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REVIEW

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Modeling genetic epilepsies in a dish

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Abstract

Human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells, provide a powerful platform for mechanistic studies of disorders of neurodevelopment and neural networks. hPSC models of autism, epilepsy, and other neurological disorders are also advancing the path toward designing and testing precision therapies. The field is evolving rapidly with the addition of genome-editing approaches, expanding protocols for the two-dimensional (2D) differentiation of different neuronal subtypes, and three-dimensional (3D) human brain organoid cultures. However, the application of these techniques to study complex neurological disorders, including the epilepsies, remains a challenge. Here, we review previous work using both 2D and 3D hPSC models of genetic epilepsies, as well as recent advances in the field. We also describe new strategies for applying these technologies to disease modeling of genetic epilepsies, and discuss current challenges and future directions.

KEYWORDS

brain organoid, epilepsy, genome editing, human pluripotent stem cells, neurological disorder, seizure disorder

1 | INTRODUCTION

Epilepsy is one of the most common neurological disorders with a prevalence in the U.S. of 1.2%.[1] The hallmark feature of epilepsy is spontaneous recurrent seizures, but the disease encompasses a wide range of etiologies, behavioral manifestations, electrographic signatures, pharmacological profiles, and histopathologies.[2] Epilepsy also has a high incidence early in life and is associated with increased mortality. Approximately 30%-40% of patients cannot be adequately controlled with anti-epileptic drugs.[3] Only a small portion of medically refractory patients are surgical candidates, but most of these candidates never receive an evaluation for resective brain surgery. Unfortunately, no therapies exist for preventing epilepsy that may occur from various causes, such as after head trauma, stroke, or other neurological insult, from a genetic predisposition, or a malformation of brain development. Overall, epilepsy poses a huge burden to patients and their families.

The number of genes associated with epilepsy has exploded since the first gene linked to a clinical seizure phenotype was discovered nearly three decades ago.[4] To date, over 500 loci are linked to epilepsy,[5] but our understanding of how these mutations lead to spontaneous seizures and other epilepsy manifestations lags behind gene discovery. Traditional strategies to model genetic epilepsies have involved the use of rodent models, obtained through either genetic modification or finding spontaneous mutations within rodent colonies. Despite many advances made studying animals, these models do not fully recapitulate human disease phenotypes and mechanisms, likely because human brain development differs considerably from that of the rodent brain in many critical aspects.[6-8] In addition, many genetically modified mouse models of epilepsy fail to show spontaneous recurrent seizures (the hallmark of epilepsy) and may only exhibit signs of cortical network excitability through testing of seizure thresholds after chemoconvulsant administration. Limited scalability of rodent models also constrains the possibility of performing rapid mutation-

specific mechanistic studies and drug screening. Postmortem tissues or surgical specimens from patients are difficult to acquire and those available generally represent late-stage disease, offering limited insight into causative mechanisms. Zebrafish models of genetic epilepsies are gaining traction and are extremely amenable to high-throughput drug screening.[9] The fish models have only been useful for interrogating loss-of-function epilepsy genes, although recent advances in gene editing technologies may allow for epilepsy missense mutations to be interrogated in zebrafish models in the future.[10] Consequently, decades of research have not produced profound mechanistic insights into any of the many epilepsy syndromes, including the monogenic epilepsies.

The derivation of human embryonic stem cells (hESCs) from blastocysts by the Thomson laboratory in 1998[11] and the generation of mouse induced pluripotent stem cells (iPSCs) through somatic-cell reprogramming by Yamanaka and colleagues in 2006[12] opened the door for translational research using stem cells in disease modeling and therapeutic development. The discovery of mouse iPSCs was quickly followed by the generation of human iPSCs.[13,14] Soon after, iPSC disease modeling was developed by differentiating the cells into tissues relevant for investigating specific diseases.[15,16] Patient iPSCs carry the identical mutation(s) in the context of the patient's specific genetic background. Given the inability to acquire disease tissue for in vitro study from a human subject with a central nervous system (CNS) disorder during the subject's life, the differentiation of hPSCs to neural cells or brain-like structures offers an unprecedented opportunity to model human brain development and brain disorders.

Various protocols have been developed to differentiate hPSCs to neural cells that are most relevant to models of epilepsy, such as cortical-like excitatory neurons,[17,18] cortical-like inhibitory neurons,[19-21] dentate granule cell-like neurons[22] and astrocytes.[23] These neurons in 2D cultures form functional synapses and eventually neuronal networks. To better recapitulate the architecture of the developing human brain, efforts have been made to generate 3D cerebral organoids from iPSCs.[24,25] Some of these brain organoids have features that resemble cerebral cortex, ventral telencephalon and also hippocampal anlage.[25,26] More recent advances include the development of more uniform and defined brain region-specific organoids/spheroids.[27-32] Progress in neural differentiation protocols also makes iPSCs very appealing for stem cell-based transplantation therapies for neurological disorders, an area that is being actively studied.[33-37] The application of genome editing to hPSCs further enhances the utility of in vitro human cell models of epilepsy. Genome editing technologies, such as TALEN (transcription activator-like effector nuclease) and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 nuclease) systems can be used to modify hPSCs directly,[38,39] or simultaneously with fibroblast reprogramming.[40,41] This approach is particularly useful given the limited access to subjects with rare epilepsy mutations, or to study the effects of specific mutations in an isogenic control background.

To date, iPSCs have been generated for well over two dozen different CNS disorders[42,43] and the number continues to grow rapidly. hPSCs have been used to model a wide array of neurological disorders with nearly completely penetrant epilepsy phenotypes, such as Dravet syndrome (DS),[44,45] Angelman syndrome (AS),[46] variant Rett syndrome caused by cyclin-dependent kinase-like 5 (*CDKL5*) mutations,[47,48] and syntaxin-binding protein 1 (STXBP1)-related epileptic encephalopathy.[49,50] This approach has also been employed to model other neurodevelopmental disorders with seizures as one of the comorbidities, including tuberous sclerosis complex (TSC),[51-55] typical Rett syndrome,[56-60] fragile × syndrome,[61-63] Timothy syndrome,[64,65] 15q11.2 deletion syndrome,[66] Phelan-McDermid syndrome,[67] and Miller-Dieker syndrome.[68] More recently, hPSC cultures have been used to study microcephaly resulting from ZIKV exposure.[69] Although whole animal models still remain critical for studying various behavioral and electrophysiological features of human epilepsies, "epilepsy in a dish" models are becoming critical platforms to study disease mechanisms and to develop patient-specific therapeutic interventions.[70,71] Below we review recent advances in hPSC technology (with a focus on genome editing) and iPSC-derived 2D and 3D neural culture methods, as well as their applications for disease modeling. Finally, we discuss the challenges and potential future directions of these model systems.

2 | GENERATION AND GENE EDITING OF IPSCs

The generation of patient-specific iPSC lines enables a wide repertoire of genetic epilepsies to be modeled in culture. iPSCs are generated by the forced expression of specific transcription factors in somatic cells,[12] a process called "reprogramming," to reset the epigenetic state of the cells similar to the pluripotent state of hESCs derived from preimplantation human embryos. Most often, the reprogramming is performed on dermal fibroblasts isolated from a skin biopsy,[72] but more recently hematopoietic cells[73,74] or kidney epithelial cells derived from urine[75,76] have been used. iPSCs can self-renew and have the potential to generate cells in the three germinal lineages and their subsequent progeny. Therefore, they provide a potentially unlimited source of starting material from which they can be differentiated into various cell types for disease modeling, drug screening, toxicity studies, or regenerative therapies.

The utility of iPSCs can be further expanded by genome editing, particularly using the CRISPR/Cas9 system, which can make precise changes in cellular genomic DNA[77-80] or can be

used to modulate gene expression.[81] This two-component system consists of a Cas9 nuclease and a single guide RNA (sgRNA) that mediates DNA binding specificity. In brief, Cas9 nuclease is targeted to a genomic DNA sequence of interest where it introduces a double-strand break (DSB). The DSB triggers the endogenous DNA repair machinery to repair the break via one of two pathways: an error-prone nonhomologous end-joining (NHEJ) pathway or a homology directed repair (HDR) pathway.[82] The NHEJ pathway joins DNA ends in a manner that often introduces random insertion or deletion (indel) mutations.[83] These indels can introduce frame shifts and premature stop codons when targeted to open reading frames, thus disrupting gene function through nonsense-mediated mRNA decay (NMD) mechanisms.[84] Alternatively, when the HDR pathway is activated in the presence of a donor template with sequence homology to the region flanking the DSB,[85] the breaks can be repaired seamlessly with insertion of the donor nucleotides. Changes of either single nucleotide or large exogenous DNA sequences can be introduced into the genomic region of interest when it is included in the repair template.

Using CRISPR/Cas9 genome editing, any gene of interest, in theory, can be deleted or altered to interrogate its role in biological processes, while circumventing difficulties in obtaining patient tissues and allowing for comparison with an isogenic control.[86] Loss of function (LOF) "virtual patient" lines can be generated through the NHEJ pathway. Patient-specific mutations, including inherited or de novo germline mutations, can also be edited into a control iPSC line through HDR-mediated repair when a template carrying the mutation is provided. Generating additional "virtual patient" lines from the same genetic background also allows for direct comparisons among specific patient mutations.[38] However, HDR-mediated repair is less efficient than NHEJ. The repair efficiency can vary by an order of magnitude among cells of different types or even those of the same type but of different genetic backgrounds.[87] Moreover, a potential limitation of using "virtual patient" lines in a control iPSC line is the loss of patient-specific genetic modifiers. Therefore, combining this approach with patient-specific material is often ideal for confirming cellular phenotypes associated with specific mutations. Alternatively, CRISPR/Cas9 based genome editing may be used to reverse a genetic variant of interest to the wild-type status.[88] Since genotypic variability is a major driver of phenotypic variability, isogenic controls are vital to determine causality between genotype and phenotype. Such isogenic models enable subtle phenotypes to be examined, which might not be possible when comparing cells derived from two different individuals.[89] This approach is becoming a standard practice in the field.

Another powerful application of CRISPR/Cas9 technology is the introduction of exogenous sequences into a specific genomic locus via the HDR pathway to serve as a reporter for the activity of that gene.[90] When this approach is used to fuse a fluorescent protein sequence or an epitope sequence to an endogenous open reading frame (also called endogenous/in frame tagging), the subcellular localization, and dynamics of the encoded fusion protein can be analyzed under endogenous physiological conditions.[91] In addition, the knock-in of reporter genes to label specific cell populations enables phenotypic analyses to be carried out in the cell types of interest.[92,93] In particular, the insertion of reporter genes into developmentally important loci such as FEZF2,[94] OLIG2,[95] or NKX2.1[96] has facilitated the identification and characterization of target neuronal cell types that can be used for disease modeling or transplantation. However, inserting a large sequence requires HDR with a correspondingly large, exogenously provided repair template, an inefficient process in human cells. Nevertheless, endogenous tagging offers a major improvement over conventional overexpression systems or bacterial artificial chromosome (BAC) approaches,[97] especially for live imaging[91] and functional studies.[98,99] The ability to endogenously tag proteins allows analysis of the biochemical activity of epilepsy-related proteins for which there are no validated antibodies. Combined with phenotypic analysis in patient iPSC models, this approach provides a key molecular tool to determine underlying cellular mechanisms.

