The Use of Organoids in Epilepsy Research
Webinar
(Transcript)

Priya Balasubramanian: 00:02 Welcome everyone. I'm Priya Balasubramanian. I am associate director of research at CURE Epilepsy. And thank you all for joining us today. Today is our second virtual seminar of 2021. And this is part of our Frontiers in Research Seminar series. Today's seminar is entitled The Use of Organoids in Epilepsy Research. And this seminar will discuss recent advances in techniques using three-dimensional brain organoids and why they are a useful tool to model epilepsy and desk potential therapies. This seminar series is generously supported by the Nussenbaum-Vogelstein family. This helps educate and expose researchers, clinicians, and students to exciting epilepsy research. It also provides opportunities for young investigators to interact with leaders in the field. In the past few months, we've been conducting these seminars virtually due to COVID-19 and we will continue to do so until conditions allow us to all come back together in person, hopefully soon.

Priya Balasubramanian: 01:12 Do mark your calendars for our Postdoctoral Data Blitz seminar. This is an exciting one. It's on April 21st. We'll have three postdocs share their research and they'll also have a question and answer session with them. So please do register for the seminar and try to attend. And I also want to say a big thank you to all the postdocs who sent in their abstracts for consideration. We'll reach out to the three postdocs who were selected to speak by the end of the month.

Priya Balasubramanian: 01:45 CURE Epilepsy is proud to be a leader in the epilepsy research community. For over 20 years, we have funded over 260 projects in 16 countries. As some of you might know, we have three different funding mechanisms. We have our CURE Epilepsy Award, we have the Taking Flight Award. The CURE Epilepsy Award funds more established researchers over two years, $250,000. And the Taking Flight Award funds more junior researchers who have at least three years of postdoc experience, but are yet to attain significant funding. The 2021 grant cycle for these two awards is already underway, but our Catalyst Award, which is our newest award, the RFA for that one will be announced in Q2 of this year. This is a new funding mechanism and it's intended to fund translational research with the goal of advancing new therapies to clinical application. Please mark your calendars for that.

Priya Balasubramanian: 02:55 And now it is my pleasure to welcome Dr. Jack Parent. He is the William J Herdman professor of neurology and co-director of the epilepsy program at the University of Michigan. Dr. Parent's lab is interested in studying epileptogenesis, adult

neurogenesis, brain repair and SUDEP. And to do this, they use some very cutting edge IPSC and organoid techniques, some of which you hear about today, as well as rodent and zebrafish models. Dr. Parent is also a two-time CURE Epilepsy grantee, including a 2017 innovator board in which his team used an innovative CRISPR screen and IPSC-derived neurons to identify novel gene mutations that can cause a focal cortical dysplasia like abnormalities.

Priya Balasubramanian: 03:45 Before he begins, I'd like to encourage everyone to ask questions. You can submit your questions anytime during the presentation by typing them in the Q&A tab located on the bottom of your Zoom panel and click send. And we'll do our best to get through as many of the questions as we can. Today's seminar as well as all of our future seminars are recorded and they will be available on the CURE Epilepsy website. So with that, I will turn it over to Jack. Thank you.

Dr. Jack Parent: 04:17 Thank you, Priya. So I would like to thank CURE Epilepsy and Priya for inviting me to speak to you today. I'm going to talk about our work on brain organoid models of epilepsy. And I have, let's see, no disclosures. I'm having trouble advancing. Okay, it's going slowly. So I'm going to talk to you about organoids today. So these are 3D cell culture models that have become popular for modeling human organs in a dish. And they are intermediate between 2D cell culture, which are more simplistic models, and the complexity of in vivo organisms. And for human organoids, for some tissues, you can use adult tissue stem cells, for example, for making intestinal organoids. But for other tissues such as brain, you have to start with human pluripotent stem cells. So that means either somatic cells that are reprogrammed to induce pluripotent stem cells or human embryonic stem cells derived from blastocyst that are then cultured in three dimensions.

Dr. Jack Parent: 05:43 And from a brain organoid perspective, obviously there's limited access to live human brain tissue. So this gives you a way to study human brain tissue in a dish in three dimensions. 2D human pluripotent stem cell derived cultures have limited maturation. You can only culture them for several months and a network complexity, whereas brain organoids can be cultured for years and develop putatively more complex neural networks. And then for some brain neurodevelopmental diseases, it's important to use human models because there are important differences between mouse and human brain development.

Dr. Jack Parent: 06:35 So this slide shows some of those differences. So in addition to the obvious differences with mice being lissencephalic and their brains much smaller, the stem cell niche is different. So for the developing cortex, the dorsal ventricular zone gives rise to excitatory neurons in the developing cortex. These are radio glial stem cells in the ventricular zone. And then they also give rise to intermediate progenitors that are in the subventricular zone. And compared to rodent, primates have a massively expanded subventricular zone with a cell type outer radio glial or basil radio glia that are not really present in rodent. But these subventricular zone cells are thought to give rise to the superficial layers of the neocortex and are in part responsible for the expansion of the primate neocortex. And the whole process of neurogenesis occurs in less than a week in mice and is protracted to over three months in human.

