Human cerebral organoids — a new tool for clinical neurology research

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Abstract | The current understanding of neurological diseases is derived mostly from direct analysis of patients and from animal models of disease. However, most patient studies do not capture the earliest stages of disease development and offer limited opportunities for experimental intervention, so rarely yield complete mechanistic insights. The use of animal models relies on evolutionary conservation of pathways involved in disease and is limited by an inability to recreate human-specific processes. In vitro models that are derived from human pluripotent stem cells cultured in 3D have emerged as a new model system that could bridge the gap between patient studies and animal models. In this Review, we summarize how such organoid models can complement classical approaches to accelerate neurological research. We describe our current understanding of neurodevelopment and how this process differs between humans and other animals, making human-derived models of disease essential. We discuss different methodologies for producing organoids and how organoids can be and have been 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.

Neurological diseases are the leading cause of disability and the second leading cause of death worldwide[1]. Preventive strategies and interdisciplinary treatment regimens are improving outcomes of neurological conditions[2], 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)[3] required a sample size of >10 million people who were

monitored over decades[3]. 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[4]. 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 research in patients and model organisms (FIG. 1). With the rise of 3D in vitro models, known as organoids, complex disease processes can now be studied in a human context.

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 contrast the benefits and limitations of organoids with those of animal models and 2D human models, and highlight recent advances that provide an extensive

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Key points

- 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.

toolbox for neurological research. We introduce different approaches for in vitro 3D disease modelling and address how organoids have been used to study various conditions. Finally, we illustrate how organoids could be integrated into existing research frameworks of patient and animal studies.

Neurodevelopment across species
Conserved principles
The human brain differs notably from that of common laboratory animals, such as rodents, not only with respect to overall size, but also in the contributions of different cell types and the six-layered architecture and folding (gyrification) of the cortex. Nevertheless, major events in neurodevelopment follow principles that are conserved between rodents, primates and humans.

Regardless of the species, neurons in the brain originate from a pool of neuroepithelial cells. Neuroepithelial cells divide symmetrically at first, thereby increasing the progenitor pool, before transforming into radial glia cells (RGCs), which are the neural progenitor cells of the brain[5] and generate excitatory and inhibitory neurons. The number of neurons produced by RGCs during neurogenesis differs vastly depending on the species[6] but adheres to some general principles.

In the dorsal forebrain, two main progenitor populations — RGCs and intermediate progenitor cells (IPCs) — are established during development (reviewed elsewhere[7]) and produce neurons via two mechanisms: direct neurogenesis, in which one RGC produces one neuron via asymmetric division[8]; and indirect neurogenesis, in which RGCs first divide asymmetrically to generate an IPC, from which two neurons are produced via symmetric division[9]. Apical RGCs (aRGCs) divide in the ventricular zone, whereas IPCs divide in the subventricular zone. The ratio between the two modes of neurogenesis influences the number of neurons that a progenitor pool can generate, as intermediate populations such as IPCs strongly increase the neuronal output from a limited number of RGCs[10]. Accordingly, indirect neurogenesis accounts for most of the neuronal production in mammals[8,9] and the proportion of neurons generated in this way is greatest in large gyrated brains, such as the human brain[11]. Newly generated neurons migrate along the basal processes of RGCs to populate the developing cortex in an inside–out manner — the deepest layers of the cortex are produced first and upper layer excitatory neurons are produced later[12].

Whereas excitatory neurons are exclusively generated in the dorsal forebrain, cortical interneurons in rodents, primates and humans are thought to derive mainly from the ganglionic eminences (GEs) in the ventral forebrain[13,14]. The GEs also develop from a ventricular zone, but their organization differs from dorsal areas in that they include abundant non-epithelial progenitors[14]. Interneurons do not ascend along RGC fibres but undergo tangential migration (orthogonal to the RGC fibres) into the cortex, populating all layers and regions[13]. Subregions of the GEs — the caudal GE (CGE), medial GE (MGE) and lateral GE (LGE) — generate distinct interneuron subtypes[15,16] that are largely conserved at the epigenetic level across species[17].

The role of dorsal regions in generating cortical interneurons in different species is unclear. In mice, dorsal progenitors are known to generate interneurons that migrate to the olfactory bulb[18], but whether cortical interneurons can be generated from dorsal progenitors has long been debated[19]. This debate continues, as studies published in 2022 provided evidence that dorsal progenitors generate interneurons in humans[20] but not in mice[21]. Overall, the ventral areas differ greatly from the dorsal regions in terms of organization, timing and neuron specification, adding another layer of complexity to neurogenesis.

Neurodevelopment in the primate brain
Differences between species affect cell type composition and neurodevelopment. In comparison with other animals, the primate brain contains several distinct types of progenitors and neurons (FIG. 2), individual excitatory and inhibitory lineages are amplified, and other processes inside and outside the cerebrum differ. Specific genetic changes underlie these species differences.

Expansion of excitatory neurons. Neurodevelopment in primates and other gyrencephalic species relies heavily on an additional progenitor cell type that substantially increases neuron production: outer RGCs (oRGCs). oRGCs reside in the outer subventricular zone and, although their contribution to neuronal production in lissencephalic animals is negligible, they account for the majority of neurons in humans, non-human primates and some other species[22,23] (FIG. 2a). In primates, the initial pool of oRGCs is expanded by a temporary shortening of the cell cycle, which results in a large population in the outer subventricular zone[24]. Rodents and other lissencephalic animals have very few oRGCs[25–27], a difference that is due to several human-specific aspects of oRGC development, expansion and proliferation[28–36].

The biology and development of oRGCs can be recapitulated in human cerebral organoids (hCOs)[37]. Studies of such organoids and primary tissues have revealed that the mTOR signalling pathway in oRGCs from humans

differs even from that in chimpanzees and macaques[35]. In addition to increasing the number of neurons, oRGCs provide a scaffold for migrating newly generated neurons. 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 oRGCs[38] (FIG. 2a). Thus, oRGCs 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 elsewhere[39]). 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[40] (FIG. 2b).