Despite the rapid evolution of genome editing, the approach still has multiple technical challenges that prevent efficient genome editing in established iPSCs.[100] First, unwanted off-target mutations may occur. Although recent whole-genome sequencing results show that off target editing by CRISPR/Cas9 is very low in human stem cells,[101-103] this problem has been further reduced through additional strategies such as designing sgRNAs with very high specificity scores, using a modified Cas9 nuclease with improved binding specificity to sgRNAs, testing sgRNAs with different lengths and PAM sequences, and the delivery of purified gRNA and Cas9 protein as a CAS9-ribonucleoprotein (RNP) complex. Second, a clonal line with homogeneous genetic background is difficult to obtain from established iPSCs that do not recover well from single-cell clonal isolation processes.[104] The addition of a rho-associated coiled-coil kinase (ROCK) inhibitor,[104] commercially available supplements for single cell isolation, or physiologic oxygen (2%) culture conditions[105] have been reported to enhance clone recovery during this harsh selection process. Some of these treatments also likely reduce the development/accumulation of spontaneous chromosomal aberrations over prolonged time in culture.[105]

An alternative strategy to overcome the obstacle of making clonal genome-edited iPSC lines is to perform simultaneous reprogramming and CRISPR/Ca9 gene editing from somatic tissues. This approach avoids multiple rounds of clonal picking, which enables the generation of multiple edited and unedited iPSC lines simultaneously. It also requires considerably less time and resources compared to

conventional multistep protocols that can take at least several months to complete.[40,41] However, this approach is much more suitable for gene knockout than for HDR-based mutational insertion/correction, which is the less efficient of the two processes. Third, the efficiency of seamless genome editing of specific mutations or correction of patient mutations through HDR in the absence of selectable markers is very low. It can be painstakingly difficult to isolate an edited clonal line, which often makes it time- and cost-prohibitive. However, several factors may be used to improve the process. One is the delivery of purified gRNA and Cas9 protein as CAS9-ribonucleoprotein (RNP) complex to avoid the inconsistency of in vivo protein transcription and translation. This approach improves the efficiency of genome editing in human stem cells[78,106,107] and reduces off-target mutations associated with plasmid transfection and indels in the allele that does not need to be modified given the reduced temporal availability of the Cas9 protein.[106] Chemically or structurally modified sgRNAs that bind to Cas9 nuclease more tightly and are less prone to degradation may also significantly enhance the efficiency of CRISPR/Cas9 mediated genome editing.[108,109] Lastly, compounds that either inhibit the NHEJ pathway (ie, SCR7 and RS-1) or enhance HDR efficiency (ie, L755507, brefeldin A, E1B55K and E4orf6) can improve the efficiency of mutational insertion/correction in human stem cells, although the in vitro toxicity of these small molecules and proteins still requires extensive evaluation (recently reviewed in Reference 100).

More recently, hPSC lines with stable or inducible expression of Cas9 protein through lentiviral integration have been developed that allow genome-wide screening. The screening is done by delivering thousands of gRNAs (several gRNA libraries are available) into stem cells with high efficiency.[110,111] For human use, it will be essential to reduce CRISPR off-target effects, improve editing efficiency, and exploit novel delivery strategies at a low cost and with high safety (not further discussed here). Nevertheless, the CRISPR/Cas9 based genome-editing system has become a powerful platform for helping investigators determine how a specific mutation affects cellular function and for generating high-throughput platforms for drug screening in a dish.

3 | TWO-DIMENSIONAL NEURAL DIFFERENTIATION

Differentiating hPSCs into disease-relevant neural cell types offers great potential for studying the development and progression of neurological disorders. One of the goals of such studies involving genetic epilepsies is to generate cortical networks in vitro that closely resemble those found in vivo. Various protocols have been developed to derive neural cells from hPSCs that are most relevant to epilepsy, including cortical-like excitatory neurons,[17,18] cortical-like inhibitory neurons (γ-aminobutyric acid [GABA]-expressing interneurons),[19-21] and hippocampal-like neurons.[22,112] Recent developments in optimizing the protocols of neural differentiation also make it possible to generate astrocytes,[113-115] midbrain dopamine neurons[116,117] and spinal motor neurons[118,119] from hPSCs. Most of these protocols involve the application of small molecules to hPSC monolayers or the forced expression of specific transcription factors. Small molecule differentiation can recapitulate some in vivo neurodevelopmental processes such as inducing ventricular zone (VZ)-like structures and transcriptional signatures. However, the resulting neural cultures are often heterogeneous and vary between cell lines.[120] Compared to small molecule differentiation, induced differentiation using transcription factors allows rapid generation of more homogeneous cultures that exhibit reproducible neuronal properties (2 weeks vs 4-6 weeks for small molecule differentiation).[121] However, the induced differentiation approach bypasses normal signaling pathways during neural development such that some developmental phenotypes in hPSC models of neurodevelopmental disorders may be difficult to interpret or completely absent.[122] Below, we focus on the differentiation of forebrain cortical-like excitatory and inhibitory neurons that are relevant to the epilepsies.

3.1 | Small molecule differentiation

Based upon knowledge of the molecular cues underlying embryonic brain development, various protocols have been developed to direct hPSCs toward specific neuronal cell lineages. To differentiate iPSCs into forebrain neurons, one of two categories of protocols is typically used: dual inhibition of the SMAD signaling pathways (BMP and TGF-β) in a monolayer culture,[17,20,123,124] or embryoid body (EB) differentiation.[19,125-129] SMAD signaling is inhibited with proteins such as Noggin and Dickkopf-1, or small molecule inhibitors, such as SB-431542, LDN193189, and dorsomorphin, to promote the differentiation of PSCs into a forebrain neural lineage. In the monolayer protocols, iPSCs are plated as an adherent monolayer followed by rapid induction of a neuroepithelial sheet through dual inhibition of the SMAD signaling pathways (Noggin and SB-431542 used in Reference 17, SB-431542 and dorsomorphin used in Reference 124, SB-431542 and LDN193189 used in Reference 123). In EB protocols, hPSCs are allowed to form aggregates in suspension in the absence of exogenous growth factors or small molecules. EBs are then plated onto an adherence-promoting substrate and cultured with dual SMAD inhibitors, promoting formation of definitive neuroectoderm.[126,128,129] Alternatively, the EB method has been modified by the direct application of dual SMAD inhibitors into hPSC aggregate culture, and then cell aggregates are plated as adherent cultures.[125,127] After

definitive neuroectoderm develops and organizes into neural rosette-like structures, the neural rosettes are selected for continued growth and differentiation. Following the rosette selection, cortical development of the monolayer cultures is coupled with sequential expression of markers for the appropriate cortical laminar projection neurons.

Compared to the monolayer protocol, EB aggregate protocols generate a higher yield and purity of neurons,[130] and also result in slightly more mature neurons with longer neurites.[120] Notably, electrophysiological analysis did not reveal significant differences between these two differentiation protocols and indicated that human neurons cultured in vitro require prolonged culture durations to reach synaptic maturity.[120] Most protocols generate functional but still immature cortical neurons after ~4-6 weeks from the start of the differentiation. One recent report showed that early-born cortical neurons with functional electrophysiological properties could be generated within 8-16 days with combinatorial application of six pathway inhibitors,[123] although this has not yet been replicated. Variations among the differentiation protocols can result in a bias toward development of either excitatory or inhibitory neurons, depending upon modulation of the sonic hedgehog (SHH) pathway after the development of a primitive neuroepithelium. For example, one can generate GABAergic interneurons by patterning the neuroepithelium into a homogenous population of NKX2.1-expressing MGE-like progenitors in about 6 weeks using SHH and/or the smoothened activator purmorphamine to activate the SHH pathway.[19,20]

3.2 | Transcription factor induced differentiation

Compared to the small molecule differentiation method, a quicker way to generate neurons from hPSCs is to force the expression of lineage-determining transcription factors. We refer here to these transcription factor-induced neurons as iNeurons. The induced expression of Neurogenin-2 (NGN2), for example, allows one to generate functional excitatory neurons within 2 weeks.[121] Analysis of these neurons shows an overall similar transcriptional expression profile except for lower expression of deep-layer neuronal markers, such as TBR1,[131] compared to that of those derived from EBs. These NGN2-induced neurons display short-term plasticity, synaptic function and modulation, and the ability to functionally integrate when transplanted into the mouse brain.[121,131] Transient expression of a combination of the transcription factors ASCL1 and DLX2, or these two factors plus LHX6 and a specific microRNA generates functional GABAergic interneurons from hPSCs with a high degree of synaptic maturation.[132,133] These inhibitory iNeurons consist of various populations of GABAergic neurons that express subtype-specific markers and display inhibitory synaptic function. They can also integrate into host synaptic circuits after grafting in vivo.[132] When co-cultured with NGN-2 excitatory iNeurons, the generation of these defined populations of functionally mature human GABAergic iNeurons provides a new platform for the study of diseases affecting inhibitory synaptic transmission A remaining challenge is that none of the current protocols reliably generates parvalbumin-expressing fast-spiking interneurons, a cell type critical for many epilepsies and other neurological and psychiatric disorders.

4 | THREE-DIMENSIONAL HUMAN BRAIN ORGANOID MODELS

The human brain is one of the most complex organs in the animal kingdom, and has more types of neurons and regional diversity than that of other mammals.[6,134] One critical aspect of human brain development is the generation of an outer subventricular zone (oSVZ), which contains a large population of outer radial glia (oRG; also known as basal RG—bRG) progenitor cells. oRG are present in large numbers in gyrencephalic primates[135-137] and are considered pivotal to the evolutionary increase of human cortex surface size and complexity.[138] The oRG population is largely absent during mouse brain development.[139] Therefore, human cellular models provide a unique opportunity to study this cell population. Even more compelling would be to study these and other neural progenitors in the developing human brain using a cell culture system that models the 3D architecture of human forebrain neural progenitor zones and the developing cortical mantle. A recently developed cell culture technology, known as cerebral organoids, brain organoids, or cortical spheroids, now enables this approach. The general method involves growing hPSC aggregates in suspension such that they differentiate into self-organizing cerebral-like 3D neural structures.[24,25] Depending upon the specific protocol, the structures resemble diverse regions of brain and recapitulate several key in vivo features of brain organogenesis. Thus, they are attractive models for studies of unique aspects of brain development, and allow modeling of human brain disorders in 3D. A growing number of protocols have been developed to differentiate organoids that partly recapitulate specific CNS regions such as the hippocampus,[140] midbrain,[141,142] hypothalamus,[29] cerebellum,[143] thalamus,[31] oligodendrocytes/white matter,[144] anterior pituitary,[145] and retina.[146] Here we highlight recent advances in generating forebrain cerebral organoids that contain cortical glutamatergic neurons and cortical interneurons.

Although neurons in monolayer cultures can develop functional synapses and form neuronal networks, traditional 2D cultures are still limited in their ability to recapitulate human brain cytoarchitechture and to be maintained for the extended time periods necessary for human neural

development. Neurons in brain organoids arguably show greater neuronal maturation than those from 2D monolayer-derived neurons.[28] The decreased maturation potential of 2D cell cultures may reflect a lack of the complex extracellular environment necessary to generate appropriate cell-cell connections and extracellular matrix interactions. This shortcoming and the ability for prolonged (> 1.5 years) culturing of organoids make 3D tissue-like structures a more promising model to achieve mature human brain-like networks. The 3D culture models are not just more heterogeneous and complex than 2D neural cultures, but also more physiologically relevant to structures of human brain in terms of the spatial organization of tissues and electrophysiological properties of neural cells.[147-152] Cerebral organoids generate a laminar organization that partly recapitulates human cortical development, such as defined multilayer progenitor zone organization (VZ and inner/outer SVZ) and diverse neural cell types with identities of potentially all six layers of developing neocortex. Many studies have described the appearance of astrocytes[28,29,153] and less frequently oligodendrocytes,[154] in cortical organoids during later stages of differentiation. More recently, human oligodendrocyte-lineage containing brain organoids were developed to generate oligodendrocytes that are transcriptionally similar to human fetal oligodendrocytes and mature both morphologically and electrophysiologically over time.[144,154,155]

Protocols for generating cerebral organoids from hPSCs are evolving rapidly in this fast moving field. These protocols often require two processes: the establishment of neural identity and the recapitulation of 3D structural organization. One approach for generating cerebral organoids is known as the "intrinsic protocol" based on the self-organizing potential of stem cells. As a result, each organoid is typically comprised of diverse cell types found in forebrain, hindbrain, and retina.[25,26] More recently, this self-organizing approach has been modified to generate region-specific brain organoids. The method depends on the specific temporal application of extrinsic signaling molecules (reviewed in Reference 149) that pattern EBs and neural progenitors to adopt the fate of the desired brain region.[27-29,31,32] For generating forebrain cortical organoids, hPSCs are allowed to develop into EB-like aggregates, which have three germ layer differentiation potential. Subsequent suspension culture of EBs in a minimal medium, in the presence of SMAD signaling inhibitors, inhibits mesoderm and endoderm formation and instead promotes the formation of neural rosettes, a polarized organization of neuroepithelial cells and the later source of diverse neural progenitors. EBs are then embedded in a Matrigel scaffold[25,29,156] or cultured in medium with a low concentration of Matrigel[30,157,158] to support the self-organization of the neuroepithelium and to induce the correct polarity signals for the development of an apicobasally polarized neuroepithelial bud. The neuroepithelium further develops into structures that are remarkably similar to the VZ and SVZ regions of the developing human brain. These include an oSVZ region that contains oRG cells that are crucial for human cortical expansion.[6] The support by Matrigel in some protocols seems to be critical for the building of in vitro brain structures.[29] Later agitation, such as those provided by spinning bioreactors,[29] or mechanically cutting the organoids[30] to remove the necrotic center promotes the development of much larger and more uniform cortical organoids. The organoids contain fluid-filled cavities that resemble brain ventricles,[25] and generate neurons with identities of deep and superficial layers of neocortex. A simplified approach for cortex-specific organoid generation that does not require extracellular matrix support and media agitation also yields cortical spheroids with well-defined segregation of superficial and deep cortical layers.[28,31,32]

