# Module:
# Biological Foundations of Mental Health

## Week 2
## Building blocks of the brain

### Topic 3
### Exploring mental health using stem cells: What are iPSCs? – part 3 of 3

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**Lecture transcript**

**Slide 3**

So this is the final session in this series about iPS cells-- induced pluripotent stem cells-- and how we can generate them and use them as cellular models.

At the start of the session, I suggested to you a thought experiment. And the thought experiment was this-- wouldn't it be nice if we could reach back in development and study how developmental disorders, like autism, actually arose in the brain of children in utero? Who are going to go on and get a disorder? I suggested that we could use these cells that I've been telling you about-- these iPS cells-- to do precisely that. So in this final section, I want to ask and address the question-- how can we use these cellular models to study the aetiology of a disorder like autism?

**Slide 4**

So this is a slide I've already shared with you. And it describes the developmental profile that we get from iPS cells as we try and turn them into nerve cells. And I've told you how we start with iPS cells. We neuralise them to turn them into neural progenitor cells, and then we slowly, but surely, differentiate them to young neurons and, later, mature neurons.

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So how can we use cultures like this to start to address the questions around the aetiology of neurodevelopmental disorders? The first thing we can do is fairly obvious. We can take-- we can make iPS lines from lots of different individuals-- both from cohorts of patients that have got particular disabilities or particular neurovariants and from controls-- so from neurotypical individuals. We can grow them in the way I've described to you-- making neurons. And we can compare. And we can ask-- can we see any differences in the way the patient minds grow from the way-- the way the control minds grow? So that's one way we can approach disease aetiology using iPS cells.

A second way would be to induce mutations in the iPS cells. So you'll be aware there are a number of ways now of inducing genetic variation-- so genome editing as it's usually called-- into cells in vitro. So the CRISPR-cas9 system is probably the most popular at the present time. But there are others, like ZNFs and TALENs, that allow you to do similar things. So we could do that. And we know that a number of different genetic variants are associated with different disorders. So we can induce precisely those variants into the iPS cells and ask what difference does it make now to how they develop into the type of neurons they give rise to?

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A third thing we could do would be to study environmental risk factors. So, for example, we know that in autism, there's a big increase in risk of a mum giving rise to a child who goes on to get autism if she suffers from influenza during her first trimester. And we think we understand that that's induced by cytokines-- pro-inflammatory cytokines-- produced by the mother in response to the viral infection. And those cytokines are able to exert an influence across the placenta on the development of the foetus. Well, in principle, we can do that-- we can study that. We can ask if we expose these iPS cells to similar cytokines, what difference does it make to their development? And can we see anything in that difference that we recognise as being possibly part of the aetiology of the disease?

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So those are just three examples of the kind of studies that we could undertake. So let me ask-- well, what kind of phenotypes might you expect to see? In other words, what kind of assays are we going to run on these cells to detect differences? So one obvious thing we can do is look at gene expression. So we can take lines from patients or controls, or we can induce mutations, or we can expose the cells to environmental risk factors, and then ask-- what difference does that make to the expression of genes as the cells develop, both at the earlier stages of neuro development-- like these neural rosettes-- or at the later stages of young neurons and more mature neurons?

We can also obviously look at physiology. So I've told you that these cells eventually become physiologically active-- so electrophysiologically active. They develop the kind of channels and the kind of receptors that you would normally expect to see in human neurons. Well, we can ask-- do any-- in any of these types of studies, do we see any difference in the types of electrophysiological properties that the cells develop?

And a third difference we might look for might be what we might call morphogenetic difference. In other words, I talked about the histogenesis that happens through this developmental profile. Well, is that histogenesis altered in cells from patients versus controls? Do the cells grow a same size and shape as they should? Do they form neurons in the same way as they should? Do those neurons start to wire up and form the appropriate structures as they would in normal circumstances? So there's an example of some of the studies that we can do and some of the possible phenotypes we might see.

**Slide 7**

So I want to finish this series of presentations by sharing with you just one example. So there are a number of published examples out there now, and this is one paper where they used iPS-derived neurons to study the pathophysiology of a disorder called Timothy syndrome. Now, I don't want to talk about Timothy syndrome in any great detail, except to say that Timothy syndrome is known to be caused by a particular mutation in a particular gene. And the gene in question is this gene we-- C-A-C-N-A-1-C. We tend to refer to it as CACNA1C. And this gene encodes a calcium channel-- this particular calcium channel. And the gene encodes the alpha-one subunit of this calcium channel. And we know from a whole series of other studies that calcium channels are very, very important in signal transduction in neurons, but also in other cells, in fact.