The subplate is important in the organization of circuits that connect the two hemispheres and the cortex to the thalamus[41]. 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 elsewhere[41]). Similarly, defects of the subplate can cause structural malformations, such as agenesis of the corpus callosum[42]. 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 scaffold[43].

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 rodents[11,44] (FIG. 2b). These neurons form intratelencephalic connections and have been linked with human-specific cognitive abilities[45,46]. 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 unchanged[30,32], 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
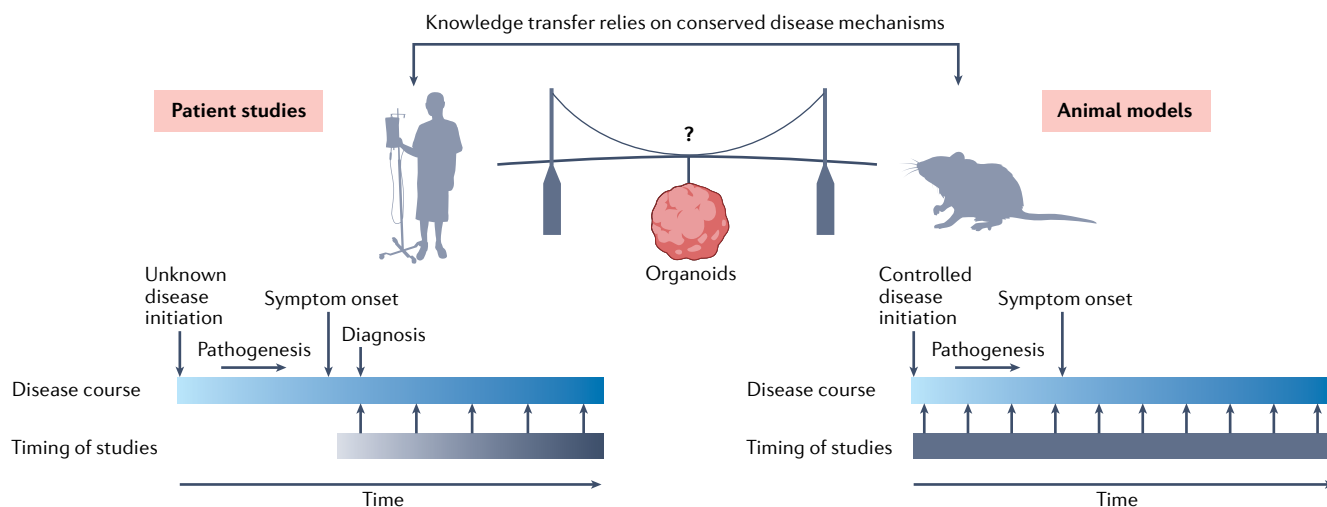

Fig. 1 | Organoids can bridge patient and animal studies to advance our understanding of neurological disease. Studies in patients (left), such as sequencing, neuropathology or patient-derived xenograft models, provide a snapshot of disease at a given time point. Furthermore, these studies are usually 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.
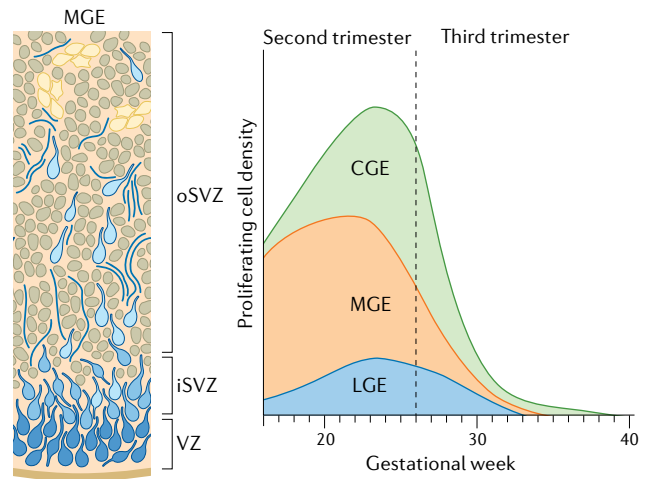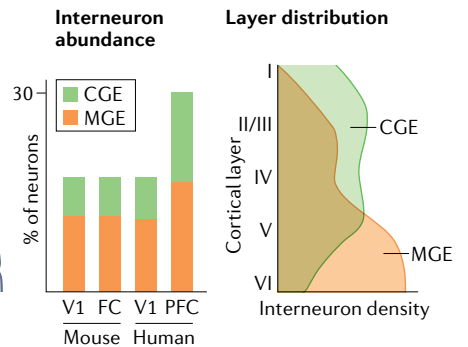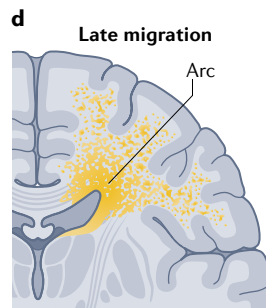
a Radial glia cell development
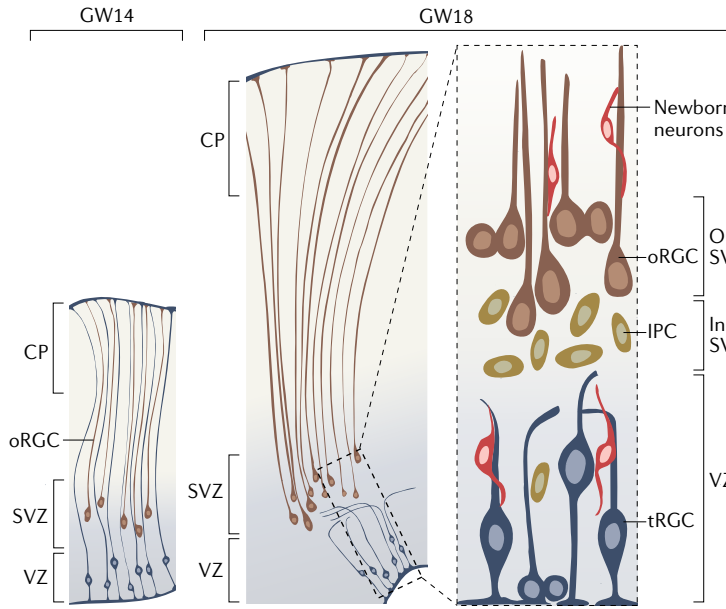
b Expansion of layers II/III
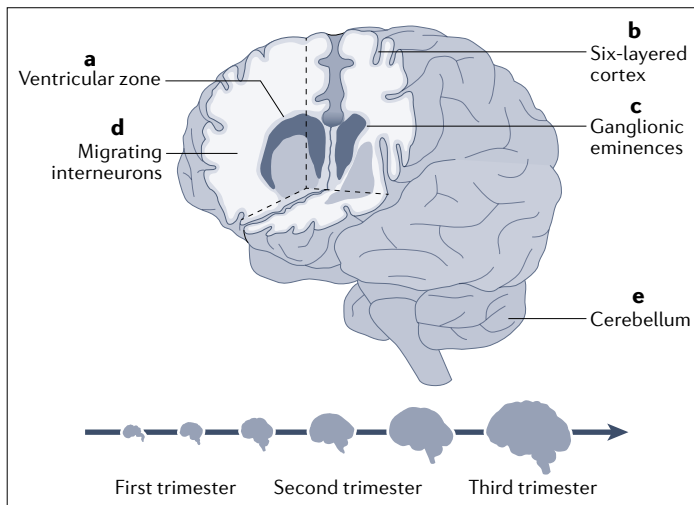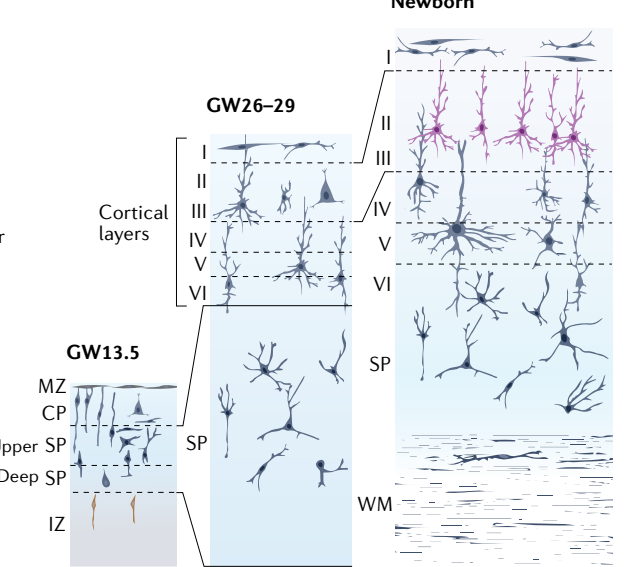
c Ganglionic eminence development