Much of the initial effort was focused on cortical organoids, emphasizing the development of glutamatergic cortical neurons. Few interneurons are found in standard cerebral organoids/cortical spheroids[28,29] because the organoids are generated from protocols that promote dorsal cell fates through inhibition of WNT and SHH pathways and thus lack tissue resembling the ventral ganglionic eminences. To rectify this problem, ventralized organoids containing GABAergic interneurons have been generated. Similar to the generation of GABA interneurons in 2D culture, the EBs can be pattered to ventral forebrain organoids/spheroids by combined activation of the SHH pathway and WNT inhibition,[26,27,32,159] or SHH pathway activation alone.[30] These ventral organoids develop ventricular zone (VZ)-like structures expressing *NKX2-1* and later generate GABAergic interneuron subtypes. They also can be fused to dorsalized glutamatergic organoids in vitro.[27,32,159] The fused forebrain assemblies demonstrate the migration of interneurons from the ventralized (GE-like) organoid into the dorsalized organoid and establishment of circuits with glutamatergic cells, mirroring normal developmental processes observed in the fetal forebrain.[27,32,159]

In terms of neuronal function, organoids can be grown for more extended periods of time compared to 2D cultures, allowing for increased neuronal maturation. Initial studies showed the presence of slow neuronal calcium waves and action potentials induced by current injections.[25,28,29] Both glutamatergic and GABAergic synaptic transmission is present in cortical organoids.[29] Evoked action potential firing rates and excitatory synaptic inputs increase substantially in fused forebrain organoids that are comprised of glutamatergic and GABAergic neurons in a more physiological ratio.[27] Prolonged culture (up to 20 months) of human cortical spheroids also promotes astrocytic maturation.[153] Recent studies showed that spontaneous neuronal network activity could be detected in 8-month-old brain organoids through extracellular recordings with high-density silicon microelectrodes.[156] Loss of this activity in

the presence of neurotransmitter receptor antagonists suggests the presence of synaptic networks in the organoids. Thus, this technology offers new opportunities to study both neuronal dynamics in developing human brain-like structures and the neural circuitry underlying disease mechanisms of epilepsy and other neurological disorders.

5 | ASSAYS FOR MEASURING HPSC-DERIVED NEURONAL ACTIVITY AND DIFFERENTIATION

Various methods can be used to determine the function of neurons generated from hPSCs. Traditional electrophysiologic techniques, such as patch-clamp recordings, provide insight into intrinsic cellular and synaptic properties that may lead to epilepsy.[44,67] Such studies can be performed on 2D neuronal cultures, but they also can be applied to acute slices or dissociated cells from organoid cultures. Moreover, electrophysiological activity of hPSC-derived neurons transplanted into the rodent brain may be assayed in ex vivo brain slices.[36,160] However, patch clamp techniques are labor intensive, and typically only permit recording from individual cells at a single time point during development. As an alternative, tissue culture plates with embedded multielectrode arrays (MEAs) may be used to measure extracellular field potentials of neuronal cultures.[161-163] MEAs allow for longitudinal real-time measurements of spontaneous activity from dozens of neurons under normal culture conditions. This technique also allows for repeated recordings from different cell types grown under various experimental conditions in multiple-well format. MEA recordings are often combined with drug application. For example, one study used such recordings to identify a drug that blocks hyperexcitability in ALS patient iPSC-derived motor neurons.[164]

Another approach for assaying functional activity of iPSC-derived neurons is to use optical Ca^{2+} imaging to detect transient increases of calcium concentration in active neurons. This imaging is often accomplished by using either bulk loading of chemical-based fluorescent calcium indicators,[64] or by expressing genetically encoded indicators of Ca^{2+} concentration such as GCaMP6.[165-167] For example, in studying Timothy syndrome caused by a mutation in the $Ca_v1.2$ calcium channel, the investigators used the Fura-2 dye to show that the phenotype of dendrite retraction is independent from calcium flux through the channel.[64] Calcium oscillations can be monitored to show network formation through automated and non-invasive imaging in 96- or 384-well plates.[168-171] Calcium imaging has been performed in either intact or dissociated cerebral organoids to demonstrate that spontaneous surges of intracellular calcium correspond to electrical activity.[25,28,32] In addition, in vivo two-photo calcium imaging systems can be applied for longitudinally measuring neuronal network activities of grafted organoids in rodent brain.[172]

Although Ca^{2+} imaging is applied to multiple cells simultaneously, it typically lacks the temporal resolution to reliably distinguish individual action potentials and does not reliably measure local field potentials. In contrast, high-density silicon microelectrodes have recently been developed to obtain scalable spatially oversampled neural recordings in live mammalian brain. Each probe comprises up to 1000 electrode pads on five shanks with 200 recording sites per shank.[173] This approach makes it possible to record large populations of single unit activity from multiple brain structures in freely moving animals. Application of this technology to 8-month-old human brain organoids enabled the detection of spontaneously active neuronal networks.[156]

In addition to electrophysiologic and calcium imaging studies, image-based techniques are available to study the morphology of iPSC-derived neurons. Measuring neurite outgrowth in culture is perhaps the most common phenotypic assay to study neuronal maturation in vitro.[174] In addition to neurite length, the complexity of neurite branching, number of extensions per cell, and number of neurites per cell can be measured by image segmentation. Sholl analysis[175] is often used and provides the number of dendrite intersections against the radial distance from the soma center.[176] Recent developments in automated live-cell imaging systems allow for longitudinal and high-throughput phenotypic screens of neurite outgrowth of iPSC-derived neurons in monolayer cultures.[177-180] Assessing neuronal morphological changes is helpful for understanding the mechanisms of neurological disorders, as well as quantifying the effects of potential drug treatments.[181]

Lastly, molecular characterization such as transcriptome analysis offers a powerful strategy to provide valuable insight into complex biological systems, including the CNS, using molecular signatures. Conventional bulk transcriptome analysis using RNA sequencing (bulk RNA-seq) provides the average transcriptional patterns for entire cell populations. In contrast, single-cell RNA sequencing (scRNA-seq) enables high-throughput (up to thousands of cells per experiment) analysis of an entire transcriptome at a single-cell level.[182-184] scRNA-seq often involves four major steps: isolating single cells, capturing their transcripts, generating sequencing libraries in which the transcripts are mapped to individual cells, and analyzing and interpreting scRNA-seq data. Due to technical noise (ie, low amount of starting material and high drop out rate) and inherent biological variation, scRNA-seq often produces nosier and more variable data than bulk RNA-seq.[185] In bulk RNA-seq experiments, batch effects can be minimized by preparing sequencing libraries from biological replicates in parallel and then sequencing them simultaneously. However, in scRNA-seq experiments, this is not feasible, as cells from

different conditions are often captured and prepared independently due to the lower throughput of this platform.[186] Appropriate controls and a minimum of three biological replicates per condition are recommended, and also functional validation is an important step to assess the relevance of these data to the biological context.[186]

Although plenty of improvements are in need, scRNA-seq is best suited to efficiently reveal the complex cellular diversity in brain organoids,[187] track the trajectories of distinct cell lineages in development,[27,32,156] and to understand organoid-to-organoid variability.[156,158] Camp and colleagues were the first to directly compare cerebral organoids with human early fetal neocortex using scRNA-seq based on the Fluidigm system and found that in vivo cell lineages and gene expression programs were largely recapitulated in the organoid cortical regions.[187] A major limitation of this study was the low throughput of scRNA-seq technology (less than 1000 cells per experiment). Quadratto et al. substantially advanced the characterization of self-patterned whole-brain organoids using a higher throughput scRNA-seq microdroplet-based microfluidics platform.[156] Using this Drop-seq based method the authors sequenced over 80 000 cells from 31 brain organoids and found that neurons in multiple lineages progressively matured over time at the transcriptome level. Notably, the authors also reported significant organoid-to-organoid variability related to bioreactor-based batch effects identified in the scRNA-seq analysis,[156] which was confirmed by a recent study using a similar approach to generate self-patterned multi-region brain organoids.[158]

6 | iPSC MODELS OF GENETIC EPILEPSIES

iPSC models of genetic epilepsies include those in which epilepsy is the defining feature/symptom, such as DS or STXBP1-Related Epileptic Encephalopathy, and disorders in which epilepsy is a variable feature associated with other neurodevelopmental abnormalities, including TSC, Rett syndrome, Fragile X syndrome and Timothy syndrome. Many of the recent studies of epilepsy-associated genetic diseases have focused on those with an onset in infancy or early childhood given the relative ease of modeling defects in early neural development with hPSCs. Although an epileptic-like phenotype is not as well-defined using in vitro models as it is in animal models, in vitro models have been used to show increased spontaneous action potential firing rates and increased bursting and synchrony as correlates of epileptic-like activity. Patient-derived iPSC models for many of these diseases have also demonstrated altered neuronal morphology including soma size, neurite outgrowth, synapse formation, and dendritic spine length.[50,188,189] 2D iPSC models have been particularly important for analyzing parameters such as gene expression, cell morphology, neuronal excitability, and synapse formation since 2D cultures are easy to manipulate and readily available.[190-192] Several reviews have summarized the modeling of genetic epilepsies and other related neurological disorders in monolayer neurons.[71,193] However, 3D brain organoid modeling, with its more complex laminar organization, cell diversity, and mature neural networks, opens the door to new possibilities for modeling epilepsy related disorders. Benefits and disadvantages of different model systems for human brain disorders have been summarized recently (summarized in Figure 1).[148] In subsequent sections of this review, we describe studies using 2D or 3D hPSC-derived cultures to model the abovementioned genetic epilepsies and related neurodevelopmental disorders with a focus on human brain-specific phenotypes.

6.1 | Dravet syndrome

Dravet syndrome (DS) is a severe childhood epileptic encephalopathy that typically presents in infancy with treatment-resistant, prolonged seizures, and subsequent developmental delay and intellectual disability.[194] In over 80% of cases, the cause is a de novo heterozygous mutation in the *SCN1A* gene, which encodes the voltage-gated sodium channel (VGSC) $Na_v1.1$, leading to haploinsufficiency.[195] Opening of VGSCs results in an influx of sodium ions necessary for the firing of action potentials, making haploinsufficiency of this sodium channel seem unlikely to cause epilepsy. Prior heterozygous knockout or human mutant *SCN1A* knock-in mouse models suggested an answer to this apparent paradox with the finding of selective decreases in interneuron excitability predicted to cause disinhibition and cortical hyperexcitability.[196,197] However, recent work with DS patient iPSCs and additional mouse studies support a more complex pathophysiology.

