (LGE) and caudal ganglionic eminence (CGE) generate interneurons throughout neurogenesis (right) but the peak of neurogenesis in the CGE is later than in other regions and persists until the end of gestation. d | Interneuron migration to the cortex. In humans, this process persists until the first years of life, with large corridors of interneurons migrating in the so-called Arc into the forebrain (left). The proportion of interneurons in the human brain is larger than that in rodents — interneurons constitute up to 30% of all neurons in association cortices (centre), such as the prefrontal cortex (PFC), compared with around 15% in the human sensory cortices (V1) or mouse association (frontal cortex (FC)) or sensory cortices (V1). In addition, the contribution of the CGE interneurons is greater in the human brain than in rodent brain. MGE and CGE interneurons differ in their final positioning in the cortex, with MGE interneurons (yellow) predominantly in deep and CGE interneurons (green) in the expanded upper layers (right). e | Cerebellum development. The rhombic lip (RL) generates granule cell progenitors that migrate to the external granule layer (green) and unipolar brush cells (UBCs, purple) that migrate into the cerebellar lobes. In the developing human cerebellum, the RL contains a VZ (blue) and an SVZ (red). The SVZ is established at approximately GW 11, after which the RL is internalized by GW 17 in humans; this internalization does not occur in other non-human primates. Part c, left panel adapted with permission from Ref. 53, UCSF. Part d, left panel adapted with permission from Ref. 24, Wiley. Part e adapted from Ref. 72, Springer Nature Limited.

Like the proliferation in the GEs, the migration of interneurons into the cortex occurs over an extended period in humans — including the postnatal period — in comparison with other animals42. Postnatal cortical interneuron migration has been observed in mice39 but to a lesser extent than in humans and other gyrencephalic animals39. In humans, interneurons migrate into the cortex from the MGE and CGE in a structure called the Arc (Fig. 2d) until well into the first year of life46. In addition, migratory streams that lead to the olfactory bulb in rodents are redirected to the cortex in humans and contribute to the diversity of cortical interneurons41.

This post-migratory cortical interneuron population differs between primates and rodents in several ways (Fig. 2o). GABAergic interneurons are more abundant in the human brain than in the mouse brain42, and even more so in areas that mediate higher cognitive function, such as association cortices; in these regions, interneurons account for up to ~30% of all neurons compared with <15% in mice44. Similarly, the distribution of interneuron subtypes differs. MGE-derived interneurons are found in deep layers across species and populate the mouse cortex uniformly, but CGE-derived interneurons are enriched in the upper layers in primates and humans (Fig. 2o), more so in areas associated with higher cognition, such as the prefrontal cortex, where they account for up to 50% of all interneurons44. In addition, interneurons in subcortical structures seem to differ, as demonstrated by the discovery of a primate-specific striatal interneuron type41.

Overall, the extended period of neurogenesis in humans influences not only progenitor organization and biology, but also the identity, composition and distribution of interneurons throughout the human brain. Use of organoids enables these unique aspects of development to be studied. For example, the migratory dynamics of interneurons over developmental time42–44 can be monitored, as can the relevance of specific cell types and their developmental trajectories to diseases such as Timothy syndrome47 and tuberous sclerosis48.

Human-specific cerebellar development. Human-specific developmental processes also occur in the cerebellum and are implicated in disease. The human cerebellum has a much greater surface area than that in other animals — ~750-fold greater than that in mice and tenfold greater than that in non-human primates49. The differences in size can be attributed to differences in cerebellar progenitor zones. In addition to the cerebellar ventricular zone that produces GABAergic neurons, the developing cerebellum contains a progenitor zone called the rhombic lip, the function of which differs between humans and mice. Rhombic lip progenitors generate excitatory neurons and expand into their own ventricular zone and subventricular zone in humans and non-human primates but not in mice. In a process unique to humans, the rhombic lip is also internalized after mid-gestation76,77 (Fig. 2e). This process is relevant to developmental disorders, as rhombic lip dysfunction has been associated with cerebellar vermis hypoplasia, also known as Dandy–Walker malformation12,78, and has been identified as the source of medulloblastoma group 3 and 4 — a severe childhood
brain tumour. Human cerebellar organoid models that include rhombic lip progenitors have recently been developed, enabling investigation of cerebellar development in humans.

**The molecular basis of differences.** Numerous studies have been conducted to advance our understanding of the molecular basis of the evolutionary differences that distinguish humans from other species. Regulation of some conserved pathways is altered, resulting in changes in Robo2, mTOR, PDGF and Notch signalling, as well as morphological transitions that affect the organization of the ventricular zone and increases in progenitor numbers. In addition, several human-specific gene duplications and variants have been identified that alter the functions of NOTCH2NL, TBC1D3 (Ref. 37), ARHGAP11B (Ref. 38), PPP1R17 (Ref. 39) and human-specific enhancers, such as HARE5 (Ref. 40), and alter microRNA expression, all of which are involved in progenitor expansion and regulation. Similarly, gene variants have been discovered that change neuronal maturation, spine morphology and connectivity.

Differences between human and rodent brain development result not only from evolutionary gains in primates but also from secondary losses of acquired traits in rodents. A prominent example is cortical gyration, which occurs in most amniotes. Lissencephaly and reduced brain size in rodents is caused by secondary loss of miR-3607 (Ref. 40), which is expressed in evolutionarily more distant animals, such as ferrets, that exhibit gyrencephaly and similar neural progenitor cells to humans. This example illustrates that diverse evolutionary mechanisms have led to the developmental differences that make us human. Organoids enable recapitulation of human developmental milestones and enable manipulation of developmental processes to understand how the complex human brain has emerged and what goes wrong in diseases of development.

**Brain organoids for modelling**

Disease modelling with 3D human organoids has the potential to bridge the gap between conventional animal models and humans. Organoids have been developed for numerous organ systems (reviewed in detail elsewhere) and are generally derived from pluripotent stem cells (PSCs), although some non-brain organoids can be generated from adult stem cells or fetus-derived cultures. With respect to brain organoids, Sasa’s group pioneered the 3D in vitro culture of optic cup structures and cortical tissue, and our group developed the cerebellar organoid model and pioneered its use for disease modelling. Organoids have a high degree of self-organization and contain progenitor cells and differentiated cell types. Numerous variations of these organoid models have been developed, and essentially every part of the human brain can now be recapitulated. Non-human brain organoid models exist, but we focus on hCOs.

**Human brain modelling**

Generation of hCOs starts with embryonic stem cells or induced PSCs (iPSCs). The stem cells aggregate into embryoid bodies, which are incubated in a medium that restricts fate to the neural lineage before being embedded into an extracellular matrix or transferred to a proliferative medium that supports progenitor expansion. Upon formation of radially organized neural progenitors in ventricular zone-like rosettes, an organoid is established. During these initial weeks of culture, hCOs mostly comprise progenitor regions that expand symmetrically. The first neurons appear around the ventricular zone after ~20 days. Organoids can be cultivated for long periods; postnatal characteristics develop after >1 year.

The cellular composition of hCOs is determined by the diversity of the initial progenitor pool. Unguided protocols involve no extracellular signalling molecules, resulting in a mixture of regional identities. Guided protocols involve addition of morphogens to induce or restrict specific fates, leading to so-called restricted hCOs with more homogeneous cell populations. These approaches can be used to generate different hCOs depending on the research question. Furthermore, fusion of differentially patterned hCOs into assemboids enables analysis of interactions between regions, such as interneuron migration from ventral to dorsal hCOs or axonal projections. Together, these possibilities result in a comprehensive toolbox for studying disease processes during human neurodevelopment (Fig. 5).