e Cerebellum development

d Late migration

Interneuron abundance

Layer distribution
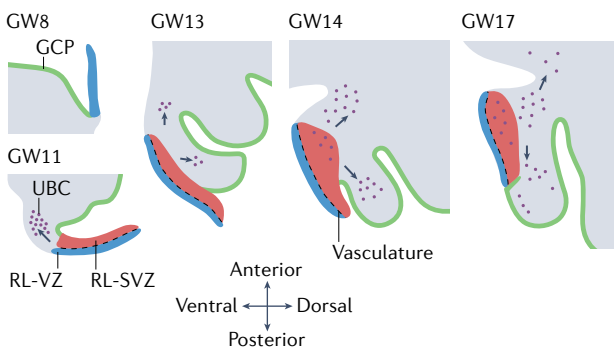
of the human brain has a strong effect on the timeline of interneuron generation (reviewed elsewhere[47]). In mice, neurogenesis in the ventral GE regions occurs from around embryonic day 10.5 (E10.5) to E16 (REFS.[48,49]), and the timing differs slightly between the MGE, LGE and CGE[50]. By contrast, ventral neurogenesis in humans peaks around mid-gestation[14] and continues until the end of pregnancy — proliferation occurs in the MGE earlier than in the CGE[51,52] (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 (VZ) 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 RGCs (oRGCs, brown) emerge in the subventricular zone (SVZ) and connect only to the pial surface via their basal process. Subsequently (at GW18, 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 GW13.5 with GW26–29) — during mid-gestation, the SP becomes larger than the CP and cortical layers I to VI 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 GW26–29 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)[54]. In addition, the contribution of 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 GW11, after which the RL is internalized by GW17 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.[282], Wiley. Part **e** adapted from REF.[72], Springer Nature Limited.

of immature neurons that contribute to neuron production during the second trimester, thereby increasing interneuron numbers in the human brain[53] (FIG. 2c). Sustained proliferation of cells that express doublecortin suggests that, as in the dorsal forebrain, ventral areas rely on intermediate progenitor types to increase neuronal output[14,53].

Interneuron identities are determined by complex genetic programmes that largely overlap in humans and mice[13,17] but important differences have emerged. Some genes that are specific to interneuron subtypes in mice are less specific or not expressed in humans[54]; for example, the serotonin receptor subunit *Htr3a*, one of the main markers of CGE interneurons in mice, is not expressed by CGE interneurons in humans or other primates[54,55]. Conversely, in humans and other gyrencephalic animals, such as ferrets, the calcium-binding protein secretagogin is expressed by late-migrating CGE interneurons[56], whereas this population is absent in rodents[57,58].

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 animals[47]. Postnatal cortical interneuron migration has been observed in mice[59] but to a lesser extent than in humans and other gyrencephalic animals[56]. 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 life[60]. 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 interneurons[61].

This post-migratory cortical interneuron population differs between primates and rodents in several ways (FIG. 2d). GABAergic interneurons are more abundant in the human brain than in the mouse brain[62], 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 mice[54]. 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. 2d), more so in areas associated with higher cognition, such as the prefrontal cortex, where they account for up to 50% of all interneurons[54]. In addition, interneurons in subcortical structures seem to differ, as demonstrated by the discovery of a primate-specific striatal interneuron type[54].

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 time[63–66] can be monitored, as can the relevance of specific cell types and their developmental trajectories to diseases such as Timothy syndrome[63,67] and tuberous sclerosis[68].

