WEBVTT

NOTE duration:"00:25:39.5300000"

NOTE recognizability:0.918

NOTE language:en-us

NOTE Confidence: 0.946004114285714

00:00:00.000 --> 00:00:01.876 So where I transition into next speaker,

NOTE Confidence: 0.946004114285714

00:00:01.880 --> 00:00:03.052 which is Flora Vacarina,

NOTE Confidence: 0.946004114285714

00:00:03.052 --> 00:00:04.517 I'm going to introduce her.

NOTE Confidence: 0.946004114285714

00:00:04.520 --> 00:00:07.586 So Flora Flora received the MD

NOTE Confidence: 0.946004114285714

00:00:07.586 --> 00:00:10.629 from the University of Padua in

NOTE Confidence: 0.946004114285714

00:00:10.629 --> 00:00:12.910 Italy and then she spent a few

NOTE Confidence: 0.946004114285714

00:00:12.910 --> 00:00:14.859 years and europharma call as a

NOTE Confidence: 0.946004114285714

00:00:14.859 --> 00:00:16.514 neuro pharmacology fellow at NIH,

NOTE Confidence: 0.946004114285714

00:00:16.520 --> 00:00:18.000 where she completed internship

NOTE Confidence: 0.946004114285714

00:00:18.000 --> 00:00:19.480 and residency in psychiatry.

NOTE Confidence: 0.946004114285714

00:00:19.480 --> 00:00:23.365 Then she completed internship and residency,

NOTE Confidence: 0.946004114285714

00:00:23.365 --> 00:00:28.040 residency in psychiatry at Yale and.

NOTE Confidence: 0.946004114285714

00:00:28.040 --> 00:00:30.032 Then she did a research fellowship

NOTE Confidence: 0.946004114285714

00:00:30.032 --> 00:00:31.360 in developmental genetic of

NOTE Confidence: 0.946004114285714

00:00:31.418 --> 00:00:32.918 the Yale Child Study Center,

NOTE Confidence: 0.946004114285714

00:00:32.920 --> 00:00:35.194 where she subsequently became an assistant

NOTE Confidence: 0.946004114285714

00:00:35.194 --> 00:00:37.680 associate and then a full professor.

NOTE Confidence: 0.946004114285714

00:00:37.680 --> 00:00:41.552 And in 2010 she was appointed as a

NOTE Confidence: 0.946004114285714

00:00:41.552 --> 00:00:43.948 Harris Harris professor as a child Study

NOTE Confidence: 0.946004114285714

00:00:43.948 --> 00:00:45.676 Center for Development of Neuroscience

NOTE Confidence: 0.946004114285714

00:00:45.676 --> 00:00:48.034 at Yale University School of Medicine.

NOTE Confidence: 0.946004114285714

00:00:48.040 --> 00:00:50.091 And since 2009 she has been the

NOTE Confidence: 0.946004114285714

00:00:50.091 --> 00:00:52.508 director of the program in the

NOTE Confidence: 0.946004114285714

00:00:52.508 --> 00:00:53.975 neurodevelopment and regression.

NOTE Confidence: 0.946004114285714

00:00:53.980 --> 00:00:55.568 And I'm very happy.

NOTE Confidence: 0.946004114285714

00:00:55.568 --> 00:00:58.342 So he's also my great collaborator and

NOTE Confidence: 0.946004114285714

00:00:58.342 --> 00:01:00.300 I'm happy looking forward to talk.

NOTE Confidence: 0.946962533333333

00:01:01.340 --> 00:01:02.300 Thank you, Alexei.

NOTE Confidence: 0.916662025

00:01:07.660 --> 00:01:09.976 Okay. Well, thank you very much.

NOTE Confidence: 0.916662025

00:01:09.980 --> 00:01:13.370 Let's move on.

NOTE Confidence: 0.916662025

00:01:13.370 --> 00:01:17.234 So let me start by saying that

NOTE Confidence: 0.916662025

00:01:17.234 --> 00:01:20.438 humans are a mosaic of germline

NOTE Confidence: 0.916662025

00:01:20.438 --> 00:01:23.190 and somatic genomic variations.

NOTE Confidence: 0.916662025

00:01:23.190 --> 00:01:25.038 And we're all very different from

NOTE Confidence: 0.916662025

00:01:25.038 --> 00:01:26.750 each other for different reasons.

NOTE Confidence: 0.916662025

00:01:26.750 --> 00:01:30.866 And similarly, genetic risk for human

NOTE Confidence: 0.916662025

00:01:30.866 --> 00:01:33.430 disease rarely map to a single gene.

NOTE Confidence: 0.916662025

00:01:33.430 --> 00:01:36.310 I'm not going to say that this is unheard of.

NOTE Confidence: 0.916662025

00:01:36.310 --> 00:01:38.587 We just heard two very good examples of that,

NOTE Confidence: 0.916662025

00:01:38.590 --> 00:01:39.870 but it's a rare phenomenon.

NOTE Confidence: 0.916662025

00:01:39.870 --> 00:01:43.664 Most most disorders map to multiple genes,

NOTE Confidence: 0.916662025

00:01:43.670 --> 00:01:44.528 risk genes.

NOTE Confidence: 0.916662025

00:01:44.528 --> 00:01:49.495 So one effort of our lab is being to find

NOTE Confidence: 0.916662025

00:01:49.495 --> 00:01:52.359 convergence in biological mechanisms.

NOTE Confidence: 0.916662025

00:01:52.360 --> 00:01:54.680 In complex developmental disorders,

NOTE Confidence: 0.851589798

00:01:56.960 --> 00:01:59.040 of course, this present challenges,

NOTE Confidence: 0.851589798

00:01:59.040 --> 00:02:00.798 particularly when you're studying the brain.

NOTE Confidence: 0.851589798

00:02:00.800 --> 00:02:03.581 So let me explain to you what I mean

NOTE Confidence: 0.851589798

00:02:03.581 --> 00:02:07.420 by convergence. So here you see.

NOTE Confidence: 0.851589798

00:02:07.420 --> 00:02:10.340 The promoter with the gene downstream of it.

NOTE Confidence: 0.851589798

00:02:10.340 --> 00:02:13.312 And that could be thought of a convergence,

NOTE Confidence: 0.851589798

00:02:13.312 --> 00:02:15.542 right? Because the promoter eventually

NOTE Confidence: 0.851589798

00:02:15.542 --> 00:02:17.422 synthesize regulates product synthesis

NOTE Confidence: 0.851589798

00:02:17.422 --> 00:02:19.338 which regulates cellular function.

NOTE Confidence: 0.851589798

00:02:19.340 --> 00:02:21.560 But you can see up here, there could

NOTE Confidence: 0.851589798

00:02:21.560 --> 00:02:23.540 be a number of different mutations,

NOTE Confidence: 0.851589798

00:02:23.540 --> 00:02:26.078 right, that could actually lead to

NOTE Confidence: 0.851589798

00:02:26.078 --> 00:02:28.960 the exactly similar or same phenotype.

NOTE Confidence: 0.851589798

00:02:28.960 --> 00:02:30.605 And this could be a close by

NOTE Confidence: 0.851589798

00:02:30.605 --> 00:02:32.320 like it could be in a promoter,

NOTE Confidence: 0.851589798

00:02:32.320 --> 00:02:33.848 but it could be very far away like

NOTE Confidence: 0.851589798

00:02:33.848 --> 00:02:35.694 in an enhancer that could be hundreds

NOTE Confidence: 0.851589798

00:02:35.694 --> 00:02:37.742 of kilo base away sometimes or it

NOTE Confidence: 0.851589798

00:02:37.742 --> 00:02:39.202 could be in different chromosomes

NOTE Confidence: 0.851589798

00:02:39.202 --> 00:02:40.680 for the transcription factor that

NOTE Confidence: 0.851589798

00:02:40.680 --> 00:02:41.880 binds to that enhancer.

NOTE Confidence: 0.851589798

00:02:41.880 --> 00:02:44.568 And so this very disparate different

NOTE Confidence: 0.851589798

00:02:44.568 --> 00:02:46.879 mutation could actually lead to

NOTE Confidence: 0.851589798

00:02:46.879 --> 00:02:49.119 essentially the very same phenotype.

NOTE Confidence: 0.851589798

00:02:49.120 --> 00:02:50.023 So how are,

NOTE Confidence: 0.851589798

00:02:50.023 --> 00:02:52.580 how are we going to deal with that?

NOTE Confidence: 0.851589798

00:02:52.580 --> 00:02:56.172 And one way to study this is exactly

NOTE Confidence: 0.851589798

00:02:56.172 --> 00:02:59.142 what we've been talking about today

NOTE Confidence: 0.851589798

00:02:59.142 --> 00:03:02.704 is to study personal genomes and the

NOTE Confidence: 0.851589798

00:03:02.704 --> 00:03:05.830 way these personal genomes can develop

NOTE Confidence: 0.851589798

00:03:05.918 --> 00:03:08.220 in vitro in different ways and what's

NOTE Confidence: 0.851589798

00:03:08.220 --> 00:03:09.860 the influence on this development.

NOTE Confidence: 0.851589798

00:03:09.860 --> 00:03:12.653 So we've been using induced pretty buttons

NOTE Confidence: 0.851589798

00:03:12.653 --> 00:03:14.899 themselves to generate these organoids,

NOTE Confidence: 0.851589798

00:03:14.900 --> 00:03:17.618 which are 3D aggregates of neuro

NOTE Confidence: 0.851589798

00:03:17.618 --> 00:03:21.050 progenitors that can be interrogated using.