The experimental timeline depends on whether the focus is on early progenitor biology and disease susceptibility, neuronal maturation, migration and activity, or late non-neuronal populations, and can last for more than 1 year. Organoids are constantly accessible during these long experiments, enabling flexible application of compounds, modification of culture conditions and/or genetic perturbations.

**Approaches to disease**

Organoids can be used to study disease processes in two main ways. The first is to study the effects of known risk factors for diseases. Widespread use of diagnostic genomic sequencing and the rise of genome-wide association studies have identified a plethora of genetic variants that are associated with diseases, including structural brain defects, such as microcephaly, and neurodevelopmental disorders, such as autism spectrum disorders (ASD). Organoids can be used to investigate the causal relationships between these risk variants and defined cellular phenotypes. Furthermore, generating organoids from patient-derived iPSCs enables replication of the patient-specific genomic background.

The second use of organoids to study disease is to investigate the mechanisms that underlie development of diseases with a known cause. For example, many monogenic diseases have been identified, including early infantile epileptic encephalopathies, neurocutaneous syndromes, lysosomal storage disorders and neurodegenerative disorders, but the link between developmental processes and phenotypes often remains poorly understood. Organoids can be used to dissect the mechanisms of pathogenic mutations in human tissue (Fig. 6). Similarly, organoids can be used to screen potential therapeutic agents.
Assessing disease traits with organoids

Major structural defects such as microcephaly were the first developmental disorders to be studied in organoids. The cardinal symptom of primary microcephaly (referred to as microcephaly primary hereditary (MCPH)) is a small head circumference. The condition is caused by various genes, the functions of which converge on several common pathways, such as DNA replication and repair and centrosome biology. Our understanding of how associated gene alterations cause MCPH has relied heavily on mouse models, which have revealed changes to important aspects of basic neurodevelopment (reviewed elsewhere). For example, mice with mutations in Mcph1, the first gene to be associated with MCPH, have a smaller cerebral cortex than wild-type mice, and the model revealed a premature switch from symmetric to asymmetric cell division, which reduces the initial aRGC pool size and underlies the phenotype.

Some genes associated with MCPH, including CDK5RAP2 and ASPM, have been linked to the evolutionary expansion of the brain in primates, indicating that not all MCPH-related genes are entirely conserved between humans and rodents. Cdk5rap2-knockout mice do have a smaller brain but the phenotype varies between mouse strains. By contrast, CDK5RAP2 mutations in hCOS recapitulate MCPH-like effects and have demonstrated that the size of the progenitor pool is reduced owing to premature neurogenic divisions. Similarly, ASPM mutation, which is the most common cause of MCPH, results in drastic brain size reductions in humans but causes only a moderate and variable phenotype in mice. This disparity could result from differences in expression of ASPM in the subventricular zone between mice and humans, making the rodents less susceptible to the mutation. Accordingly, ferrets, which have a prominent subventricular zone similar to that in humans, develop microcephaly upon ASPM mutation. Features of microcephaly also developed in hCOS that were generated from iPSCs from patients with ASPM mutations. These findings demonstrate that when mutations cause disease through processes that are not conserved between humans and rodents, alternatives to mouse models are needed.

In a study published in 2020, organoids were used to assess the effects of MCPH-associated genes. The methodology developed for this study enables loss-of-function analysis for multiple genes in parallel. Instead of developing one organoid model per gene, mosaic organoids were created in which one gene per cell was mutated using the CRISPR–Cas9 system. Each individual starter cell was also uniquely barcoded so that the number of daughter cells generated could be measured. This enabled measurement of the effect of each MCPH-associated gene on proliferation in the...
Organoids. This approach demonstrated that 32 genes caused a reduction in proliferation, consistent with the reduced proliferation during brain development seen in microcephaly, and their effects were independently validated in homogeneous, single-gene knockout organoids by investigating lineage development and organoid size. Phenotypic patterns differed between variants, and the pathways affected included some that have previously been associated with microcephaly and some novel pathways. One novel pathway identified regulates brain size in association with endoplasmic reticulum stress and caused microcephaly through dysregulation of extracellular matrix proteins.

This study showed that the effects of genes associated with a defined developmental phenotype can be efficiently screened through use of hCOs. This method can be used to address a wide range of underlying mechanisms and pathways. It can also establish causality between risk genes and disease phenotypes, which is critical not only for advancing diagnosis and treatment but also for future mechanistic studies.

Low-risk and unknown variants in organoids

ASD is highly heritable but has complex genetic causes (reviewed elsewhere). Other than in ASD-related monogenetic syndromes, high-risk de novo mutations have only a small role in the disease; instead, an individual's risk is usually determined by a combination of multiple common and new variants. Over 100 genes have been associated with ASD, many of which converge on pathways that regulate transcription or synaptogenesis.

In this context, the use of organoids generated from patient-derived iPSCs provides a major advantage over animal models and the study of single risk gene knockouts in organoids, as it enables investigation of the effects of numerous combined, low-risk mutations, some of which might not be known, while accounting for genetic background.

One such study has been done to investigate morphological and transcriptomic abnormalities in organoids generated from iPSCs from four patients with ASD and their neurotypical relatives. Each patient had severe idiopathic ASD with macrocephaly without a known causal genetic mutation. However, the organoid study revealed an imbalance of excitatory and inhibitory neurons and synapses in all four. This imbalance has previously been proposed as a mechanism of ASD and is supported by computational and transcranial magnetic stimulation studies in patients.

A similar approach was used with organoids derived from patients with ASD and healthy relatives to identify and compare neurodevelopmental pathophysiological processes in ASD with and without macrocephaly. The disease mechanism differed between patients with and without macrocephaly but converged within each cohort despite distinct individual genetic backgrounds.

In ASD with macrocephaly, progenitor proliferation and excitatory cortical plate neurons were increased, whereas increases in the early-generated preplate neurons (the opposite pattern) were seen in ASD without macrocephaly. Nevertheless, some alterations were shared between the two ASD cohorts, mostly in the regulation of nr3c1. These studies demonstrate that the use of organoids to model the combination of risk variants within individuals can identify convergent pathways involved in pathogenesis. The findings have revealed that the pathways involved in ASD are important in processes that are unique to or amplified in humans, demonstrating the value of human model systems.

In addition to studying patient-specific phenotypes, understanding the role of risk genes in healthy neurodevelopment is crucial for identifying the cell types that are affected in disease. Transcriptomic analysis of brains from patients with ASD have revealed that processes in the mid-fetal period underlie ASD pathogenesis, thereby narrowing the set of genes that could be involved to those that are expressed during this window. Large-scale efforts have generated databases of somatic mutations, gene expression and epigenomics in brain development and disease, and these resources are invaluable for determining the temporal window during which risk genes are expressed. Use of high-throughput single-cell RNA sequencing techniques with tissue from people with ASD has identified cell-type-specific effects on upper layer projection neurons. hCOs can be used to investigate neurodevelopment in vitro and complete the developmental timeline of cell-type specific processes. Numerous studies.
have been done to examine single-cell gene expression and chromatin accessibility in hCOs. Importantly, organoids enable very dense sampling over time to understand the temporal dynamics of risk gene expression as well as cell type specificity. Identification of sensitive periods for different cell types can facilitate the development of aetiological theories. Coupling of high-throughput screening technologies with in vitro single-cell profiling in organoids promises to provide even greater insight into the effects of risk variants.