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 primates[69]. 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-gestation[70,71] (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 malformation[71,72], and has been identified as the source of medulloblastoma group 3 and 4 — a severe childhood

brain tumour[73,74]. Human cerebellar organoid models[75] that include rhombic lip progenitors[76] 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[77]. Regulation of some conserved pathways is altered, resulting in changes in Robo[28], mTOR[35], PDGF[34] and Notch signalling[31], as well as morphological transitions that affect the organization of the ventricular zone and increases in progenitor numbers[78]. In addition, several human-specific gene duplications and variants have been identified that alter the functions of *NOTCH2NL*[29,36], *TBC1D3* (REF.[33]), *ARHGAP11B*[30,32], *PPP1R17* (REF.[79]) and human-specific enhancers, such as *HARE5* (REF.[80]), and alter microRNA expression[81–83], all of which are involved in progenitor expansion and regulation. Similarly, gene variants have been discovered that change neuronal maturation, spine morphology[84,85] and connectivity[86].

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 gyrification, which occurs in most amniotes[87]. Lissencephaly and reduced brain size in rodents is caused by secondary loss of miR-3607 (REF.[88]), which is expressed in evolutionarily more distant animals, such as ferrets, that exhibit gyrencephaly and similar neural progenitor cells to humans[22,88,89]. 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[78].

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[90,91]) 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[92,93]. With respect to brain organoids, Sasai's group pioneered the 3D in vitro culture of optic cup structures[94,95] and cortical tissue[96], and our group developed the cerebral organoid model and pioneered its use for disease modelling[37]. 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[97]. 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[98].

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[37]. Guided protocols involve addition of morphogens to induce or restrict specific fates, leading to so-called restricted hCOs with more homogeneous cell populations[99,100]. These approaches can be used to generate different hCOs depending on the research question. Furthermore, fusion of differentially patterned hCOs into assembloids enables analysis of interactions between regions[63,64], such as interneuron migration from ventral to dorsal hCOs[63–66] or axonal projections[101,102]. Together, these possibilities result in a comprehensive toolbox for studying disease processes during human neurodevelopment (FIG. 3).

The experimental timeline depends on whether the focus is on early progenitor biology[78] and disease susceptibility[103,104], neuronal maturation, migration and activity, or late non-neuronal populations[105,106], and can last for more than 1 year. Organoids are constantly accessible during these long experiments, enabling flexible application of compounds[66,107], modification of culture conditions[107,108] and/or genetic perturbations[109].

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[110], and neurodevelopmental disorders, such as autism spectrum disorders (ASD)[111]. 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 monogenetic diseases have been identified, including early infantile epileptic encephalopathies[112,113], neurocutaneous syndromes[114], lysosomal storage disorders[115] and neurodegenerative disorders[116], 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. 4). Similarly, organoids can be used to screen potential therapeutic agents.
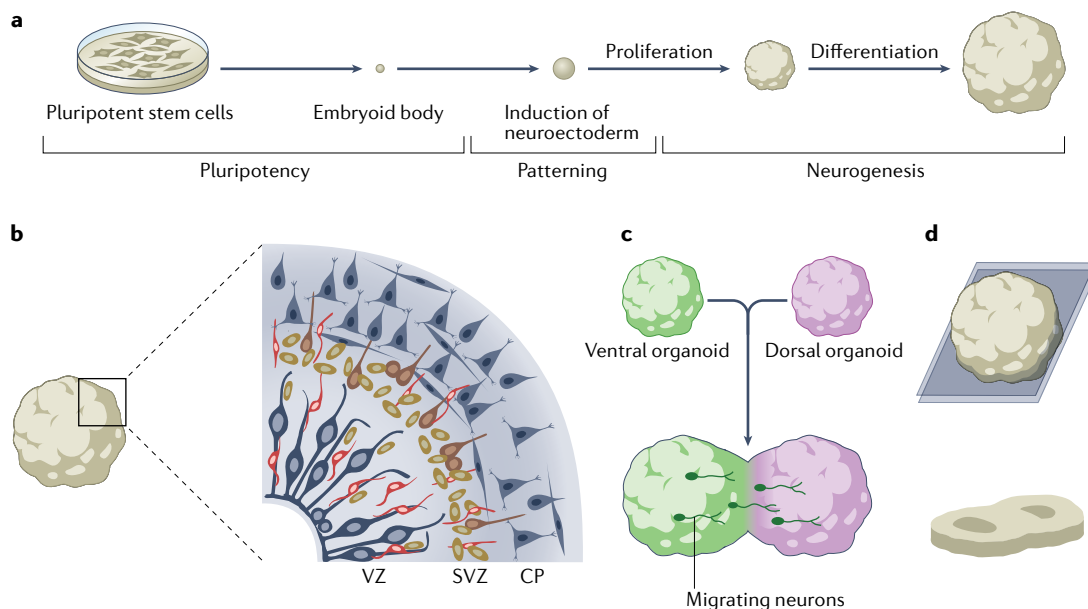
Fig. 3 | **Production and use of organoid models. a** | Organoids are generated from pluripotent stem cells, either embryonic or induced, that are grown in adherent 2D culture. These cells are aggregated in low-attachment plates to produce embryoid bodies, after which induction of neuroectoderm occurs. Organoid progenitors proliferate symmetrically in the first weeks, followed by neuron production and differentiation. During the initial culture period, organoids can be patterned to develop into representations of specific brain regions. Various protocols enable patterning for dorsal[96,99,100,161,283–285] or ventral forebrain[63–65], thalamus[101], hypothalamus[161], midbrain[161,240,286], hindbrain[287] and cerebellum[75,76]. **b** | Mature organoids recapitulate developmental hallmarks of the human brain, including ventricular zone (VZ) structures that contain apical radial glia, subventricular zone (SVZ) areas that contain intermediate progenitors and outer radial glia, and an emerging cortical plate (CP) that contains neurons. **c** | Restricted organoids can be fused to model interactions between distinct brain areas; for example, the tangential migration of interneurons from ventral to dorsal areas[63,64,66,67], the striatal[208] or thalamic projections[101] to the cortex, hypothalamic projections to the pituitary gland[288] or the connection of cortical neurons to muscle via the spinal cord[289]. **d** | To overcome difficulties such as restricted nutrient supply, sliced organoids — so-called air–liquid interface cerebral organoids — can be cultured.
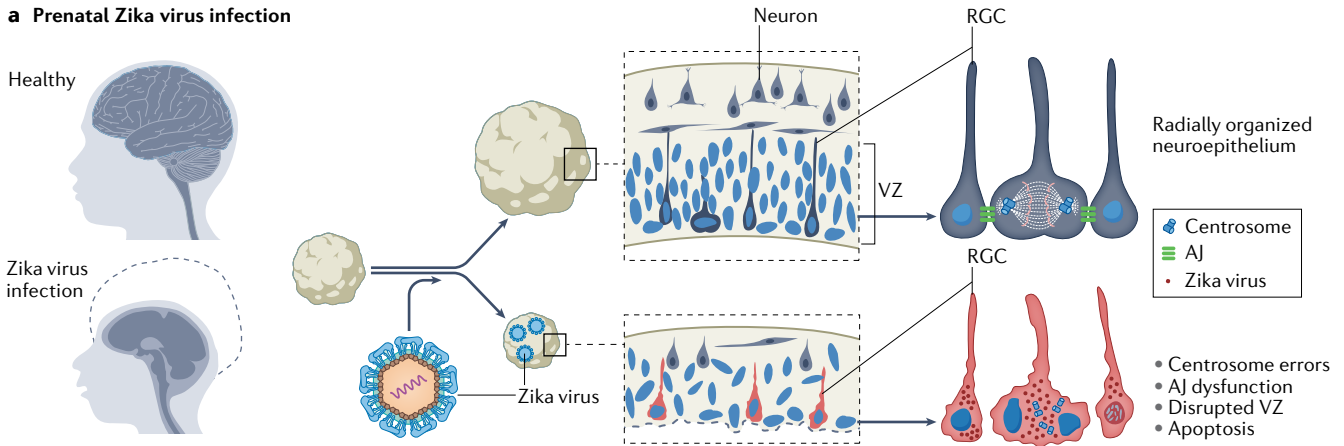
Assessing disease traits with organoids