Several groups generated iPSCs from DS patients, differentiated them into excitatory and inhibitory neurons and used whole-cell patch-clamp recordings to examine neuronal electrophysiology. One group unexpectedly showed increased (rather than the expected decrease seen in mouse interneurons) sodium current (I_{Na}) density and excitability in both presumptive excitatory and inhibitory neurons derived from two DS subjects compared to unrelated controls.[44] This result was supported by another study of one DS patient that also found increased I_{Na} in excitatory neurons.[122] However, several other groups have reported decreased I_{Na} in iPSC-derived interneurons with mutant SCN1A-linked DS,[45,157,198] and one of these studies used a more advanced protocol to generate interneurons of a medial ganglionic eminence-like origin and also showed unchanged I_{Na} in excitatory neurons.[45] The variability in results may be secondary to differences in the methods of neuronal differentiation, neuronal subtypes, or the maturity

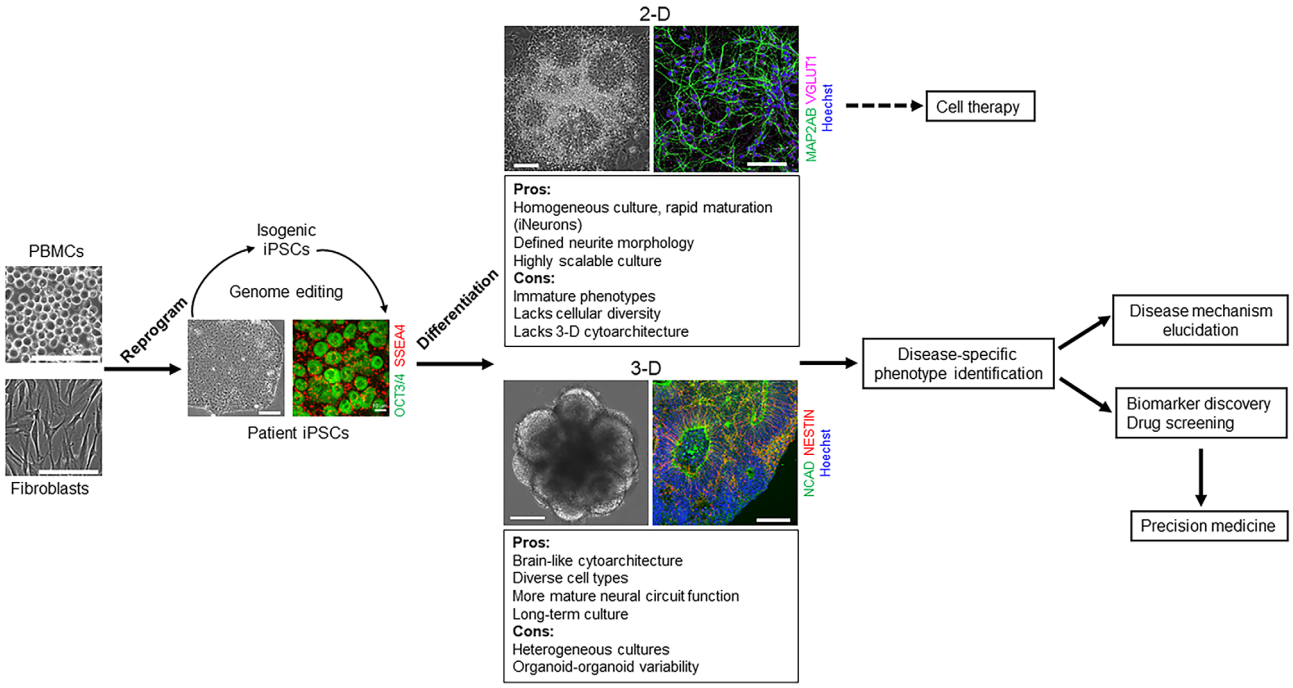

FIGURE 1 Overview of schema for studying genetic neurodevelopmental disorders using iPSC-derived 2D and 3D models. Patient somatic cells, such as peripheral blood mononuclear cells (PBMCs) or fibroblasts, are reprogrammed into iPSCs and then may be differentiated into 2D neuronal cultures or 3D brain organoids. iPSC colonies express pluripotency-associated genes such as octamer-binding transcription factor 3/4 (OCT3/4; green) and stage-specific embryonic antigent-4 (SSEA4; red). In hPSC-derived 2D neural cultures generated from a dual-smad differentiation protocol[18], neural VZ-like rosette structures (left) and cortical-like excitatory neurons (right) that express mictrotubule-associated protein 2AB (MAP2AB; green), and vesicular glutamate transporter-1 (VGLUT1; magenta) form after ~3 or 6 weeks of differentiation, respectively. In 3D cortical organoid culture generated using a published protocol (by Reference[29]), apicobasally polarized neuroepithelial buds (left) appear after 10 days of differentiation. Defined VZ-like structures with N-cadherin (NCAD; green) at the apical surface and neural progenitor cells immunolabeled for nestin (red) are seen at 34 days of differentiation (right). Although each system has unique advantages for modeling human brain disorders, hPSC-derived in vitro models provide unprecedented platforms for elucidating disease mechanism, biomarker identification, drug screening and toxicity studies, and eventually designing personalized treatments. Scale bar: 100 μm except for the image with iPSC cells immunolabeled with OCT3/4 and SSEA4 (10 μm)

of the neurons. Subsequent DS mouse model studies complicated the picture further, showing a developmental transition where initially interneurons have decreased I_{Na} and hypoexcitability, but then, due to unknown compensatory mechanisms, the interneurons normalize and excitatory neurons show increased I_{Na}[199,200] and excitability.[200] Taken together, the DS human iPSC and mouse data suggest that interneuron dysfunction initiates the epileptic encephalopathy, but resultant abnormalities in excitatory neurons may maintain the epileptic state.

6.2 | STXBP1-related epileptic encephalopathy

STXBP1-related epileptic encephalopathy is a severe epilepsy that typically begins in early infancy and results from *STXBP1* haploinsufficiency.[201] STXBP1 is a protein that is critical for effective presynaptic vesicle release. Several groups have found abnormal phenotypes in both patient-derived iPSC and genetically modified conditional hESC models of STXBP1-Related Epileptic Encephalopathy. The abnormalities include decreased SYNTAXIN1 expression/altered localization, decreased neurite outgrowth and reduced neurotransmitter release associated with heterozygous *STXBP1* LOF.[50,189] In homozygous *STXBP1* null hESC lines, severe neurodegeneration also occurs.[189]

6.3 | Tuberous sclerosis complex

TSC is a multisystem disorder that most prominently affects the skin, brain, lungs, kidneys, and heart. The disorder is caused by autosomal dominant de novo or inherited mutations in either the *TSC1* or the *TSC2* gene that encodes hamartin or tuberin, respectively.[202] The mutations are LOF and they lead to variable hamartomas in these tissues that reflect abnormal cell growth. The variability is thought to be due to the requirement for "second hit" mutations leading to loss of heterozygosity and subsequent hamartomas. TSC1 and TSC2 form part

of a heterotrimeric complex that represses the mechanistic target of rapamycin (mTOR) pathway, and LOF results in mTOR hyperactivity that causes aberrant cell growth.[203] In the brain, the main abnormal growths are cortical tubers, a type of focal cortical dysplasia (FCD), that most commonly present as seizures at any point in life from infancy to adulthood. Developmental delay, psychiatric disturbances, intellectual disability, and autism spectrum disorder are also often seen. Less frequently, subependymal giant cell astrocytomas arise in the brain and may lead to hydrocephalus.

Human PSC models of TSC have recently been developed to complement previous animal studies (see Reference 204). Costa and colleagues[188] generated heterozygous and homozygous *TSC2* knockout hESC lines via genome editing and found disease-relevant phenotypes, predominantly in homozygous lines. The phenotypes included increased neural progenitor proliferation, delayed neuronal differentiation, enhanced generation of astrocytes, and increased neuronal soma size and dendritic complexity. In terms of functional effects, *TSC2* knockout hESC-derived (mainly excitatory) neurons showed reduced evoked action potential firing and decreased spontaneous and miniature excitatory postsynaptic currents, findings that do not seem to explain the common epilepsy phenotypes in patients, although the function of cortical inhibitory-like neurons was not specifically assessed. The morphological and functional phenotypes were associated with increases in markers of mTOR pathway hyperactivity, again to a much greater degree in homozygous *TSC2* null cells, and they were reversed by the mTOR inhibitor rapamycin. Multiple groups subsequently found more subtle but similar phenotypes in haploinsufficient *TSC1* or *TSC2* patient iPSC-derived forebrain neurons or purkinje cells.[52-55]

Recently, Blair and colleagues combined brain organoid and CRISPR gene editing methods to model TSC in 3D cultures.[51] They generated hESC lines with either constitutive heterozygous or homozygous null TSC1 or TSC2 alleles, or lines with one null and one conditional *TSC2* allele, the latter to model a second hit during various stages of brain organoid development after the viral introduction of Cre to delete the second *TSC2* allele, while also activating a Cre reporter placed in a safe harbor locus. Similar to previous 2D findings in gene edited TSC lines,[188] phenotypes were present in cortical spheroids generated from homozygous null lines and largely absent in those derived from heterozygous lines. The changes included expression of markers indicating increased mTOR pathway activation, decreased neuronal and increased astrocytic differentiation, and enlarged, dysmorphic neurons and glia resembling TSC patient cortical tubers. Remarkably, focal Cre delivery to delete the conditional second allele in brain organoids from lines with constitutive haploinsufficiency led to the focal generation of tuber- or FCD-like abnormalities in the organoids. The authors replicated the findings by similarly inducing a focal knockout of a conditional allele generated from TSC patient iPSC-derived spheroids as a second hit. They also showed that early rapamycin treatment of the brain organoids prevented development of the abnormal phenotypes. Given the lack of findings in heterozygous TSC loss-of-function organoids, this elegant work provides strong support for the two-hit hypothesis of TSC pathophysiology.

6.4 | Rett syndrome

Rett syndrome (RTT) is arguably the most studied epileptic disorder using iPSCs. RTT is an X-linked neurodevelopmental disorder caused primarily by mutations in *MECP2*, which encodes a multifunctional epigenetic regulator.[205] Strikingly, 50%-90% of RTT patients have seizures.[206] iPSC models of RTT patients from several groups showed that RTT mutant neurons display a decrease in neuronal soma size, neurite outgrowth, synapse formation, and spontaneous activity, compared to wild-type MECP2-expressing neurons derived from isogenic RTT patient iPSC lines.[57,59] Marchetto and colleagues also demonstrated rescue of the *MECP2* mutation associated phenotypes via insulin-like growth factor-1 (IGF1) treatment.[59] Interestingly, mutant RTT astrocytes derived from RTT patients have adverse effects on the morphology and function of wild-type iPSC-derived neurons.[23] This finding demonstrates the critical roles played by glia in RTT pathology and the need to consider astrocytic contributions to epileptogenesis. Although postnatal functions of MECP2 have been extensively investigated, the role of MECP2 in early brain development remains poorly understood. Mellios and colleagues used RTT patient-derived iPSCs and MECP2 knockdown by shRNA to identify novel MECP2-targeted miRNAs during neuronal development. They also used MECP2-deficient and patient-derived monolayer culture and cerebral organoids to identify defects in neurogenesis, neuronal differentiation and migration.[60] They found a novel signaling pathway mediated by MECP2-targeted miRNAs that might influence neurogenesis and early brain development.[60] This study also suggests that early disruptions in brain development could result in increased susceptibility to subtle postnatal deleterious effects on brain maturation and plasticity, thus contributing to the full pathogenesis of neurodevelopmental disorders.

6.5 | Miller-Dieker syndrome

Miller-Dieker syndrome (MDS) is a severe congenital form of lissencephaly. It is caused by a heterozygous deletion of chromosome 17p13.3, which leads to a severe malformation of cortical development.[207,208] Affected children suffer from feeding difficulties, severe intellectual disability, developmental delay, and seizures. Past studies of this disease largely relied on mouse models, which have the obvious disadvantage of being naturally

lissencephalic. Although human brain organoids have not yet recapitulated gyrification, they contain most relevant cell types and structures likely to be involved in disease development. Patient iPSC-derived human brain organoids were recently used in two studies to investigate how MDS affects human progenitor subtypes (such as oRG cells) that control neuronal output and influence brain topology. One study showed a neuronal migration defect and severe apoptosis of the founder neuroepithelial stem cells in the VZ-like region. The authors also found a migration defect in the oRG cells, a cell type that is largely absent from lissencephalic rodents but critical for human neocortical expansion.[68] Similarly, Iefremova and colleagues used patient-specific forebrain organoids to investigate pathological changes associated with MDS. They found that patient-derived organoids are reduced in size, a change accompanied by a switch from symmetric to asymmetric cell division of VZ radial glial cells.[209] In addition, they identified non-cell-autonomous defects in the WNT signaling pathway. These important studies provide insight into the cellular pathogenesis of lissencephaly and demonstrate the utility of using brain organoids to model human-specific phenotypes.