**Disease mechanisms in organoids**

In addition to studying the relevance of risk genes to known phenotypes, organoids can be used to investigate novel disease mechanisms. Access to and the ability to manipulate human-specific cell types during development can provide substantial insights; in the following sections, we discuss examples of such insights into virus-related brain diseases, neurodegenerative disease and several genetic neurodevelopmental syndromes.

**Virus-associated microcephaly**

Brain organoids are an excellent tool for evaluating vulnerability to viral diseases during neurodevelopment, demonstrated by the rapid emergence of insightful models of Zika virus-associated microcephaly during the 2015 outbreak in Latin America. Maternal Zika virus infection was quickly linked to an increase in primary microcephaly, supported by the detection of virus in the amniotic fluid and the brain of a microcephalic fetus. Mouse and 2D and 3D human models were soon established to study how Zika virus infection causes microcephaly.

Neural progenitors in 2D culture were easily infected and their growth was reduced, and the concept that Zika virus targets neural progenitors was later confirmed by infection of fetus-derived neural progenitor cells but not neurons. However, microcephaly-like phenotypes require a 3D architecture and could only be recapitulated by infecting organoids (FIG. 4a). The virus-induced phenotype was specific to Zika virus; infection with Dengue virus, another member of the flavivirus family, and lymphocytic choriomeningitis virus did not reproduce the effect. Progenitors in the ventricular zone and subventricular zone were affected in these models, supporting the hypothesis that transient amplified populations of human neurodevelopment are involved in the disease. Organoids have been used to study the entry of Zika virus into neural progenitors and the mechanisms by which infection leads to microcephaly.

Use of organoids also helped to reveal how Zika virus affects RGCs, which proliferate less, produce fewer neurons and undergo apoptosis after infection, resulting in destruction of the ventricular zone architecture and the RGC scaffold. Studies of organoid and mouse models demonstrated that the Zika virus protein NS2A is responsible, as it disrupts the apical junction of RGCs, explaining the tissue architecture disturbance. Furthermore, studies in organoids and fetal brain slices also suggested that centrosome damage occurs in Zika virus-associated microcephaly, which is a common mechanism in MCPH. These studies demonstrate how organoids can be used to replicate complex tissue phenotypes that occur as a result of viral infection and to provide mechanistic insight into the effects of infection.

Use of organoids also overcame deficiencies in mouse and 2D models of Zika virus infection. In humans, but not in mice, Zika virus suppresses type I interferon (IFN) responses, meaning that ablation of IFN signalling is needed to assure infection in most mouse models. Furthermore, in human models, Zika virus attenuation of type I IFN is considerably stronger in organoids than in 2D culture, which could explain the discrepancies in infection rates between these models and shows the value of the 3D system. Organoids can also shed light on differences between neurotropic viruses. For example, infection of organoids with Zika virus or herpes simplex virus (HSV-1) revealed that both reduced type I IFN signalling but that distinct interferons mediated the IFN response in Zika virus and HSV-1 infection.

Thus, hCOs can be used to study the cellular mechanisms, dynamics, and outcomes of CNS infections, further demonstrated by studies of SARS-CoV-2 and cytomegalovirus infection in the past 2 years. 3D models recreate the in vivo infection more faithfully than 2D models, and enable investigation of the mechanisms in a tissue-like context. Furthermore, the cellular diversity and scalability of organoids enable evaluation of several viruses in different cell types at the same time.

**Alzheimer disease**

Alzheimer disease (AD) is the most common neurodegenerative disorder. The neuropathological hallmarks are amyloid-β (Aβ) plaques and neurofibrillary tangles that contain hyperphosphorylated tau. Sporadic AD is most common, but familial AD can occur, usually as a result of mutations in or duplications of APP, which encodes amyloid precursor protein (APP), or mutations in PSEN1 or PSEN2, which encode the core proteins of secretases that cleave APP to release Aβ peptides. In addition, people with Down syndrome, that is caused by an additional copy of chromosome 21, are at high risk of AD because APP is located on chromosome 21. The neuropathology of AD has been studied extensively in mouse models, but many of these models do not capture all aspects of AD or require mutations in several genes to recapitulate the full phenotype.

Human-derived models are, therefore, essential for a full understanding of the pathophysiology. The first human-derived models of AD pathogenesis were neurons that had been differentiated in 2D from iPSCs of people with sporadic AD, familial AD or Down syndrome. In these neurons, pathogenic Aβ, tau and endosome abnormalities were increased. From this system, a 3D model was developed, in which the development of Aβ and tau pathology was faster. In subsequent studies, organoids derived from iPSCs of people with familial AD spontaneously developed pathology without overexpression (FIG. 4b). Inhibition of the secretases reversed pathology in 2D models and 3D models in a time-dependent manner. The 3D models of familial AD have, therefore,
Table 1 | Summary of organoid disease models

