Giorgia Quadrato: I really the biggest thank I want to do today is to the family in the audience. What I really want to say is that your resilience and your commitment to improve the life of your loved ones is really the biggest source of inspiration for the work that we do. So really, thank you. Today, I'm going to tell you about our effort in improving modeling of human brain development and disease. In the last 70 years or so, we really as a field, we've been putting an incredible effort in trying to understand neurodevelopmental and neuropsychiatric disorders. However, we really haven't been able to come up with effective treatment for this disease and why is that? There are several reasons, but for me, one of the most important is the incredible complexity of the genetics that underlie these disorders. For example, if we think about autism, most subtype of autism are polygenic. Which means the same genetic variant in two different individuals can lead to completely different clinical manifestation. This is obviously as complicated a lot of our understanding of these disorders and our ability to come up with effective treatments. What we really need to do to understand these disorders is really being able to dive into the genetic of these disorders and basically to have access to human tissue to do so. What we ideally would like to do is to go back in time and look at how the development of the brain of a specific patient happened in the womb. Obviously, we cannot do that, but something that is quite close to this process is basically culturing 3D brain organoid derived from human pluripotent stem cells. Most of you may know that we are now able to take somatic cells from a patient, then revert the cells to pluripotency, and being able to generate the so called brain organoids. There are different protocols for generating brain organoids. Some are called unguided protocols and give us the opportunity to generate organoid that contain many region of the brain. Then there are some guided protocols that allow us to generate region specific brain organoid. Now, how do these organoids look like? Well, you can see here there is a grad student in my lab holding a flask with brain organoids, and this is how they look like. They are about 1, 2 millimeter big in diameter, and they look like lattice basically. As I mentioned, we can generate organoid from different membrane region. The model that is mostly used to recapitulate neurodevelopmental disorders are cortical organoids and why cortical? Well, we know that the human cerebral cortex is the structure in the brain that is responsible for the execution of a higher order function, including movement, speech, communication, intellect, sensory perception. These are the very function that make us human and they are also the function that are often disrupted in the developmental disorders. Now, there are multiple protocol to generate cortical organoids. There is one that we use in the lab that has been published by the Arlotta Lab and really builds on the work of Yoshiki society that is the pioneer of this field. Basically, what you can see is that when we generate cortical organoids, we are able to generate basically the majority of the cell types that we see in the human fetal cortex. These organisms are pretty reproducible, and so they really make for a good model system to understand these disorders. In the lab, we have been focused on studying Syngap1, that is one of the top autism risk gene. We know that the novel pathogenic variants in Syngap1 lead to intellectual disability, epilepsy, global developmental delay. We are really focused on this gene also because we have very close ties with the SynGAP Research Fund. We know these families, and we really care about understanding more about the biology of this gene and what was known about Syngap1 was mostly known by work and research done in excitatory neurons of mouse. When we started working on this protein, we wanted to understand a bit more about what Syngap does in human neurons. Marcella Birtele and Ashley Del Dosso their wonderful postdoctoral fellow and the PhD student in the lab, they decided to look at the expression of Syngap1 in the human fetal cortex. They look at expression of Syngap1 in different cell type of the cortex. They were very surprised to see that Syngap1 is not only expressing neurons, but they found also expression of Syngap1 in progenitor cells. This was never been described, and so they wanted to make sure that this observation was indeed correct. They decided to look at the expression of Syngap1 in cortical neurons. They really wanted to look at the expression of the protein, not only of the gene transcript. Here you can see a section of a brain organoid. You can see that much like it happened in our brain, these organoids have ventricles. Here that are fluid filled cavities surrounded by progenitors and then neurons. You can see that Syngap for sure is expressed in neurons, in this black part of the section, but it's also indeed expressed very