NOTE Confidence: 0.851589798

00:03:21.050 --> 00:03:23.450 Different assay at the genomic level,

NOTE Confidence: 0.851589798

00:03:23.450 --> 00:03:25.970 at the transcriptomic level and

NOTE Confidence: 0.851589798

00:03:25.970 --> 00:03:28.250 at the epgenomic level, right.

NOTE Confidence: 0.851589798

00:03:28.250 --> 00:03:30.686 And and hopefully we could also look

NOTE Confidence: 0.851589798

00:03:30.686 --> 00:03:33.832 at the 3D DNA confirmation to try to

NOTE Confidence: 0.851589798

00:03:33.832 --> 00:03:37.030 put all this together and come back and

NOTE Confidence: 0.851589798

00:03:37.030 --> 00:03:39.856 and derive a model for intersection

NOTE Confidence: 0.851589798

00:03:39.856 --> 00:03:43.240 between genes and phenotypes.

NOTE Confidence: 0.851589798

00:03:43.240 --> 00:03:45.824 So this sounds easy.

NOTE Confidence: 0.851589798

00:03:45.824 --> 00:03:47.116 It's not.

NOTE Confidence: 0.851589798

00:03:47.120 --> 00:03:49.178 Let me first go through what organoids

NOTE Confidence: 0.851589798

00:03:49.178 --> 00:03:51.475 are and why do we want to use them.

NOTE Confidence: 0.851589798

00:03:51.480 --> 00:03:54.360 So obviously they are a longitudinal

NOTE Confidence: 0.851589798

00:03:54.360 --> 00:03:56.280 model of brain development,

NOTE Confidence: 0.851589798

00:03:56.280 --> 00:03:59.160 so they respect individual

NOTE Confidence: 0.851589798

00:03:59.160 --> 00:04:01.320 genetic background largely,

NOTE Confidence: 0.851589798

00:04:01.320 --> 00:04:02.760 which is very important.

NOTE Confidence: 0.851589798

00:04:02.760 --> 00:04:05.413 And let's not forget they can be

NOTE Confidence: 0.851589798

00:04:05.413 --> 00:04:07.117 developed from living people,

NOTE Confidence: 0.851589798

00:04:07.120 --> 00:04:10.592 so we can still try to do

NOTE Confidence: 0.851589798

00:04:10.592 --> 00:04:12.080 correlations between phenotypes.

NOTE Confidence: 0.851589798

00:04:12.080 --> 00:04:16.560 And in vitro development of these systems,

NOTE Confidence: 0.851589798

00:04:16.560 --> 00:04:18.078 this is how they look like.

NOTE Confidence: 0.851589798

00:04:18.080 --> 00:04:20.318 We've been growing them for over

NOTE Confidence: 0.851589798

00:04:20.318 --> 00:04:22.943 10 years now and we found ways

NOTE Confidence: 0.851589798

00:04:22.943 --> 00:04:25.393 to grow lots of them in batches.

NOTE Confidence: 0.851589798

00:04:25.400 --> 00:04:26.712 If you section them,

NOTE Confidence: 0.851589798

00:04:26.712 --> 00:04:28.352 they have these complex layers

NOTE Confidence: 0.851589798

00:04:28.352 --> 00:04:29.480 of progenitors.

NOTE Confidence: 0.851589798

00:04:29.480 --> 00:04:31.160 This is, these are cortical organoids.

NOTE Confidence: 0.851589798

00:04:31.160 --> 00:04:32.800 So you see cortical progenitors,

NOTE Confidence: 0.851589798

00:04:32.800 --> 00:04:34.910 you see immature neurons piling

NOTE Confidence: 0.851589798

00:04:34.910 --> 00:04:37.490 up here on the external side.

NOTE Confidence: 0.851589798

00:04:37.490 --> 00:04:40.730 And then if you grow them for very long time,

NOTE Confidence: 0.851589798

00:04:40.730 --> 00:04:42.410 you eventually see something that looks

NOTE Confidence: 0.851589798

00:04:42.410 --> 00:04:44.250 more similar to the actual cortex.

NOTE Confidence: 0.851589798

00:04:44.250 --> 00:04:45.008 In Cortex,

NOTE Confidence: 0.851589798

00:04:45.008 --> 00:04:47.661 you see layer five and six developing

NOTE Confidence: 0.851589798

00:04:47.661 --> 00:04:49.487 before layer two and three.

NOTE Confidence: 0.851589798

00:04:49.490 --> 00:04:51.210 So it's an inverse development.

NOTE Confidence: 0.851589798

00:04:51.210 --> 00:04:53.698 You can see that at one month you

NOTE Confidence: 0.851589798

00:04:53.698 --> 00:04:56.244 see layer 6 neurons here on the

NOTE Confidence: 0.851589798

00:04:56.244 --> 00:04:58.530 outside of the progenitors in blue.

NOTE Confidence: 0.851589798

00:04:58.530 --> 00:05:01.020 And then at five months you

NOTE Confidence: 0.851589798

00:05:01.020 --> 00:05:03.449 start seeing not only layer 6,

NOTE Confidence: 0.851589798

00:05:03.450 --> 00:05:06.266 but also layer two and three in red.

NOTE Confidence: 0.851589798

00:05:06.270 --> 00:05:08.646 So really the development of this

NOTE Confidence: 0.851589798

00:05:08.646 --> 00:05:10.230 system seems to recapitulate,

NOTE Confidence: 0.851589798

00:05:10.230 --> 00:05:13.790 at least in great lines,

NOTE Confidence: 0.851589798

00:05:13.790 --> 00:05:15.908 what's happening in the real brain.

NOTE Confidence: 0.947441742857143

00:05:15.910 --> 00:05:17.989 And eventually you even have glial cells.

NOTE Confidence: 0.947441742857143

00:05:17.990 --> 00:05:20.782 You see here astrocytes of 5 1/2 months

NOTE Confidence: 0.947441742857143

00:05:20.782 --> 00:05:23.226 that develop in the organoids as well.

NOTE Confidence: 0.947441742857143

00:05:23.230 --> 00:05:24.889 So a long time ago, but.

NOTE Confidence: 0.947441742857143

00:05:24.889 --> 00:05:27.262 Five years ago we asked the crucial

NOTE Confidence: 0.947441742857143

00:05:27.262 --> 00:05:29.027 question right to what extent

NOTE Confidence: 0.947441742857143

00:05:29.027 --> 00:05:31.061 organoids look beautiful but do they

NOTE Confidence: 0.947441742857143

00:05:31.061 --> 00:05:33.505 are really similar to the real brain?

NOTE Confidence: 0.947441742857143

00:05:33.510 --> 00:05:36.363 So we did a paper where we took three

NOTE Confidence: 0.947441742857143

00:05:36.363 --> 00:05:39.498 fetal specimen and had a cortical

NOTE Confidence: 0.947441742857143

00:05:39.498 --> 00:05:42.720 specimen for those fetal specimen at

NOTE Confidence: 0.947441742857143

00:05:42.821 --> 00:05:45.065 about 1617 postconceptional weeks.

NOTE Confidence: 0.947441742857143

00:05:45.065 --> 00:05:47.990 And then we developed organo.

NOTE Confidence: 0.947441742857143

00:05:47.990 --> 00:05:50.006 We developed in just pretty bottom

NOTE Confidence: 0.947441742857143

00:05:50.006 --> 00:05:52.454 stem sets from skin fibroblast from

NOTE Confidence: 0.947441742857143

00:05:52.454 --> 00:05:54.530 those specimen generated organoid.

NOTE Confidence: 0.947441742857143

00:05:54.530 --> 00:05:57.346 Can analyze them over a time course of

NOTE Confidence: 0.947441742857143

00:05:57.346 --> 00:06:00.596 three time courses and compare them to the

NOTE Confidence: 0.947441742857143

00:06:00.596 --> 00:06:03.410 isogenic cortices and what we found here.

NOTE Confidence: 0.947441742857143

00:06:03.410 --> 00:06:05.354 You can see these are the

NOTE Confidence: 0.947441742857143

00:06:05.354 --> 00:06:07.210 actual samples at the bottom.

NOTE Confidence: 0.947441742857143

00:06:07.210 --> 00:06:09.485 These are the cortex themselves and these

NOTE Confidence: 0.947441742857143

00:06:09.485 --> 00:06:11.789 are the organoids over three time points.

NOTE Confidence: 0.947441742857143

00:06:11.790 --> 00:06:15.170 Compared to a large data sets of

NOTE Confidence: 0.947441742857143

00:06:15.170 --> 00:06:18.070 gene expression across human stages,

NOTE Confidence: 0.947441742857143

00:06:18.070 --> 00:06:21.294 you can see that while the brains the

NOTE Confidence: 0.947441742857143

00:06:21.294 --> 00:06:24.649 cortices of this specimen are a snapshot

NOTE Confidence: 0.947441742857143

00:06:24.649 --> 00:06:27.532 of development because they map exactly

NOTE Confidence: 0.947441742857143

00:06:27.532 --> 00:06:31.150 to a 1617 post conceptual weak cortex,

NOTE Confidence: 0.947441742857143

00:06:31.150 --> 00:06:34.030 the organoids are a range.

NOTE Confidence: 0.947441742857143

00:06:34.030 --> 00:06:36.328 Here they present a range of

NOTE Confidence: 0.947441742857143

00:06:36.328 --> 00:06:37.860 similarities that go back.