| Disease model                      | Approach                                                                 | Findings                                                                 | Refs.                   |
|------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|-------------------------|
| **Structural defects**             |                                                                          |                                                                         |                         |
| Microcephaly                       | Patient iPSCs; gene editing (including single-gene mutations in CDK5RAP2, ASPM and WDR62); screening of risk genes | Reduced organoid size; reduction and exhaustion of neural progenitors     | 10,109,111, 120,121,254 |
| Macrocephaly                       | Patient iPSCs (Sandhoff disease, HEXB mutation); gene editing (PTEN knockout) | Expanded progenitor pools; enlarged organoids                             | 255,270                 |
| Seckel syndrome                    | Patient iPSCs carrying CPAP mutations                                     | Premature differentiation owing to aberration of cilia dynamics          | 206                     |
| Lissencephaly (Miller–Dieker syndrome) | Patient iPSCs with 17p13.3 deletion                                       | Reduced organoid size; morphological phenotypes owing to radial glial cell architecture changes | 204,205                 |
| **Infectious/inflammatory disease** |                                                                          |                                                                         |                         |
| Zika virus                         | Zika virus infection of organoids                                         | Reduced organoid size owing to infection; apoptosis of neural progenitors | 103,104, 160–162,277    |
| SARS-CoV-2                         | SARS-CoV-2 infection of organoids                                         | Neurotropism of choroid plexus                                           | 170–172                 |
| Cytomegalovirus                    | Cytomegalovirus infection of organoids                                    | Reduced organoid size owing to infection via PDGFRα and EGFR              | 106,170–173             |
| Herpes simplex virus               | Herpes simplex virus infection of organoids                              | Reduced organoid size owing to infection; apoptosis of neural progenitors | 104,205                 |
| Aicardi–Goutières syndrome         | Patient iPSCs; gene editing of TREV1                                     | Reduced organoid size; neurotoxicity owing to secreted interferons        | 259                     |
| **Neurodegeneration**              |                                                                          |                                                                         |                         |
| Alzheimer disease                  | Patient iPSCs; gene editing (PSEN1, PSEN2, APOE4); organoids and 3D co-cultures | Amyloid-β plaques; neurofibrillary tau tangles; endosome abnormalities; effects on microglia | 180,181,185, 186,187,189 |
| Frontotemporal dementia            | Patient iPSCs carrying tau-Val337Met mutation                             | Splicing dysregulation; autophagy–lysosomal pathway dysfunction; excitotoxicity; apoptosis | 194                     |
| Parkinson disease                  | Patient iPSCs; gene editing (LRRK2, GBA1 and SNCA)                        | α-Synuclein oligomers; Lewy body-like aggregates; dopaminergic neuron loss | 195–197                 |
| Hereditary spastic paraplegia      | Patient iPSCs carrying SPG11 mutations                                    | Reduced organoid size; proliferation defects; premature neurogenesis      | 200                     |
| Huntington disease                 | Patient iPSCs                                                             | Defects in specification and organization of progenitors                  | 201                     |
| Creutzfeld–Jakob disease           | Prion infection of organoids                                             | Assessment of prion seeding capability in organoids revealed de novo prion propagation | 108,202                 |
| Amyotrophic lateral sclerosis      | Patient iPSCs, organoid slice model                                       | Changes in transcriptomics, unfolded protein response and DNA repair      | 203                     |
| **Neurodevelopmental and neuropsychiatric disorders** |                                                                 |                                                                         |                         |
| Angelman syndrome                  | Patient iPSCs carrying microdeletion; gene editing (UBE3A)                 | Increased synchronicity of network activity owing to increase in BK channels | 209                     |
| Timothy syndrome                   | Patient iPSCs carrying CACNA1C mutation                                   | Altered interneuron migration; increased salutation frequency and decreased salutation length | 63,67                   |
| Tuberculous sclerosis complex      | Patient iPSCs carrying heterozygous TSC2 mutation; gene editing (TSC1 and TSC2) | Over-proliferation of interneuron lineage from CLIP cells; development of cortical tubers and tumours | 68,202,204              |
| DiGeorge syndrome (22q11.2 deletion syndrome) | Patient iPSCs carrying 22q11.2 deletion                                   | Increased spontaneous activity owing to loss of DGCR8 at 22q11.2           | 203                     |
| Neurofibromatosis type 1           | Patient iPSCs carrying 17q11.2 deletion; gene editing (NF1)               | Increased proliferation; maturation deficits                               | 200,201                 |
| Rett syndrome                      | Patient iPSCs; small hairpin RNA knockdown                                | Dysregulation of microRNA; synaptic dysregulation; increased synchronicity of network activity owing to mutant interneurons | 232,234–236             |
| Autism spectrum disorder           | Patient iPSCs, gene editing (ARID1B, KMT5B, CHD8 and CNTNAP2)             | Increased production of neurons owing to accelerated cell cycle; dysregulation of early gene regulatory networks and ventral and dorsal lineages | 114,115,119, 208,209    |
| Schizophrenia                      | Patient iPSCs carrying DISC1 mutation; gene editing (DISC1)               | Disruption of cell cycle; architecture disturbance                        | 270,271                 |
| Down syndrome                      | Patient iPSCs with trisomy 21                                            | Increased production of interneurons                                      | 272                     |
| Fragile X syndrome                 | Patient iPSCs                                                             | Accelerated neurogenesis through iPSCs; changes in gene expression and developmental trajectories | 207                     |
| Periventricular heterotopia        | Patient iPSCs carrying DCSH1 or FAT4 mutations                            | Disturbance of radial glial cell architecture, leading to migratory defects | 273                     |
enabled more accurate modelling of Aβ and tau pathology, and the use of iPSCs from people with familial AD and Down syndrome enables generation of patient-specific models. Beyond familial AD, several risk genes have been identified in sporadic AD, and organoids can be used to study the role of these genes in AD pathophysiology. Many of the risk genes identified are expressed in microglia, and the importance of microglia in AD pathology is supported by evidence of neuroinflammation in a 3D culture model that combined neurons, astrocytes and microglia. The strongest risk factor for sporadic AD is the APOE ε4 allele, and in 2D and organoid models that included microglia, Aβ deposits were cleared less efficiently by microglia that expressed APOE ε4 than by those that expressed APOE ε3. Interestingly, expression of APOE ε4 in cerebral organoids resulted in development of AD pathology at a later stage (6 months) than did APP duplication or mutation of PSEN1 or PSEN2 (2 months), suggesting that different pathogenesis time lines can be replicated.

The brain vasculature is another non-neuronal player in AD pathogenesis, and single-cell profiling of vasculature from people with AD has shown that AD risk genes are abundantly expressed in brain endothelial cells in humans but not in mice. Vascular cells can be incorporated into organoids, enabling investigation of some blood–brain barrier properties in the context of Aβ pathology. However, the lack of functional vascularization in organoids remains a major limitation of current studies. Nevertheless, existing models — especially 3D tissue models — have provided important insights into AD pathology.

**Other neurodegenerative diseases**

Although tau tangles are a hallmark of AD, mutations in MAPT, which encodes tau, can cause frontotemporal dementia (FTD-tau). This disease is heterogeneous, but dysregulation of glutamatergic signalling from excitatory neurons is thought to cause the symptoms. 2D models of neurons derived from iPSCs of people with a MAPT mutation have identified impaired cytoskeletal remodeling of the axon initial segment, which results in activity changes. In addition, dysregulation of the autophagy–lysosomal pathway has been identified in FTD-tau. However, 2D models could not disentangle the developmental regulation of these processes. In a study published in 2021, a comprehensive brain organoid model for FTD was developed by use of several patient iPSC lines and isogenic controls, and this model revealed a selective loss of excitatory neurons in the late stages of development. This loss was initiated by disruption of the autophagy–lysosomal pathway, which promoted tau aggregation. Furthermore, expression of the splicing regulator ELAVL4 was increased and co-localized with tau in stress granules, resulting in splicing dysregulation and impaired excitatory neuron function. This dysregulation of excitatory neuron development resulted in excitotoxicity and apoptosis in the late stages of development (Fig. 4c). Thus, the extended duration of organoid development enabled the different steps in the development of FTD-tau pathology to be identified.

Organoids have also been used to model other neurodegenerative disorders, including Parkinson disease and Creutzfeldt–Jakob disease (TABLE 1). Despite the fact that symptomatic neurodegenerative diseases generally occur in older people, organoids can be used to study the early pathogenic processes. More complex systems that include microglia and vasculature will enable more accurate modelling of the neuroinflammatory aspects of these diseases. Final disease stages are unlikely to be replicated in organoid models, but their use to uncover early disease processes could lead to new approaches for drug development.

**Neurodevelopmental syndromes**

In contrast to neurodegenerative disorders, most neurodevelopmental syndromes are initiated during prenatal development. For many of these syndromes, single, causal gene mutations have been defined; the combination of these mutations and iPSC and organoid modelling allows powerful mechanistic studies, as organoids can
recapitulate the emergence of phenotypes during neurodevelopment in a human system. Fascinating work of this kind has been done in diverse diseases, such as neurocutaneous syndromes (neurofibromatosis201,202) and tuberous sclerosis complex (TSC)203,204, DiGeorge syndrome205, genetic lissencephaly (Miller–Dicker syndrome206,207 and Seckel syndrome208), fragile X syndrome209, Phelan–McDermid syndrome210, Timothy syndrome211,212 and Angelman syndrome213 (TABLE 1). In the following sections, we focus on three disorders in which organoid studies have led to new aetiological theories: Timothy syndrome, Angelman syndrome and TSC.