Dr. Jack Parent: 07:44 So to model human brain development, investigators have come up with a brain organoid methods. And they're really two approaches to make brain organoids. One is called unguided differentiations. So all of them rely on aggregating human pluripotent stem cells into embryoid bodies. And then with unguided differentiation, the default is to make four brain-like tissues versus using small molecules to guide differentiation to make brain region specific organoids. So with unguided differentiation, it's nice because you can get lots of different brain tissue, but the problem with it is you get lots of different brain tissue and it's very inconsistent and variable. So, with a few exceptions, most groups have gone to directed differentiation to make region specific organoids. And another important approach that you can use with these region specific organoids is to combine them so you can pattern them toward two different brain regions and fuse the two to make so-called assembloids. And I'll speak more about that approach in a minute.

Dr. Jack Parent: 09:03 In addition, advances that are ongoing include co-culturing the human pluripotent stem cells with other human cell types that aren't present in the organoids, like microglia or microvascular endothelial cells, or even transplanting them into immunocompromised mice to use the mice as an incubator where they vascularize the organoid as it grows in vivo.

Dr. Jack Parent: 09:32 So, there are challenges with this approach that'll go into. I mentioned batch-to-batch consistency and variability in the organoids. Obviously it's missing non-neural cell types. The center as they grow can become a necrotic, which is typical, even if you're in a spinning bioreactor that doesn't prevent the center from dying. The maturation is slow because it follows

human developmental timeframes, which as you know take many months to develop. There's a limited neuronal subtype repertoire and regionalization in brain organoids compared to human fetal tissue. And then Arnold [Creaksdean 00:10:19] showed that brain organoids are enriched in glycolytic and ER stress pathways compared to fetal brain. Although I think this PR problem is overstated because a recent paper by Dan Geschwind's group that just came out in nature neuroscience found actually that there's a fair amount of glycolytic and ER stress pathway activation in human fetal tissue. And that the developmental trajectory is similar between the organoids and human fetal tissue in these pathways. So I think that's not really a main major issue. And then with the original organoids, there's no subcortical connections.

Dr. Jack Parent: 10:58 But, there are advances that are overcoming these challenges. So the consistency, as I mentioned, is improving with the small molecule patterning. I also mentioned that you can co-culture with the missing non-neural cell types. To get rid of the necrotic center, you can cut the organoids, or as I said, you can grab them in vivo into mice and they'll become vascularized. And in terms of no subcortical connections with assembloids, you can combine region specific organoids to get the connectivity. This is important for epilepsy research. So for example, the Park Lab at Yale has patterned organoids to excitatory cortex and thalamus, and then fuse them and you get reciprocal thalamo-cortical and cortico-thalamic projections in these fusion organoids that obviously for certain types of epilepsy would be an important model to use.

Dr. Jack Parent: 12:04 And then even more relevant is combining excitatory and inhibitory progenitors. So as many of you know, the stem cells that give rise to the excitatory cortical neurons are in a different location than those that give rise to the interneuron. So the excitatory neurons arise from the dorsal ventricular zone and migrate radially to the cortex, but the inhibitory progenitors come from the ganglionic eminences. So the ventral forebrain, the sub-pallial forebrain. And they arise in the medial and caudal ganglionic eminences and migrate tangentially to inhabit the cortex.

Dr. Jack Parent: 12:45 So to model that in organoid, several groups including Sergiu Pasca's developed fusions of excitatory cortical organoids and what he calls human sub pallial spheroids or ganglionic eminence organoids. And then fuses the two, and what you can see here with a GFP interneuron label, [Dlx1/2 00:13:07] reporter. When you fuse the labeled sub-pallium with the excitatory cortex over time, the green cells migrate into the

excitatory cortex to form networks of excitatory and inhibitory neurons. And obviously for studying epilepsy, you want both excitatory and inhibitory neurons in your networks. And this method has already been used to study several neurodevelopmental disorders with implications for epilepsy. For example, the Pasca lab's paper looked at Timothy Syndrome and found some findings. And there is a nice paper that's on bio archive right now from Ran Samarasinghe and Ben Novitch at UCLA describing their fusion organoid studies of Rett Syndrome. It has really nice functional data that I encourage you to look at.

Dr. Jack Parent: 14:02 So how are we going to use brain organoids to study epilepsy? So the main use right now is for looking at genetic epilepsies. Because you're starting with human-induced pluripotent stem cells that carry a gene variance of interest in epilepsy from patients that are derived from fibroblast blood cells from the patient that you've reprogrammed, and so it's natural to study these genetic epilepsies with the human pluripotent stem cells and organoids. And you can look at structural brain development network function, test drugs. People are starting to do genetic screens in the organoids.