strongly here in the progenitor region. Basically we discovered that Syngap1 is indeed expressed at the apical end fit of these radial glial cells. In collaboration with Marcelo Coba at USC, we were also able to do proteomics for Syngap1 and look also at the complex and protein interaction of Syngap1 in progenitor cells in organ day 7 that mostly form contain only progenitor cells. We were able to confirm the expression of Syngap1 in progenitors. This was also confirmed by looking at the expression of Syngap1 in human fetal tissue. You can see that Syngap1 has a very similar pattern of expression with TJP1 that is as well expressed at the apical length fit of these progenitors. Obviously the question was, what is Syngap1 doing in this progenitor? It's not supposed to be there. We did bulk sequencing of organisms generated by patient with Syngap1 haploinsufficiency. They have 50% of the level of Syngap1 that we see in control. We were able to repair this variant and then look at what processes were dysregulated, and we found that mostly the processes that are deregulated are related to cytoskeletal remodeling or cell adhesion. Also, looking at Syngap1 interactors into progenitors, we saw basically the same picture. These progenitor cells, radial glia cells, are very special cells. They have two processes, one apical one and one basal one. The apical process reaches the ventricle of the human brain and then the basal process extends towards the PL surface. We really wanted to have a model to understand whether the cytoskeletal remodeling of these processes was altered. We team up with Randy Ashton at University of Wisconsin Medicine. Yes, this rosette system. You can see in this rosette system, we are able to see how these radial glia cells extend from the apical to the basal with this apical basal polarity. Sure enough we found that in patients with Syngap1 haploinsufficiency, we see that there is a disruption in the way these rosette form. Here you can see that in control lines, we see that these ventricles form and here basically they are mostly absent. When they are there, you can see that the morphologies is pretty altered. This is a quantification, again, showing this reduction in the number of rosette and when they are there, the perimeter and circularities is actually altered. As I mentioned before, these disorders are very much dependent by the genetic background. We decided to edit this variant in another genetic background, so in a control line, and sure enough, we found that the same phenotype was recapitulated also in a different genetic background. We also look at another variant. Actually, we disrupted the Ras GAP domain of the Syngap protein, and we found that the phenotype was again recapitulated, suggesting that the Ras GAP enzymatic domain of Syngap1 is involved in this early developmental process. Now, Syngap1 is a very complex protein. Here you can see Syngap1 has different domains. What we're interested in doing now is to extend our investigation to other variants and really try to understand other different domains. What is the function of other different domains during early development? This is something that we are now trying to do in the lab. Going back to the organoid model, that is a model that is more advanced compared to the rosette that I showed you before, normally, again, here is a section of a brain organoid, and you can see there is a separation between progenitors and neurons in control organoids. In Syngap haploinsufficient organoids, you can see that this separation is not as clear anymore. You can see that some of the neurons are actually still into the progenitor levels. Then you can see that the morphology overall is disrupted. We look at the division mode of these radial glia, and we found that in haploinsufficient lines, we saw a disruption in the way these radial cells that are the progenitors of the entire cortex, they do not divide properly, and so there is an increasing differentiative division, as you can see in here. Overall, when we look with single cell sequencing, specifically at the number of these cells in the organoids and the transcriptional profile of these cells, we found that there was a decrease in number and then up regulation of terms associated with differentiation, down regulation of terms associated with mitosis, suggesting that these radial cells have an impaired developmental trajectory. If we look at then the structural features, structural and functional features of neurons in these haploinsufficient organoids, we found that indeed, there is an evidence of faster maturation of neurons in this culture. This increase and faster maturation in haploinsufficient organoids is actually something that is a very good biomarker to test potential therapies. Now basically we have tested some antisense oligonucleotide that are able to rescue the level of Syngap doing transcript and protein. We found that these antisense oligonucleotide are indeed also able to rescue the level of network activity. Now we are partnering with some biotechs to