Major structural defects such as microcephaly were the first developmental disorders to be studied in organoids[37]. The cardinal symptom of primary microcephaly (referred to as microcephaly primary hereditary (MCPH)) is a small head circumference[117]. 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[110]. 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[118]). For example, mice with mutations in *Mcph1*, the first gene to be associated with MCPH[119], have a smaller cerebral cortex than wild-type mice[120,121], and the model revealed a premature switch from symmetric to asymmetric cell division, which reduces the initial aRGC pool size and underlies the phenotype[121].

Some genes associated with MCPH, including *CDK5RAP2* and *ASPM*, have been linked to the evolutionary expansion of the brain in primates[122,123], 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[124]. By contrast, *CDK5RAP2* mutations in hCOs recapit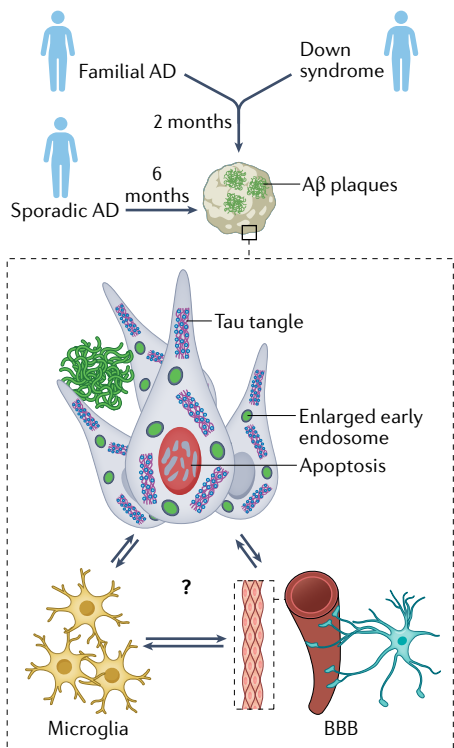ulate MCPH-like effects and have demonstrated that the size of the progenitor pool is reduced owing to premature neurogenic divisions[37]. Similarly, *ASPM* mutation, which is the most common cause of MCPH, results in drastic brain size reductions in humans[125] but causes only a moderate[126–128] and variable[129] 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[118]. Accordingly, ferrets, which have a prominent subventricular zone similar to that in humans, develop microcephaly upon *ASPM* mutation[130]. Features of microcephaly also developed in hCOs that were generated from iPSCs from patients with *ASPM* mutations[131]. 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 172 MCPH-associated genes[109]. 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
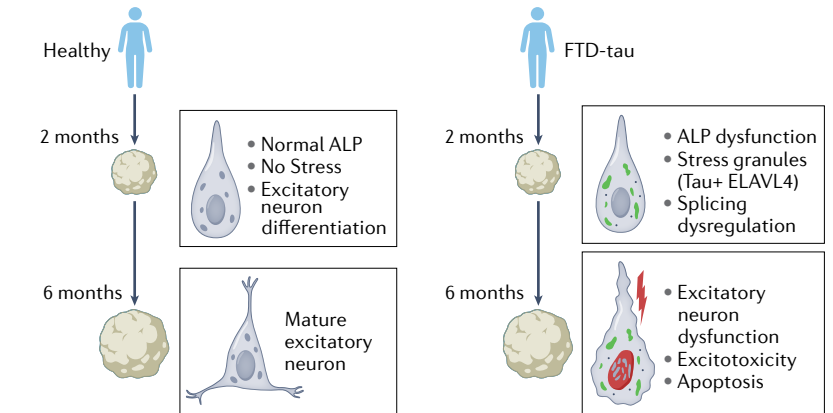
a Prenatal Zika virus infection