Dr. Jack Parent: 14:44 Another use that I'm going to bring up today is looking at anti-seizure medicine teratogenicity in brain organoids. And then a question that we may want to discuss at the end is whether it will be useful for acquired epilepsies. It's not clear at present, but I think it's moving in that direction as the technology improves. And at the end, I'll talk about hippocampal organoids that may be interesting in this regard. So for genetic epilepsies, as all of you know with next-gen sequencing, there's been an explosion in our identification of epilepsy genes and we need a better models to study these epilepsies. And that includes zebrafish, rodent, and human [inaudible 00:15:36] stem cell models, both 2D, and what I'll focus on today, the 3D brain organoid models.

Dr. Jack Parent: 15:41 And I'm going to focus on two genes today; STRADA and PCDH19. So what we'll talk about in the first part of my talk, I'm going to go over our work on Polyhydramnios, Megalencaphaly and Dymptomatic Epilepsy Syndrome, or PMSC, also known as Pretzel Syndrome, which is due to homozygous loss of function deletions in the STRADA gene, and it's an mTORopathy. And then I'm going to tell you about a new brain organoid method that my lab has generated called SOSRS, self-organized single rosettes spheroids, and our use of that model to study anti-seizure medicine associated neural tube defects, and another common genetic epilepsy, Protocadherin-19 Cluster Epilepsy. And then I'll end briefly talking about other epilepsies that have

been modeled with brain organoids and how to test network function in organoids.

Dr. Jack Parent: 16:38 So, all of you know mTOR pathways are very important in malformations of cortical development and epilepsy. The classic disorder is tuberous sclerosis complex, which results in cortical tubers and loss of function mutations lead to mTOR hyperactivity. But there are many other parts of the pathway that lead to disorders such as megalencaphaly, hemimegalencaphaly, and focal cortical dysplasia. Two important studies have looked at this pathway with brain organoids. Rudy [inaudible 00:17:15] lab published in cell stem cell work on PTEN knockout organoids. And Helen Beta's group at Cal had a really elegant study looking at using organoids to look at mechanisms of tuberous sclerosis complex.

Dr. Jack Parent: 17:32 Today, I'm going to tell you about our work studying another aspect of mTOR pathway disorders, STADA. STRADA alpha gene and megalencaphaly. So PMSC syndrome is caused by homozygous deletion or mutations in the STADA alpha gene. And most commonly it occurs in Old Order Mennonite communities in Pennsylvania and Ohio due to a founder deletion in consanguinity. And these kids have macrocephaly and megalencaphaly, severe epilepsy, neurocognitive defects. They have areas of focal cortical dysplasia. And Peter Crino showed that their brains have evidence of mTOR hyperactivity shown here with increased Phospho-S6 staining. And his group treated several of these kids with everolimus and were able to partially reverse or halt progression of the disorder.

Dr. Jack Parent: 18:39 So we collaborated with Peter and his group. Whitney reprogrammed or collected fibroblasts for several of these kids and sent them to us. And we generated human pluripotent stem cells to study. To grow the organoids, we used a method developed by Guo-li Ming and Hongjun Song called the Spin-Ω method, where they use this mini bioreactor in 12 well plates and this is the protocol with dual SMAD inhibitors and some Wnt agonists. And over time, these human pluripotent stem cells form a beautiful excitatory cortical organoids that are very consistent. So a former MSTP student in the lab, Xixi Plummer went down to Guo-li and Hongjun's lab and was able to learn the technique. It forms very consistent cortical organoids. And Louis Dang, a pediatric epileptologist in the lab is using this to study PMSC. And we were excited right off the bat because right away you can see that compared to ... So we had two patients with PMSE and two controls that we generated organoids from. Right away you can see the PMSE organoids are bigger and it looks like it's modeling megalencaphalyl.

Dr. Jack Parent: 20:09 So the first thing Louis did was to look at mTOR hyperactivity in his organoids, and he stained for phosphyl-6 and phosphyl4E-BP1. And compared to the controls, there was market increase in phosphyl-6 and phosphyl4E-BP1 in the PMSE organoids, which is quantified here. So as expected, they show evidence of mTOR hyperactivity. And when Louis looked at the morphology, and this is in the first two weeks, he saw that the PMSE organoids were much as quantified here by surface area between days eight and 12. At each time point, they were significantly larger and they had this budding phenotype. So you can look and see these round structures and these are neural rosettes, so they're analogous to the developing neural tube. And compared to the control there, the neural rosettes were smaller but much more abundant and budding off the exterior of the organoid. And Louis quantified the buds per organoid, and it was markedly increased in the PMSE organoids.