try to test in our system therapeutics using this network activity as basically biomarker. We are very excited about this more translational spin for this project. To summarize this first part of the talk, what I'll show you today is that we were able to discover a function for Syngap1 and the presence of Syngap1 in the radial glial progenitors, human radar progenitors, and here Syngap1 as an important function as scaffolding protein. Haploinsufficiency of Syngap1 leads to disruption in the division mode of the radial glia and in overall in accelerated maturation of cortical production neuron. Now, when we look at Syngap expression in these progenitor cells, we also look at, as I mentioned before, the proteins that are interacting with Syngap in these progenitors. We found that several other synaptic proteins that interact with Syngap in neurons are also present in progenitors. Now we are very interested in testing this hypothesis of whether a complex similar to the complex that we see in neurons for preserving the integrity of the scaffolding of the postsynaptic density of the neuron could also be present to preserve the scaffolding of radial glial cells. This concept has also been actually materialize in a sculpture by this local artist that is called Jenn Kwak is a local artist here in Los Angeles. She has represented, as you can see here this concept, showing that a complex that is used in neurons to regulate synaptic transmission is actually recycled here in this other cell type, in this case, these radial glial cells. Now I want to switch gear for the last part of my talk and talk about the new direction that we have taken in the lab, mostly inspired by conversation with Syngap families. When we talk with Syngap families, what they told us was that, obviously, cognitive impairment in their children is obviously heartbreaking and disruptive, but there is something else that is also very disruptive for their family, which is gastrointestinal symptoms. Marcella Birtele that wonderful postdoc in the lab. She actually just got a faculty position accepted at faculty position at UC Irvine, so she will be starting her own lab next year. Marcella Birtele really got passionate about this question about understanding whether Syngap1 could also affect another type of neuron, the enteric neurons. She did a first analysis looking at the expression of autism gene in the adult enteric nervous system. She found that Syngap1 is indeed expressing in enteric neurons, along with a bunch of other gene that you can see here highlighted. The enteric neurons come from the neural crest developmentally. They come mostly from the vagal neural crest, and some these come also from the sacral neural crest. What Marcella, she basically became very interested in understanding a bit more about these cells. We also found that indeed these autism gene are also found not only in adult intestinal neurons, but also in fetal enteric neuron. This is actually an analysis done by the Woolsey lab showing that you can see that when you look at different type of intestinal cells, there is this increase in the expression of autism genes in enteric progenitors and in enteric neurons. This was very interesting for us. Here you see some data collected by the Simons Foundation and Citizens in which you see that this is not unique to Syngap1. Many other gene associated with autism also show the same show that basically patient cohort with autism gene variants have this gastrointestinal this motility problem. In the case of Syngap1 and also other genes, the main issue was actually with constipation. Marcella decided to develop a system to look at dysfunction in enteric neurons. She really built on the work of Lauren Studer. She was able to generate these enteric spheroids that are able to generate the cellular diversity that we see in the enteric nervous system. This is all unpublished, very preliminary, but what she found is that much like we see for cortical neurons, also enteric neurons with Syngap1 haploinsufficiency show this faster maturation. This is pretty interesting. Then the question now is try to understand if this increased maturation has also effect on the intestinal epithelium. In collaboration with Jason Spence, we are trying to understand a bit more about how Syngap affect, for example, the niche or other intestinal cell type. But something that Marcella has been also very much focusing on has been trying to develop this assembloid model that is an assembloid model, a fusion between human, smooth muscles and enteric neurons. What she has found is that indeed, she's able to see contraction in these spheroid of human smooth muscle. What she's now doing is try to understand how contractility is also altered in co culture with Syngap cell lines. Then Marcella's idea is to bring this project to her own lab and screen many other gene associated with autism spectrum disorders. Just to conclude the second part, I show you that autism genes are enriched in the enteric nervous system. I also show you how Syngap1 is able to regulate maturation. Not only in cortical neurons, but

also in this peripheral neurons. Then we really think that this assembloid between enteric nervous system and smooth muscle can also be a very simple model to screen for gut motility and for therapies to improve gut motilities. With this, I'd like to thank all my lab members. I think I highlighted the people that were involved in this specific project, our collaborators, and then specifically the SynGAP Research Fund, NIH and all other funding agency for support, and then thank you for listening.

Dr. Lilia Iakoucheva: Hi, Giorgia. I don't know if you can see me. This is Lilia from UCSD.

Giorgia Quadrato: Hi, Lilia. How are you.

Dr. Lilia Iakoucheva: Good. Thank you for the very interesting talk. I have two questions, basically. When you see this disorganization of this ventricular zones of the rosettes in the Syngap organoids, and faster maturation into radial glia, into neurons. Do you think also migration is involved? Do you know whether radial glia matures into neurons faster in mutant organoids, and then neurons cannot move out. They're stuck in the radial glia area. Is that the case? Then following that, my second question is when you did your ASO rescue, what exactly did you do it in organoids and what exactly phenotypes were you able to rescue? Were you able to rescue the slow down the maturation then or migration or if you know what exactly phenotypes. Thank you.