6.6 | PTEN macrocephaly

The phosphatase and tensin homolog (PTEN)-protein kinase B (PKB)/AKT cascade regulates human cortical development,[210,211] and PTEN heterozygous loss-of-function mutations have been found in patients with macrocephaly.[212,213] In the human cerebral organoid system, deletion of the *PTEN* gene by CRISPR/Cas9-mediated genome editing increases AKT activity in human neural progenitors, promotes cell cycle re-entry, and transiently delays neuronal differentiation.[69] These alterations result in a pronounced expansion of the radial glia and intermediate progenitor cells. In this study, both mouse and human brain organoids were used to model macrocephaly and showed that the lack of PTEN led to a significant increase in size (recapitulating macrocephaly), but only knockout human organoids displayed substantially increased surface folding. The phenotypic differences might reflect inherent species differences in signaling regulation, cellular response, or brain cytoarchitecture. The use of human brain organoids thus can produce human-specific phenotypes and provide unique insights into underlying disease mechanisms.

7 | ZIKV INFECTION

Although not a genetic cause of epilepsy, congenital ZIKV virus infection is known to cause microcephaly and severe brain abnormalities in infants,[214-216] and infected subjects also have a high risk of epilepsy.[217] Recent studies using human brain organoids to model ZIKV infection have demonstrated that it induces the death of neural progenitor cells and impairs organoid formation, providing certain mechanistic insights into disease pathogenesis.[29,218,219] However, none of these studies recapitulated the key developmental features of the human cortex such as surface area and folding. In contrast, genetically modified PTEN mutant brain organoids show increased surface area and folding, and were used to model the destructive effect of ZIKV infection on these key features of the human cortex in vitro.[69] In this study, ZIKV infection led to the widespread initiation of apoptosis at the onset of surface folding and later severely impaired organoid growth both in size and surface folding. Furthermore, recent work has exploited brain organoid technology in developing drugs or screening therapeutic compounds that can mitigate the destructive actions of ZIKV infection on fetal brain.[30,220,221] Importantly, these findings further illustrate the strength of the human brain organoid system in modeling complex structural malformations of the developing human brain, and in recapitulating human neural progenitor-specific pathology.

8 | LIMITATIONS, CHALLENGES, AND FUTURE DIRECTIONS

While the use of hPSC-based models offers many advantages, modeling epilepsy and other neurological disorders with hPSCs also presents several challenges. One issue, mentioned earlier, concerns the variability of iPSC lines within and between subjects. Using isogenic controls generated by gene editing along with multiple lines per condition partially alleviates this problem. Another critical question is how closely hPSC-derived neurons reflect those that are affected in human epilepsies. Because hPSC-derived neurons are differentiated in vitro for only weeks to months, they typically represent an earlier developmental stage compared to in vivo development. If the onset of seizures is associated with a gene expressed at a later stage of brain development, hPSC derived neurons may not reflect the critical gene alteration in patient brains. Secondly, although transcriptional profiles of 2D hPSC monolayer neuronal cultures show remarkable preservation of in vivo neurodevelopmental signatures from embryonic to early fetal corticogenesis, these neurons do not mature beyond fetal stages in vitro.[222] Different compounds or conditions have been used to accelerate the rate of maturation, including gamma secretase inhibitors (DAPT and compound E) that block notch signaling, neurotrophic factors, and co-culture with either human or rodent astrocytes.[223] The lack of maturation may also be alleviated partially by using improved culture media, such as BrainPhys,[224] and with the development of mature neuronal reporters that label purely mature neurons for electrophysiological study. Moreover, these approaches may be combined with NGN2-directed differentiation, yielding a more homogeneous population of mature neurons.[121]

A major limitation of organoid models is the relative immaturity of neuronal circuitry compared to their in vivo counterpart. Transcriptional profiles of cortical organoids that are cultured up to 100 days in vitro recapitulate those of about 16-24 week postconception human brain.[28,187] More recent studies suggest that the prolonged culture of human brain organoids leads to further maturation beyond early developmental stages.[156,225] One study found spontaneous action potentials and defined synaptic junctions in 8-month old brain organoids, but not in younger ones, suggesting that long-term culturing can promote functional neuronal connectivity.[156] Human cortical circuits require the assembly of both glutamatergic neurons that are generated in the dorsal forebrain and GABAergic interneurons that are produced in the ventral forebrain. In the dorsal-ventral fused organoids described earlier, interneurons that migrate into cortical spheroids fired action potentials at twice the rate of cells in unfused ventral spheroids.[27] This finding suggests that prepatterned organoids can be fused to promote the formation of functional synapses and neuronal maturation. Grafting of cerebral organoids into rodent brain in vivo also yielded progressive differentiation and maturation.[172] Another recent approach used to enhance maturation and decrease cell death within brain organoids involves culturing them at a liquid-air interface, which improves neuronal survival and morphology with extensive axon outgrowth following long-term in vitro culture.[225] However, many human cortical circuits develop postnatally, and may take years to mature in vivo. In particular, the development of parvalbumin fast-spiking interneurons in 2D or 3D hPSC-derived neuronal cultures has been challenging. Thus, significant challenges of circuit maturation in brain organoids remain.

Another obstacle to the application of hPSCs for disease modeling involves their limited ability to reproducibly recapitulate the diversity of neural cell types and regional specificity found in the human brain. There are numerous neuronal subtypes in human brain, organized by brain region, layer, connectivity, neurotransmitter content, and transcriptional profiles. These various neuronal subtypes make diverse contributions to the epileptic phenotype. In addition, although neurons in 3D brain organoids have more complex anatomic connectivity and neuronal networks than 2D monolayer neurons do, many nonneuronal cell types found in the brain such as oligodendrocytes, meningeal cells, microglia and brain microvascular endothelial cells remain challenging to incorporate into brain organoid cultures in vitro.[26,28,226] This is largely due to the lack of surrounding supportive tissues and endogenous developmental and patterning cues in the organoids. The "intrinsic" organoids mainly depend on the ability of stem cells to spontaneously acquire neural identity and to self-organize into brain-like structures. This promotes the generation of somewhat diverse brain-like regions and cell populations.[26] Although such diversity is appealing, it often leads to inconsistent outcome and batch effects that may conceal critical phenotypes. Thus, reproducibility and consistency are substantial challenges in these self-organizing brain organoid protocols[182]

On the other hand, recent "extrinsic" protocols create brain-region specific organoids whereby patterning is restricted by externally added morphogens, and these have shown promise in improving reproducibility. For example, the dorsal forebrain organoids that were recently developed by Velasco et al., reliably generate a rich diversity of cell types similar to those in the human cerebral cortex and show consistent reproducibility in the cell types produced in these region-specific organoids.[158] Although these methods restrict the ability to generate complex brain structures compared to the "intrinsic" protocols, this shortcoming can be partially overcome by assembling different types of organoid-derived structures in vitro, generating so-called "assembloids." For example, fusion of human cortical organoids and human subpallial organoids creates functional human forebrain structures that contain both glutamatergic excitatory neurons and GABAergic inhibitory interneurons.[27,32,159] A similar assembloid approach has been used to reconstruct thalamo-cortical circuitry from hPSCs.[31] These approaches are especially significant for investigating epileptic-like phenotypes of interconnections between excitatory and inhibitory neurons, or those involving cortical-thalamic/thalamocortical networks. Tissue engineering and biomaterials will be also important for adding structural features that are normally present in human brain into organoids. For example, one group has combined hPSC-derived neural progenitor cells, endothelial cells, mesenchymal stem cells, and microglia/macrophage precursors on chemically defined polyethylene glycol hydrogels to enable these cells to develop into 3D neural structures and to model cellular interactions within the developing brain.[227] Although still preliminary, this work supports the feasibility of integrating nonneural cells or tissues into brain organoids to investigate more complicated cellular interactions in the developing brain. This approach is especially important for investigations of neurodevelopmental disorders, including epilepsies, that require these cellular interactions during developmental processes such as neuronal migration, synaptogenesis, maturation, and network homeostasis.

With increasing knowledge of human cortical development and progress in the development of differentiation protocols, there have been a growing number of methods that researchers can use to interrogate cellular properties of interest in brain organoids. Although there is not yet a standard practice in this field, the sampling of multiple organoids per experiment at multiple developmental time points, ideally also with organoids derived from multiple iPSC lines, is optimal to

TABLE 1 Sample size and functional assays in selected studies using bulk and/or scRNA-seq

Study	Organoid type	hPSC lines	Bulk/scRNA-seq	Sample size in bulk or scRNA-seq	Disease modeling	Major functional assay
[187]	Self-organized	2 controls	scRNA-seq (Fluidigm system)	333 cells (five organoids in five time points)	Not applicable	Not applicable
[28]	hCS	7 controls	Bulk	Three lines at two time points	Not applicable	2D calcium imaging, slice & 3D electrophysiology
[29]	Forebrain; Midbrain and Hypothalamic organoid	4 controls	Bulk	Three samples at three time points and two samples at one time point	ZIKV	Slice calcium imaging & electrophysiology; Proliferation
[32]	hCO; hMGEO	3 controls	Bulk & scRNA-seq (Chromium system)	Bulk: not clear scRNA-seq: 59235 cells (two replicates at two time points)	Not applicable	3D Calcium imaging; slice electrophysiology
[27]	hCS; hSS	7 controls; 7 patients	scRNA-seq (BD Resolve system & Smart-seq2)	11 838 cells at one time point from multiple oragnoids	Timothy syndrome	2D & 3D calcium imaging; slice electrophysiology
[153]	hCS	4 controls	Bulk & scRNA-seq (Smart-seq2)	Bulk: 3-15 hCSs per experiment; scRNA-seq: 710 cells (three-seven hCSs per time point; two lines and five time points)	Not applicable	2D calcium imaging, cytotoxicity assay
[156]	Self-organized	1 control	scRNA-seq (Drop-seq)	scRNA-seq: 80000 cells (31 organoids; multiple batches)	Not applicable	3D electrophysiology (high-density silicon microelectrodes)
[30]	Human cortical and ventral telencephalic organoid	4 controls	Bulk	12 samples (three replicates from four time points)	ZIKV	3D Calcium imaging; slice electrophysiology
[225]	ALI-CO	2 controls	scRNA-seq (10x genomics)	13 280 cells from six ALI organoids of two hCOs	Not applicable	3D MEA; Slice electrophysiology
[31]	hCO; hMGEOs; hThO	2 control	scRNA-seq (Chromium system)	10-12 hr THOs (11 277 cells at two time points)	Not applicable	3D calcium imaging; slice electrophysiology
[158]	Self-organized; hCS	4 controls	scRNA-seq (10x genomics)	166 242 cells from 21 organoids (at least three organoids per batches)	Not applicable	Not applicable

Abbreviations: hCS, human cortical spheroid; hSS, human subpallium spheroid; hCO, human cortical-like organoid; hMGEO, human medial ganglionic eminence (MGE)-like organoid; hThO, human thalamus-like brain organoid; ALI-CO, air-liquid interface cerebral organoids; MEA, multielectrode arrays; 2D, dissociated cells from organoids; 3D, intact organoids.

mitigate outlier effects. It will be also important for the disease features to be replicated by multiple laboratories in order to support their true relevance. While forming a "standard" protocol is unrealistic, detailed reporting of differentiation methods in published studies is an imperative practice (see Table 1 for examples of the numbers of organoids and/or cell lines analyzed in various selected studies).

9 | CONCLUSIONS

The need to create alternative models of human epilepsy has been highlighted by the difficulties/failures to translate findings from animal models to clinical therapies. Although there are still many challenges ahead, hPSC technology provides the unprecedented ability to model genetic epilepsies by growing many renewable human cells in a dish and differentiating them into disease-relevant neural cell types. In particular, these models are well suited to investigate disease mechanisms and for drug screening to discover new precision therapies to treat many severe genetic epilepsies and related disorders. The approaches are facilitated by the relative ease of differentiating hPSCs to brain tissues that partially recapitulate early human neurodevelopment in vitro. A variety of hPSC-based models have already provided insight into mechanisms for several of the genetic epilepsies. Advances in the development of hPSC models will arise from improvements in methods to derive specific neuronal subtypes and neural circuitry in 2D and 3D cultures. Progress in the development of efficient and reliable genome editing methods for hPSC lines will no doubt play a role in these advances. Lastly, improved functional assessment of neural activity and exciting higher throughput drug screening capabilities are on the horizon. Given the rapid advances already realized, hPSC-derived models hold strong promise for providing a better understanding of human brain development, disease mechanisms, and new epilepsy therapies by bridging the gap between model systems and patients.

