Review Article

Modeling congenital brain malformations with brain organoids: a narrative review

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Background and Objective: During embryonic development, the dysregulation of the proliferation and differentiation of neuronal progenitors triggers congenital brain malformations. These malformations are common causes of morbidity and mortality in patients younger than 2 years old. Animal models have provided considerable insights into the etiology of diseases that cause congenital brain malformations. However, the interspecies differences in brain structure limit the ability to transfer these insights directly to studies of humans. In recent years, brain organoids generated from human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) using a 3-dimensional (3D) culture system have been used to resemble the structure and function of a developing human brain. Therefore, we aimed to summarize the different congenital brain malformations that have been modeled by organoids and discuss the ability of this model to reveal the cellular and molecular mechanisms of congenital brain malformations.

Methods: A comprehensive search was performed using PubMed and Web of Science's Core Collection for literature published from July 1, 2000 to July 1, 2022. Keywords included terms related to brain organoids and congenital brain malformations, as well as names of individual malformations.

Key Content and Findings: The self-assembled 3D aggregates have been used to recapitulate structural malformations of human brains, such as microcephaly, macrocephaly, lissencephaly (LIS), and periventricular nodular heterotopia (PH). The use of disease-specific brain organoids has revealed unprecedented details of mechanisms that cause congenital brain malformations.

Conclusions: This review summarizes the establishment and development of brain organoid technologies and provides an overview of their applications in modeling congenital brain malformations. Although several hurdles still need to be overcome, using brain organoids has greatly expanded our ability to reveal the pathogenesis of congenital brain malformations. Compared with existing methods, the combination with cutting-edge technologies enables a more accurate diagnosis and development of increasingly personalized targeted therapy for patients with congenital brain diseases.

Keywords: Brain organoid; congenital brain malformations; neurodevelopment; human pluripotent stem cells

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Introduction

The human brain is one of the most complex organs in the body, and its development occurs through a series of processes, including neurogenesis, cell migration, neuronal maturation, and neural circuit formation (1). The nervous system originates from the ectoderm, with the neural tube and neural crest giving rise to the central and peripheral nervous systems, respectively. The neocortex development starts with the expansion of neuroepithelial cells at the ventricular zone (VZ), which gives rise to radial glial (RG) cells. RG cells have the capacity for self-renewal to expand the neural progenitor cells (NPCs) pool and can differentiate into intermediate progenitor cells or neurons directly (2,3). The newborn neurons then migrate to their final destinations in the different layers of the cerebral cortex (4,5). Dysfunctions of early neurodevelopment, including neurogenesis and neural migration, have been associated with structural malformations of the human brain.

Congenital brain malformations comprise a diverse group of neurodevelopmental disorders that occur during early embryonic development and are caused by genetic or environmental factors (6). The pathogenesis of congenital brain malformations remains largely unclear due to the lack of disease models based on the human genetic background. Most studies choose rodent animals as the disease model to explore the pathophysiology of human brain malformations. However, rodent animal brains are lissencephaly (LIS) and short of outer radial glial cells (oRGs), posing a formidable barrier to recapitulate human brain diseases (7). Moreover, single-cell transcriptomics showed extensive differences in cell types and gene expression between humans and rodents (8). This emphasizes the importance of species-specific models.

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), opened a new avenue to model human diseases *in vitro* (9). hPSCs-derived 3-dimensional (3D) aggregates named brain organoids have similarities with human brains in cell types, cytoarchitectures, and spatial architectures. Therefore, brain organoids have become potential tools for exploring the mechanism of congenital human brain malformations (10).

Here, we summarize the development of methodologies to differentiate human brain organoids and their applications in brain disease modeling, especially for congenital brain malformations, such as microcephaly, macrocephaly, LIS, and periventricular nodular heterotopia (PH). Furthermore, we also discuss future perspectives for advancing current brain organoid technologies to expand their applications. We present the following article in accordance with the Narrative Review reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-239/rc).

Methods

We performed a comprehensive search using PubMed and Web of Science's Core Collection for literature published from July 1, 2000 to July 1, 2022. Keywords included terms related to the technology (brain organoid/cerebral organoid) and the disease (congenital brain malformations/structural brain abnormalities), as well as names of individual malformations (microcephaly/macrocephaly/LIS/PH). The detailed search strategy is summarized in *Table 1*. The literature research was supplemented by reviewing references within individual papers.