NOTE Confidence: 0.947441742857143

00:06:37.860 --> 00:06:40.779 Not only to the 16 postconceptional week,

NOTE Confidence: 0.947441742857143

00:06:40.780 --> 00:06:43.378 but back to April postconceptional week,

NOTE Confidence: 0.947441742857143

00:06:43.380 --> 00:06:45.255 and even possibly earlier for

NOTE Confidence: 0.947441742857143

00:06:45.255 --> 00:06:47.554 stages for which we don't have

NOTE Confidence: 0.947441742857143

00:06:47.554 --> 00:06:49.660 human brain to compare them to.

NOTE Confidence: 0.947441742857143

00:06:49.660 --> 00:06:53.350 So organoids are a way to look in back

NOTE Confidence: 0.947441742857143

00:06:53.350 --> 00:06:55.940 from stem cells to later developmental,

NOTE Confidence: 0.947441742857143

00:06:55.940 --> 00:06:58.460 fetal and late fetal developmental stages.

NOTE Confidence: 0.947441742857143

00:06:58.460 --> 00:07:01.300 So let me show you a few slides on an

NOTE Confidence: 0.947441742857143

00:07:01.381 --> 00:07:04.460 ongoing study on autism spectrum disorder.

NOTE Confidence: 0.947441742857143

00:07:04.460 --> 00:07:07.580 This is a data set of

NOTE Confidence: 0.953112388888889

00:07:10.060 --> 00:07:12.881 14 families where we take the problem

NOTE Confidence: 0.953112388888889

00:07:12.881 --> 00:07:15.020 with autism spectrum disorder and

NOTE Confidence: 0.953112388888889

00:07:15.020 --> 00:07:17.576 we compare to the unaffected Father.

NOTE Confidence: 0.953112388888889

00:07:17.580 --> 00:07:20.316 And we do this to avoid a spuriously

NOTE Confidence: 0.953112388888889

00:07:20.316 --> 00:07:21.870 negative background comparison between

NOTE Confidence: 0.953112388888889

00:07:21.870 --> 00:07:24.294 groups that may be very different.

NOTE Confidence: 0.953112388888889

00:07:24.300 --> 00:07:27.548 So we do intra family comparisons here.

NOTE Confidence: 0.953112388888889

00:07:27.550 --> 00:07:29.350 And another thing we're doing,

NOTE Confidence: 0.953112388888889

00:07:29.350 --> 00:07:32.430 we separated head circumference size.

NOTE Confidence: 0.953112388888889

00:07:32.430 --> 00:07:34.680 So we separated patients into

NOTE Confidence: 0.953112388888889

00:07:34.680 --> 00:07:37.430 macrocephalic which have a larger brain,

NOTE Confidence: 0.953112388888889

00:07:37.430 --> 00:07:40.265 larger brain size versus those that don't.

NOTE Confidence: 0.953112388888889

00:07:40.270 --> 00:07:43.310 And the reason for doing that is that

NOTE Confidence: 0.953112388888889

00:07:43.310 --> 00:07:46.705 about 20% of people with all these

NOTE Confidence: 0.953112388888889

00:07:46.705 --> 00:07:48.630 marmacrocephalic and they're often

NOTE Confidence: 0.953112388888889

00:07:48.630 --> 00:07:52.330 have higher severity of symptoms.

NOTE Confidence: 0.953112388888889

00:07:52.330 --> 00:07:55.102 So here you see a single cell data sets

NOTE Confidence: 0.953112388888889

00:07:55.102 --> 00:07:58.207 of this that we generated in this study.

NOTE Confidence: 0.953112388888889

00:07:58.210 --> 00:08:00.210 Each dot representing this U

NOTE Confidence: 0.953112388888889

00:08:00.210 --> 00:08:02.210 map represent a single cell,

NOTE Confidence: 0.953112388888889

00:08:02.210 --> 00:08:04.314 and they're grouped by

NOTE Confidence: 0.953112388888889

00:08:04.314 --> 00:08:05.366 transcriptome similarities,

NOTE Confidence: 0.953112388888889

00:08:05.370 --> 00:08:07.683 and this is one of the largest data sets,

NOTE Confidence: 0.953112388888889

00:08:07.690 --> 00:08:10.014 if not the largest of organoids by

NOTE Confidence: 0.953112388888889

00:08:10.014 --> 00:08:12.010 single cell sequencing represents about

NOTE Confidence: 0.947633378666667

00:08:14.410 --> 00:08:15.862 650,000 / 650,000 cells.

NOTE Confidence: 0.947633378666667

00:08:15.862 --> 00:08:18.040 And you can see that they're

NOTE Confidence: 0.947633378666667

00:08:18.115 --> 00:08:20.310 grouped into various cell types.

NOTE Confidence: 0.947633378666667

00:08:20.310 --> 00:08:21.990 And here you see at the

NOTE Confidence: 0.947633378666667

00:08:21.990 --> 00:08:23.110 bottom radial glial cells,

NOTE Confidence: 0.947633378666667

00:08:23.110 --> 00:08:24.330 there is a trajectory

NOTE Confidence: 0.947633378666667

00:08:24.330 --> 00:08:25.550 between radial glial cells,

NOTE Confidence: 0.947633378666667

00:08:25.550 --> 00:08:27.254 intermediate progenitors and

NOTE Confidence: 0.947633378666667

00:08:27.254 --> 00:08:28.958 eventually cortical excitatory

NOTE Confidence: 0.947633378666667

00:08:28.958 --> 00:08:31.230 neuron and inhibitory neuron.

NOTE Confidence: 0.947633378666667

00:08:31.230 --> 00:08:34.342 And they're annotated by canonical markers.

NOTE Confidence: 0.947633378666667

00:08:34.342 --> 00:08:37.190 And one thing I like to point out

NOTE Confidence: 0.947633378666667

00:08:37.261 --> 00:08:40.276 that over time you see that there is a

NOTE Confidence: 0.947633378666667

00:08:40.276 --> 00:08:42.634 trajectory where the progenitors decrease

NOTE Confidence: 0.947633378666667

00:08:42.634 --> 00:08:45.069 in quantity and neurons increase.

NOTE Confidence: 0.947633378666667

00:08:45.070 --> 00:08:47.908 Which is what's to be expected.

NOTE Confidence: 0.947633378666667

00:08:47.910 --> 00:08:51.234 And let me highlight an important

NOTE Confidence: 0.947633378666667

00:08:51.234 --> 00:08:53.814 distinction of two particular cell

NOTE Confidence: 0.947633378666667

00:08:53.814 --> 00:08:56.760 groups that in this organ or that

NOTE Confidence: 0.947633378666667

00:08:56.760 --> 00:08:58.304 reflect actual development and

NOTE Confidence: 0.947633378666667

00:08:58.304 --> 00:09:00.610 one is the pre plate during early

NOTE Confidence: 0.947633378666667

00:09:00.610 --> 00:09:02.460 neurogenesis and the cortical plate

NOTE Confidence: 0.947633378666667

00:09:02.460 --> 00:09:05.189 which will form the actual cerebral cortex.

NOTE Confidence: 0.947633378666667

00:09:05.190 --> 00:09:08.050 So the pre plate is a transient layer

NOTE Confidence: 0.947633378666667

00:09:08.050 --> 00:09:10.810 of cells that develop very early.

NOTE Confidence: 0.947633378666667

00:09:10.810 --> 00:09:13.852 And serves as has various developmental

NOTE Confidence: 0.947633378666667

00:09:13.852 --> 00:09:16.770 functions but then eventually disappears.

NOTE Confidence: 0.947633378666667

00:09:16.770 --> 00:09:18.804 And then soon after that you

NOTE Confidence: 0.947633378666667

00:09:18.804 --> 00:09:20.610 have the actual cortical plate,

NOTE Confidence: 0.947633378666667

00:09:20.610 --> 00:09:23.298 the six layer cortical plate developing

NOTE Confidence: 0.947633378666667

00:09:23.298 --> 00:09:26.455 from the same regular real cells and we

NOTE Confidence: 0.947633378666667

00:09:26.455 --> 00:09:29.570 have both type of cells in this organoids.

NOTE Confidence: 0.947633378666667

00:09:29.570 --> 00:09:31.873 And of course another thing that I

NOTE Confidence: 0.947633378666667

00:09:31.873 --> 00:09:34.009 want to highlight is the viability.

NOTE Confidence: 0.947633378666667

00:09:34.010 --> 00:09:35.786 This is given the large data

NOTE Confidence: 0.947633378666667

00:09:35.786 --> 00:09:36.970 set that we have,

NOTE Confidence: 0.947633378666667

00:09:36.970 --> 00:09:39.370 we could actually assess that.

NOTE Confidence: 0.947633378666667

00:09:39.370 --> 00:09:41.904 You can see by color each color

NOTE Confidence: 0.947633378666667

00:09:41.904 --> 00:09:45.156 represent a cell type and one.

NOTE Confidence: 0.947633378666667

00:09:45.156 --> 00:09:47.604 One source of variability of course is age.

NOTE Confidence: 0.947633378666667

00:09:47.610 --> 00:09:53.010 And you see light green are TD0 and in in

NOTE Confidence: 0.947633378666667

00:09:53.010 --> 00:09:56.650 darker green TD30 and TD60 various stages.

NOTE Confidence: 0.947633378666667

00:09:56.650 --> 00:09:58.874 And so of course that TD0 you have

NOTE Confidence: 0.947633378666667

00:09:58.874 --> 00:10:00.687 more progenitor regular cells in pink

NOTE Confidence: 0.947633378666667

00:10:00.687 --> 00:10:02.487 and later you have more neurons,

NOTE Confidence: 0.947633378666667

00:10:02.490 --> 00:10:05.316 but still there is a large

NOTE Confidence: 0.947633378666667

00:10:05.316 --> 00:10:07.200 variability between different preps.