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REFERENCES

1. Zack MM, Kobau R. National and state estimates of the numbers of adults and children with active epilepsy - United States, 2015. *MMWR Morb Mortal Wkly Rep*. 2017;66:821-825.
2. Grone BP, Baraban SC. Animal models in epilepsy research: legacies and new directions. *Nat Neurosci*. 2015;18:339-343.
3. Brodie MJ, Barry SJ, Bamagous GA, Norrie JD, Kwan P. Patterns of treatment response in newly diagnosed epilepsy. *Neurology*. 2012;78:1548-1554.
4. Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. *Cell*. 1990;61:931-937.
5. Noebels J. Pathway-driven discovery of epilepsy genes. *Nat Neurosci*. 2015;18:344-350.
6. Miller DJ, Bhaduri A, Sestan N, Kriegstein A. Shared and derived features of cellular diversity in the human cerebral cortex. *Curr Opin Neurobiol*. 2019;56:117-124.
7. Rakic P. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci*. 1995;18:383-388.
8. Zeng H, Shen EH, Hohmann JG, et al. Large-scale cellular-resolution gene profiling in human neocortex reveals species-specific molecular signatures. *Cell*. 2012;149:483-496.
9. Griffin A, Krasniak C, Baraban SC. Advancing epilepsy treatment through personalized genetic zebrafish models. *Progress in Brain Research*. 2016;226:195-207.
10. Prykhozhij SV, Fuller C, Steele SL, et al. Optimized knock-in of point mutations in zebrafish using CRISPR/Cas9. *Nucleic Acids Res*. 2018;46:e102.
11. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145-1147.
12. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663-676.
13. Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol*. 2007;25:1177-1181.
14. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861-872.
15. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008;321:1218-1221.
16. Park IH, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. *Cell*. 2008;134:877-886.
17. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol*. 2009;27:275-280.
18. Shi Y, Kirwan P, Livesey FJ. Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nat Protoc*. 2012;7:1836-1846.
19. Liu Y, Liu H, Sauvey C, Yao L, Zarnowska ED, Zhang SC. Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. *Nat Protoc*. 2013;8:1670-1679.
20. Maroof AM, Keros S, Tyson JA, et al. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. *Cell Stem Cell*. 2013;12:559-572.
21. Nicholas CR, Chen J, Tang Y, et al. Functional maturation of hPSC-derived forebrain interneurons requires an extended

timeline and mimics human neural development. *Cell Stem Cell*. 2013;12:573-586.
22. Yu DX, Di Giorgio FP, Yao J, et al. Modeling hippocampal neurogenesis using human pluripotent stem cells. *Stem Cell Reports*. 2014;2:295-310.
23. Williams EC, Zhong X, Mohamed A, et al. Mutant astrocytes differentiated from Rett syndrome patients-specific iPSCs have adverse effects on wild-type neurons. *Hum Mol Genet*. 2014;23:2968-2980.
24. Eiraku M, Watanabe K, Matsuo-Takasaki M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell*. 2008;3:519-532.
25. Lancaster MA, Renner M, Martin CA, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501:373-379.
26. Lancaster MA, Knoblich JA. Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc*. 2014;9:2329-2340.
27. Birey F, Andersen J, Makinson CD, et al. Assembly of functionally integrated human forebrain spheroids. *Nature*. 2017;545:54-59.
28. Pasca AM, Sloan SA, Clarke LE, et al. Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat Methods*. 2015;12:671-678.
29. Qian X, Nguyen HN, Song MM, et al. Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell*. 2016;165:1238-1254.
30. Watanabe M, Buth JE, Vishlaghi N, et al. Self-organized cerebral Organoids with human-specific features predict effective drugs to combat Zika virus infection. *Cell Rep*. 2017;21:517-532.
31. Xiang Y, Tanaka Y, Cakir B, et al. hESC-derived thalamic Organoids form reciprocal projections when fused with cortical Organoids. *Cell Stem Cell*. 2019;24:487-497 e7.
32. Xiang Y, Tanaka Y, Patterson B, et al. Fusion of regionally specified hPSC-derived Organoids models human brain development and interneuron migration. *Cell Stem Cell*. 2017;21:383-398 e7.
33. Hargus G, Cooper O, Deleidi M, et al. Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proc Natl Acad Sci U S A*. 2010;107:15921-15926.
34. Lu P, Woodruff G, Wang Y, et al. Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. *Neuron*. 2014;83:789-796.
35. Mansour AA, Goncalves JT, Bloyd CW, et al. Erratum: an in vivo model of functional and vascularized human brain organoids. *Nat Biotechnol*. 2018;36:772.
36. Oki K, Tatarishvili J, Wood J, et al. Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. *Stem Cells*. 2012;30:1120-1133.
37. Wang S, Bates J, Li X, et al. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell*. 2013;12:252-264.
38. Miyaoka Y, Chan AH, Judge LM, et al. Isolation of single-base genome-edited human iPS cells without antibiotic selection. *Nat Methods*. 2014;11:291-293.
39. Soldner F, Laganiere J, Cheng AW, et al. Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. *Cell*. 2011;146:318-331.
40. Howden SE, Maufort JP, Duffin BM, Elefanty AG, Stanley EG, Thomson JA. Simultaneous reprogramming and gene correction of patient fibroblasts. *Stem Cell Reports*. 2015;5:1109-1118.
41. Tidball AM, Dang LT, Glenn TW, et al. Rapid generation of human genetic loss-of-function iPSC lines by simultaneous reprogramming and gene editing. *Stem Cell Reports*. 2017;9:725-731.
42. Marchetto MC, Brennand KJ, Boyer LF, Gage FH. Induced pluripotent stem cells (iPSCs) and neurological disease modeling: progress and promises. *Hum Mol Genet*. 2011;20:R109-R115.
43. Srikanth P, Young-Pearse TL. Stem cells on the brain: modeling neurodevelopmental and neurodegenerative diseases using human induced pluripotent stem cells. *J Neurogenet*. 2014;28:5-29.
44. Liu Y, Lopez-Santiago LF, Yuan Y, et al. Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. *Ann Neurol*. 2013;74:128-139.
45. Sun Y, Pasca SP, Portmann T, et al. A deleterious Nav1.1 mutation selectively impairs telencephalic inhibitory neurons derived from Dravet syndrome patients. *elife*. 2016;5.
46. Chamberlain SJ, Chen PF, Ng KY, et al. Induced pluripotent stem cell models of the genomic imprinting disorders Angelman and Prader-Willi syndromes. *Proc Natl Acad Sci U S A*. 2010;107:17668-17673.
47. Livide G, Patriarchi T, Amenduni M, et al. GluD1 is a common altered player in neuronal differentiation from both MECP2-mutated and CDKL5-mutated iPS cells. *Eur J Hum Genet*. 2015;23:195-201.
48. Ricciardi S, Ungaro F, Hambrock M, et al. CDKL5 ensures excitatory synapse stability by reinforcing NGL-1-PSD95 interaction in the postsynaptic compartment and is impaired in patient iPSC-derived neurons. *Nat Cell Biol*. 2012;14:911-923.
49. Patzke C, Han Y, Covy J, et al. Analysis of conditional heterozygous STXBP1 mutations in human neurons. *J Clin Invest*. 2015;125:3560-3571.
50. Yamashita S, Chiyonobu T, Yoshida M, et al. Mislocalization of syntaxin-1 and impaired neurite growth observed in a human iPSC model for STXBP1-related epileptic encephalopathy. *Epilepsia*. 2016;57:e81-e86.
51. Blair JD, Hockemeyer D, Bateup HS. Genetically engineered human cortical spheroid models of tuberous sclerosis. *Nat Med*. 2018;24:1568-1578.
52. Li Y, Cao J, Chen M, et al. Abnormal neural progenitor cells differentiated from induced pluripotent stem cells partially mimicked development of TSC2 neurological abnormalities. *Stem Cell Reports*. 2017;8:883-893.
53. Nadadhur AG, Alsaqati M, Gasparotto L, et al. Neuron-glia interactions increase neuronal phenotypes in tuberous sclerosis complex patient iPSC-derived models. *Stem Cell Reports*. 2019;12:42-56.
54. Sundberg M, Tochitsky I, Buchholz DE, et al. Purkinje cells derived from TSC patients display hypoexcitability and synaptic deficits associated with reduced FMRP levels and reversed by rapamycin. *Mol Psychiatry*. 2018;23:2167-2183.
55. Zucco AJ, Pozzo VD, Afinogenova A, Hart RP, Devinsky O, D'arcangelo G. Neural progenitors derived from tuberous sclerosis complex patients exhibit attenuated PI3K/AKT signaling and delayed neuronal differentiation. *Mol Cell Neurosci*. 2018;92:149-163.

56. Ananiev G, Williams EC, Li H, Chang Q. Isogenic pairs of wild type and mutant induced pluripotent stem cell (iPSC) lines from Rett syndrome patients as in vitro disease model. *PLoS ONE*. 2011;6:e25255.
57. Cheung AY, Horvath LM, Grafodatskaya D, et al. Isolation of MECP2-null Rett syndrome patient hiPS cells and isogenic controls through X-chromosome inactivation. *Hum Mol Genet*. 2011; 20:2103-2115.
58. Kim KY, Hysolli E, Park IH. Neuronal maturation defect in induced pluripotent stem cells from patients with Rett syndrome. *Proc Natl Acad Sci U S A*. 2011;108:14169-14174.
59. Marchetto MC, Carromeu C, Acab A, et al. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell*. 2010;143:527-539.
60. Mellios N, Feldman DA, Sheridan SD, et al. MeCP2-regulated miRNAs control early human neurogenesis through differential effects on ERK and AKT signaling. *Mol Psychiatry*. 2018;23: 1051-1065.
61. Liu J, Koscielska KA, Cao Z, et al. Signaling defects in iPSC-derived fragile X premutation neurons. *Hum Mol Genet*. 2012; 21:3795-3805.
62. Sheridan SD, Theriault KM, Reis SA, et al. Epigenetic characterization of the FMR1 gene and aberrant neurodevelopment in human induced pluripotent stem cell models of fragile X syndrome. *PLoS ONE*. 2011;6:e26203.
63. Urbach A, Bar-Nur O, Daley GQ, Benvenisty N. Differential modeling of fragile X syndrome by human embryonic stem cells and induced pluripotent stem cells. *Cell Stem Cell*. 2010;6: 407-411.
64. Krey JF, Pasca SP, Shcheglovitov A, et al. Timothy syndrome is associated with activity-dependent dendritic retraction in rodent and human neurons. *Nat Neurosci*. 2013;16:201-209.
65. Pasca SP, Portmann T, Voineagu I, et al. Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nat Med*. 2011;17:1657-1662.
66. Yoon KJ, Nguyen HN, Ursini G, et al. Modeling a genetic risk for schizophrenia in iPSCs and mice reveals neural stem cell deficits associated with adherens junctions and polarity. *Cell Stem Cell*. 2014;15:79-91.
67. Shcheglovitov A, Shcheglovitova O, Yazawa M, et al. SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. *Nature*. 2013;503:267-271.
68. Bershteyn M, Nowakowski TJ, Pollen AA, et al. Human iPSC-derived cerebral organoids model cellular features of Lissencephaly and reveal prolonged mitosis of outer radial glia. *Cell Stem Cell*. 2017;20:435-449 e4.
69. Li Y, Muffat J, Omer A, et al. Induction of expansion and folding in human cerebral Organoids. *Cell Stem Cell*. 2017;20:385-396 e3.
70. EpiPM Consortium. A roadmap for precision medicine in the epilepsies. *Lancet Neurol*. 2015;14:1219-1228.
71. Parent JM, Anderson SA. Reprogramming patient-derived cells to study the epilepsies. *Nat Neurosci*. 2015;18:360-366.
72. Vangipuram M, Ting D, Kim S, Diaz R, Schule B. Skin punch biopsy explant culture for derivation of primary human fibroblasts. *J Vis Exp*. 2013;77:e3779.
73. Derosa BA, Van Baaren JM, Dubey GK, et al. Derivation of autism spectrum disorder-specific induced pluripotent stem cells from peripheral blood mononuclear cells. *Neurosci Lett*. 2012; 516:9-14.
74. Okita K, Yamakawa T, Matsumura Y, et al. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. *Stem Cells*. 2013;31:458-466.
75. Wang L, Wang L, Huang W, et al. Generation of integration-free neural progenitor cells from cells in human urine. *Nat Methods*. 2013;10:84-89.
76. Zhou T, Benda C, Duzinger S, et al. Generation of induced pluripotent stem cells from urine. *J Am Soc Nephrol*. 2011;22:1221-1228.
77. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013;339:819-823.
78. Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J. RNA-programmed genome editing in human cells. *elife*. 2013;2: e00471.
79. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. *Science*. 2013;339:823-826.
80. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc*. 2013;8:2281-2308.
81. Gilbert LA, Horlbeck MA, Adamson B, et al. Genome-scale CRISPR-mediated control of gene repression and activation. *Cell*. 2014;159:647-661.
82. Featherstone C, Jackson SP. DNA double-strand break repair. *Curr Biol*. 1999;9:R759-R761.
83. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu Rev Biochem*. 2010;79:181-211.
84. Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nat Biotechnol*. 2013;31:230-232.
85. Jasin M, Rothstein R. Repair of strand breaks by homologous recombination. *Cold Spring Harb Perspect Biol*. 2013;5: a012740.
86. Santos DP, Kiskinis E, Eggan K, Merkle FT. Comprehensive protocols for CRISPR/Cas9-based gene editing in human pluripotent stem cells. *Curr Protoc Stem Cell Biol*. 2016;38:5B 6 1-5B 6 60.
87. Miyaoka Y, Berman JR, Cooper SB, et al. Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing. *Sci Rep*. 2016;6:23549.
88. Schwank G, Koo BK, Sasselli V, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell*. 2013;13:653-658.
89. Sandoe J, Eggan K. Opportunities and challenges of pluripotent stem cell neurodegenerative disease models. *Nat Neurosci*. 2013; 16:780-789.
90. Hockemeyer D, Jaenisch R. Induced pluripotent stem cells meet genome editing. *Cell Stem Cell*. 2016;18:573-586.
91. Roberts B, Haupt A, Tucker A, et al. Systematic gene tagging using CRISPR/Cas9 in human stem cells to illuminate cell organization. *Mol Biol Cell*. 2017;28:2854-2874.
92. Doyon JB, Zeitler B, Cheng J, et al. Rapid and efficient clathrin-mediated endocytosis revealed in genome-edited mammalian cells. *Nat Cell Biol*. 2011;13:331-337.
93. Nakano T, Ando S, Takata N, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell*. 2012;10:771-785.