So in this paper, the authors generated iPS cells from patients with Timothy syndrome and also from other individuals-- control individuals-- who didn't have Timothy syndrome-- didn't have this mutation in the CACNA1C gene. And then they differentiated the iPS cells into neurons using a procedure quite similar to the one that I've described to you. And then they asked the question that I posed earlier-- namely, can we see any differences in the neurons that have been derived from the Timothy syndrome patients versus those derived from controlled individuals? And the first thing to look for, obviously, was can they see any difference in the behaviour of the calcium channel? And they found that they could.

So what you're looking at here now is calcium flux in either control cells or cells derived from the

Timothy syndrome individuals. And you can immediately see there's a difference associated with this calcium channel mutation. And it clearly is the calcium channel because if you treat with this inhibitor of calcium flux-- inhibitor of the calcium channel you rectify it.

So this is very, very reassuring in the sense that these iPS cells show a phenotype that's precisely what you would predict, given that these cells carry this mutation of the calcium channel that we know is associated with the disease. So that's good and that's very reassuring. But at the same sense, it's slightly predictable. We've taken cells here that have got a mutation in the calcium channel-- and, lo and behold, if you look at that calcium channel, it behaves in a mutated fashion. So you could say it is no surprise here-- this is just telling you precisely what you would have predicted.

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But in this paper, they go and use these cells to do something a bit further. So what they look at here is histogenesis-- this process of building tissue that, as I've said, the iPS cells are really very good at. So what they tried to generate was cortical neurons-- cerebral cortical neurons.

So let me just use this histological section over here to remind you of some features of cortical histogenesis. So this is a piece-- a section through a human cerebral cortex. This is the pial surface out here. And this would be the ventricular zone. And here is the white matter and here is the grey matter. What you can immediately see from this image-- what, of course, you already know-- is that the cerebral cortex is a very strongly laminated structure. You can see the layers very, very clearly within this piece of cortical tissue.

So in-- these iPS cells-- these differentiated iPS cells-- they-- these authors use different markers to try and ask have they got the normal distribution of either upper-layer cortical neurons, like these layer two-three cells here, compared to what are called infragranular cortical neurons-- these deeper layer five-six neurons.

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And using different markers that label different neurons of different types, they are able to show that, in the Timothy syndrome cells-- here shown in red-- there were fewer cells labelled with the markers for lower neurons. And more of the cells labelled with markers indicating they were supragranular-- so the upper-layer neurons. So in other words, the neurons derived from the Timothy syndrome iPSCs had a greater propensity to make upper-layer neurons, and a reduced propensity to make lower-layer neurons.

Now when the authors looked at this in a bit more detail, what they discovered is, within this lower-layer neuronal population, there was another disturbance. Namely, that a smaller proportion of the lower-layer cells showed expression of gene SATB2. Now that rung bells. That seemed important, because we know that these SATB2-positive cells, amongst these lower-layer neurons, are a particular type of neuron.

So by and large, these lower-layer cortical neurons can take on either one of two fates. They either become subcortically projecting neurons. Or they become what we call callosal projecting neurons. Now the subcortical projecting neurons project, as the name suggests, to other regions of the brain-- the so-called subcortical regions. And those could be the thalamus, the striatum, the cerebellum, the spinal cord.

But the SATB2-positive cells belong to the other population. They belong to the callosal population. And the callosal population get their name from the fact that they project across the corpus callosum to the cerebral cortex on the other hemisphere. So what this observation says is that neurons from the Timothy syndrome patients have a lower proportion of the SATB2-positive cells-- that is, a lower proportion of the callosal projecting neurons.

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Now what the authors were also able to do is look at transgenic mice. And these were transgenic mice that had been engineered to carry precisely the mutation that is found in Timothy syndrome. So these are mice with the same Timothy syndrome mutation. And when they look at the cortical structure in these mice, what they find is exactly the same thing as is being observed here. Namely, that the number of SATB2-positive cells in the lower layer of the cortex of the mouse is fewer in the Timothy syndrome mutated mouse than in the control mice. They see exactly the same histogenic phenotype in the mice as they see in the cells that carry the calcium channel mutation.

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So what do we make of that? Let's summarise what we've just seen. So what we're looking at here is cells that carry a mutation in this CACNA1C gene and comparing them with cells that don't have that mutation. What we see is that the cells that have the CACNA1C mutation have a calcium channel deficit. Now that's reassuring, but it's not surprising-- the CACNA1C is, after all, a calcium channel protein. But what we've seen beyond that is that those cells have an altered expression in the deep cortical neuron population of this sacB2 gene. And that has led in the mice to an altered callosal projection. In other words, an altered histogenic phenotype. So the development of the cortex seems to be quite different in these cells. So this is an example of where we've been able to use iPS cells derived from patients and ask some very fundamental questions about the impact that the mutation associated with their disease on brain development.

