

CURE Webinar
Panels & Exomes: Diagnostic Yield and Detection of Childhood Epilepsy
(Transcript)

- Brandon Laughlin: [00:00:05](#) Hello, everyone. Welcome to today's webinar. My name is Brandon Laughlin with Citizens United for Research in Epilepsy or CURE. And I'd like to thank everyone for joining us for today's presentation brought to you by CURE and sponsored by Invitae. CURE is very proud to host this webinar on the importance of early and accurate genetic testing in epilepsy. Many people don't know the cause of their epilepsy but genetic research is changing that. CURE, the leading private funder of epilepsy research in the world founded its signature program, the Epilepsy Genetics Initiative, or EGI to really help broaden our understanding of the genetic causes of epilepsy, leading us toward more personalized medicine and bringing us one step closer to a cure.
- Brandon Laughlin: [00:00:54](#) The vision is to improve the ways that we prevent the diagnosis and we treat this devastating disease EGI's centralized database holds the genetic data of people with epilepsy, and that data will be analyzed and then re-analyzed until the cause of a patient's epilepsy is found. Findings will then be reported to the patient's treating physician. And this data will be made available to advance cutting edge research projects.
- Brandon Laughlin: [00:01:20](#) To date EGI has actually enrolled over 700 epilepsy patients and their family from around the world providing a novel genetic diagnosis in 10 of these families, as well as identifying a new gene not previously implicated with epilepsy. For more information on becoming an EGI enrollment site or referring a patient to the EGI project, please contact our team at EGI@CUREepilepsy.org. Or 1-844-EGI-CURE.
- Brandon Laughlin: [00:01:49](#) Today, we have Dr. Joseph Sullivan from the University of California, San Francisco and Dr. Swaroop Aradhya from Invitae here to discuss Panels and exomes: The diagnostic yield and detection of childhood epilepsy. Dr. Sullivan is an associate professor of neurology and pediatrics at the University of California San Francisco, and the director of the UCSF Pediatric Epilepsy Center at Benioff Children's Hospital. As a pediatric epileptologist, he cares for all children with various forms of epilepsy, but has specific clinical and research interests in Dravet syndrome, PCDH19 related epilepsy and the evaluation of children for epilepsy surgery.
- Brandon Laughlin: [00:02:34](#) Dr. Sullivan is a member of the American Epilepsy Society and serves on a number of boards including the chair of the PCDH19 Alliance, Scientific Advisory Board, chair of the Epilepsy Foundation of Northern California's board of directors and chair

of the Pediatric Epilepsy Research Consortium steering committee. He is also a member of the Medical Advisory Board for the Dravet Syndrome Foundation. He is a graduate of Union College and Albany Medical College. He completed a pediatric residency at the Children's Memorial Hospital of Northwestern University in Chicago, where he spent an additional year as pediatric chief resident. He then completed his neurology, child neurology and epilepsy training at the hospital of the University of Pennsylvania, and the Children's Hospital of Philadelphia, before taking his first faculty position at UCSF in 2007.

Brandon Laughlin: [00:03:28](#) And Dr. Aradhya is a board certified laboratory geneticist who has helped shape professional practices and technology applications in clinical genetic testing over the past 15 years. He joined Invitae to help bring genetics into mainstream medicine by innovating laboratory technologies, fostering advances in evidence based clinical standards, and building mechanisms to empower individuals globally to access their genetic information. He completed his medical genetics training in 2007 at Stanford University, and received his PhD in human genetics in 2001 at Baylor College of Medicine.

Brandon Laughlin: [00:04:08](#) Over the course of his career, he has participated in the International Human Genome Project, and helped characterize the molecular basis for various genetic disorders. He currently participates in the clean gen resource project, serves on the board of directors of the American Board of Medical Genetics, and is adjunct faculty at Stanford University.

Brandon Laughlin: [00:04:31](#) Before we begin, I just want to go over a couple of housekeeping items. First, please submit any questions you have anytime during the presentation by just typing them into the questions pane of the GoToWebinar control panel, and then clicking send. Zahra from Invitae will read them aloud to our speakers during the Q&A portion of today's program. Also, today's webinar will be recorded and will be made available for on-demand viewing on both the CURE and Invitae websites. I'll now go ahead and turn it over to Dr. Sullivan, and then Dr. Aradhya will follow. I want to thank everybody for joining us today.

Dr. Joseph Sullivan: [00:05:09](#) Thank you, Brandon, for the wonderful introduction. And thank you to CURE and Invitae for making this webinar possible. And thank you to all the attendees wherever you are and whatever time zone for calling in. So as Brandon said, I'm a pediatric epileptologist. So the title of my talk here is The role of the epileptologist in the exploding world of genetics patients, panels and practice.