94. Ruby KM, Zheng B. Gene targeting in a HUES line of human embryonic stem cells via electroporation. *Stem Cells*. 2009;27:1496-1506.
95. Xue H, Wu S, Papadeas ST, et al. A targeted neuroglial reporter line generated by homologous recombination in human embryonic stem cells. *Stem Cells*. 2009;27:1836-1846.
96. Goulburn AL, Alden D, Davis RP, et al. A targeted NKX2.1 human embryonic stem cell reporter line enables identification of human basal forebrain derivatives. *Stem Cells*. 2011;29:462-473.
97. De Dieuleveult M, Yen K, Hmitou I, et al. Genome-wide nucleosome specificity and function of chromatin remodellers in ES cells. *Nature*. 2016;530:113-116.
98. Savic D, Partridge EC, Newberry KM, et al. CETCh-seq: CRISPR epitope tagging ChIP-seq of DNA-binding proteins. *Genome Res*. 2015;25:1581-1589.
99. Xiong X, Zhang Y, Yan J, et al. A scalable epitope tagging approach for high throughput ChIP-Seq analysis. *ACS Synth Biol*. 2017;6:1034-1042.
100. Zhang Z, Zhang Y, Gao F, et al. CRISPR/Cas9 genome-editing system in human stem cells: current status and future prospects. *Mol Ther Nucleic Acids*. 2017;9:230-241.
101. Smith C, Gore A, Yan W, et al. Whole-genome sequencing analysis reveals high specificity of CRISPR/Cas9 and TALEN-based genome editing in human iPSCs. *Cell Stem Cell*. 2014;15:12-13.
102. Suzuki K, Yu C, Qu J, et al. Targeted gene correction minimally impacts whole-genome mutational load in human-disease-specific induced pluripotent stem cell clones. *Cell Stem Cell*. 2014;15:31-36.
103. Veres A, Gosis BS, Ding Q, et al. Low incidence of off-target mutations in individual CRISPR-Cas9 and TALEN targeted human stem cell clones detected by whole-genome sequencing. *Cell Stem Cell*. 2014;15:27-30.
104. Watanabe K, Ueno M, Kamiya D, et al. A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat Biotechnol*. 2007;25:681-686.
105. Forsyth NR, Musio A, Vezzoni P, Simpson AH, Noble BS, Mcwhir J. Physiologic oxygen enhances human embryonic stem cell clonal recovery and reduces chromosomal abnormalities. *Cloning Stem Cells*. 2006;8:16-23.
106. Kim S, Kim D, Cho SW, Kim J, Kim JS. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Res*. 2014;24:1012-1019.
107. Lin S, Staahl BT, Alla RK, Doudna JA. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. *elife*. 2014;3:e04766.
108. Dang Y, Jia G, Choi J, et al. Optimizing sgRNA structure to improve CRISPR-Cas9 knockout efficiency. *Genome Biol*. 2015;16:280.
109. Hendel A, Bak RO, Clark JT, et al. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. *Nat Biotechnol*. 2015;33:985-989.
110. Cao J, Wu L, Zhang SM, et al. An easy and efficient inducible CRISPR/Cas9 platform with improved specificity for multiple gene targeting. *Nucleic Acids Res*. 2016;44:e149.
111. Chen Y, Cao J, Xiong M, et al. Engineering human stem cell lines with inducible gene knockout using CRISPR/Cas9. *Cell Stem Cell*. 2015;17:233-244.
112. Sarkar A, Mei A, Paquola ACM, et al. Efficient generation of CA3 neurons from human pluripotent stem cells enables modeling of hippocampal connectivity in vitro. *Cell Stem Cell*. 2018;22:684-697 e9.
113. Canals I, Ginisty A, Quist E, et al. Rapid and efficient induction of functional astrocytes from human pluripotent stem cells. *Nat Methods*. 2018;15:693-696.
114. Li X, Tao Y, Bradley R, et al. Fast generation of functional subtype astrocytes from human pluripotent stem cells. *Stem Cell Reports*. 2018;11:998-1008.
115. Tchieu J, Calder EL, Guttikonda SR, et al. NFIA is a gliogenic switch enabling rapid derivation of functional human astrocytes from pluripotent stem cells. *Nat Biotechnol*. 2019;37:267-275.
116. Kriks S, Shim JW, Piao J, et al. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature*. 2011;480:547-551.
117. Swistowski A, Peng J, Liu Q, et al. Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. *Stem Cells*. 2010;28:1893-1904.
118. Hu BY, Zhang SC. Differentiation of spinal motor neurons from pluripotent human stem cells. *Nat Protoc*. 2009;4:1295-1304.
119. Karumbayaram S, Novitch BG, Patterson M, et al. Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem Cells*. 2009;27:806-811.
120. Chandrasekaran A, Avci HX, Ochalek A, et al. Comparison of 2D and 3D neural induction methods for the generation of neural progenitor cells from human induced pluripotent stem cells. *Stem Cell Res*. 2017;25:139-151.
121. Zhang Y, Pak C, Han Y, et al. Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron*. 2013;78:785-798.
122. Schafer ST, Paquola ACM, Stern S, et al. Pathological priming causes developmental gene network heterochronicity in autistic subject-derived neurons. *Nat Neurosci*. 2019;22:243-255.
123. Qi Y, Zhang XJ, Renier N, et al. Combined small-molecule inhibition accelerates the derivation of functional cortical neurons from human pluripotent stem cells. *Nat Biotechnol*. 2017;35:154-163.
124. Shi Y, Kirwan P, Smith J, Robinson HP, Livesey FJ. Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. *Nat Neurosci*. 2012;15(477–86):S1.
125. Kim DS, Lee JS, Leem JW, et al. Robust enhancement of neural differentiation from human ES and iPS cells regardless of their innate difference in differentiation propensity. *Stem Cell Rev*. 2010;6:270-281.
126. Li XJ, Zhang SC. In vitro differentiation of neural precursors from human embryonic stem cells. *Methods Mol Biol*. 2006;331:169-177.
127. Reinhardt P, Glatza M, Hemmer K, et al. Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling. *PLoS ONE*. 2013;8:e59252.
128. Zeng H, Guo M, Martins-Taylor K, et al. Specification of region-specific neurons including forebrain glutamatergic neurons from human induced pluripotent stem cells. *PLoS ONE*. 2010;5:e11853.
129. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*. 2001;19:1129-1133.

130. Muratore CR, Srikanth P, Callahan DG, Young-Pearse TL. Comparison and optimization of hiPSC forebrain cortical differentiation protocols. *PLoS ONE*. 2014;9:e105807.
131. Pak C, Danko T, Zhang Y, et al. Human neuropsychiatric disease modeling using conditional deletion reveals synaptic transmission defects caused by heterozygous mutations in NRXN1. *Cell Stem Cell*. 2015;17:316-328.
132. Sun AX, Yuan Q, Tan S, et al. Direct induction and functional maturation of forebrain GABAergic neurons from human pluripotent stem cells. *Cell Rep*. 2016;16:1942-1953.
133. Yang N, Chanda S, Marro S, et al. Generation of pure GABAergic neurons by transcription factor programming. *Nat Methods*. 2017;14:621-628.
134. Geschwind DH, Rakic P. Cortical evolution: judge the brain by its cover. *Neuron*. 2013;80:633-647.
135. Fietz SA, Kelava I, Vogt J, et al. OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nat Neurosci*. 2010;13:690-699.
136. Hansen DV, Lui JH, Parker PR, Kriegstein AR. Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature*. 2010;464:554-561.
137. Martinez-Cerdeno V, Cunningham CL, Camacho J, et al. Comparative analysis of the subventricular zone in rat, ferret and macaque: evidence for an outer subventricular zone in rodents. *PLoS ONE*. 2012;7:e30178.
138. Lui JH, Hansen DV, Kriegstein AR. Development and evolution of the human neocortex. *Cell*. 2011;146:18-36.
139. Wang X, Tsai JW, Lamonica B, Kriegstein AR. A new subtype of progenitor cell in the mouse embryonic neocortex. *Nat Neurosci*. 2011;14:555-561.
140. Sakaguchi H, Kadoshima T, Soen M, et al. Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. *Nat Commun*. 2015;6:8896.
141. Jo J, Xiao Y, Sun AX, et al. Midbrain-like Organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. *Cell Stem Cell*. 2016;19:248-257.
142. Monzel AS, Smits LM, Hemmer K, et al. Derivation of human midbrain-specific organoids from neuroepithelial stem cells. *Stem Cell Reports*. 2017;8:1144-1154.
143. Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep*. 2015;10:537-550.
144. Marton RM, Miura Y, Sloan SA, et al. Differentiation and maturation of oligodendrocytes in human three-dimensional neural cultures. *Nat Neurosci*. 2019;22:484-491.
145. Ozone C, Suga H, Eiraku M, et al. Functional anterior pituitary generated in self-organizing culture of human embryonic stem cells. *Nat Commun*. 2016;7:10351.
146. Eiraku M, Takata N, Ishibashi H, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature*. 2011;472:51-56.
147. Amin ND, Pasca SP. Building models of brain disorders with three-dimensional Organoids. *Neuron*. 2018;100:389-405.
148. Chen HI, Song H, Ming GL. Applications of human brain Organoids to clinical problems. *Dev Dyn*. 2019;248:53-64.
149. Di Lullo E, Kriegstein AR. The use of brain organoids to investigate neural development and disease. *Nat Rev Neurosci*. 2017;18:573-584.
150. Kelava I, Lancaster MA. Dishing out mini-brains: current progress and future prospects in brain organoid research. *Dev Biol*. 2016;420:199-209.
151. Kretzschmar K, Clevers H. Organoids: modeling development and the stem cell niche in a dish. *Dev Cell*. 2016;38:590-600.
152. Wang H. Modeling neurological diseases with human brain Organoids. *Front Synaptic Neurosci*. 2018;10:15.
153. Sloan SA, Darmanis S, Huber N, et al. Human astrocyte maturation captured in 3D cerebral cortical spheroids derived from pluripotent stem cells. *Neuron*. 2017;95:779-790 e6.
154. Kim H, Xu R, Padmashri R, et al. Pluripotent stem cell-derived cerebral Organoids reveal human Oligodendrogenesis with dorsal and ventral origins. *Stem Cell Reports*. 2019;12:890-905.
155. Madhavan M, Nevin ZS, Shick HE, et al. Induction of myelinating oligodendrocytes in human cortical spheroids. *Nat Methods*. 2018;15:700-706.
156. Quadrato G, Nguyen T, Macosko EZ, et al. Cell diversity and network dynamics in photosensitive human brain organoids. *Nature*. 2017;545:48-53.
157. Kadoshima T, Sakaguchi H, Nakano T, et al. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc Natl Acad Sci U S A*. 2013;110:20284-20289.
158. Velasco S, Kedaigle AJ, Simmons SK, et al. Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature*. 2019;570:523-527.
159. Bagley JA, Reumann D, Bian S, Levi-Strauss J, Knoblich JA. Fused cerebral organoids model interactions between brain regions. *Nat Methods*. 2017;14:743-751.
160. Espuny-Camacho I, Michelsen KA, Gall D, et al. Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. *Neuron*. 2013;77:440-456.
161. Mack CM, Lin BJ, Turner JD, Johnstone AF, Burgoon LD, Shafer TJ. Burst and principal components analyses of MEA data for 16 chemicals describe at least three effects classes. *Neurotoxicology*. 2014;40:75-85.
162. Obien ME, Deligkaris K, Bullmann T, Bakkum DJ, Frey U. Revealing neuronal function through microelectrode array recordings. *Front Neurosci*. 2014;8:423.
163. Pine J. Recording action potentials from cultured neurons with extracellular microcircuit electrodes. *J Neurosci Methods*. 1980;2:19-31.
164. Wainger BJ, Kiskinis E, Mellin C, et al. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. *Cell Rep*. 2014;7:1-11.
165. Grienberger C, Konnerth A. Imaging calcium in neurons. *Neuron*. 2012;73:862-885.
166. Hamel EJ, Grewe BF, Parker JG, Schnitzer MJ. Cellular level brain imaging in behaving mammals: an engineering approach. *Neuron*. 2015;86:140-159.
167. Peron S, Chen TW, Svoboda K. Comprehensive imaging of cortical networks. *Curr Opin Neurobiol*. 2015;32:115-123.
168. Collier TJ, Srivastava KR, Justman C, et al. Nortriptyline inhibits aggregation and neurotoxicity of alpha-synuclein by enhancing