NOTE Confidence: 0.947633378666667

00:10:07.200 --> 00:10:08.760 And so how do we deal with that?

NOTE Confidence: 0.947633378666667

00:10:08.760 --> 00:10:11.514 That's that's an important phenomenon in

NOTE Confidence: 0.947633378666667

00:10:11.514 --> 00:10:15.047 in this field that we need to understand

NOTE Confidence: 0.947633378666667

00:10:15.047 --> 00:10:17.880 and we need to possibly study, right.

NOTE Confidence: 0.947633378666667

00:10:17.880 --> 00:10:19.880 So I was talking about

NOTE Confidence: 0.947633378666667

00:10:19.880 --> 00:10:21.080 variability earlier on.

NOTE Confidence: 0.947633378666667

00:10:21.080 --> 00:10:21.554 So,

NOTE Confidence: 0.947633378666667

00:10:21.554 --> 00:10:24.398 so what's you this variability what,

NOTE Confidence: 0.947633378666667

00:10:24.400 --> 00:10:25.138 what is,

NOTE Confidence: 0.947633378666667

00:10:25.138 --> 00:10:28.090 what is the origin of this variability and

NOTE Confidence: 0.947633378666667

00:10:28.167 --> 00:10:31.119 so one thing obviously could be a number

NOTE Confidence: 0.947633378666667

00:10:31.119 --> 00:10:35.550 of factors like reprogramming like you know.

NOTE Confidence: 0.947633378666667

00:10:35.550 --> 00:10:37.236 And anything that has to do

NOTE Confidence: 0.947633378666667

00:10:37.236 --> 00:10:38.950 with batch effect of cultures.

NOTE Confidence: 0.947633378666667

00:10:38.950 --> 00:10:40.546 And of course some of this may

NOTE Confidence: 0.947633378666667

00:10:40.546 --> 00:10:41.230 have an effect,

NOTE Confidence: 0.947633378666667

00:10:41.230 --> 00:10:43.234 but we largely excluded them and

NOTE Confidence: 0.947633378666667

00:10:43.234 --> 00:10:45.629 it seems that one important source

NOTE Confidence: 0.947633378666667

00:10:45.629 --> 00:10:48.109 of viability is genetic background,

NOTE Confidence: 0.947633378666667

00:10:48.110 --> 00:10:50.954 because if we culture IPS line

NOTE Confidence: 0.947633378666667

00:10:50.954 --> 00:10:53.930 organoid from the same individual.

NOTE Confidence: 0.947633378666667

00:10:53.930 --> 00:10:55.660 Even if they're cultured in

NOTE Confidence: 0.947633378666667

00:10:55.660 --> 00:10:57.044 different batches or differentiation,

NOTE Confidence: 0.947633378666667

00:10:57.050 --> 00:10:58.650 they're still displaying more

NOTE Confidence: 0.947633378666667

00:10:58.650 --> 00:11:01.050 similarity than all the other ones.

NOTE Confidence: 0.947633378666667

00:11:01.050 --> 00:11:04.004 So we believe that any background is

NOTE Confidence: 0.947633378666667

00:11:04.004 --> 00:11:06.928 an important driver of these differences.

NOTE Confidence: 0.947633378666667

00:11:06.930 --> 00:11:07.674 And these differences,

NOTE Confidence: 0.947633378666667

00:11:07.674 --> 00:11:09.280 of course, they're not random.

NOTE Confidence: 0.947633378666667

00:11:09.280 --> 00:11:11.616 If we have different percentage, say,

NOTE Confidence: 0.947633378666667

00:11:11.616 --> 00:11:14.046 excitatory neuron or inhibitory neurons,

NOTE Confidence: 0.947633378666667

00:11:14.050 --> 00:11:17.446 it's not just a serendipitous phenomenon.

NOTE Confidence: 0.947633378666667

00:11:17.450 --> 00:11:19.470 And you can see here, for example,

NOTE Confidence: 0.947633378666667

00:11:19.470 --> 00:11:21.430 that is highly correlated.

NOTE Confidence: 0.947633378666667

00:11:21.430 --> 00:11:23.884 Back down here with expression of

NOTE Confidence: 0.947633378666667

00:11:23.884 --> 00:11:25.990 certain genes in progenital cells.

NOTE Confidence: 0.947633378666667

00:11:25.990 --> 00:11:28.923 So the reason why we have different

NOTE Confidence: 0.947633378666667

00:11:28.923 --> 00:11:31.669 percentage of a excitatory neuron

NOTE Confidence: 0.947633378666667

00:11:31.670 --> 00:11:33.535 and inhibitory neuron is because

NOTE Confidence: 0.947633378666667

00:11:33.535 --> 00:11:35.400 there is a different programming

NOTE Confidence: 0.947633378666667

00:11:35.466 --> 00:11:37.246 of this transcription factors in

NOTE Confidence: 0.947633378666667

00:11:37.246 --> 00:11:39.026 the progenital cells in those

NOTE Confidence: 0.935831114285714

00:11:39.090 --> 00:11:41.550 spreads. So they do reflect

NOTE Confidence: 0.935831114285714

00:11:41.550 --> 00:11:43.550 differences within each organoid,

NOTE Confidence: 0.935831114285714

00:11:43.550 --> 00:11:45.950 within each organ that's derived from

NOTE Confidence: 0.935831114285714

00:11:45.950 --> 00:11:48.140 a particular person and perhaps.

NOTE Confidence: 0.935831114285714

00:11:48.140 --> 00:11:50.515 Reflects intrinsic difference in in

NOTE Confidence: 0.935831114285714

00:11:50.515 --> 00:11:53.419 the development of each one of us.

NOTE Confidence: 0.935831114285714

00:11:53.420 --> 00:11:56.393 So what happens if we compare people

NOTE Confidence: 0.935831114285714

00:11:56.393 --> 00:11:58.258 with autism with their father?

NOTE Confidence: 0.948639715

00:12:06.250 --> 00:12:09.088 So when we compared gene expression,

NOTE Confidence: 0.948639715

00:12:09.090 --> 00:12:10.902 single cell gene expression

NOTE Confidence: 0.948639715

00:12:10.902 --> 00:12:13.167 between problems and their father,

NOTE Confidence: 0.948639715

00:12:13.170 --> 00:12:16.090 we got the first surprise and that was

NOTE Confidence: 0.948639715

00:12:16.090 --> 00:12:18.422 that when we analyzed macrocephalic

NOTE Confidence: 0.948639715

00:12:18.422 --> 00:12:21.166 and normal cephalic separately.

NOTE Confidence: 0.948639715

00:12:21.170 --> 00:12:23.240 We found that they don't intersect

NOTE Confidence: 0.948639715

00:12:23.240 --> 00:12:25.170 or they intersect very minimum.

NOTE Confidence: 0.948639715

00:12:25.170 --> 00:12:27.695 That means that the differential

NOTE Confidence: 0.948639715

00:12:27.695 --> 00:12:29.715 gene expression is largely

NOTE Confidence: 0.948639715

00:12:29.715 --> 00:12:31.928 specific to which AST subgroup,

NOTE Confidence: 0.948639715

00:12:31.930 --> 00:12:34.130 and you can see examples of that here.

NOTE Confidence: 0.948639715

00:12:34.130 --> 00:12:35.183 So for example,

NOTE Confidence: 0.948639715

00:12:35.183 --> 00:12:37.289 in red you see differential gene

NOTE Confidence: 0.948639715

00:12:37.289 --> 00:12:38.809 expressions that are increased,

NOTE Confidence: 0.948639715

00:12:38.810 --> 00:12:41.026 in blue that are decreased and the one

NOTE Confidence: 0.948639715

00:12:41.026 --> 00:12:43.015 that are increased in macrosympalic

NOTE Confidence: 0.948639715

00:12:43.015 --> 00:12:45.310 which reflect largely dorsal cortical

NOTE Confidence: 0.948639715

00:12:45.310 --> 00:12:47.590 plate neurons and their progenitors.

NOTE Confidence: 0.948639715

00:12:47.590 --> 00:12:51.020 And a decrease in transcript or inhibitory

NOTE Confidence: 0.948639715

00:12:51.020 --> 00:12:53.898 neuron are actually not the same that

NOTE Confidence: 0.948639715

00:12:53.898 --> 00:12:55.470 are in fact they're the opposite.

NOTE Confidence: 0.948639715

00:12:55.470 --> 00:12:58.067 So if these are increase in macrocephalic,

NOTE Confidence: 0.948639715

00:12:58.070 --> 00:13:00.464 those are decreased and the enormous

NOTE Confidence: 0.948639715

00:13:00.464 --> 00:13:03.024 ephalic also don't have any significant

NOTE Confidence: 0.948639715

00:13:03.024 --> 00:13:05.700 change in interneurons and that's reflected

NOTE Confidence: 0.948639715

00:13:05.700 --> 00:13:08.789 also by the relative abundance of cells.

NOTE Confidence: 0.948639715

00:13:08.790 --> 00:13:10.202 So in macrocephalic individuals

NOTE Confidence: 0.948639715

00:13:10.202 --> 00:13:12.824 you see an increase in these dorsal

NOTE Confidence: 0.948639715

00:13:12.824 --> 00:13:14.147 cortical plate neurons,

NOTE Confidence: 0.948639715

00:13:14.150 --> 00:13:15.794 a decrease in preplate.