Dr. Jack Parent: 21:26 So what are the buds comprised of? He stained them for neural stem cells with PAX6 and neurons with a neuron-specific beta tubulin. And he found the PMSE organoids had more of this PAC6 and less neurons, suggesting that it was a delay in differentiation and that the stem cells, instead of becoming neurons, were continuing to proliferate. And I'm not showing this, but he also found an increase in [KI67 00:21:55] marker cell proliferation in the PMOC organoids.

Dr. Jack Parent: 22:02 Louis looked at later time points, week eight on top and week 12 on the bottom. And he looked at the neural stem cells labeled with PAX6 and then early and somewhat later born neurons, CTIP2 and SATB2. And at a week eight, there was still a differentiation defect because there were less of these CTIP2 positive neurons in PMSE compared to control. There weren't many SATB2 neurons at this eight week time point in either group. But by 12 weeks, the PMSE organoids caught up and there were no differences in any of the cell types. They're all making deep and more superficial layer cortical neurons.

Dr. Jack Parent: 22:47 But interestingly, at the same time point, Louis looked at HOPX staining. And HOPX is a marker that's specific for these outer radial glia that I mentioned earlier that are expanded in the primate brain, including humans, and are thought to give rise to superficial cortical neurons. So he measured neuron specific beta tubulin, and this was not changed at 12 weeks. So the PMSE organoids caught up in making neurons, but they had many more of these HOPX+ outer radial glia. And Arnold Krickstein has shown that these outer radial glia are high in markers of mTOR pathway. And he also found them in human focal cortical dysplasia. So the prediction is if they're more out

radial glia, then there should be an expansion of the superficial cortical layer neurons. So Louis looked at this at day 113, and he stained for CTIP2, which is a deep layer marker, and also BRN2 and SATB2 more superficial layer markers. And compared to the controls, the PMSE had an expansion of these more superficial cortical layers, suggesting this may be another mechanism for the megalencaphaly in this syndrome.

Dr. Jack Parent: 24:04 I mentioned that these patients have areas of focal dysplastic cortex, and as you know, compared to normal cortex, there's dislamination in cortical dysplasia along with astrogliosis and dysmorphic neurons in a unique cell type, at least in a focal cortical dysplasia type 2B called balloon cells. And these balloon cells express markers of progenitors, astrocytes and neurons all at the same time. So Louis started looking for some of these changes. When he stained with GFAP, he found these really enlarged and abnormal looking astrocytes in the PMSE organoids that weren't present in the control. There was quite a lot of variability in the number of astrocytes in a given organoid. And so he didn't see a significant increase in total astrogliosis as Hellen Beta pad in her TSE organoids, but we think maybe that's partly due to the variability. But when he looked for balloon cells, which incidentally have never been convincingly demonstrated in rodent models with focal cortical dysplasia, but in the control organoids, he stained for GFAP, HOPX and MAP2 and did not see any bizarre large cells.

Dr. Jack Parent: 25:24 But in the PMSE organoids at this time point, there were many enlarged cells. And if we blow up this pair, they're expressing all three markers and they look very abnormal. Some of them were bi-nucleated, which is classic for balloon cells. And if we compare to resected human FCD tissue, these bi-nucleated cells look very similar to what's been seen in human focal cortical dysplasia, and these balloon cells look very similar to the classic balloon cells in human FCD tissue. So we think this model is a high fidelity model of malformations of cortical development. And it will be interesting to look at function and see if we see seizure like activity in the PMSE model.

Dr. Jack Parent: 26:16 So just to summarize this part of the talk, PMSE patient cortical organoids model megalencaphaly and have the mTOR hyperactivity and neuroepithelial budding phenotype. This phenotype is Wnt-dependent. I didn't have time to show you that, or that there's also alteration in cilia in the organoids as well as increased cell proliferation and cell death. The PMSE organoids show increased outer radial glia and potentially greater generation of superficial cortical neurons, and they have

abnormally enlarged astroglia and balloon cells, suggesting that they're a very good model of FCD.

Dr. Jack Parent: 26:55 So I'm going to move on and address this question. How do we improve upon brain organoid methods? So as I mentioned to you, the standard brain organoid protocol involves starting with aggregated cells as embroid body. So it's a 3D structure and then there's patterning and self-organization, and they form these multi-rosette organoids. And each of these rosettes shown here with their lumen stained by [inaudible 00:27:28] is analogous to the neural tube. But as you all know, when we develop, we don't have multiple neural tubes. We have a single neural plate that folds to form a single neural tube. So we start as a 2D structure with folding to make a neural tube.

Dr. Jack Parent: 27:45 So Andrew Tidball, really talented research investigator in the lab asked, can we better model this using single rosette that organoid, starting with a 2D monolayer. So what he did was culture IPS cells as a monolayer and use a cutting tool to cut them into squares, plated them in geltrex and these homogeneous roundup to form these beautiful single rosette organoids that have a single lumen in the center. This is what they look like from day nine to 18 as they grow. And the growth rate between organoids is very consistent. They grow about a micron an hour. And interestingly, if you look at the human telencephalic neural tube from E30, they're about the same size and they look very similar.