Healthy

Zika virus infection

Neuron

RGC

VZ

Zika virus

Radially organized neuroepithelium

- Centrosome ⚙ Centrosome
- AJ ≡ AJ
- Zika virus · Zika virus

RGC

- Centrosome errors
- AJ dysfunction
- Disrupted VZ
- Apoptosis

b AD

Familial AD

Down syndrome

2 months

Sporadic AD

6 months

Aβ plaques

Tau tangle

Enlarged early endosome

Apoptosis

Microglia   ?   BBB

c Frontotemporal dementia

Healthy

2 months

6 months

- Normal ALP
- No Stress
- Excitatory neuron differentiation

Mature excitatory neuron

FTD-tau

2 months

6 months

- ALP dysfunction
- Stress granules (Tau+ ELAVL4)
- Splicing dysregulation

- Excitatory neuron dysfunction
- Excitotoxicity
- Apoptosis

d Timothy syndrome

Ventral   Dorsal

Cytoskeleton

↑Saltation frequency

↓Saltation length

P P P P
P P P P

P P P
CREB

Ca²⁺   Ca$_V$1.2

GABA receptor

e Angelman syndrome

Healthy   Angelman syndrome

UBE3A

Accumulation

UBE3A

Degradation

fAHP

Membrane potential   Time

Ca²⁺ spikes

[Ca²⁺]

Time

Cell 1
Cell 2
Cell 3
Cell 4

f Tuberous sclerosis complex

$TSC2^{+/-}$ Early

CLIP cell

↑↑ mTOR $TSC2^{+/-}$

Late

Tuber phenotype

Tumour phenotype

Giant cell
Dysmorphic IN

CLIP cell
Giant cell

◀ Fig. 4 | **Investigating neurological disease mechanisms with organoids. a** | Prenatal Zika virus infection causes microcephaly (left), characterized by a drastic reduction in brain size and head circumference. Studies in organoids have revealed the mechanisms involved (right). In healthy organoids (top), radial glia cells (RGCs) in the ventricular zone (VZ) are radially organized and have tight apical junctions (AJ; blue ovals represent nuclei of RGCs for which the cell body is not shown for clarity). In organoids infected with Zika virus, AJs between RGCs are destroyed and centrosome errors occur, leading to disruption of the VZ. Apoptosis of RGCs accounts for the reduced neuronal output and the size defect. **b** | Organoids can be generated from patients with familial Alzheimer disease (AD), sporadic AD or Down syndrome. AD pathology — including amyloid-β (Aβ) plaques, tau tangles and enlarged early endosomes — develops in these organoids and neuronal apoptosis is increased. This pathology develops more quickly in organoids derived from people with familial AD or Down syndrome (2 months) than in organoids derived from people with sporadic AD (6 months). These organoid models are starting to incorporate interactions of neurons with microglia and the blood–brain barrier (BBB), which could provide further insights into disease mechanisms. **c** | Frontotemporal dementia with tau pathology (FTD-tau) is caused by mutations in the *MAPT* gene, which encodes tau. In organoids derived from people with FTD-tau, dysfunction of the autophagy–lysosomal pathway (ALP) occurs early and the excitatory lineage splicing regulator ELAVL4 co-localizes with tau in stress granules, resulting in splicing dysfunction, aberrant development of the excitatory lineage and consequent neuronal dysfunction, excitotoxicity and apoptosis. **d** | Timothy syndrome is a neurodevelopmental disease caused by mutations in the $Ca_V1.2$. Work on assembloids of ventral and dorsal organoids derived from patients with Timothy syndrome revealed inefficient saltatory migration movements of interneurons. Increased Ca^{2+} influx via the mutant $Ca_V1.2$ channel altered the cytoskeleton to reduce the length of saltatory movements and remodelled GABA receptors to increase the frequency of saltatory movements. **e** | In Angelman syndrome, loss of the *UBE3A* gene that encodes a ubiquitin protein ligase leads to accumulation of big potassium channels in neurons (top). 2D and organoid experiments have shown that neurons in Angelman syndrome have increased fast components of the after hyperpolarization (fAHP, middle). In organoids derived from patients with Angelman syndrome, synchronicity of calcium events was greater than in organoids from healthy people (bottom). **f** | Organoids derived from people with tuberous sclerosis complex (TSC) recapitulated the tuber and tumour phenotypes and demonstrated that abnormalities of caudal late interneuron progenitor (CLIP) cells underlie the disease. CLIP cells are vulnerable to heterozygous *TSC2* mutations, which leads to their over-proliferation that initiates formation of tubers (dysmorphic interneurons (IN) and giant cells) or tumours. Part **f** adapted with permission from REF.[68], Kelli Holoski.

organoid. 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[109].

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[132]). Other than in ASD-related monogenetic syndromes, high-risk de novo mutations have only a small role in the disease[133]; 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[130]. 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[134], 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[135]. 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[136] and is supported by computational[137] and transcranial magnetic stimulation studies[138] 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[139]. 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 oRGCs. 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[140,141], 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[142], gene expression[143] and epigenomics[144,145] in brain development[146] and disease[147], and these resources are invaluable for determining the temporal window during which risk genes are expressed. Use of high-throughput single-cell RNA (scRNA) sequencing techniques with tissue from people with ASD has identified cell type-specific effects on upper layer projection neurons[148]. hCOs can be used to investigate neurodevelopment in vitro and complete the developmental timeline of cell-type specific processes[149]. Numerous studies

have been done to examine single-cell gene expression and chromatin accessibility in hCOs[35,99,139,145,149–154]. 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[155]. 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 (TABLE 1). 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[156,157]. Maternal Zika virus infection was quickly linked to an increase in primary microcephaly, supported by the detection of virus in the amniotic fluid[158] and the brain of a microcephalic fetus[159]. Mouse and 2D and 3D human models were soon established to study how Zika virus infection causes microcephaly[103,160–168].

Neural progenitors in 2D culture were easily infected and their growth was reduced[163], and the concept that Zika virus targets neural progenitors was later confirmed by infection of fetus-derived neural progenitor cells but not neurons[168]. However, microcephaly-like phenotypes require a 3D architecture and could only be recapitulated by infecting organoids[103,160,161] (FIG. 4a). The virus-induced phenotype was specific to Zika virus; infection with Dengue virus[103], another member of the flavivirus family, and lymphocytic choriomeningitis virus[165] 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[161,165,166,168]. 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[103,160,161], resulting in destruction of the ventricular zone architecture and the RGC scaffold[168]. 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[167]. Furthermore, studies in organoids and fetal brain slices also suggested that centrosome damage occurs in Zika virus-associated microcephaly[165,168], which is a common mechanism in MCPH[110]. 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[169]. 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[104]. 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[104].