reconfiguration of the monomeric form. *Neurobiol Dis*. 2017; 106:191-204.
169. Jin M, O'nuallain B, Hong W, et al. An in vitro paradigm to assess potential anti-Abeta antibodies for Alzheimer's disease. *Nat Commun*. 2018;9:2676.
170. Rana P, Luerman G, Hess D, Rubitski E, Adkins K, Somps C. Utilization of iPSC-derived human neurons for high-throughput drug-induced peripheral neuropathy screening. *Toxicol In Vitro*. 2017;45:111-118.
171. Tortoriello G, Morris CV, Alpar A, et al. Miswiring the brain: Delta9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway. *EMBO J*. 2014;33:668-685.
172. Mansour AA, Goncalves JT, Bloyd CW, et al. An in vivo model of functional and vascularized human brain organoids. *Nat Biotechnol*. 2018;36:432-441.
173. Scholvin J, Kinney JP, Bernstein JG, et al. Close-packed silicon microelectrodes for scalable spatially oversampled neural recording. *IEEE Trans Biomed Eng*. 2016;63:120-130.
174. Yeyeodu ST, Witherspoon SM, Gilyazova N, Ibeanu GC. A rapid, inexpensive high throughput screen method for neurite outgrowth. *Curr Chem Genomics*. 2010;4:74-83.
175. Scholl AJ. Prostatectomy; an evaluation of methods. *Surg Clin North Am*. 1954;34:1719-1728.
176. Binley KE, Ng WS, Tribble JR, Song B, Morgan JE. Sholl analysis: a quantitative comparison of semi-automated methods. *J Neurosci Methods*. 2014;225:65-70.
177. Bamba Y, Kanemura Y, Okano H, Yamasaki M. Visualization of migration of human cortical neurons generated from induced pluripotent stem cells. *J Neurosci Methods*. 2017;289:57-63.
178. Camarena V, Cao L, Abad C, et al. Disruption of Mbd5 in mice causes neuronal functional deficits and neurobehavioral abnormalities consistent with 2q23.1 microdeletion syndrome. *EMBO Mol Med*. 2014;6:1003-1015.
179. Cavaliere F, Cerf L, Dehay B, et al. In vitro alpha-synuclein neurotoxicity and spreading among neurons and astrocytes using Lewy body extracts from Parkinson disease brains. *Neurobiol Dis*. 2017;103:101-112.
180. Oudin MJ, Gajendra S, Williams G, Hobbs C, Lalli G, Doherty P. Endocannabinoids regulate the migration of subventricular zone-derived neuroblasts in the postnatal brain. *J Neurosci*. 2011;31:4000-4011.
181. Sherman SP, Bang AG. High-throughput screen for compounds that modulate neurite growth of human induced pluripotent stem cell-derived neurons. *Dis Model Mech*. 2018;11:dmm031906.
182. Brazovskaja A, Treutlein B, Camp JG. High-throughput single-cell transcriptomics on organoids. *Curr Opin Biotechnol*. 2019; 55:167-171.
183. Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp Mol Med*. 2018;50:96.
184. Picelli S. Single-cell RNA-sequencing: the future of genome biology is now. *RNA Biol*. 2017;14:637-650.
185. Chen G, Ning B, Shi T. Single-cell RNA-Seq technologies and related computational data analysis. *Front Genet*. 2019;10:317.
186. Olsen TK, Baryawno N. Introduction to single-cell RNA sequencing. *Curr Protoc Mol Biol*. 2018;122:e57.
187. Camp JG, Badsha F, Florio M, et al. Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. *Proc Natl Acad Sci U S A*. 2015;112:15672-15677.
188. Costa V, Aigner S, Vukcevic M, et al. mTORC1 inhibition corrects neurodevelopmental and synaptic alterations in a human stem cell model of tuberous sclerosis. *Cell Rep*. 2016;15:86-95.
189. Patzke C, Sudhof TC. The conditional KO approach: Cre/lox technology in human neurons. *Rare Dis*. 2016;4:e1131884.
190. Flaherty EK, Brennand KJ. Using hiPSCs to model neuropsychiatric copy number variations (CNVs) has potential to reveal underlying disease mechanisms. *Brain Res*. 2017;1655:283-293.
191. Soliman MA, Aboharb F, Zeltner N, Studer L. Pluripotent stem cells in neuropsychiatric disorders. *Mol Psychiatry*. 2017;22: 1241-1249.
192. Wen Z, Christian KM, Song H, Ming GL. Modeling psychiatric disorders with patient-derived iPSCs. *Curr Opin Neurobiol*. 2016;36:118-127.
193. Tidball AM, Parent JM. Concise review: exciting cells: modeling genetic epilepsies with patient-derived induced pluripotent stem cells. *Stem Cells*. 2016;34:27-33.
194. Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O. Severe myoclonic epilepsy in infancy: Dravet syndrome. *Adv Neurol*. 2005;95:71-102.
195. Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest*. 2005;115:2010-2017.
196. Ogiwara I, Miyamoto H, Morita N, et al. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J Neurosci*. 2007;27:5903-5914.
197. Yu FH, Mantegazza M, Westenbroek RE, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci*. 2006;9:1142-1149.
198. Liu Q, Gao R, Li J, et al. Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering. *Biotechnol Biofuels*. 2017;10:1.
199. Favero M, Sotuyo NP, Lopez E, Kearney JA, Goldberg EM. A transient developmental window of fast-spiking interneuron dysfunction in a mouse model of Dravet syndrome. *J Neurosci*. 2018;38:7912-7927.
200. Mistry AM, Thompson CH, Miller AR, Vanoye CG, George AL Jr, Kearney JA. Strain- and age-dependent hippocampal neuron sodium currents correlate with epilepsy severity in Dravet syndrome mice. *Neurobiol Dis*. 2014;65:1-11.
201. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet*. 2008;40:782-788.
202. Au KS, Williams AT, Roach ES, et al. Genotype/phenotype correlation in 325 individuals referred for a diagnosis of tuberous sclerosis complex in the United States. *Genet Med*. 2007;9:88-100.
203. Hasbani DM, Crino PB. Tuberous sclerosis complex. *Handb Clin Neurol*. 2018;148:813-822.
204. Blair JD, Bateup HS. New frontiers in modeling tuberous sclerosis with human stem cell-derived neurons and brain organoids. *Dev Dyn*. 2019.
205. Ip JPK, Mellios N, Sur M. Rett syndrome: insights into genetic, molecular and circuit mechanisms. *Nat Rev Neurosci*. 2018;19: 368-382.
206. Dolce A, Ben-Zeev B, Naidu S, Kossoff EH. Rett syndrome and epilepsy: an update for child neurologists. *Pediatr Neurol*. 2013; 48:337-345.

207. Matarese CA, Renaud DL. Classical (type I) lissencephaly and Miller-Dieker syndrome. *Pediatr Neurol*. 2009;40:324-325.
208. Nagamani SC, Zhang F, Shchelochkov OA, et al. Microdeletions including YWHAE in the Miller-Dieker syndrome region on chromosome 17p13.3 result in facial dysmorphisms, growth restriction, and cognitive impairment. *J Med Genet*. 2009;46:825-833.
209. Iefremova V, Manikakis G, Krefft O, et al. An Organoid-based model of cortical development identifies non-cell-autonomous defects in Wnt signaling contributing to Miller-Dieker syndrome. *Cell Rep*. 2017;19:50-59.
210. Lee JH, Huynh M, Silhavy JL, et al. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nat Genet*. 2012;44:941-945.
211. Mirzaa GM, Riviere JB, Dobyns WB. Megalencephaly syndromes and activating mutations in the PI3K-AKT pathway: MPPH and MCAP. *Am J Med Genet C Semin Med Genet*. 2013;163C:122-130.
212. Butler MG, Dasouki MJ, Zhou XP, et al. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. *J Med Genet*. 2005;42:318-321.
213. Marchese M, Conti V, Valvo G, et al. Autism-epilepsy phenotype with macrocephaly suggests PTEN, but not GLIALCAM, genetic screening. *BMC Med Genet*. 2014;15:26.
214. Driggers RW, Ho CY, Korhonen EM, et al. Zika virus infection with prolonged maternal Viremia and fetal brain abnormalities. *N Engl J Med*. 2016;374:2142-2151.
215. Li H, Saucedo-Cuevas L, Shresta S, Gleeson JG. The neurobiology of Zika virus. *Neuron*. 2016;92:949-958.
216. Mlakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. *N Engl J Med*. 2016;374:951-958.
217. Pastula DM, Yeargin-Allsopp M, Kobau R. Enhanced epilepsy surveillance and awareness in the age of Zika. *JAMA Neurol*. 2017;74:631-632.
218. Cugola FR, Fernandes IR, Russo FB, et al. The Brazilian Zika virus strain causes birth defects in experimental models. *Nature*. 2016;534:267-271.
219. Garcez PP, Loiola EC, Madeiro Da Costa R, et al. Zika virus impairs growth in human neurospheres and brain organoids. *Science*. 2016;352:816-818.
220. Xu M, Lee EM, Wen Z, et al. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nat Med*. 2016;22:1101-1107.
221. Zhou T, Tan L, Cederquist GY, et al. High-content screening in hPSC-neural progenitors identifies drug candidates that inhibit Zika virus infection in fetal-like Organoids and adult brain. *Cell Stem Cell*. 2017;21:274-283 e5.
222. Stein JL, De La Torre-Ubieta L, Tian Y, et al. A quantitative framework to evaluate modeling of cortical development by neural stem cells. *Neuron*. 2014;83:69-86.
223. Tang X, Zhou L, Wagner AM, et al. Astroglial cells regulate the developmental timeline of human neurons differentiated from induced pluripotent stem cells. *Stem Cell Res*. 2013;11:743-757.
224. Bardy C, Van Den Hurk M, Eames T, et al. Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. *Proc Natl Acad Sci U S A*. 2015;112:E2725-E2734.
225. Giandomenico SL, Mierau SB, Gibbons GM, et al. Cerebral organoids at the air-liquid interface generate diverse nerve tracts with functional output. *Nat Neurosci*. 2019;22:669-679.
226. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science*. 2014;345:1247125.
227. Schwartz MP, Hou Z, Propson NE, et al. Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. *Proc Natl Acad Sci U S A*. 2015;112:12516-12521.

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