Dr. Jack Parent: 28:42 So we're very excited about this model. If Andrew patterns them toward dorsal excitatory phenotype at early time points, they express neural stem cell markers like PAX6 and Nesting. By day 22, they're expressing phospho vimentin, which labeled dividing radial glia. And you can see the radial glia dividing at the atypical lumen, which models inter kinetic nuclear migration, where the stem cells are in S-phase out here and then they migrate in and divide at the lumen and then go back out. And then they're also dividing cells in the sub ventricular zone. And then the neurons develop on the outside as labeled with double [inaudible 00:29:27] here. By day 42, we're already getting different cortical layers that express CTIP2 and SATB2 with the stem cells in the center, and they start making these HOPX+ outer radial CLIA as well.

Dr. Jack Parent: 29:42 Carmen Varela, a grad student in the lab, worked with Andrew to pattern these as ventral inhibitory organoids as well. And they express using SAD, smooth and agonist. They express markers of mGE like NKX2.1, OLIG2, which labels the progenitors of the interneurons. And by day 56, they're making

somatostat and then GABA interneuron markers. And we're doing fusions with these as well. So we think this is going to be useful for studying a number of diseases.

Dr. Jack Parent: 30:17 And to look at reproducibility in these organoids, we started doing some single cell RNAseq and this is in one month SOSRS. And so Andrew sent four separate SOSRS for single cell RNAseq and looked at the overlap and they made seven different clusters, including mitotic radial glia, cortical hem and choroid plexus, cortical radial glial cells, cortical neurons and intermediate progenitors. Surprisingly, we found some LGE in GABAergic neurons, but when we look at 90 days, these drop out, so we think there are only an early feature. And the different organoids overlap quite a bit in the different cell types. So it's a very consistent method.

Dr. Jack Parent: 31:03 So one of the things Andrew wanted to study with these is neural tube defects. So as you know, there are many teratogens that cause neural tube defects. And since he gets a beautiful neural tube like structure with his SOSRS, he wanted to see if he could model this. He used two compounds that affect something called the apical constriction pathway. So when columnar epithelium go from a flat monolayer to a tube-like structure, they constrict their atypical domains. And with rock inhibitor or blebbistatin, you can see that this nice apical lumens stained with zonula occludens. One in the untreated gets totally altered with these teratogens. And Andrew measured apical surface area, or lumen to SOSRS area and they were both increased with rock inhibitor treatment. Similarly, the lumen to SOSRS area increased with blebbistatin as well as a decrease in the circularity of the lumen.

Dr. Jack Parent: 32:15 So Andrew next said, "Can we study anti-seizure medicine neural tube defects with these?" And valproic acid is the most implicated seizure medicine in neural tube defects. It's known to inhibit HDAC and folic acid pathways, and also cause oxidative stress. And there's about a 10 to 20 fold increase in neural tube defects, including spinal bifida in children of mothers on valporic acid. In mice, if you give them valproic acid to model neural tube defects, they get exencephaly, a very severe phenotype. So Andrew looked at exposure of valproic acid to the SOSRS. Again, green is a zone of glutens one. Blue is just a nuclear marker. And you can see disruption of this apical lumen with expansion and irregularity. And doing a dose response, he found a small effect by 100 micromolar valproic acid and a larger effect of 200 micromolar in terms of lumen to SOSRS area and 200 micromolar also affected circularity. So we

think this is going to be a very useful approach to model neural tube defects due to teratogens.

Dr. Jack Parent: 33:37 To look at mechanism, Andrew tested whether HDAC inhibitors or inhibitors of the folic acid metabolism have the same effect. And so this is abnormal lumen to SOSRS area with 200 and 400 micromolar valproic acid, but within HDAC inhibitor TSA, he could not replicate it or with aminopterin which blocks folic acid metabolism. So it suggests these may not be the mechanisms of valproic acid, although with the caveat that valporic acid affects several classes of HDAC inhibitor more than what TSA blocks. So there's still work to be done there, but we're encouraged by this model and it may be useful to screen new anti-seizure medicines.

Dr. Jack Parent: 34:26 So we also wanted to use the SOSRS to study a genetic epilepsy. And we chose a very interesting epilepsy to look at first called X-linked Clustering Epilepsy or XCE. This is caused by mutations in the PCDH19 gene, which encodes protocadherin 19. It causes one of the most common genetic epilepsies, developmental and epileptic encephalopathy type nine. Used to be called epilepsy and females with mental retardation and also female clustering epilepsy, but because mosaic males are also affected, it's probably better to call it X-linked Clustering Epilepsy. So these children have varied phenotypes of early onset epilepsy, intellectual disability, and neuropsychiatric features that come on later. And the patients typically have seizure clusters that are often provoked by fever. Their seizures can be focal or generalized. And what's really unique about this disorder, it affects heterozygous females or mosaic males, but hemizygous males are unaffected. And in most x-linked disorders, males are more severely affected. So that raises the question: why are hemizygous males spared?