Thus, hCOs can be used to study the cellular mechanisms, dynamics, and outcomes of CNS infections, further demonstrated by studies of SARS-CoV-2 (REFS.[170–172]) and cytomegalovirus infection[173] 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[174]. 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[175,176]. 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[177] or Down syndrome[178,178]. 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[179]. In subsequent studies, organoids derived from iPSCs of people with familial AD spontaneously developed pathology without overexpression[180,181] (FIG. 4b). Inhibition of the secretases reversed pathology in 2D models[177,178] and 3D models[179,180,182] 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	37,109,131, 253,254
Macrocephaly	Patient iPSCs (Sandhoff disease, *HEXB* mutation); gene editing (*PTEN* knockout)	Expanded progenitor pools; enlarged organoids	255,256
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, 160–168,257
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	104,170–173
Herpes simplex virus	Herpes simplex virus infection of organoids	Reduced organoid size owing to infection; apoptosis of neural progenitors	104,258
Aicardi–Goutières syndrome	Patient iPSCs; gene editing of *TREX1*	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	180,181,183, 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	260
Huntington disease	Patient iPSCs	Defects in specification and organization of progenitors	261
Creutzfeldt–Jakob disease	Prion infection of organoids	Assessment of prion seeding capability in organoids revealed de novo prion propagation	198,262
Amyotrophic lateral sclerosis	Patient iPSCs, organoid slice model	Changes in transcriptomics, unfolded protein response and DNA repair	263
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 saltation frequency and decreased saltation length	63,67
Tuberous 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	68,202,264
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	251,265–267
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	134,135,139, 268,269
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

Table 1 (cont.) | **Summary of organoid disease models**

Disease model	Approach	Findings	Refs.
Neurodevelopmental and neuropsychiatric disorders (cont.)			
WWOX-related epileptic encephalopathy	Patient iPSCs; gene editing (WWOX)	Increased neuronal activity; increased number of interneurons	274
Pitt–Hopkins syndrome	Patient iPSCs carrying TCF4 mutation	Impaired WNT signalling leading to reduced progenitor proliferation and neuron production	275
Myotonic dystrophy type 1	Patient iPSCs with 600 or 1,200 CTG repeats in DMPK	Microcephaly phenotype; reduction in glutamatergic neurons; increases in glia	276
Brain tumours			
Glioblastoma	Gene editing; transplantation of glioma stem cells; patient-derived samples	Tumour induction and infiltration of healthy organoid tissue	277–281
Environmental effects			
Hypoxia	Incubation of organoids in low-oxygen environment	Endoplasmic reticulum stress and unfolded protein response, leading to microcephaly phenotype	108

BK channel, voltage-dependent big potassium channel; CLIP, caudal late interneuron progenitor; EGFR, endothelial growth factor receptor; iPSC, induced pluripotent stem cell; PDGFRα, platelet-derived growth factor receptor-α.

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[177–181,183].

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[184,185], 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[186]. 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 (REF.[187]). Interestingly, expression of APOE*ε4 in cerebral organoids resulted in development of AD pathology at a later stage (6 months)[187] than did APP duplication or mutation of PSEN1 or PSEN2 (2 months)[180], 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[188]. Vascular cells can be incorporated into organoids, enabling investigation of some blood–brain barrier properties in the context of Aβ pathology[189]. However, the lack of functional vascularization in organoids remains a major limitation of current studies (FIG. 4b). 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)[190]. This disease is heterogeneous, but dysregulation of glutamatergic signalling from excitatory neurons is thought to cause the symptoms[191]. 2D models of neurons derived from iPSCs of people with a MAPT mutation have identified impaired cytoskeletal remodelling of the axon initial segment, which results in activity changes[192]. In addition, dysregulation of the autophagy–lysosomal pathway has been identified in FTD-tau[193]. 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[194], 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[195–197] and Creutzfeldt–Jakob disease[198] (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[182,199].

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 (neurofibromatosis[200,201] and tuberous sclerosis complex (TSC)[68,202]), DiGeorge syndrome[203], genetic lissencephaly (Miller–Dieker syndrome[204,205] and Seckel syndrome[206]), fragile X syndrome[207], Phelan–McDermid syndrome[208], Timothy syndrome[63,67] and Angelman syndrome[209] (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 $Ca_V1.2$ (REF.[210]). In mice, high expression of the mutant channel is lethal, but low expression[211] 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, $Ca_V1.2$ mutation resulted in a loss of channel inactivation and increased intracellular calcium[212]. Furthermore, work in 2D human models and in mice showed that the mutation leads to an activity-dependent but calcium-independent retraction of dendrites[213]. In addition to their functional and morphological roles, L-type calcium channels have been implicated in regulation of tangential migration[214], and assembloids 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 assembloids, enabling modelling of migration in disease[63,64,66]. In Timothy syndrome, this assembloid model revealed a cell-autonomous decrease in the length of individual saltations and an increase in the frequency of saltations[63]. Use of these assembloids 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 signalling[67] (FIG. 4d). Previous use of organoids to study the effects of neurotransmitter signalling on interneuron migration has identified similar effects of GABA receptor inhibition[66], 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 assembloids. 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 symptoms[215]. 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 brain[216] and linked to the neuron-specific expression of a neighbouring gene[217].

Several mouse models have been generated to advance our understanding of the disease mechanisms, and model animals have motor dysfunction and susceptibility to seizures[218]. An underlying excitatory–inhibitory imbalance was identified[219], with specific defects in interneurons[220,221]. In neurons derived from iPSCs of people with Angelman syndrome, parental imprinting is established during in vitro development, paving the way for human models[222]. In one such model, maturation of mutated 2D differentiated neurons was delayed[223]. 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 identified[224].