NOTE Confidence: 0.948639715

00:13:15.794 --> 00:13:17.849 And in the normal cephalic,

NOTE Confidence: 0.948639715

00:13:17.850 --> 00:13:20.405 you if you have the opposite phenomenon.

NOTE Confidence: 0.948639715

00:13:20.410 --> 00:13:22.130 So this was quite puzzling,

NOTE Confidence: 0.948639715

00:13:22.130 --> 00:13:24.450 quite interesting and do we

NOTE Confidence: 0.948639715

00:13:24.450 --> 00:13:26.770 have an explanation of that.

NOTE Confidence: 0.948639715

00:13:26.770 --> 00:13:30.847 So here just to show you that even by

NOTE Confidence: 0.948639715

00:13:30.847 --> 00:13:32.810 immunocytochemistry we reproduce these

NOTE Confidence: 0.948639715

00:13:32.810 --> 00:13:35.850 differences that I just described.

NOTE Confidence: 0.948639715

00:13:35.850 --> 00:13:38.902 So what we think this reflects is

NOTE Confidence: 0.948639715

00:13:38.902 --> 00:13:40.758 actually a different difference

NOTE Confidence: 0.948639715

00:13:40.758 --> 00:13:42.950 in the actual pathogenesis.

NOTE Confidence: 0.948639715

00:13:42.950 --> 00:13:45.308 Because if I go back to the pre plate

NOTE Confidence: 0.948639715

00:13:45.308 --> 00:13:47.750 and cord and those are cortical plate

NOTE Confidence: 0.948639715

00:13:47.750 --> 00:13:49.844 enormous epalic people that we have

NOTE Confidence: 0.948639715

00:13:49.844 --> 00:13:52.361 an increase in pre plate neurons that

NOTE Confidence: 0.948639715

00:13:52.361 --> 00:13:54.804 basically say what does it say that

NOTE Confidence: 0.948639715

00:13:54.877 --> 00:13:57.247 this radio glia says prematurely exit

NOTE Confidence: 0.948639715

00:13:57.247 --> 00:13:59.967 the cell cycle generate more pre plate.

NOTE Confidence: 0.948639715

00:13:59.967 --> 00:14:01.862 These are transient population and

NOTE Confidence: 0.948639715

00:14:01.862 --> 00:14:05.009 there is less of course progenitor that

NOTE Confidence: 0.948639715

00:14:05.009 --> 00:14:07.324 generating the subsequent cortical plate.

NOTE Confidence: 0.948639715

00:14:07.330 --> 00:14:08.512 Whereas the microcephalic

NOTE Confidence: 0.948639715

00:14:08.512 --> 00:14:10.088 of the opposite phenomenon,

NOTE Confidence: 0.948639715

00:14:10.090 --> 00:14:11.760 this progenitor generates fewer pre

NOTE Confidence: 0.948639715

00:14:11.760 --> 00:14:14.170 plate and there is more progenitors,

NOTE Confidence: 0.948639715

00:14:14.170 --> 00:14:17.376 there is more later on to generate

NOTE Confidence: 0.948639715

00:14:17.376 --> 00:14:19.250 an exuberant cortical plate

NOTE Confidence: 0.948639715

00:14:19.250 --> 00:14:22.510 neuron generation and so why?

NOTE Confidence: 0.948639715

00:14:22.510 --> 00:14:24.267 Why do we think this is important?

NOTE Confidence: 0.948639715

00:14:24.270 --> 00:14:26.069 Why is this something that's of interest?

NOTE Confidence: 0.948639715

00:14:26.070 --> 00:14:29.950 Because obviously this could reflect

NOTE Confidence: 0.948639715

00:14:29.950 --> 00:14:32.776 differences in the actual pathogenesis of

NOTE Confidence: 0.948639715

00:14:32.776 --> 00:14:35.710 what we call homogeneously autism people.

NOTE Confidence: 0.948639715

00:14:35.710 --> 00:14:38.454 They may actually be not reflecting the

NOTE Confidence: 0.948639715

00:14:38.454 --> 00:14:40.589 same pathogenic phenomenon in development.

NOTE Confidence: 0.948639715

00:14:40.590 --> 00:14:42.786 So just to summarize this part,

NOTE Confidence: 0.948639715

00:14:42.790 --> 00:14:43.395 organoid.

NOTE Confidence: 0.948639715

00:14:43.395 --> 00:14:47.025 Reproduce the lineages and cell type,

NOTE Confidence: 0.948639715

00:14:47.030 --> 00:14:48.514 at least the major one that we

NOTE Confidence: 0.948639715

00:14:48.514 --> 00:14:49.750 see in protocol development.

NOTE Confidence: 0.948639715

00:14:49.750 --> 00:14:52.210 There is great variability that in

NOTE Confidence: 0.948639715

00:14:52.210 --> 00:14:53.850 genetic programs of differentiation

NOTE Confidence: 0.948639715

00:14:53.913 --> 00:14:56.954 across individual and there are two

NOTE Confidence: 0.948639715

00:14:56.954 --> 00:14:59.509 different formal ASD that perhaps

NOTE Confidence: 0.948639715

00:14:59.510 --> 00:15:01.990 are different in pathogenesis

NOTE Confidence: 0.948639715

00:15:01.990 --> 00:15:04.790 and potentially they could have

NOTE Confidence: 0.948639715

00:15:04.790 --> 00:15:06.870 potential implications of treatment.

NOTE Confidence: 0.948639715

00:15:06.870 --> 00:15:09.180 So the next question was why?

NOTE Confidence: 0.948639715

00:15:09.180 --> 00:15:10.896 Why do we have these differences?

NOTE Confidence: 0.948639715

00:15:10.900 --> 00:15:12.940 What the transcriptome is without?

NOTE Confidence: 0.948639715

00:15:12.940 --> 00:15:14.910 What's the origin of these

NOTE Confidence: 0.948639715

00:15:14.910 --> 00:15:15.698 transcriptomic differences?

NOTE Confidence: 0.948639715

00:15:15.700 --> 00:15:17.280 And this brought up,

NOTE Confidence: 0.948639715

00:15:17.280 --> 00:15:18.860 this is ongoing studies,

NOTE Confidence: 0.948639715

00:15:18.860 --> 00:15:22.199 still unpublished bring us to

NOTE Confidence: 0.948639715

00:15:22.199 --> 00:15:24.110 the next step which is the non

NOTE Confidence: 0.948639715

00:15:24.175 --> 00:15:26.060 coding element of the genome.

NOTE Confidence: 0.948639715

00:15:26.060 --> 00:15:28.220 So as you know those are

NOTE Confidence: 0.948639715

00:15:28.220 --> 00:15:29.948 the portion of the genome,

NOTE Confidence: 0.948639715

00:15:29.948 --> 00:15:31.316 the regular gene expression.

NOTE Confidence: 0.948639715

00:15:31.320 --> 00:15:33.434 So to analyze those what we did,

NOTE Confidence: 0.902717811666667

00:15:33.440 --> 00:15:36.038 we took the non holding genome

NOTE Confidence: 0.902717811666667

00:15:36.038 --> 00:15:38.640 segmented by using cheap seek data,

NOTE Confidence: 0.902717811666667

00:15:38.640 --> 00:15:40.032 chromatic immunoprecipitation

NOTE Confidence: 0.902717811666667

00:15:40.032 --> 00:15:42.120 in various regions,

NOTE Confidence: 0.902717811666667

00:15:42.120 --> 00:15:44.409 mainly enhancers, promoters,

NOTE Confidence: 0.902717811666667

00:15:44.409 --> 00:15:48.224 repressed regions and mixed regions.

NOTE Confidence: 0.902717811666667

00:15:48.230 --> 00:15:51.512 And then correlated them with gene

NOTE Confidence: 0.902717811666667

00:15:51.512 --> 00:15:54.589 derived the data sets of about

NOTE Confidence: 0.902717811666667

00:15:54.590 --> 00:15:56.390 173,000 gene linked enhancers.

NOTE Confidence: 0.902717811666667

00:15:56.390 --> 00:15:58.190 So took the enhancers,

NOTE Confidence: 0.902717811666667

00:15:58.190 --> 00:16:01.347 linked them to genes and then perform

NOTE Confidence: 0.902717811666667

00:16:01.347 --> 00:16:03.760 correlation analysis where we could

NOTE Confidence: 0.902717811666667

00:16:03.760 --> 00:16:05.732 actually correlate the enhancer

NOTE Confidence: 0.902717811666667

00:16:05.732 --> 00:16:07.494 activity to the transcription factor

NOTE Confidence: 0.902717811666667

00:16:07.494 --> 00:16:09.390 that was bound to that enhanced.

NOTE Confidence: 0.902717811666667

00:16:09.390 --> 00:16:12.456 So correlation between activity of an

NOTE Confidence: 0.902717811666667

00:16:12.456 --> 00:16:15.654 enhancer and and transcription factor RN,

NOTE Confidence: 0.902717811666667

00:16:15.654 --> 00:16:18.150 A/C levels for those.

NOTE Confidence: 0.902717811666667

00:16:18.150 --> 00:16:19.488 The transcription factor,

NOTE Confidence: 0.902717811666667

00:16:19.488 --> 00:16:22.164 the bound to it and correlation

NOTE Confidence: 0.902717811666667

00:16:22.164 --> 00:16:23.982 between the enhancers and

NOTE Confidence: 0.902717811666667

00:16:23.982 --> 00:16:25.710 the downstream link chain.