Dr. Jack Parent: 35:41 So what does protocadherin 19 do? It's a member of the delta non-clustered protocadherins, which is part of the cadherins super family. So these are proteins that are involved in cell-cell interactions. They have large extracellular domains and cadherin interaction domains. PCDH19 is prominently expressed in the nervous system, is thought to underlie cell migration in synaptogenesis, may be involved in radial glial proliferation and GABAergic transmission, may also regulate the neurosteroid allopreganolone transcriptionally.

Dr. Jack Parent: 36:20 And the reason that we think females and mosaic males are affected but not hemizygous males is due to something called the cellular interference hypothesis. So the idea is that in a normal individual, you're developing neurons, interact through

protocadherin 19. In hemizygous males, there is only nonfunctional PCDH19, and the cells are still able to interact. But in affected females where there's random X-inactivation, you have two populations of cells. Some of the cells are only expressing the wildtype ilial that has normal PCDH19 and other cells are expressing only the mutant ilial that has dysfunctional PCDH19. And these cells cannot interact with each other during brain development, and they segregate. And that would be true of mosaic males as well with two populations. And this may explain the variability of the disorder due to the degree of random X inactivation in different brain regions.

Dr. Jack Parent: 37:33 So we reasoned that the SOSRS model would be a really good way to test this hypothesis and what Wei Niu, an assistant research scientists in the lab, and Sandra Mojica-Perez, a technician lab, did was they generated CRISPR knockout lines for PCDH19 from human pluripotent stem cells as well as isogenic controls. And they labeled them with GFP and RFP. And the idea, again, is if you mix all wildtype, everything's normal, if you mix all mutant, everything's normal. But if you have one population of wildtype and one population of mutant, then you get the disease. And so here Wei and Sandra mixed GFP labeled wildtype with RFP labeled wildtype. They mixed normally as you can see here. They mixed GFP knockout and RFP knockout and they mixed normally when they generated the SOSR. But when they mixed wildtype and knockout, you can see that the wildtype cells in red and the knockout cells in green did not interact and said they segregated in the radial glial ventricular zone domain shown here.

Dr. Jack Parent: 38:50 So this supports the cellular interference hypothesis. To make a model of mosaic males, Wei and Sandra did a CRISPR knockout of PCDH19 in male IPS cells. And in this case, they had unlabeled cells and GFP labeled cells that they mixed. When they mixed wildtype and wildtype, both male cells everything was homogeneous. But when they mixed wildtype and knockout cells, over time this segregation again developed with the unlabeled wild type cells segregating from the GFP labeled knockout cells. So again, we have it in the mixed females and the mosaic male model.

Dr. Jack Parent: 39:39 They next looked at development of the cortical layer neurons. And so the early born neurons expressed CTIP2. So you can ignore the center. That's another area of cortex that, for technical reasons, went into the lumen. But the outside is the early born neuron layer. And when wildtype and wildtype are mixed, it's a nice homogeneous layer. But with mixing wildtype and knockout, there's expansion of this layer. It's disorganized,

and we think this is either due to premature differentiation of the early born neurons or a migration defect in them. And we're currently trying to differentiate between the two.

Dr. Jack Parent: 40:20 So this is exciting to us, but I think the power of the organoid approach is also in combining it with mouse models. And so Julie Ziobro, a post-doc in the lab, developed a mouse model of the X-linked Clustered Epilepsy. She obtained a knockout line from Hisashi Umemori at Boston Hospital, and also a GFP reporter that's on the X chromosome from Sundeep Kalantry who's in human genetics at Michigan, such that when she crossed males with Xgfp and female PCDH19 knockout, the female offspring that are heterozygous will have GFP only on the cells that express the wildtype PCDH19 ilial and the unlabeled cells will be knockout cells.

Dr. Jack Parent: 41:14 And so Julie crossed the gfp reporter with either knockout or wildtype females. And when she crossed it with wildtype females, again, one ilial is gfp, another ilial is unlabeled. And because of random X inactivation, you have two populations but they mix quite readily. But when she crossed it with the knockout, the knockout cells shown in blue don't interact appropriately with the gfp labeled cells that express the wildtype ilial, similar to what we saw in the organoids. This was not surprising because Paul Thomas had used a similar approach of HA-tag PCDH19 cells to make a mouse model that he published in Neuron. His mice did not have spontaneous seizures but he did see an increase in interictal epileptiform activity in the mice. What Julie did is apply a hyperthermic seizure protocol to 16-day-old mice and looked at seizure threshold by temperature and seizure severity and found a lower temperature for seizure onset in the heterozygous knockout mice, compared to the control and more severe seizures. So only the heterozygous knock-outs had Racine scale of four or five seizures, and none of the wildtype did.