**Timothy syndrome.** Timothy syndrome is caused by an autosomal dominant mutation in *CACNA1C*, which encodes the α-subunit of the L-type calcium channel Ca$_{\alpha}$1.2 (REF. 210). In mice, high expression of the mutant channel is lethal, but low expression211 leads to ASD-like characteristics, although the model did not replicate severe cardiac disease and other symptoms associated with Timothy syndrome. This limitation of the mouse model prompted investigation of *CACNA1C* mutations in a human context.

In a 2D model of neurons derived from iPSCs of patients with Timothy syndrome, Ca$_{\alpha}$1.2 mutation resulted in a loss of channel inactivation and increased intracellular calcium212. Furthermore, work in 2D human models and in mice showed that the mutation leads to an activity-dependent but calcium-independent retraction of dendrites211. In addition to their functional and morphological roles, L-type calcium channels have been implicated in regulation of tangential migration214, and organoids of ventral and dorsal organoids have been used to study this migration in Timothy syndrome. Interneuron migration follows a saltatory movement pattern that is preserved in these organoids, enabling modelling of migration in disease215. In Timothy syndrome, this organoid model revealed a cell-autonomous decrease in the length of individual saltations and an increase in the frequency of saltations216. Use of these organoids also demonstrated that the migratory defects are mediated by two different pathways: saltation length is controlled by L-type calcium channel-dependent regulation of the actin skeleton via calcium, whereas saltation frequency is modulated by GABA receptor signalling217 (FIG. 4d). Previous use of organoids to study the effects of neurotransmitter signalling on interneuron migration has identified similar effects of GABA receptor inhibition218, emphasizing the robustness of the model across laboratories.

This work demonstrates that disease-relevant phenotypes of interneuron migration can be studied by using patient iPSCs to generate organoids. The accessibility and high-throughput nature of organoid cultures enables application of drugs and changes in the external environment (for example, by changing media composition), ultimately providing a platform to generate mechanistic insights into a range of pathways.

**Angelman syndrome.** Angelman syndrome is a rare genetic disorder characterized by intellectual deficits and epilepsy, among other symptoms219. It is caused by loss of function of the imprinted *UBE3A* gene, which encodes E3 ubiquitin protein ligase that ubiquitinates proteins for subsequent degradation. The loss of function can be caused by deletions or large mutations of the maternal allele, paternal uniparental disomy or imprinting defects, and leads to defects in ion channel processing. The imprinting that results in maternal-only expression of *UBE3A* is unique to the brain220 and linked to the neuron-specific expression of a neighbouring gene221.

Several mouse models have been generated to advance our understanding of the disease mechanisms, and model animals have motor dysfunction and susceptibility to seizures218. An underlying excitatory–inhibitory imbalance was identified219, with specific defects in interneurons220,221. In neurons derived from iPSCs of people with Angelman syndrome, parental imprinting is established during in vitro development, paving the way for human models222. In one such model, maturation of mutated 2D differentiated neurons was delayed222. Thus, mice and 2D models enabled several aspects of the disease to be studied, but how mutations in *UBE3A* cause these phenotypes remained unclear. Accumulation of UBE3A substrates was proposed as the mechanism, although a single responsible protein had not been identified224.

Subsequent work in which organoids were used in combination with 2D and mouse models of Angelman syndrome has identified the responsible protein225. Comparison of 2D-differentiated neurons that derived from healthy people and people with Angelman syndrome revealed an increase in the magnitude and duration of the fast component of after-hyperpolarization of the action potential (FIG. 4e). This difference was caused by elevated expression of voltage-dependent big potassium (BK) channels in neurons from patients with Angelman syndrome. BK channels were shown to be a substrate of UBE3A, and deletion or point mutation of *UBE3A* decreased degradation of these channels. Studies in organoids reproduced these findings and enabled calcium imaging, which revealed increased synchronicity of neuronal activity in Angelman syndrome. Inhibition of BK channels with paxilline reversed all these phenotypes in organoids. Furthermore, the findings in human models led to the demonstration that increased BK channel currents are conserved in Angelman syndrome mouse models, and that paxilline treatment could ameliorate seizure susceptibility in these mice. Overall, this study demonstrates how 2D and organoid models can be used to uncover mechanistic targets of disease and how these findings can be validated in mouse models if mechanisms are conserved.

**Tuberous sclerosis.** TSC is caused by mutations in *TSC1* or *TSC2*, which encode the proteins hamartin and tuberin that together regulate cell growth. The disease is characterized by tumours and lesions in multiple organs226. In the brain, tumours develop near the lateral ventricle wall as subependymal nodules that can progress into subependymal giant cell astrocytomas (SEGAs), and tubers — local disorganized regions with dysplastic cells that are thought to cause epilepsy.
— develop in the cortex. Tumours and tubers develop during prenatal development226. Use of organoids has demonstrated that human-specific processes underlie the pathogenesis of TSC68.

Heterozygous mouse models of TSC lack morphological abnormalities227 but homozygous deletion of Tsc1 or Tsc2 is lethal. By contrast, inducible knockouts of Tsc1 or Tsc2 in mice can recapitulate several characteristics of the disease, such as hyperexcitability228, nodular tumours and dysmorphic cells229–231. This discrepancy in phenotypes led to the assumption that TSC is caused by loss of heterozygosity (LOH) — that is, loss of the healthy allele — in a two-hit process225. Furthermore, in a human spheroid model of TSC that was induced to form only dorsal forebrain tissue, LOH was also required for excitatory neuron dysregulation, similar to the findings in mice202. However, although LOH is common in SEGAs, it is rare in cortical tubers231. Thus, how pathogenesis could involve heterozygous cells remained unclear209. One possibility was that disease initiation in heterozygous cells involves cell types that are not present in the dorsal forebrain.

In a study published in 2022, we generated unguided organoids from multiple patients with TSC and isogenic controls20 (Fig. 4f). In line with previous findings, heterozygous organoids remained unchanged during early stages of development. However, at later stages, tumour lesions developed from heterozygous interneuron progenitors, revealing the disease-initiating population. When grown in different culture conditions, organoids derived from patients with TSC could also recapitulate cortical tuber phenotypes25. Subsequent scRNA profiling and comparison with sequencing data from fetal tissue indicated specific dysregulation of CGE interneuron progenitors known as caudal late interneuron progenitors, or CLIP cells, during mid-gestation as the underlying cause of the phenotypes. CLIP cells generate tumours and give rise to dysmorphic interneurons and so-called giant cells that initiate tuber pathology. Both lesions arise from heterozygous cells, as CLIP cells are vulnerable to heterozygous TSC2 mutations. Only in tumour lesions is the second allele lost through copy-neutral LOH as the disease progresses. Excitatory dysmorphic neurons emerge later than dysmorphic interneurons, indicating that they are secondary effects of the presence of dysmorphic interneurons69.

Thus, the TSC organoid model explains the differences in mutual profiles between tumours and tubers but also establishes that both derive from CLIP cells. This vulnerable disease-initiating population could only be studied with a human-based model that recreates the protracted development of the human brain with its unique cell types.