NOTE Confidence: 0.902717811666667

00:16:25.710 --> 00:16:28.230 And by doing that we built the

NOTE Confidence: 0.902717811666667

00:16:28.230 --> 00:16:30.613 regular and what we mean by regular

NOTE Confidence: 0.902717811666667

00:16:30.613 --> 00:16:33.335 is a map of this gene enhancer

NOTE Confidence: 0.902717811666667

00:16:33.335 --> 00:16:35.747 transcription factor interaction.

NOTE Confidence: 0.902717811666667

00:16:35.750 --> 00:16:37.435 And we could identify two

NOTE Confidence: 0.902717811666667

00:16:37.435 --> 00:16:38.783 different type of enhancers,

NOTE Confidence: 0.902717811666667

00:16:38.790 --> 00:16:41.090 ones we call activating enhancers

NOTE Confidence: 0.902717811666667

00:16:41.090 --> 00:16:42.930 because they're positively correlated

NOTE Confidence: 0.902717811666667

00:16:42.930 --> 00:16:44.729 with the downstream genes.

NOTE Confidence: 0.902717811666667

00:16:44.730 --> 00:16:47.172 And whereas the repressing enhancers are

NOTE Confidence: 0.902717811666667

00:16:47.172 --> 00:16:49.311 those that are negatively correlated

NOTE Confidence: 0.902717811666667

00:16:49.311 --> 00:16:51.981 with the downstream genes and you see

NOTE Confidence: 0.902717811666667

00:16:51.981 --> 00:16:53.763 an example of this phenomenon here.

NOTE Confidence: 0.902717811666667

00:16:53.770 --> 00:16:56.524 So this is the regulatory graph for emx one.

NOTE Confidence: 0.902717811666667

00:16:56.530 --> 00:16:58.738 This is one of the genes that was

NOTE Confidence: 0.902717811666667

00:16:58.738 --> 00:17:00.610 up regulated in microcephalic AST.

NOTE Confidence: 0.902717811666667

00:17:00.610 --> 00:17:02.218 And you can see that there

NOTE Confidence: 0.902717811666667

00:17:02.218 --> 00:17:03.290 is this enhancer here

NOTE Confidence: 0.88948307

00:17:05.970 --> 00:17:07.890 693906 which is the major

NOTE Confidence: 0.88948307

00:17:07.890 --> 00:17:09.810 enhancers that activates emx one.

NOTE Confidence: 0.88948307

00:17:09.810 --> 00:17:11.980 This is the correlation coefficients.

NOTE Confidence: 0.88948307

00:17:11.980 --> 00:17:12.940 There are other,

NOTE Confidence: 0.88948307

00:17:12.940 --> 00:17:15.700 but they're less less powerful at

NOTE Confidence: 0.88948307

00:17:15.700 --> 00:17:18.350 activating this gene transcription and

NOTE Confidence: 0.88948307

00:17:18.350 --> 00:17:21.364 this enhances upstream of this enhances.

NOTE Confidence: 0.88948307

00:17:21.364 --> 00:17:23.980 There are five transcription factors okay,

NOTE Confidence: 0.88948307

00:17:23.980 --> 00:17:25.846 and four are inhibiting this enhancer

NOTE Confidence: 0.88948307

00:17:25.846 --> 00:17:28.134 and one IOM which is another gene

NOTE Confidence: 0.88948307

00:17:28.134 --> 00:17:30.096 that was appregulated in ASD models,

NOTE Confidence: 0.88948307

00:17:30.100 --> 00:17:32.472 sophalic is actually activating

NOTE Confidence: 0.88948307

00:17:32.472 --> 00:17:34.446 that enhanced so.

NOTE Confidence: 0.88948307

00:17:34.446 --> 00:17:38.342 So that's one example of going

NOTE Confidence: 0.88948307

00:17:38.342 --> 00:17:40.598 upstream of gene expression and trying

NOTE Confidence: 0.88948307

00:17:40.598 --> 00:17:43.270 to find out what's happening above.

NOTE Confidence: 0.88948307

00:17:43.270 --> 00:17:45.010 And then another thing that we've

NOTE Confidence: 0.88948307

00:17:45.010 --> 00:17:46.595 been doing is actually looking

NOTE Confidence: 0.88948307

00:17:46.595 --> 00:17:48.435 at the transcription factor that

NOTE Confidence: 0.88948307

00:17:48.435 --> 00:17:49.907 drives the type specification.

NOTE Confidence: 0.88948307

00:17:49.910 --> 00:17:53.094 So we looked at the single cell rnac.

NOTE Confidence: 0.88948307

00:17:53.100 --> 00:17:56.460 Derived gene markers that are

NOTE Confidence: 0.88948307

00:17:56.460 --> 00:17:58.300 specific for certain cell types,

NOTE Confidence: 0.88948307

00:17:58.300 --> 00:18:00.980 say excitatory neuron for example,

NOTE Confidence: 0.88948307

00:18:00.980 --> 00:18:02.666 you see they're not here or

NOTE Confidence: 0.88948307

00:18:02.666 --> 00:18:04.100 inhibitory neuron or radial glia.

NOTE Confidence: 0.88948307

00:18:04.100 --> 00:18:06.055 And then found those transcription

NOTE Confidence: 0.88948307

00:18:06.055 --> 00:18:08.010 factors that actually can explain

NOTE Confidence: 0.88948307

00:18:08.073 --> 00:18:10.245 or are correlated with this cell

NOTE Confidence: 0.88948307

00:18:10.245 --> 00:18:11.693 type specific gene expression.

NOTE Confidence: 0.88948307

00:18:11.700 --> 00:18:13.912 And we derive sets of transcription factor

NOTE Confidence: 0.88948307

00:18:13.912 --> 00:18:16.259 for example that can activate all neurons.

NOTE Confidence: 0.88948307

00:18:16.260 --> 00:18:18.240 You see these are all neurons.

NOTE Confidence: 0.88948307

00:18:18.240 --> 00:18:20.165 And repressing already or perhaps

NOTE Confidence: 0.88948307

00:18:20.165 --> 00:18:22.779 the activator of a certain type of

NOTE Confidence: 0.88948307

00:18:22.779 --> 00:18:24.627 excitatory in human or a certain

NOTE Confidence: 0.88948307

00:18:24.627 --> 00:18:26.598 type of inhibitory in human.

NOTE Confidence: 0.88948307

00:18:26.600 --> 00:18:27.354 So this,

NOTE Confidence: 0.88948307

00:18:27.354 --> 00:18:29.993 this really improves our ability to go

NOTE Confidence: 0.88948307

00:18:29.993 --> 00:18:32.091 upstream of cell type differences and

NOTE Confidence: 0.88948307

00:18:32.091 --> 00:18:35.113 try to find out what are the upstream

NOTE Confidence: 0.88948307

00:18:35.113 --> 00:18:37.717 mechanism that regulate those cell types.

NOTE Confidence: 0.88948307

00:18:37.720 --> 00:18:41.786 And finally this is the

NOTE Confidence: 0.88948307

00:18:41.786 --> 00:18:43.794 regular of macrocephalic AST.

NOTE Confidence: 0.88948307

00:18:43.800 --> 00:18:46.523 So what we did here we displayed

NOTE Confidence: 0.88948307

00:18:46.523 --> 00:18:47.690 in the regular.

NOTE Confidence: 0.88948307

00:18:47.690 --> 00:18:49.426 The differential gene expression

NOTE Confidence: 0.88948307

00:18:49.426 --> 00:18:50.728 in macrocephalic ASD,

NOTE Confidence: 0.88948307

00:18:50.730 --> 00:18:52.754 and you see here a few genes that

NOTE Confidence: 0.88948307

00:18:52.754 --> 00:18:54.868 are upstream that are actually their

NOTE Confidence: 0.88948307

00:18:54.868 --> 00:18:56.412 transcription factor that regulate

NOTE Confidence: 0.88948307

00:18:56.412 --> 00:18:58.290 exactly for neuron development.

NOTE Confidence: 0.88948307

00:18:58.290 --> 00:19:01.485 You see emx one that I talked about before,

NOTE Confidence: 0.88948307

00:19:01.490 --> 00:19:04.956 and you see this analysis 693906

NOTE Confidence: 0.88948307

00:19:04.956 --> 00:19:06.886 that as you saw before,

NOTE Confidence: 0.88948307

00:19:06.890 --> 00:19:08.494 is regulated by IOMS,

NOTE Confidence: 0.88948307

00:19:08.494 --> 00:19:10.900 which is also regulated in ASD.

NOTE Confidence: 0.88948307

00:19:10.900 --> 00:19:13.204 And then these are connected to

NOTE Confidence: 0.88948307

00:19:13.204 --> 00:19:14.740 other transcription factor through

NOTE Confidence: 0.88948307

00:19:14.800 --> 00:19:16.753 enhancing that as you can see here

NOTE Confidence: 0.88948307

00:19:16.753 --> 00:19:18.700 the red means they're activated.

NOTE Confidence: 0.88948307

00:19:18.700 --> 00:19:21.220 So we're really going upstream and

NOTE Confidence: 0.88948307

00:19:21.220 --> 00:19:22.900 explaining this gene expression

NOTE Confidence: 0.88948307

00:19:22.969 --> 00:19:25.059 differences by the activity of

NOTE Confidence: 0.88948307

00:19:25.059 --> 00:19:25.895 those enhances.

NOTE Confidence: 0.88948307

00:19:25.900 --> 00:19:27.676 So we have enhancers that activate

NOTE Confidence: 0.88948307

00:19:27.676 --> 00:19:29.908 genes and we have enhancers like this

NOTE Confidence: 0.88948307

00:19:29.908 --> 00:19:32.218 one here that are downstream of genes.