Giorgia Quadrato: Thank you, Lilia. These are great questions as always. You're right. Radial glia cells are very important for guiding the migration and degeneration of the cortical layers. I show you this imaging, which basically we see that the neurons are actually stuck in the progenitor layers. We haven't really got very deep into that type of analysis, but we think that that's probably the case, and in fact, we think that it could be that this displacement of neurons in humans could also lead to problem in network connectivity, circuit assembly. That's certainly the case. The organisms are not the best model to investigate positioning in the cortical layer because as you know, these cortical layers are not really well formed and maintained. We didn't really go there much. But there is work from Gavin Rumba showing that even in mouse, they have seen some problem with the positioning of neurons. I think you're absolutely right. That's a feature. In terms of antisense oligonucleotide, what we have done so far is we have really looked at whether we can rescue level of protein and transcript and we can. Then we have been focused mostly on network activity bring down the level to the control level. We wanted to have an essay that is high throughput and easy. We have done that with calcium imaging, but we want to scale it up with microelectrode arrays. The idea is to have something that is very high throughput, but it's very interesting question to see if all the other phenotypes are also rescued. We haven't done it, but we should be doing and we'll do it. Thank you.

Jonathan Sebat: Have your study participants been solely individuals with autism with coexisting cognitive disabilities, i.e, intellectual disability, or have you also sampled individuals with autism with average or above average intelligence?

Giorgia Quadrato: This is a great question. In fact, I have to say our studies have been only focused on Syngap haploinsufficient patients. These patients have seizures, they have global developmental delay. We have only been really focused on Syngap so far, but we want to expand our analysis to Syngap interactors, though. This is what we are going to do. We are mostly focused on intellectual disability.

Jonathan Sebat: Hi, Giorgia. This is Jonathan Sebat at UCSD. Thank you. As you mentioned, Syngap1 seizures are common in Syngap1. Do you know if they respond well to treatment or are they treatment resistant? What do you know about how organoids respond to anticonvulsants? I have a second part to the question as well, but finish that one first.

Giorgia Quadrato: What I can say is that there is a lot of heterogeneity in this patient, and so there are patient that respond well, patient that don't respond at all. We haven't test these in organoids.

We should do it. Sorry, the second part of the question.

Jonathan Sebat: The second part of the question is relating in terms of the synaptic effects of Syngap1 and the epilepsy in particular, is there a way to relate that to the enteric nervous system? Is so can you say something about gut motility that learning from the brain and then trying to extrapolate that to what's going on in the gut?

Giorgia Quadrato: I really not sure how to address this question. I think this is exactly what we really want to understand how this faster maturation may affect motility because I have to say, we don't really know in the first place how this process happened and how they happen in a normal physiological condition. There is really not much known. We are trying to study these neural factor junction and try to understand a bit more in control condition and then what happens, in the case of Syngap specifically. We want to understand also, for other autism genes, there is some convergence. I love the question, very creative and I don't have an answer, but yeah, thank you. I'll be thinking more about it. I like the design.

Jonathan Sebat: We have an online question. What was responsible for the accelerated maturation of cortical projections?

Giorgia Quadrato: What's responsible? What basically we think is happening is that there is the radial glial cells, the division mode is altered, and there is this increase in differentiative division. In general, the developmental trajectory is altered. This leads to the faster maturation. I have to say, I haven't stressed it, but there is also a number of issues that are related to neurons and to the synapsis. There are alteration that we believe are may not be development related. They are also synaptic dysfunction that happen. For example, there are changes in the morphology of dendritic spines composition of the postsynaptic density. This is something that is neuronal. But then what we think is happening is that the phenotype we see is really dependent by basically the division model of the radial glia.

Jonathan Sebat: Well, thank you, Dr. Quadrato for our wonderful presentation for taking time to be with us today.

Giorgia Quadrato: Thank you.