Current limitations of organoid models

The value of organoid models for investigating human brain development and disease has been demonstrated in numerous studies. Nevertheless, several limitations of these models must be considered. Organoids grow quickly during lengthy periods of culture and their size exceeds the limit for passive diffusion of oxygen, causing formation of necrotic tissue in the core. Whether this compromises the overall utility of the model232 or merely affects individual cells remains under debate233,234. Meta-analysis of scRNA data235 and multi-omics data236 suggest that in vitro conditions create an artificial stressed state in a defined set of cells while the remaining tissue remains unaffected. To improve nutrient supply, vascularized organoids have been developed with co-culture236,237, induction209 and transplantation238 methods, and cultured organoid slices have also been used208,239. However, these techniques currently lack the scalability to replace regular organoid cultures and require improvements.

Besides improving culture conditions, a major focus in recent years has been to create increasingly precise organoid models of specific brain areas. Protocols have been developed for models of the forebrain, midbrain230,231, spinal cord231, cerebellum75,76 and other brain regions240. These approaches are likely to improve the reproducibility and robustness of models by minimizing the inherent variability of the progenitor pool. However, disease modelling with restricted organoids can only recreate phenotypes in these constrained regions, necessitating careful experimental planning.

A major advantage of human in vitro models is that they replicate human-specific cell types. Currently, however, brain organoids are largely made up of neural tissue and lack non-neuronal cell types. One such cell type is oligodendrocytes, which myelinate axons in the CNS. These cells differentiate in distinct developmental stages from RGs in the human brain231 and have complex roles in additional processes, such as interneuron migration234. Oligodendrocytes can be induced and matured in organoids, but specific culture conditions are required209,210,211. Consequently, these cells are rare in standard protocols. They can form spontaneously in organoids208, although controlled mixing procedures provide greater control over the ratio of cell types237,241–243. Methods for generating holistic 3D cultures that have the same high throughput as traditional methods are required to build more precise models and to study processes such as demyelination and neuroinflammation. Such approaches could also increase the lifetime and maturity of in vitro cultures.

Organoids are electrically active and can even reproduce complex functional networks and disease phenotypes209,250–252, so have been used to study activity-related processes. However, one of the most important limitations of organoids is that the tissue architecture and organization that influence activity patterns in vivo are missing. This limitation is illustrated by comparing organoids with normal cortical architecture. The markers expressed by neurons that are generated in organoids recapitulate the serial specification of layer identity in excitatory neurons and the distinct fates of upper-layer and deep-layer neurons are established, but their spatial organization does not recapitulate that in the brain. Separation of layer markers was improved in one study published in 2020 (REF. 239) but the fine-tuned architecture of the six-layered human cortex is still far from being recreated. Similarly, although distinct brain regions can be produced and fused in vitro, they do not replicate the intricate arrangement of the human brain.
Organoids in neurological research

Human 3D organoid models have contributed to advances in our understanding of human brain development and neurological diseases. Considering the benefits and limitations of these model systems, organoids will undoubtedly become an important tool for neurological research, especially when paired with other approaches (FIG. 5). Close collaboration with clinical experts is required to ensure that the versatility possible with gene editing in organoids and iPSC-derived models is used to capture medically relevant information. For example, organoids can be used to explore the effects of disease-associated gene variants in the context of specific cellular phenotypes. Ultimately, this application could directly benefit patient care by providing information about the disease risk associated with specific traits.

However, induced pluripotent stem cells (iPSCs) from patients can also be used to establish patient-derived organoids (step 5). These models can be used for drug testing (step 6) that leads to improved therapies. Cells from these organoid models can also be transplanted (step 7) into animals for in vivo evaluation of human cell types (step 8). All of these organoid-based models can provide insights into disease mechanisms (step 9). The increased understanding of disease and improvements in therapies that result feed back into patient care and patient research.

Thus, existing and future novel organoid disease models could be used to assess and inform the relevance of new variants.

Accurate disease models are needed to identify patient-relevant phenotypes and pathways, and patient-specific organoid models can facilitate development of such models. As in the studies of MCPH discussed above, use of such models to understand the effects of single mutations can inform strategies to screen for other risk genes. This approach will be important for establishing powerful new screening models. In the case of conserved developmental processes, the impact of a mutation can be reliably investigated in vivo in genetically engineered mouse models, but combining insights from human organoid models and mouse models enables more reliable and robust modelling of conserved processes.

Transplantation of organoid-derived cells into rodent brains is another excellent tool for determining the in vivo effects and clinical importance of specific human cell types. Organoids can be used to study human-specific cell types during development,
and orthotopic transplantation could enable studies of in vivo processes and intricate cell type interactions. The combination of patient-derived organoids, risk gene assessment and additional models, such as transplantation models, will be powerful for studying disease mechanisms. However, development of accurate aetiological theories and improvements in treatment necessitate crosstalk with patient research. Investigation of disease in patients with noninvasive approaches, sequencing, neuropathological studies and patient-derived in vitro or xenograft models is invaluable for elucidating acute disease processes. These methods can provide insights into the disease process in people, although mostly catch snapshots of disease progression. By contrast, organoids are excellent for studying disease initiation and early pathophysiology. Only by combining these approaches will we be able to ensure that organoid research focuses on disease-relevant developmental processes and enrich our understanding of neurological disease.

**Conclusion**

Brain organoids enable the generation and perturbation of human tissue in vitro, providing an unprecedented opportunity to decipher neurological diseases. Such models are becoming increasingly relevant as our understanding of the unique processes that contribute to brain development in humans increases. Through analyses that would not be possible in animal models or in patients, human in vitro models have provided insights into previously inaccessible stages of development, leading to groundbreaking studies of neurodevelopmental, neurodegenerative and infectious disorders that have showcased the versatility of organoid models. We anticipate that the development of more complex organoid systems in the coming years, together with patient research and animal models, will further advance our understanding of disease development and ultimately help to improve patient treatment.

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Malik, S. et al. Neurogenesis continues in the third trimester of pregnancy and is suppressed by premature birth. J. Neurosci. 33, 411–423 (2013).
53. Paredes, M. F. et al. Nests of dividing neuralroblasts sustain interneuron production for the developing human brain. Science 367, eabk2346 (2022).
54. Kriener, F. M. et al. Innovations present in the primate interneuron repertoire. Nature 586, 626–269 (2021).
55. Hodge, R. D. et al. Conserved cell types with divergent features in human versus mouse cortex. Nature 573, 61–66 (2019).
56. Ellis, J. K. et al. Ferret brain possesses young interneuron collections equivalent to human postnatal migratory streams. J. Comp. Neurol. 527, 2389–2406 (2020).
57. Raju, C. S. et al. Secretagogen is expressed by developing neocortical GABAergic neurons in humans but not mouse neocortex, and a sizeable part of human neocortex remains, with a high density of GABAergic cell bodies. Cereb. Cortex 28, 1946–1958 (2018).
58. Shi, Y. et al. Mouse and human share conserved transcriptional programs for interneuron development. Science 374, ea66441 (2021).
59. Inta, D. et al. Neurogenesis and widespread forebrain migration of distinct GABAergic neurons from the postnatal subventricular zone. Proc. Natl Acad. Sci. USA 105, 20994–20999 (2008).
60. Paredes, M. F. et al. Extensive migration of young neurons into the human frontal lobe. Science 354, aaf7075 (2016).
61. Schmitz, M. T. et al. The development and evolution of interneurons in primate cerebrum. Nature 603, 871–877 (2022).
62. Loomba, S. et al. Connectome comparison of mouse and human neocortex. J. Neurosci. 377, eab9294 (2022).
63. Birey, F. et al. Assembly of functionally integrated human forebrain spheroids. Nature 545, 54–59 (2017).