NOTE Confidence: 0.88948307

00:19:32.220 --> 00:19:34.340 So for example this enhancers

NOTE Confidence: 0.88948307

00:19:34.340 --> 00:19:36.460 downstream activated by Neuro D2,

NOTE Confidence: 0.88948307

00:19:36.460 --> 00:19:40.126 by eoms and by Vhlh E22 which

NOTE Confidence: 0.88948307

00:19:40.126 --> 00:19:41.298 are all up regulated.

NOTE Confidence: 0.88948307

00:19:41.300 --> 00:19:44.408 So this is a self reinforcing network

NOTE Confidence: 0.88948307

00:19:44.408 --> 00:19:48.014 that is giving us some ideas of what's

NOTE Confidence: 0.88948307

00:19:48.014 --> 00:19:51.235 going on in the development of of

NOTE Confidence: 0.88948307

00:19:51.235 --> 00:19:53.615 this organized in these patients,

NOTE Confidence: 0.88948307

00:19:53.620 --> 00:19:55.060 but you might ask.

NOTE Confidence: 0.88948307

00:19:55.060 --> 00:19:57.220 Why do we care about this?

NOTE Confidence: 0.88948307

00:19:57.220 --> 00:19:59.776 Why do we want to show all these enhancers?

NOTE Confidence: 0.88948307

00:19:59.780 --> 00:20:02.097 What the reason is this again right?

NOTE Confidence: 0.88948307

00:20:02.100 --> 00:20:05.620 Because how do we know that one enhancer,

NOTE Confidence: 0.88948307

00:20:05.620 --> 00:20:06.362 say here,

NOTE Confidence: 0.88948307

00:20:06.362 --> 00:20:08.588 activate a certain genes if we

NOTE Confidence: 0.88948307

00:20:08.588 --> 00:20:10.139 don't make a regular?

NOTE Confidence: 0.88948307

00:20:10.140 --> 00:20:11.932 We need to make a regular in

NOTE Confidence: 0.88948307

00:20:11.932 --> 00:20:13.632 order to actually make these

NOTE Confidence: 0.88948307

00:20:13.632 --> 00:20:15.376 connections meaningful and possible.

NOTE Confidence: 0.88948307

00:20:15.380 --> 00:20:16.544 And not only that,

NOTE Confidence: 0.88948307

00:20:16.544 --> 00:20:18.290 we need to make this regular

NOTE Confidence: 0.88948307

00:20:18.354 --> 00:20:19.814 mean different individuals in

NOTE Confidence: 0.88948307

00:20:19.814 --> 00:20:22.004 as many individual as we can.

NOTE Confidence: 0.88948307

00:20:22.010 --> 00:20:24.523 In order to be able to figure

NOTE Confidence: 0.88948307

00:20:24.523 --> 00:20:25.600 out this relationship

NOTE Confidence: 0.941511456666667

00:20:25.675 --> 00:20:28.547 and to figure out how the non coding

NOTE Confidence: 0.941511456666667

00:20:28.547 --> 00:20:30.888 genome relates to the coding regions,

NOTE Confidence: 0.941511456666667

00:20:30.890 --> 00:20:33.480 we already found some intersection

NOTE Confidence: 0.941511456666667

00:20:33.480 --> 00:20:35.552 between our differential express

NOTE Confidence: 0.941511456666667

00:20:35.552 --> 00:20:38.022 genes and the spy genes and other

NOTE Confidence: 0.941511456666667

00:20:38.022 --> 00:20:40.130 data sets of ASD risk genes.

NOTE Confidence: 0.941511456666667

00:20:40.130 --> 00:20:42.370 But these are coding genes we need to

NOTE Confidence: 0.941511456666667

00:20:42.370 --> 00:20:44.806 do this work for the non coding part,

NOTE Confidence: 0.941511456666667

00:20:44.810 --> 00:20:49.550 so that's being our next step so.

NOTE Confidence: 0.941511456666667

00:20:49.550 --> 00:20:50.550 I don't have much time,

NOTE Confidence: 0.941511456666667

00:20:50.550 --> 00:20:54.732 but let me say that perhaps differential

NOTE Confidence: 0.941511456666667

00:20:54.732 --> 00:20:58.029 regular activity can explain one day ASD,

NOTE Confidence: 0.941511456666667

00:20:58.030 --> 00:20:59.298 differential gene expression and

NOTE Confidence: 0.941511456666667

00:20:59.298 --> 00:21:01.923 self state and can point to no coding

NOTE Confidence: 0.941511456666667

00:21:01.923 --> 00:21:03.909 element that are enacting these changes.

NOTE Confidence: 0.941511456666667

00:21:03.910 --> 00:21:05.788 And in the last few slides,

NOTE Confidence: 0.941511456666667

00:21:05.790 --> 00:21:07.589 let me go back to something that

NOTE Confidence: 0.909840637

00:21:10.150 --> 00:21:12.790 Andrea Chenko spoke to you just

NOTE Confidence: 0.909840637

00:21:12.790 --> 00:21:15.582 a few minutes ago. And in fact,

NOTE Confidence: 0.909840637

00:21:15.582 --> 00:21:18.412 we by chance have this very same figure

NOTE Confidence: 0.909840637

00:21:18.412 --> 00:21:20.944 here about Peter Lawrence French flag,

NOTE Confidence: 0.909840637

00:21:20.950 --> 00:21:22.366 which basically says that

NOTE Confidence: 0.909840637

00:21:22.366 --> 00:21:23.763 gradients are important, right?

NOTE Confidence: 0.909840637

00:21:23.763 --> 00:21:25.761 And these are the collab young

NOTE Confidence: 0.909840637

00:21:25.761 --> 00:21:28.089 collaborators in his lab and my lab

NOTE Confidence: 0.909840637

00:21:28.089 --> 00:21:29.985 that they've made possible this project.

NOTE Confidence: 0.909840637

00:21:29.990 --> 00:21:32.188 And we're all very grateful to them.

NOTE Confidence: 0.909840637

00:21:32.190 --> 00:21:34.152 But basically they built this Chamber

NOTE Confidence: 0.909840637

00:21:34.152 --> 00:21:36.551 which allows us to look at the

NOTE Confidence: 0.909840637

00:21:36.551 --> 00:21:38.266 orthogonal effect of two gradients,

NOTE Confidence: 0.909840637

00:21:38.270 --> 00:21:39.278 and we intagonist,

NOTE Confidence: 0.909840637

00:21:39.278 --> 00:21:40.958 which is posteriorizing the organoids

NOTE Confidence: 0.909840637

00:21:40.958 --> 00:21:43.714 and a Sonic a joke agonies, which is.

NOTE Confidence: 0.909840637

00:21:43.714 --> 00:21:46.366 Ventralizing them and these are the

NOTE Confidence: 0.909840637

00:21:46.366 --> 00:21:48.604 organoid cultures in this area and this

NOTE Confidence: 0.909840637

00:21:48.604 --> 00:21:50.123 is a slide that you already showed,

NOTE Confidence: 0.909840637

00:21:50.130 --> 00:21:51.490 so I'm not going too much in detail.

NOTE Confidence: 0.909840637

00:21:51.490 --> 00:21:53.440 We're reading the gradient by

NOTE Confidence: 0.909840637

00:21:53.440 --> 00:21:54.610 using gene expression.

NOTE Confidence: 0.909840637

00:21:54.610 --> 00:21:57.584 And just let me say that it's

NOTE Confidence: 0.909840637

00:21:57.584 --> 00:22:01.106 fantastic what we see because dorsal

NOTE Confidence: 0.909840637

00:22:01.106 --> 00:22:04.389 genes which are up here like TBR

NOTE Confidence: 0.909840637

00:22:04.389 --> 00:22:07.042 one and Fox G1 are expressing the

NOTE Confidence: 0.909840637

00:22:07.042 --> 00:22:09.048 dorsal portion of the chambers.

NOTE Confidence: 0.909840637

00:22:09.050 --> 00:22:11.030 And not in the ventral and

NOTE Confidence: 0.909840637

00:22:11.030 --> 00:22:12.928 cortical genes like Phase 2 E MX2,

NOTE Confidence: 0.909840637

00:22:12.930 --> 00:22:14.840 they're expressing the anterior chamber

NOTE Confidence: 0.909840637

00:22:14.840 --> 00:22:18.809 which is C5D is anterior and C1 is posterior.

NOTE Confidence: 0.909840637

00:22:18.810 --> 00:22:22.290 And then ventral gene like nkx

NOTE Confidence: 0.909840637

00:22:22.290 --> 00:22:23.690 2.1 which you see here in mouse,

NOTE Confidence: 0.909840637

00:22:23.690 --> 00:22:26.105 it's a ventral gene in the basal

NOTE Confidence: 0.909840637

00:22:26.105 --> 00:22:28.346 ganglia instead is expressed in

NOTE Confidence: 0.909840637

00:22:28.346 --> 00:22:30.676 the basal ganglia in the ventral

NOTE Confidence: 0.909840637

00:22:30.676 --> 00:22:32.488 regions of the of the chamber.

NOTE Confidence: 0.909840637

00:22:32.490 --> 00:22:34.420 So this Chamber really seems

NOTE Confidence: 0.909840637

00:22:34.420 --> 00:22:37.610 to be able to make.

NOTE Confidence: 0.909840637

00:22:37.610 --> 00:22:38.560 Brain regions,

NOTE Confidence: 0.909840637

00:22:38.560 --> 00:22:39.985 specific brain regions

NOTE Confidence: 0.909840637

00:22:39.985 --> 00:22:41.885 different from one another,

NOTE Confidence: 0.909840637

00:22:41.890 --> 00:22:43.934 and we only need 5 days of

NOTE Confidence: 0.909840637

00:22:43.934 --> 00:22:45.490 exposure to this gradient.