Brain organoids methodologies

In general, there are two approaches to generating brain organoids: unguided and guided methods (*Figure 1A*). The protocol of generating unguided brain organoids, or cerebral organoids, has continued to advance in the last decade. The first embryonic stem cell (ESC)-derived cortical neuroepithelia, which was considered a 2.5-dimensional (2.5 D) brain model, was established in 2008 using a technique known as SFEBq [serum-free floating culture of embryoid body (EB)-like aggregates] (11). The self-organized tissue showed apical-basal polarization and mimicked structural aspects of the human cerebral cortex. Then, Eiraku *et al.* (12) recapitulated optic-cup development with dissolved Matrigel, which was the first instance of neural tissue being fully self-organized *in vitro*. In 2013, Lancaster *et al.* (13) developed the first 3D culture system for cerebral organoids. Neuroectoderm was generated from EBs and embedded in droplets of Matrigel, which could provide a scaffold for complex tissue growth. Then, the droplets were transferred to a spinning bioreactor or orbital shaker to continue the culture, and human brain organoids formed within two months (*Figure 1B*). This method generated unguided brain organoids, or cerebral organoids, which exhibited a variety of cell lineage identities, including forebrain, midbrain, and hindbrain.

Although the cell-type diversity in cerebral organoids

Table 1 The search strategy summary

Items	Specification
Date of search	The first search was conducted on November 1, 2021. The second search was conducted on July 1, 2022
Databases and other sources searched	PubMed and Web of Science's core collection
Search terms used	Combinations of terms related to the technology (brain organoid/cerebral organoid) and the disease (congenital brain malformation/structural brain abnormalities), as well as names of individual malformations (microcephaly/macrocephaly/lissencephaly/periventricular nodular heterotopia) appearing in article title, abstract or medical subject headings
Timeframe	Published between July 2000 to July 2022
Inclusion and exclusion criteria	All study types published in the English language were included
Selection process	The search was conducted by two authors (XSJ and XLJ) independently. Any disagreement between the two authors was resolved by a third author (MX)