In this study, spindles of different brain regions were combined to form assembloids, which were used to model Timothy syndrome.
64. Babloyantz, A. et al. Interneuron progenitors promote brain tumors and microcephaly through a direct genetic pathway. Cell Rep. 14, 649–660 (2016).
65. Xiang, Y. et al. Fusion of regionally specified interneuron progenitors promotes brain tumors and microcephaly. Proc. Natl Acad. Sci. U.S.A. 116, 24969–24974 (2019).
66. Chinnappa, K. et al. Secondary loss of miR-3607 during species evolution. Cell 175, 229–241 (2018).
67. Schmidt, E. R. E. et al. A human-specific modifier of postnatal subventricular zone migration of distinct GABAergic neurons from the mouse postnatal migratory streams. Neuron 109, 1255–1262 (2019).
68. Nowakowski, T. J. et al. Regulation of cell-type-specific transcriptomes by microRNA networks during human brain development. Nat. Neurosci. 21, 1784–1792 (2018).
69. Tomasello, U. et al. mir-137 and mir-122, two outer subventricular zone non-coding RNAs, regulate basal progenitor expansion and neuronal differentiation. Cell Rep. 38, 110381 (2022).
70. Charron, C. et al. Cerebral organoids have the same cell cycle conditions and proliferative capacities of cerebral cortical organoids. Cell 174, 912–922 (2018).
71. Kim, J., Koo, B. K. & Knoblich, J. A. Human organoids: model systems for human biology. Nat. Rev. Cell Biol. 21, 571–584 (2020).
72. Rasmussen, N. S. & Knoblich, J. A. Human organoids: new strategies and methods for analyzing human development and disease. Cell 185, 2756–2769 (2022).
73. Hendriks, D., Artegaian, B. H., de Sousa Lopes, S. C. & Clevers, H. Establishment of human fetal hepatocyte organoids that assemble a complete liver and knockout in organoids from cultures human liver. Nat. Protoc. 16, 182–217 (2021).
74. Bonfanti, P., Dusart, M., Hynes, R. O. & D’Arcy, M. Generation of human and mouse fetal pancreatic progenitors are modulated by epidermal growth factor. Stem Cell Dev. 24, 1766–1778 (2015).
75. Emrich, M. et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 472, 51–56 (2011).

This study is the first to describe in vitro generation of self-organized neural tissue by generating optic-cup cultures from mouse embryonic stem cells.
76. Nakano, T. et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell 10, 771–785 (2012).
77. Kadowa, T. et al. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES-cell-derived neocortex. Proc. Natl Acad. Sci. U.S.A. 112, 20284–20289 (2015).
78. This study demonstrates self-organization of cortical tissue that forms 3D human neocortex in vitro from human embryonic stem cells.
79. Lancaster, M. A. & Knoblich, J. A. Generation of cerebral organoid Müllerian stem cells. Nat. Protoc. 9, 2359–2340 (2014).
80. In this study, 3D cultures derived from human pluripotent stem cells were used for the first time to model disease and recapitulate human-specific features of brain development.
81. Gordon, A. et al. Long-term maturation of human cortical organoids matches key early postnatal transitions. Nat. Neurosci. 24, 530–542 (2021).
82. Velasco, S. et al. Individual brain organoids reproducibly form cell diversity of the human cerebral neocortex. Nat. Neurosci. 22, 661–679 (2019).
83. Yoon, S.-J. et al. Reliability of human cortical organoid generation. Nat. Methods 16, 75–78 (2019).
84. Xiang, Y. et al. MSC-derived cerebral organoids form reciprocal projections when fused with cortical organoids. Stem Cell 24, 487–497 e7 (2019).
85. Sorensen, C. L. et al. Cerebral organoids at the air–liquid interface generate neural and functional cortex. Nat. Neurosci. 22, 661–679 (2019).
86. Gance, P. P. et al. Zika virus impacts growth in human neospheres and brain organoids. Science 352, 816–818 (2016).

This study shows that Zika virus can infect human cortical progenitors in cerebral organoids and cause microcephaly-like phenotypes.
reviews

123. Bae, B. Jayaraman, D. & Walsh, C. A. Genetic changes shaping the human brain. Dev. Cell 32, 425–434 (2015).

124. Luzarraga, S. B. et al. CellScraper regulates centrosome function and centrosome segregation in neuronal progenitors. Development 137, 1907–1917 (2010).

125. Desir, J., Cassart, M., David, P., Bogart, P. V. & Abbouche, M. Microarray data with ASPM mutation shows simplified cortical gyration with antero-posterior gradient pre- and post-natally. Am. J. Med. Genet. Part A 143, 1430–1443 (2008).

126. Pulvers, J. N. et al. Mutations in mouse Aspm (abnormal spindle-like microcephaly associated) cause not only microcephaly but also major defects in the germ cell. Proc. Natl. Acad. Sci. 107, 16595–16600 (2010).

127. Fujimori, A. et al. Disruption of Aspm causes microcephaly with abnormal neuronal differentiation. Brain Dev. 36, 661–669 (2014).

128. Jayaraman, D. et al. Microcephaly proteins Wdr62 and Aspm define a mother centrosome regulating centriole biogenesis, apical complex, and cell fate. Neuron 92, 815–828 (2016).

129. Capecci, M. R. & Pouzet, A. ASPM regulates symmetric stem cell division by tuning cyclin E ubiquitination. Nat. Commun. 6, 8765 (2015).

130. Satterfield, K. F. et al. Large-scale exome sequencing study implicates developmental and functional changes in the neurobiology of autism. Cell 180, 568–584 e25 (2020).

131. Li, R. et al. Inferring cortical development with organoid culture in vitro and modeling abnormal spindle-like (ASPM related primary) microcephaly disorders. Development 148, 823–835 (2021).

132. Lord, C. et al. Autism spectrum disorder. Nat. Rev. Dis. Primers 5, 20 (2020).

133. Gaugler, T. et al. Genetic risk for autism resides with common variation. Nat. Genet. 46, 881–885 (2014).

134. Poulton, B. et al. Autism genes converge on asynchronous development of shared neuron classes. Nature 620, 268–273 (2022).

135. In this study, organoid models with different ASD-related mutations were generated and revealed convergent developmental changes.

136. Mariam, J. et al. FOXP1-dependent dysregulation of GABA glutamate segregation in autism spectrum disorders. Cell 162, 375–390 (2015).

137. Use of induced pluripotent stem cells of patients with ASD in the study identified convergent mechanisms in idiopathic ASD.

138. Rippon, G., Brock, J., Brown, C. & Boucher, J. Disorder-related connectivity in the autistic brain: changes between the “amygdalascope” and “amygdalascope”. Int. J. Psychophysiol. 65, 164–172 (2007).

139. Rosenberg, A., Patterson, J. S. & Angelaki, D. E. Mechanisms of human cortical development. Science 344, 602–608 (2014).

140. In this study, cerebral organoids were used together with other model systems to investigate the effects of Zika virus infection.

141. In this study, neurons, astrocytes and microglia were combined to study AD.

142. Lin, Y.-T. et al. APOE4 causes widespread microglial and cellular alterations associated with Alzheimer’s disease phenotypes in human iPSC-derived brain cell types. Nature 585, 1141–1154 e7 (2021).