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Let me summarise where I think we are then. So what I've told you is that we can use these cellular models to study neurodevelopmental disorders. What I want to suggest to you is that these models that I've described have some real advantages compared to other ways that we might study such disease. But I also want to tell you that they've got some disadvantages. So this is a balance.

So, amongst the pros-- amongst the advantages-- the first thing to say is obviously these are human. So we're used to having to study neural development in animal models. But what these cells are are actually true human cells. And I showed you that plate earlier, filled with cortical neurons, and pointed out that this was an enormous advantage that we've never had before-- the ability to actually study human neurons developing in a culture dish.

So one advantage is they're human. The second is they have what we call good construct validity. And that was-- a good example was the Timothy syndrome paper I cited to you. These cells have precisely the mutation that we find in patients. Of course they do-- they're actually derived from those patients. And so the mutation that drives the disease-- precisely that mutation-- is there available in the iPS cells. So this was what we would describe as good construct validity for the disease. And that's one of the assets of this model.

Another point I would make is that we've got good controls. So we're able to compare the development of the cells with the mutation with cells derived from individuals that don't have the mutation. So we're very well controlled for that mutation in the analysis. I should say that we can go beyond that. I pointed out that we can engineer these cells-- so we can either introduce mutations into cells or, indeed, we can use CASPR/Cas9-type genome editing to remove mutations from cells. And that way we can actually use same cells with the same genetic background with or without particular genetic variants.

The system, I would argue, is very tractable. So these are not the easiest cells to grow in culture. But, nonetheless, they are cells that one can readily culture. And that makes this a very approachable system that can be used-- as it currently is being used worldwide-- to study these developmental processes.

There is an enormous interest in the pharmaceutical industry in these models, because they seem like they would be amenable to high throughput screening. So we'll be able to put these cells into drug discovery assays and use them to discover novel drug targets, novel therapeutics for some of the disorders that we've been discussing. And the cells that are genetically and phenotypically manipulable in the way that I've described to you.

**Slide 13**

So those are the advantages of this cellular system. Nonetheless, it's important to point out there are some problems that we've yet to totally overcome-- some limitations to the system. The first I point is the variability. So I've pointed out that we can take cells from different individuals, and compare their performance and the kind of assays that we've been talking about. But, of course, one of the problems is that no two individuals are the same, either genetically or epigenetically. And so, if we just took cells from maybe half a dozen different control individuals-- so neurotypical individuals who didn't have any neurodevelopmental diagnosis at all-- we'd nonetheless find some degree of variability between them. And that variability would be more than we'd expect to see in similar cells taken from, say, mice. Where we're able to control the genome and we know that every mouse in a colony is genetically identical-- something that obviously isn't true about individual humans.

The second problem is a more pervasive one. And that is that the disorders that we're studying generally are going to be disorders of the system properties of the brain. What do I mean by that? What I mean is that a disorder, like schizophrenia or autism or ADHD, isn't just going to be a property of a single population of neurons-- let alone a single set of molecules within a single population of neurons. Rather they're going to be disorders of properties that emerge from the brain working as a whole. And those properties are currently going to be inaccessible to us. So one of the challenges with the iPS system is to try and build models that will actually have system properties inbuilt. I showed you the beautiful picture of Madeline Lancaster's where she's able to grow the cerebral organoids. And I think we're going to have to do more to try and push those forward-- to try and get us a whole brain, if you will, or as close to whole brain as we can to start to understand how phenotypic differences exist in that sort of gross level.

I pointed out that development of iPS cells into neurons is reassuringly slow. It's good that it's slow, because human development is slow. But it's infuriating that it's slow if you want to get on and do experiments. There's no question that the fact that you have to wait so long for your cells to develop is a disadvantage in a practical logistic sense.

And then the final point is really an important one. Most of the disorders-- in fact, all the disorders I've been telling you about are really characterised by altered behaviour. These are behavioural phenotypes. An autistic child is defined as being autistic purely on the basis of clinically observed behavioural differences. Now our problem is these cells don't behave-- or at least they don't show the kind of behaviour that we'd be interested in getting from patients. And so it's going to remain an article of faith the extent to which the cellular and molecular phenotypes that we start to observe-- and that I've told you about-- actually relate to actual human behaviour. So we're going to continue to require good clinical phenotyping, good clinical data. We're going to continue to require animal studies. Mice might not be human, but they do at least have observable behaviour that you can try and relate to human behaviour.

So although this is a new model of neurodevelopmental disorders, I think it's got tremendous utility. It's going to be a long time before it completely supersedes the other behavioral-based assays and behavioral-based systems that we're going to need access to. So thank you for listening. That's my presentation-- or series of presentations-- on cellular models of neurodevelopment. I hope it was enjoyable.

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