Dr. Jack Parent: 42:39 What Julie also found was decreased parvalbumin+ interneurons in area CA1 and she's now looking for deficits in synaptogenesis and at the network effects. But we think it's going to be really useful to combine both of these models. So both the mice and the SOSRS successfully modeled the self-segregation phenotype and cellular interference in X-linked Clustered Epilepsy. Additional phenotypes include decreased hyperthermic seizure threshold and less CA1 [inaudible 00:43:09] interneurons in the mice and altered PCDH19 and cadherin sub-cellular localization, which I didn't have time to show you in the SOSRS as well as a migration or differentiation defect. And so we're combining these models now. Because of

what she found in the mouse, we're exploring the ventral inhibitory SOSRS as well as the fusion SOSRS, looking at synaptogenesis and network function in both models. And we're also testing a potential ASO therapy starting with the organoid model and optimizing it, and then we'll go into the mice. So I think they're very complimentary approaches.

Dr. Jack Parent: 43:47 So in the brief remaining time, I'm just going to talk about some other findings from other groups using human brain organoids to model epilepsy as well as looking at function in the organoids. So as I mentioned earlier, Sergiu Pasco's lab has studied Timothy Syndrome with fusion organoids, and Helen Beta looked at tuberous sclerosis complex and found some abnormalities. There's also work on periventricular nodular heterotopia in organoids look with findings of defective radial progenitor cells and older migration in specific neuronal subpopulation. Rudy [inaudible 00:44:29] group found defects in organoids generated from Angelman Syndrome patients. His group also did the PTEN model in organoids. Several groups have studied LIS1, Miller Dieker Syndrome in organoids with interesting phenotypes. And then Alison [inaudible 00:44:53] recently published work in Rett Syndrome. And as I mentioned the Novitch lab has a nice paper on bio archive studying Rett Syndrome and fusion organoids.

Dr. Jack Parent: 45:05 So studying epilepsy with organoids, one obviously wants to look at function. And the field is still trying to figure out the best way to look at network function in the organoids. So our group's done a few different things. So we've dissociated 90 day SOSRS and looked at patch-clamp recordings, and we find spontaneous and evoked activity. Wei Niu in the lab has plated the organoids on multi electrode arrays to look at a local field potential activity, a nice paper by the [inaudible 00:45:40] lab in cell stem cell a couple of years ago showed in six to eight month old organoids, they develop really nice network activity that models fetal brain development using multi electrode arrays.

Dr. Jack Parent: 45:58 With our collaborator, Yukun Yuan and [inaudible 00:46:00] lab, we made acute slices from the organoids and looked at action potentials and post synaptic currents. And I mentioned Ranmal and Ben at UCLA collaborating with Istvan Mody and Peyman Golshani. What they're doing is using patch electrodes but putting them in to the organoids, a fusion organoid ere to record local field potentials. And when they combine a fusion of cortical and ganglionic eminence, so the intern arm progenitors to get a mixed excitatory inhibitory networks, they get lots of oscillatory activity in different frequency bands that resemble what you would see in a human brain, which is really exciting.

And they found that these oscillations are altered in Rett Syndrome. And they've also done calcium imaging, two-photon calcium imaging with GCaMP6 looking at neuronal activity and looking for synchronous discharges in several epilepsy models.

Dr. Jack Parent: 47:14 The other thing Ron was doing, which is really exciting is making human hippocampal organoids by patterning them with different small molecules. So here, he has generated organoids from control IPS cells and those from a patient with SCN8A developmental and epileptic encephalopathy. And these markers, ZBTB20 is highly expressed in CA1 pyramidal cells. Neuropilen 2 is in DG and CA3 more than CA1. And PROX1 is a marker of dentate granule cells, and he sees expression of all these markers. Obviously it doesn't have the organization of a normal hippocampus, and so there's going to be a lot of work to optimize this. But when he used those electrode LFP recordings in fusion organoids, so the GE gives rise to hippocampal inner neurons. So he fused the hippocampus with GE and when he does a few potential recordings, he sees these sharp wave ripple discharges which are important for hippocampus information processing. And then the SCN8A mutant, there's a loss of these sharp wave ripples. So it seems like this approach is going to be useful not only for studying seizure mechanisms, but also perhaps for some of the cognitive comorbidities in some of these genetic epilepsies.

Dr. Jack Parent: 48:40 And in terms of looking at seizures, Ranmal looked at two photon imaging of GCaMP6 in an SCN8A fused hippocampal cortical organoid and you can see, it's running a little slowly, but you can see these bursts of synchronous activity in the organoids that model seizure-like discharges. So I think this is a really exciting area. And I think when we get better at the hippocampal organoids, we can start thinking about modeling acquired epilepsies like from a head trauma and other disorders.