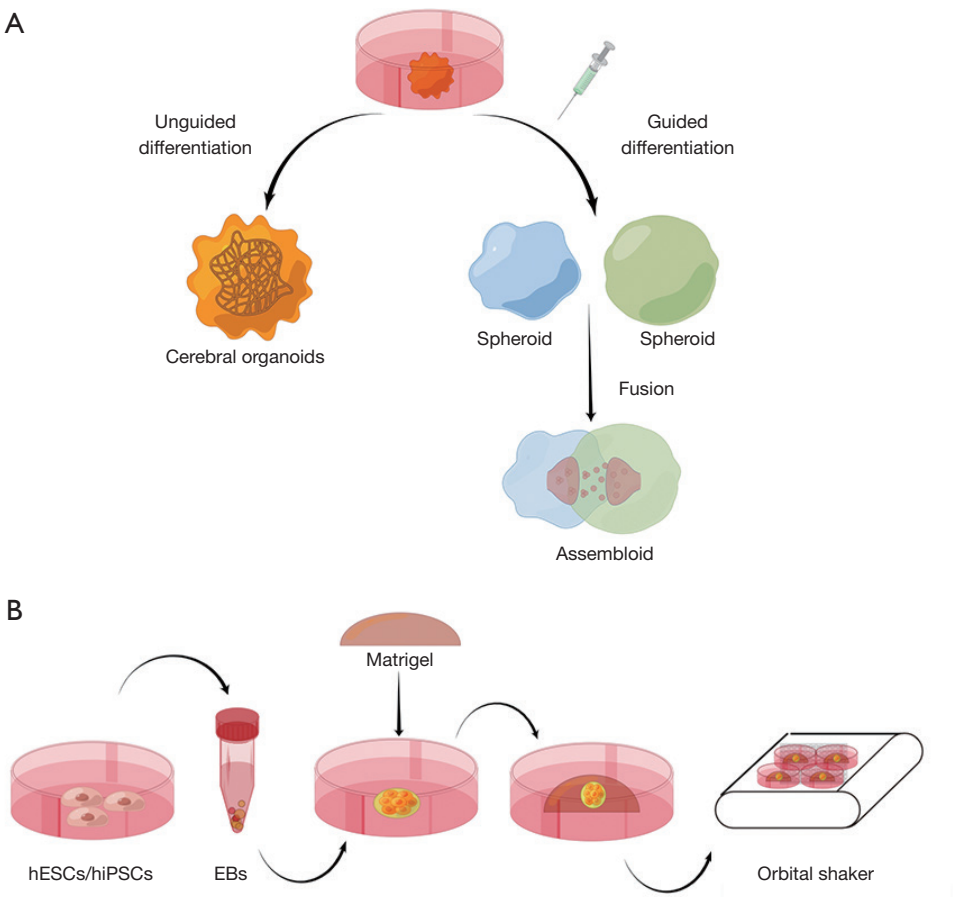

Figure 1 Current brain organoid methodologies. (A) A schematic of the 3D culture system. (B) Guided and unguided methodologies to generate brain organoids. hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; EBs, embryoid bodies; 3D, 3-dimensional. By Figdraw (www.figdraw.com).

sheds light on the interactions of different brain regions, the differentiation processes are fully dependent on cell spontaneous and intrinsic signals, leading to high variability and heterogeneity in different cell lines. Thus, small molecules were added during the differentiation process to form certain brain regions. For example, SB-431542, IWR1, and Dorsomorphin were added to induce forebrain organoids formation (14). WNT3A, SHH, and Purmorphamine were used to guide thalamic organoids (15). Recently, distinct region-specific organoids were fused to form an "assembloid" to study interregional interactions. For instance, Xiang *et al.* (16) studied interneuron migration by fusing medial ganglionic eminence organoids with cortical organoids. Furthermore, vascularized human brain organoids were generated by co-culturing them with endothelial cells or introducing hESCs expressing the endothelial-inducing transcription factor EVT2 (17,18). These advances provided precision tools to model brain development and more complex neurological disorders.

Modeling congenital brain malformations using organoids

So far, brain organoids have been used to study various kinds of congenital brain malformations. We mainly discuss the pioneering studies in microcephaly, macrocephaly, LIS, and PH (*Table 2*; *Figure 2*).

Microcephaly

Microcephaly is defined as a head circumference of 3 standard deviations (SDs) or more below the mean for the gestational age and gender (37). Primary microcephaly is mostly caused by autosomal recessive mutations. So far, 27 genes have been reported to be involved in microcephaly. Centriole and spindle biogenesis and impaired DNA damage represent the two most common mechanisms that are negatively affected (38). Furthermore, perinatal viral infections account for up to 50% of cases of primary microcephaly (39), including Zika virus (ZIKV), herpes simplex virus 1 (HSV-1), and human cytomegalovirus (HCMV) (40).

Brain organoids have helped reveal the mechanisms of microcephaly and illuminated the surprising roles of centrosomes and primary cilia in regulating neurogenesis. Brain organoids derived from microcephaly patients with mutations in centrosome-associated genes such as *CDK5RAP2* showed premature neuronal differentiation and a smaller size (13,22,24,26). Gabriel *et al.* (19) developed iPS-derived organoids which harbored a splice-site mutation in *CPAP* and observed longer cilia, retarded cilium disassembly, and delayed cell cycle re-entry leading to premature differentiation of NPCs. They suggested that cilia disassembly, cell cycle progression, and neuronal differentiation were causally related to each other, and cilium played a crucial role in NPCs maintenance. Li *et al.* (20) modeled the cellular defects of *ASPM*-dependent microcephaly by 3D brain organoids. They showed fewer oRGs which could not be recapitulated with rodents due to limited oRGs in their brains.

In addition to genetic conditions, brain organoids have been used to model microcephaly caused by neurotrophic pathogens. For example, ZIKV experimental exposure in brain organoids induced increased apoptosis and decreased proliferation of NPCs, leading to reduced organoid size (28,41,42). Recently, the upregulation of type I interferons was observed in ZIKV and HSV-1-infected organoids, which was not seen in 2D cultures, highlighting the superiority of brain organoids in disease modeling (43). Based on virus-infected brain organoids, several studies have tested compounds and drugs such as hippeastrine hydrobromide and duramycin for treating infected brain organoids, which rescued structural defects (44-48).

Macrocephaly

Macrocephaly is characterized by a head circumference of 2 SDs or more above the mean for the gestational age and gender (49). It can be used interchangeably with megalencephaly, a distinct term suggesting increased growth of the cerebral structure without hydrocephaly (50).