143. In this study, a mini-bioreactor culture system was developed to grow brain organoids and used to show that Zika virus infection can cause microcephaly phenotypes in organoids.

144. In this study, neurons, astrocytes and microglia were combined to study AD.

145. Park, J. et al. A 3D human tri-culture system modeling neurodegeneration and neuroinflammation in Alzheimer’s disease. Nat. Neurosci. 21, 941–951 (2018).

146. In this study, neurons, astrocytes and microglia were combined to study AD.

147. Ramani, A. et al. SARS-CoV-2 targets neurons of 3D human brain organoids. EMBO J. 39, e110623 (2020).

148. In this study, neurons, astrocytes and microglia were combined to study AD.

149. Sun, G. et al. Modeling human cytomegalovirus-induced microcephaly in human brain organoids. Cell Rep. Med. 1, 100002 (2020).

150. In this study, a 3D human brain organoid model of frontotemporal dementia was generated and used to uncover the steps that lead to glutamatergic dysregulation.

151. Kim, H. et al. Modeling Parkinson’s disease in 3D midbrain organoids. Stem Cells Dev. 29, 1218–1229 (2020).

152. In this study, neurons, astrocytes and microglia were combined to study AD.
197. Jo, J. et al. Lewy body-like inclusions in human brain stem cells. *Nat. Commun.* 8, 1280 (2017).

200. Anastassiou, C. et al. Human iPSC-derived neurons and cerebral organoids establish differential effects of germline NF1 gene mutations. Stem Cell Rep. 14, 54–60 (2020).

203. Wegscheid, M. L. et al. Patient-derived iPSC-cerebral organoids recapitulate triad of autistic traits. *Proc. Natl Acad. Sci. USA* 112, 17668–17673 (2015).

206. Wallace, M. L., Woerden, G. M., van, Elgersma, Y., Splawski, I. et al. CaV1.2 calcium channel dysfunction of neurogenesis. *Stem Cell Rep.* 8, 219–231 (2017).

209. Bortone, D. & Polleux, F. KCC2 expression promotes disassembly to maintain neural progenitor pool. *Neuron* 53, 19–31 (2004).

212. Fiorenzano, A. et al. Single-cell transcriptomics captures features of human midbrain development for modeling distinct cortical layer formation. *Cell Stem Cell* 24, 201–209 (2019).

215. Dooves, S., Velthoven, A. J. H., van, Suciati, L. G. & Schafer, S. T. et al. Pathological priming causes emerging from cortical organoids model early human brain network development. *Cell Stem Cell* 25, 558–569.e8 (2019).

218. Ormel, P. R. et al. Microglia innately develop within into microglia during development. *Glia* 70, 1267–1288 (2022).

221. Judson, M. C. et al. GABAergic neuron-specific loss of Ube3a causes Angelman syndrome-like EEG abnormalities and enhances seizure susceptibility. *Neuron* 90, 56–69 (2016).

224. Qian, X. et al. Zika virus infection induces RNAi-mediated antiviral immunity in human stem cell-derived neurons. *J. Lipid Res.* 59, 556–566 (2018).

227. Zhang, W. et al. Modeling microcephaly with cerebral organoids reveals a WDR62–CEP170–KIF2A pathway promoting ciliary disassembly in neuroprogenitors. *Nat. Commun.* 10, 2612 (2019).

230. Wang, L. et al. Loss of NARS1 impairs progenitor proliferation in cortical organoids generated to microcephaly. *Nat. Commun.* 11, 4058 (2020).

233. Thomas, C. A. et al. Modeling of TREX1-dependent autoimmune disease using human stem cells. *e1008899* (2020).

236. Qian, X. et al. Sliced human cortical organoids reveal human oligodendrogenesis with dorsal and ventral origins. *Stem Cell Rep.* 12, 890–905 (2019).
Reviews

270. Srikant, P. et al. Shared effects of DISC1 disruption and elevated WNT signaling in human cerebral organoids. *Transl. Psychiat.* 8, 77 (2018).

271. Ye, F. et al. DISC1 regulates neurogenesis via modulating kinetochore attachment of Ndel1/Nde1 during mitosis. *Neuron* 96, 1041–1054.e5 (2017).

272. Xu, R. et al. OLIG2 drives abnormal neurodevelopmental phenotypes in human iPSC-based organoid and chimera mouse models of Down syndrome. *Cell Stem Cell* 24, 908–926.e8 (2019).

273. Klaus, J. et al. Altered neuronal migratory trajectories in human cerebral organoids derived from individuals with neuronal heterotopia. *Nat. Med.* 25, 561–568 (2019).

274. Steinberg, D. J. et al. Modeling genetic epileptic encephalopathies using brain organoids. *EMBO Mol. Med.* 13, e5610 (2021).

275. Papes, F. et al. Transcription factor 4 loss-of-function is associated with deficits in progenitor proliferation and cortical neuron content. *Nat. Commun.* 13, 2587 (2022).

276. Morelli, K. H. et al. MECP2-related pathways are dysregulated in a cortical organoid model of myotonic dystrophy. *Sci. Transl. Med.* 14, eaax2575 (2022).

277. Linkous, A. et al. Modeling patient-derived glioblastoma with cerebral organoids. *Cell Rep.* 26, 5203–5211.e5 (2019).

278. Jacob, F. et al. A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. *Cell* 180, 188–204.e22 (2020).

279. Bion, S. et al. Genetically engineered cerebral organoids model brain tumor formation. *Nat. Methods* 15, 631–639 (2018).

280. Ogawa, J., Pao, G. M., Shokhirev, M. N. & Verma, I. M. Glioblastoma model using human cerebral organoids. *Cell Rep.* 23, 1220–1229 (2018).

281. Krieger, T. G. et al. Modeling glioblastoma invasion using human brain organoids and single-cell transcriptomics. *Neuro Oncol.* 22, 1138–1149 (2020).

282. Rezaazadeh, A. et al. Periventricular nodular heterotopia in 22q11.2 deletion and frontal lobe migration. *Ann. Clin. Transl. Neurol.* 5, 1314–1322 (2018).

283. Sakaguchi, H. et al. Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. *Nat. Commun.* 6, 8896 (2015).

284. Lancaster, M. A. et al. Guided self-organization and cortical plate formation in human brain organoids. *Nat. Biotechnol.* 35, 659–666 (2017).

285. Paşca, A. M. et al. Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat. Methods* 12, 671–678 (2015).

286. Jo, J. et al. Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. *Cell Stem Cell* 19, 248–257 (2016).

287. Eura, N. et al. Brainstem organoids from human pluripotent stem cells. *Front. Neurosci.* 14, 538 (2020).

288. Kasai, T. et al. Hypothalamic contribution to pituitary functions is recapitulated in vitro using 3D-cultured human iPSCs. *Cell Rep.* 30, 18–24.e5 (2020).

289. Andersen, J. et al. Generation of functional human 3D cortico–motor assemblies. *Cell* 183, 1913–1929.e26 (2020).

This study combined cortical, spinal cord and muscle organoids to generate corticomotor assemblies.

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O.L.E. researched data for the article. Both authors made substantial contributions to discussion of the content, wrote the article and reviewed and edited the manuscript before submission.

Competing interests

J.A.K. is on the supervisory and scientific advisory board of a head bio AG (aheadbio.com) and is an inventor on several patents relating to cerebral organoids. O.L.E. declares no competing interests.

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