NOTE Confidence: 0.909840637

00:22:45.490 --> 00:22:47.374 And after that we can remove

NOTE Confidence: 0.909840637

00:22:47.374 --> 00:22:49.385 the organo from the Chamber and

NOTE Confidence: 0.909840637

00:22:49.385 --> 00:22:51.443 culture them in the usual way.

NOTE Confidence: 0.909840637

00:22:51.450 --> 00:22:53.166 But let me say one thing.

NOTE Confidence: 0.909840637

00:22:53.170 --> 00:22:53.992 Individual variation.

NOTE Confidence: 0.909840637

00:22:53.992 --> 00:22:56.364 We've been talking about that, well,

NOTE Confidence: 0.909840637

00:22:56.364 --> 00:22:58.876 it turns out the out of seven IPS

NOTE Confidence: 0.909840637

00:22:58.876 --> 00:23:01.485 line that we tried in this gradient,

NOTE Confidence: 0.909840637

00:23:01.490 --> 00:23:02.366 they're all different.

NOTE Confidence: 0.909840637

00:23:02.366 --> 00:23:04.410 They don't respond in the same way.

NOTE Confidence: 0.909840637

00:23:04.410 --> 00:23:05.930 So these are the chambers,

NOTE Confidence: 0.909840637

00:23:05.930 --> 00:23:07.674 these are the gradients.

NOTE Confidence: 0.909840637

00:23:07.674 --> 00:23:09.688 Anterior, C5, posterior C1,

NOTE Confidence: 0.909840637

00:23:09.688 --> 00:23:11.266 dorsal and ventral.

NOTE Confidence: 0.909840637

00:23:11.270 --> 00:23:12.985 And you can see that the slope,

NOTE Confidence: 0.909840637

00:23:12.990 --> 00:23:14.630 the response is similar but

NOTE Confidence: 0.909840637

00:23:14.630 --> 00:23:15.942 the slope is different.

NOTE Confidence: 0.909840637

00:23:15.950 --> 00:23:17.742 What does that suggest?

NOTE Confidence: 0.909840637

00:23:17.742 --> 00:23:20.646 That at least in this type of

NOTE Confidence: 0.909840637

00:23:20.646 --> 00:23:22.942 assay we don't behave the same way?

NOTE Confidence: 0.909840637

00:23:22.950 --> 00:23:25.195 Organized from different people respond

NOTE Confidence: 0.909840637

00:23:25.195 --> 00:23:27.929 in slightly different way and perhaps

NOTE Confidence: 0.909840637

00:23:27.929 --> 00:23:30.834 that's due to genetic background and other.

NOTE Confidence: 0.909840637

00:23:30.840 --> 00:23:32.360 Epigenetic and other phenomenon

NOTE Confidence: 0.909840637

00:23:32.360 --> 00:23:35.080 that are peculiar to each one of us,

NOTE Confidence: 0.909840637

00:23:35.080 --> 00:23:37.656 so the our brains are not constructed

NOTE Confidence: 0.909840637

00:23:37.656 --> 00:23:40.437 according to this in exactly the same way.

NOTE Confidence: 0.909840637

00:23:40.440 --> 00:23:42.760 And that's my last slide,

NOTE Confidence: 0.909840637

00:23:42.760 --> 00:23:46.952 is EU map for this Chamber or

NOTE Confidence: 0.909840637

00:23:46.952 --> 00:23:48.560 derived organoid altogether?

NOTE Confidence: 0.909840637

00:23:48.560 --> 00:23:50.751 If we combine all the organic together

NOTE Confidence: 0.909840637

00:23:50.751 --> 00:23:53.117 make single cell RN A/C can make a U map.

NOTE Confidence: 0.909840637

00:23:53.120 --> 00:23:54.950 Well, obviously it doesn't look like

NOTE Confidence: 0.909840637

00:23:54.950 --> 00:23:57.288 EU map I showed you before, right?

NOTE Confidence: 0.909840637

00:23:57.288 --> 00:23:58.920 It's not just cortex.

NOTE Confidence: 0.909840637

00:23:58.920 --> 00:24:00.558 You have palamus, you have subparium,

NOTE Confidence: 0.909840637

00:24:00.560 --> 00:24:02.320 your midbrain with dopaminenergic

NOTE Confidence: 0.909840637

00:24:02.320 --> 00:24:03.640 neurons in there.

NOTE Confidence: 0.909840637

00:24:03.640 --> 00:24:05.200 You have floor plate,

NOTE Confidence: 0.909840637

00:24:05.200 --> 00:24:07.150 you have medium structure septum

NOTE Confidence: 0.909840637

00:24:07.150 --> 00:24:09.364 with corioplexus in there developing

NOTE Confidence: 0.909840637

00:24:09.364 --> 00:24:11.116 and cortex of course.

NOTE Confidence: 0.909840637

00:24:11.120 --> 00:24:13.568 And these regions come from different

NOTE Confidence: 0.909840637

00:24:13.568 --> 00:24:15.445 regions of the Chamber, right?

NOTE Confidence: 0.909840637

00:24:15.445 --> 00:24:16.180 So the pallium,

NOTE Confidence: 0.909840637

00:24:16.180 --> 00:24:17.160 which is the cortex,

NOTE Confidence: 0.942937173333333

00:24:17.160 --> 00:24:19.686 come from the anterior regions and

NOTE Confidence: 0.942937173333333

00:24:19.686 --> 00:24:22.976 if we project them to the mouse brain

NOTE Confidence: 0.942937173333333

00:24:22.976 --> 00:24:25.660 using a software called Box Hunt.

NOTE Confidence: 0.942937173333333

00:24:25.660 --> 00:24:28.726 Again, you see that the C5 anterior

NOTE Confidence: 0.942937173333333

00:24:28.726 --> 00:24:31.209 map mostly to anterior mouse

NOTE Confidence: 0.942937173333333

00:24:31.209 --> 00:24:33.662 regions and the posterior regions.

NOTE Confidence: 0.942937173333333

00:24:33.662 --> 00:24:35.767 Instead, C1 maps to posterior

NOTE Confidence: 0.942937173333333

00:24:35.767 --> 00:24:38.083 regions of the mouse brain and

NOTE Confidence: 0.942937173333333

00:24:38.083 --> 00:24:40.372 the same is for the ventral side.

NOTE Confidence: 0.942937173333333

00:24:40.380 --> 00:24:43.329 So in conclusion.

NOTE Confidence: 0.942937173333333

00:24:43.330 --> 00:24:46.320 This is a new system that we really want to

NOTE Confidence: 0.942937173333333

00:24:46.394 --> 00:24:49.250 exploit to make our organo more credible,

NOTE Confidence: 0.942937173333333

00:24:49.250 --> 00:24:52.085 to build organoids that are more similar,

NOTE Confidence: 0.942937173333333

00:24:52.090 --> 00:24:53.917 developed in a more in a way that is

NOTE Confidence: 0.942937173333333

00:24:53.917 --> 00:24:55.808 more similar towards the actual brain.

NOTE Confidence: 0.942937173333333

00:24:55.810 --> 00:24:57.410 Human brain actually develops

NOTE Confidence: 0.942937173333333

00:24:57.410 --> 00:24:59.810 and and and that's using organo,

NOTE Confidence: 0.942937173333333

00:24:59.810 --> 00:25:02.000 it is not using tissue culture

NOTE Confidence: 0.942937173333333

00:25:02.000 --> 00:25:03.930 dishes with with factors added.

NOTE Confidence: 0.942937173333333

00:25:03.930 --> 00:25:07.507 So let me finish by highlighting the

NOTE Confidence: 0.942937173333333

00:25:07.507 --> 00:25:11.327 contribution of all my colleagues in my lab.

NOTE Confidence: 0.942937173333333

00:25:11.330 --> 00:25:13.490 All of them have greatly contributed

NOTE Confidence: 0.942937173333333

00:25:13.490 --> 00:25:14.570 to this work.

NOTE Confidence: 0.942937173333333

00:25:14.570 --> 00:25:16.706 Jessica Mariani was the first one

NOTE Confidence: 0.942937173333333

00:25:16.706 --> 00:25:18.721 who developed an organo in my

NOTE Confidence: 0.942937173333333

00:25:18.721 --> 00:25:20.562 lab back more than 10 years ago.

NOTE Confidence: 0.942937173333333

00:25:20.570 --> 00:25:23.041 And Alex and so I have greatly

NOTE Confidence: 0.942937173333333

00:25:23.041 --> 00:25:25.330 contributed to the Chamber project.

NOTE Confidence: 0.942937173333333

00:25:25.330 --> 00:25:27.448 And then of course Alexei is

NOTE Confidence: 0.942937173333333

00:25:27.450 --> 00:25:29.130 collaborated with us for many years.

NOTE Confidence: 0.942937173333333

00:25:29.130 --> 00:25:30.770 You hear him soon.

NOTE Confidence: 0.942937173333333

00:25:30.770 --> 00:25:33.230 And Andrei Levchenko and his people

NOTE Confidence: 0.942937173333333

00:25:33.306 --> 00:25:36.134 have greatly collaborated with

NOTE Confidence: 0.942937173333333

00:25:36.134 --> 00:25:37.644 us for the Chamber project.

NOTE Confidence: 0.942937173333333

00:25:37.650 --> 00:25:39.526 And with that, thank you very much.