Increased proliferation of NPCs contributes to the expansion of the human cerebral cortex (51,52). Deletion of *PTEN* generated larger cerebral organoids, which displayed an expanded NPC pool and a transient neuronal differentiation delay in the fourth week (28). Similarly, Dang *et al.* (30) developed *STRADA*-mutant human cortical organoids, and they found enlarged sizes and increased neuroepithelial budding of these disease-specific organoids after 2 weeks. These organoids also exhibited increased cell proliferation, early apoptosis, and increased extraluminal primary cilia. They also displayed increased oRGs in the twelfth week, suggesting another potential mechanism for macrocephaly.

Interestingly, a consistent positive correlation between macrocephaly and the severity of autism spectrum disorder

Table 2 Application of 3D cerebral organoid in brain malformations caused by genetic deficits

Brain malformation	Gene	PSC	Brain region modeled	Phenotype of organoids	Molecular mechanism	Ref.
Microcephaly	*CDK5RAP2*	hiPSC	Whole brain	Smaller neural tissues, premature neural differentiation	–	(13)
Microcephaly	*CPAP*	hiPSC	Whole brain	Reduced neural epithelial tissues, increased numbers, and longer cilia	Loss of CC5 domain	(19)
Microcephaly	*ASPM*	hiPSC	Cerebral cortex	Less organized neuroepithelium	–	(20)
Microcephaly	*WDR62*	hiPSC or hESC	Whole brain	Retarded cilium disassembly, long cilium, and delayed cell cycle progression	Disruption of WDR62-CEP170-KIF2A pathway	(21)
Microcephaly	*KNL1*	hESC	Whole brain	Reduced proliferation of NPCs and premature differentiation	Generation of an exonic splicing silencer site	(22)
Microcephaly	*IER3IP1*	hESC	Whole brain	Neural progenitor loss	–	(23)
Microcephaly	*PTEN*	hPSCs	Whole brain	Reduced NPCs proliferation, promoted neuronal differentiation	Reduction of AKT pathway activity	(24)
Microcephaly	*16p11.2 DUP*	hiPSC	Cerebral cortex	Smaller in size	–	(25)
Microcephaly	*AUTS2*	hiPSC	Whole brain	Reduced NPCs proliferation, disrupted NPCs polarity	Alteration in WNT-β-Catenin signaling	(26)
Macrocephaly	*PTEN*	hiPSC	Telencephalon	Increased organoid size; decreased cell cycle length; overproduction of GABAergic neurons	–	(27)
Macrocephaly	*PTEN*	hESC	Whole brain	Expanded VZ/SVZ, increased size	Increased AKT signaling	(28)
Macrocephaly	*RAN39b*	hiPSC, hESC	Whole brain	Enlarged organoid sizes, increased NPC proliferation, a transient delay of differentiation, and increased output of neuronal production	Upregulation of PI3K-AKT-mTOR signaling activity	(7)
Macrocephaly	*CHD8*	hESC	Whole brain	Enlarged organoid sizes, increased NPC proliferation, imbalanced excitatory and inhibitory neuronal production	Affected mRNA metabolism	(29)
Macrocephaly	*STRADA*	hiPSC	Cerebral cortex	Enlarged size, delayed neurogenesis, increased outer radial glia	mTOR pathway hyperactivation	(30)
Lissencephaly	*17p13.3 DEL*	hiPSC	Whole brain	Decreased vertical divisions, reduced migration speed, and overproduction of deep-layer neurons	–	(31)
Lissencephaly	*17p13.3 DEL*	hiPSC	Forebrain	Reduced size and expansion rate	Disruption of N-cadherin/β-catenin/Wnt signaling	(32)
Microlissencep-haly	*KATNB1*	hiPSC	Whole brain	$TuJ1^+$ neurons fail to migrate out of the proliferating fields	Loss of p80 causes improper MT organization	(33)
Periventricular nodular heterotopia	*OLEKHG6*	hiPSC	Whole brain	Impaired ventricular surface and induced heterotopic neurons clustering	Activation of the small GTPase RhoA	(34)
Periventricular nodular heterotopia	*DCHS1, FAT4*	hiPSC	Whole brain	Defective NPC morphology and neuronal migration dynamics	–	(35)
Periventricular nodular heterotopia	ECE2	hiPSC	Whole brain	Ectopic localization of neural progenitors and neurons	Changes in actin and microtubule cytoskeleton	(2)
Periventricular nodular heterotopia	GNG5	hiPSC	Whole brain	Premature delamination and neuronal migration defects	–	(36)

PSC, pluripotent stem cell; hiPSCs, human induced pluripotent stem cells; hESCs, human embryonic stem cells; NPC, neuronal progenitor cells; CP, cortical plate; VZ, ventricular zone; SVZ, subventricular zone; 3D, 3-dimensional.