Subsequent work in which organoids were used in combination with 2D and mouse models of Angelman syndrome has identified the responsible protein[209]. 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 organs[225]. 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 development[226]. Use of organoids has demonstrated that human-specific processes underlie the pathogenesis of TSC[68].

Heterozygous mouse models of TSC lack morphological abnormalities[227] but homozygous deletion of *Tsc1* or *Tsc2* is lethal. By contrast, inducible knockouts of *Tsc1* or *Tsc2* in mice can replicate several characteristics of the disease, such as hyperexcitability[228], nodular tumours and dysmorphic cells[229–232]. 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 process[225]. 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 mice[202]. However, although LOH is common in SEGAs, it is rare in cortical tubers[233]. Thus, how pathogenesis could involve heterozygous cells remained unclear[209]. 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 controls[68] (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 phenotypes[68]. 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 interneurons[68].

Thus, the TSC organoid model explains the differences in mutational 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 model[151] or merely affects individual cells remains under debate[98,234]. Meta-analysis of scRNA data[235] and multi-omics data[154] 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-culture[236,237], induction[189] and transplantation[238] methods, and cultured organoid slices have also been used[102,239]. 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, midbrain[197,240], spinal cord[241], cerebellum[75,76] and other brain regions[242]. 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 RGCs in the human brain[243] and have complex roles in additional processes, such as interneuron migration[244]. Oligodendrocytes can be induced and matured in organoids, but specific culture conditions are required[105,106,245]. Consequently, these cells are rare in standard protocols. They can form spontaneously in organoids[246], although controlled mixing procedures provide greater control over the ratio of cell types[237,247–249]. 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 phenotypes[209,250–252], 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
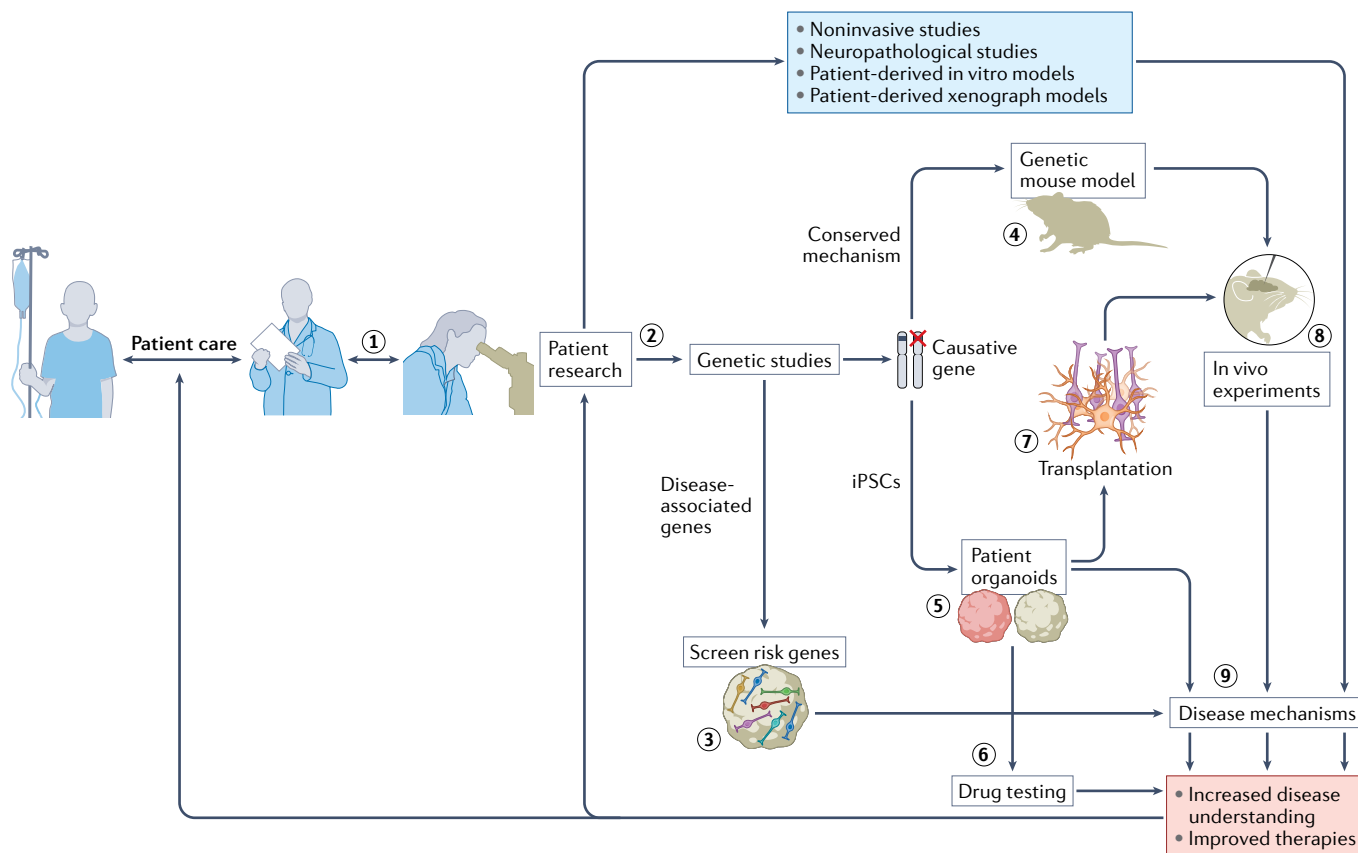
Fig. 5 | **The role of brain organoids in neurological research.** Close collaboration with medical specialists is required to increase the accuracy of organoid models (step 1). Patient research is the foundation for developing organoid models (step 2). Identification of disease-associated genes enables these genes to be screened in organoids to inform disease risk and provide insights into disease mechanisms (step 3). Identification of causative genes enables development of accurate screening platforms. When these genetic mechanisms are conserved in rodents, genetic mouse models can be developed to perform in vivo experiments (step 4).

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.

and therefore remain a reductionist model. Overall, organoids are a powerful tool for neurological research when used appropriately, but require rigorous evaluation and validation with in vivo material to realize their full potential.

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.

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[37] can inform strategies to screen for other risk genes[109]. 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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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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