Dr. Jack Parent: 49:18 So with that, I'd just like to summarize. I hope that I've convinced you that brain organoids are a powerful approach for understanding genetic epilepsies, that influence brain development. The SOSRS single rosette model is useful for studying teratogenicity of anti-seizure medicines. And I think combining mouse embryoic organoid models as we did for PCDH19 should yield mechanistic insights and novel precision therapies. Finally, improvements and organoid methods, including single rosettes, the fusion models and strategies for exploring network function, I think will advance epilepsy studies quite a bit using human cell models.

Dr. Jack Parent: 49:59 And with that, I'll acknowledge people who did the work again. Louis Dang did the PMSE brain organoid studies along with Shivansi Vaid and Grace Lin in the lab. Andrew Tidball developed the SOSRS method and got some help from Carmen Varela making the ventral SOSRS. Sandra and Wei did the PCD 19 organoid studies and Julie did the mouse studies in collaboration with Sundeep Kalantry and Hisashi Umemori at Boston Children's. And then the PMSE work was done with Peter Crino. And finally Jun Li and Qianyi Ma in human genetics helped us with the single cell RNAseq. And I showed you some data from the UCLA group. And I also want to acknowledge CURE Epilepsy and my other funding sources. So thank you for your attention, and I'd be pleased to answer any questions that you have.

Priya Balasubramanian: 51:02 Great talk, Jack. Thank you so much. So we have some time for a few questions. We've have had a couple come in, so I'd also like to remind people, if you want to send in your questions, please do send them into the Q&A tab. So Jack, one of the questions that came in is asking, did you see evidence of variable or incomplete X inactivation in the female organoids?

Dr. Jack Parent: 51:34 That's a really good question. When we started the PCDH19 studies, we started with female patient-derived IPS cells. We were doing 2D cultures. And we saw evidence for X inactivation, which we wanted. But in any given culture, we didn't know what percentage were expressing the mutant and what percentage of cells were expressing the wildtype. And it would be just too much work to try to figure that out for every experiment. And I think one of the reasons they're asking the question is with prolonged passaging, you can get erosion of X inactivation for some genes in human pluripotent stem cells. So because of that problem, we changed approaches and we took complete knockout and wildtype and mix the two in order to make a mosaic model where we didn't have to worry about escape from X inactivation. So, that's an important point.

Priya Balasubramanian: 52:40 Great. Thank you. And then there's another question here, and they're asking if, have you used electrophysiology to assess abnormal activity in the STRADA organoids, and if so, did you use any mTOR inhibitor to rescue it?

Dr. Jack Parent: 52:57 So, both very good questions. So Louis has done some experiments using rapamycin to rescue of the morphological phenotype. It rescues the megalencaphaly in the budding phenotype. In terms of the physiology, we published some work with 2D cultures looking at the physiology of the STRADA organoids. And we see some changes, but it's minor. And those

		are with dual SMAD differentiation of excitatory cortical neurons. But we think the best approach is going to be combining excitatory and inhibitory fusion organoids. And so we have not done that yet in STRADA, and that's one of the things we want to do. And looking at MEA recordings, but also putting in the patch electrodes to record local field potentials like the UCLA group is doing. I actually went on sabbatical there to learn it.
Priya Balasubramanian:	53:59	Thank you. So since we have a couple more minutes, I have a question that I was curious about. In your PMSE organoid model, you talked about how you see these different phenotypes at different ages at different time points for the organoids. You have an idea of how that might correspond to the human brain development age?
Dr. Jack Parent:	54:25	Yeah. So that's a good question. So at the time points we're looking, we probably never got later than mid second trimester stages. And to get to third trimester and even postnatal stages, you really have to go nine months a year. It really is like the time course of human development. So you really have to culture them very long. So it makes for very long experiments and unhappy grad students and postdocs.
Priya Balasubramanian:	55:03	But you can culture these organized for a very long time.
Dr. Jack Parent:	55:06	Yeah. People have cultured them for four years, even longer.
Priya Balasubramanian:	55:11	Wow. Amazing. So I would like to conclude the seminar and say a big thanks to you again, Jack, for giving us your time today and for this excellent presentation. I'd also like to say thank you to the Nussenbaum-Vogelstein family for their generous support of this Frontiers in Epilepsy Research Seminar series. And of course, thank you to all who joined us today and participated in this seminar. If you'd like more information about hosting a seminar series at your institution or about applying to one of our CURE Epilepsy grants, you can always reach out to us at research@cureepilepsy.org or visit our researcher's page on our website. And I'd like to also remind everybody to register for our next seminar. And thank you all for being here. We really value your feedback. So there's a little survey that'll pop up at the end of the seminar, so please do take that survey. And thank you again.
Dr. Jack Parent:	56:18	Thanks Priya.