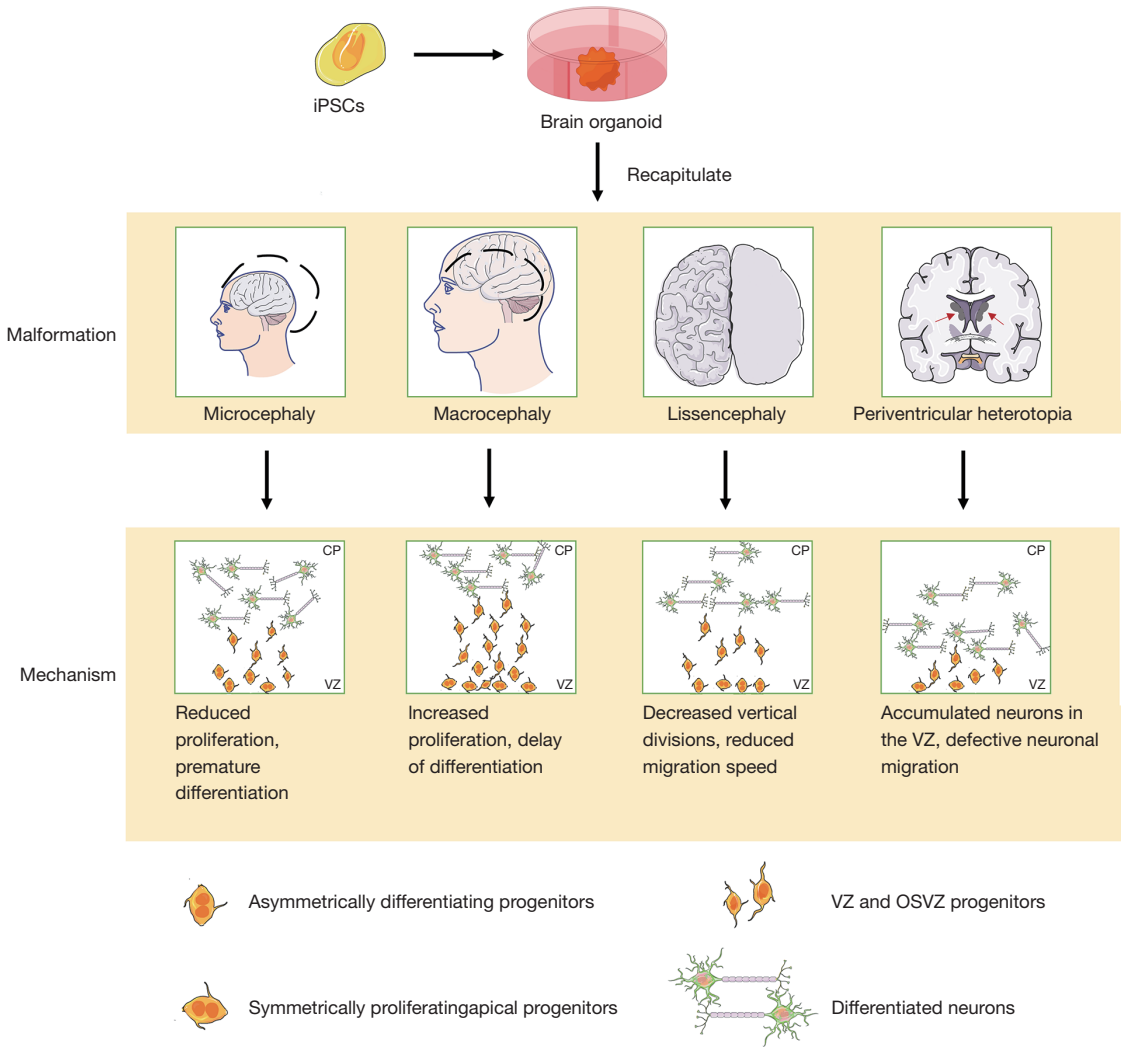

Figure 2 The use of brain organoids in modeling the mechanisms of congenital brain malformations (microcephaly, macrocephaly, lissencephaly, and periventricular heterotopia). iPSCs, induced pluripotent stem cells; CP, cortical plate; VZ, ventricular zone; OSVZ, outer subventricular zone.

(ASD) has been found using 3D neural cultures. Brain organoids derived from ASD individuals showed features of altered neurodevelopment, including decreased cell cycle length, increased cell proliferation, and unbalanced inhibitory neuron differentiation (27). Villa *et al.* (29) found that *CHD8* mutant organoids induced from patients with macrocephaly showed a cell-autonomous sustained proliferation of neural precursors. The overgrowth of neural precursors disrupted the balance between excitatory and inhibitory neuronal production, with delayed production of excitatory neurons. In terms of the mechanism, *RAB39b*-mutant organoids revealed the activation of PI3K-AKT-mTOR signaling, which contributed to macrocephaly and autistic-like behaviors (7). Altogether, brain organoids represented a novel method to investigate the molecular and anatomical paradigms of macrocephaly. The findings were consistent with the notion that individuals with macrocephaly had poor ASD clinical outcomes.

LIS

LIS, also called smooth brain, is a disorder involved in neuronal proliferation and migration characterized by a reduced number or absence of gyri and sulci (53). The

identified genetic causes of LIS include mutations in *PAFAH1B1*, *YWHAE*, *DCX*, *TUBA1A*, and *RELN*, most of which encode cytoskeletal proteins (54). Miller-Dieker syndrome (MDS), caused by a heterozygous deletion of 17p13.3 involving *PAFAH1B1* and *YWHAE*, is the most severe form of LIS.

As the mouse brain is naturally lissencephalic and short of a separate outer subventricular zone (oSVZ), the phenotypes in *Pafah1b1+/-* mice are substantially milder than in human patients. For the investigation of cell-type-specific defects in LIS, cerebral organoids were cultured from MDS patient-derived iPSCs (31). The MDS organoids recapitulated several MDS phenotypes, such as a significantly smaller size of the brain. An early defect in the expansion of the neuroepithelial cells was observed due to increased apoptosis and decreased vertical divisions, which would reduce NPCs pools. Also, the MDS neurons showed reduced track speed and straightness during migration, which could be functionally rescued by compensatory duplication of wild-type chromosome 17 (31). In another study, disruption of the N-cadherin/β-catenin/Wnt signaling axis was shown in MDS-derived organoids, leading to an imbalance of proliferation and differentiation in cortical progenitors (32). The phenotype could be ameliorated by external activation of Wnt signaling.

Although brain organoids have been successfully used to recognize the pathogenesis in human LIS, real cortical folding remains unachieved so far in cortical organoids (9). There have been efforts to engineer neuroepithelial "wrinkling" or "pseudo-folding" during early differentiation, either by inducing enhanced proliferation of NPCs through genetic manipulation or by using internal mechanical constraint in a microfluidic device (28,55-57). However, they do not lead to the formation of gyrification at the human brain level (58). According to statistics, the degree of folding scales with the surface area and the thickness of the cortical plate (CP), while organoids may be too small to achieve this (59,60). Better approaches may be needed to engineer organoids recapitulating the folding of the human neocortex.

PH

PH is another common neuronal migration disorder. It is characterized by a subset of cortical neurons that fail to locate correctly within the cerebral cortex and instead lining their sites of production as nodules (2). A few genes have been shown to cause PH, and high-throughput analysis has demonstrated extreme genetic heterogeneity (61).

O'Neill *et al.* (34) generated cerebral organoids from iPSCs derived from patients with a mutation in *PLEKHG6*. Ectopic clusters composed of neural progenitors and neurons were identified at the ventricular surfaces. The mutation also impaired the neuroepithelial lining and adherent junction belt. And functional analysis showed that the dysfunction of oRGs played an important role in the pathogenesis of PH. Similarly, mutations in *DCHS1*, *FAT4*, *ENE2*, and *GNG5* caused disrupted progenitor morphology and resulted in defective neuronal migration dynamics in brain organoids (2,35,36). Despite the altered neuronal migratory trajectories that have been demonstrated, an understanding of the molecular role of these genes during cortical development is only beginning to emerge.

Advances, limitations, and future perspectives of brain organoids

Brain organoids have proven to be a powerful system to understand congenital brain abnormalities because they provide a direct read-out for brain malformations and bridge the gap between 2D cultures and human brains. First and foremost, originating from human patients has made them better reflect the genetic background and physiology of the human brain than animal models. Also, compared with 2D cell cultures, brain organoids generate a complex architecture containing different neuronal subtypes, with proper spatial alignment and cell-cell interactions, which better recapitulates the dynamic developmental process of human brains.

Although brain organoids have greatly expanded our ability to model congenital brain malformations, there are still some hurdles to overcome (*Table 3*). First, fully organized six-layered laminar neocortical architecture and cortical folding remain unrealized, and one of the reasons is the insufficient oxygen and nutrient diffusion to the inner part of organoids. Many methodologies, such as modifications of EB size, combination with bioengineering constructs, and air-liquid interface culture, have been built to maintain oxygen and nutrient supply (57,62,63). Organoids with larger continuous cortical lobes were generated using a microscale internal scaffold to shape the organoids at the EB stage (64). Furthermore, the engineering of vascularized human brain organoids could supply oxygen and nutrient to the central region of organoids (17). Transplanting vascularized brain organoids into the mouse brain has been shown to generate functional

Table 3 Limitations, current solutions, and future perspectives in using brain organoids to study congenital brain malformations

Limitation	Current solution	Future perspective
Insufficient oxygen and nutrient diffusion	Modifications of EB size, combined with bioengineering constructs and air-liquid interface culture; vessel organoids; transplantation of organoids into animals	Vascularized organoids; organoids with six-layered laminar neocortical architecture and cortical folding
Incomplete cellular diversity	Transplant pre-differentiated cells to brain organoids; timed exposure to specific growth factors and hormones	Mature glia subpopulations in the same brain organoid
Low reproducibility	Use of micro-scaffolds, the addition of exogenous patterning factors, and the use of mini-spinning bioreactors; minimized use of variable ingredients; quantitative characterization	Increased reproducibility

EB, embryoid body.

neuronal networks and blood vessels (65).

Second, although some groups have developed astrocytes and microglia from iPSCs and transplanted them to brain organoids (66,67), morphologically and functionally mature glia subpopulations developing all in the same brain organoid are still out of reach. Recently, oligodendrocytes and myelin were generated in cortical spheroids and were used to recapitulate the pathologic process of a genetic myelin disorder (68). Future studies need to focus on complex structures underlying functional neuron-glia interactions.

Lastly, as the organoid differentiation protocols, especially the unguided methods, rely on spontaneous morphogenesis and intrinsic differentiation capacities of hPSCs, they suffer from batch-to-batch heterogeneity. Research has shown that inconsistent neural induction efficiency could be a main source of variability (69). Attempts to increase the homogeneity included the use of micro-scaffolds to arrange cells in an organ-like configuration (64), the addition of exogenous patterning factors to generate region-specific organoids (70,71), and the use of mini-spinning bioreactors with minimized volumes of variable ingredients and better-controlled conditions (72). In addition, newly generated organoids need to be qualitatively characterized to show consistent results in multiple batches (73).

Moving forward, integrating brain organoids with state-of-the-art technologies, such as lineage-coupled single-cell transcriptomics, long-term live imaging, and automated read-outs for high-throughput analyses, will help to exploit organoids to their full potential in clinical settings and translate well to patients' bedsides. The molecular diagnosis and treatment of congenital brain malformations are challenging for physicians, and most genes have not been characterized in depth. By combining CRISPR-Cas9 with lineage barcode, Esk *et al.* (23) established a loss-of-function screening in human organoids and identified 25 microcephaly-associated genes. In the aspect of treatment, the rescue of pathological phenotype in gene-corrected brain organoids provided a useful foundation for future research. Moreover, brain organoids should be used in high-throughput drug screening for congenital brain malformations. The creation of cell banks of hPSCs may provide a powerful resource for studies in this field and will enable studies to examine a larger cohort of patients (74).

Conclusions

The emergence of organoid technologies has opened new avenues for the recapitulation of human brain development, especially early neurodevelopment, and revealed the pathogenesis of congenital brain malformations. The accumulating advances enable increasingly accurate diagnoses and the development of personalized, targeted therapy for patients with congenital brain diseases. Several hurdles still need to be overcome, and future improvements will focus on combinations of *in vitro* vascularization and cutting-edge technologies.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-22-239/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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