- Dr. Joseph Sullivan: [00:05:34](#) So first, I want to go over. Just a quick outline first. Since there's probably a wide variety of practitioners from different backgrounds on the call, I do want to go over some definitions about what a seizure is, what an epilepsy is, what an epilepsy syndrome is. I then want to delve into how I as a clinician approach each individual patient to try and decide which testing those patients actually need and ultimately, comment on which patients can benefit from genetic testing. And then we will go a little bit further into showing what the yield of that testing is.
- Dr. Joseph Sullivan: [00:06:19](#) So first, what is a seizure? This may seem like a very simple question, but in fact, it is not. This is a definition that is debated at American Epilepsy Society meetings year after year. But for the purpose of this discussion, I'd like for us to refer to a seizure as a transient occurrence of signs and or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. And that is in contrast to epilepsy, which has also been debated over the years. But again, for the purpose of this talk, we are going to define it as two or more unprovoked seizures that occur greater than 24 hours apart, or if someone has a single seizure, yet the underlying ideologies or workup reveal that that patient actually has a recurrence risk of at least 80%. That that patient could also qualify for a diagnosis of epilepsy. Or if we're lucky enough to be able to make the diagnosis of an epilepsy syndrome.
- Dr. Joseph Sullivan: [00:07:18](#) And so epilepsy syndrome takes it just one step further, and it takes into account the complex signs of symptoms that define the unique epilepsy condition with different ideologies. And those main issues are the age of seizure onset, the salient seizure types, and the EEG pattern usually in between seizures or the inter actual EEG pattern. And this really is the holy grail for us in the pediatric epilepsy space, because we feel that this really improves communication among caregivers, it allows us to communicate prognosis in a better way to families. And since we're on the topic of this discussion, it really is the way that is going to pave the road to hopefully new epilepsy gene discovery.
- Dr. Joseph Sullivan: [00:08:02](#) So I've had this slide in talks probably for the last 10 years, giving talks to from medical students, to pediatric residents, to our epilepsy fellows. And it really hasn't changed despite all of the cutting edge technologies that we have in 2017, we make the initial diagnosis of epilepsy based on history, history and history. And so we really need to know what that family member or caregiver saw when that patient child or adult had their seizure.

- Dr. Joseph Sullivan: [00:08:33](#) Sometimes we still are on the fence as to whether or not it was a seizure, we're on the fence as to what type of seizure it was. So we very often will move to the next step of testing, including an EEG and MRI, usually in that order, because there are some diagnoses that we can make based on EEG that allow us to forego undergoing neuroimaging. And that is really able to classify the majority of patients. And this has actually been looked at in many studies that we actually don't have time to go into today, but I can provide those references at another time.
- Dr. Joseph Sullivan: [00:09:11](#) So then once we have this history, and once we have the EEG and MRI, we should be able to break down these patients into three broad categories. And this is the most recent classification that was published in 2010. And I don't necessarily need to read the whole slide here. But you can probably infer that a structural metabolic cause is simply that we do imaging or we have an underlying metabolic disorder that actually explains why that patient had that seizure or explains why that patient is at risk for having ongoing seizures.
- Dr. Joseph Sullivan: [00:09:44](#) As you can see, this may get a little bit into an overlap because you have a condition like tuberous sclerosis, which we know has very well defined genetics, but it also has very well defined structural abnormality. So this classification is not perfect, but I think that it serves our purposes in clinical practice.
- Dr. Joseph Sullivan: [00:10:02](#) Then secondly, we have a , just to say unknown cause. But I actually think this is great because we are basically stating that we don't know and that's actually okay. We are stating that this patient has a normal brain MRI, has an EEG that does not fit into any of the salient epilepsy syndromes. And this is the group that we are trying to investigate further to come at a more precise diagnosis, so as Brandon mentioned, we could potentially move into the world of precision medicine in epilepsy.
- Dr. Joseph Sullivan: [00:10:38](#) And then, again, some overlap a genetic epilepsy and this is the category, I'm going to show a slide here shortly that is growing at an exponential rate. And so these are the epilepsies that... Oh, no I'm going to read this because I think being the topic of the webinar, is an epilepsy that is the direct result of a known genetic defect in which seizures are the core symptom of the disorder. The knowledge regarding the genetic contributions may derive from specific molecular genetic studies, and have been well replicated and even become the basis of diagnostic tests. And for anyone who's in the epilepsy space, SCN1A and its etiologic role in Dravet syndrome is probably the most well known. Or the evidence for a central role of a genetic

component may come from appropriately designed family studies.

Dr. Joseph Sullivan: [00:11:30](#)

So this is a slide that is almost 20 years old now, and it really needs to be updated. And it uses some of the old terminology. But I show it because it really summarizes pretty much everything that I have just reviewed. We are looking at the epilepsy syndromes as they present to us and you can see the concentric circles here of the different ages that they present. Those that present above the line, were previously called idiopathic, but we didn't want to use that term anymore. And so these are some of the epilepsies that we feel have a strong genetic etiology. Those below the horizontal line are some of those that may be the structural causes. But you can see this is evolving since 1999 when this was published and a lot of those are actually moving up, above the line. So severe myoclonic epilepsy in infancy is Dravet syndrome. So that has actually moved above the line. And then you have those to the left that are focal and to the right, that are generalized.

Dr. Joseph Sullivan: [00:12:28](#)

So what is the role of genetics? So it should go without saying that there are many well done genetic studies that have shown that there's an increase of risk of epilepsy in siblings and offspring. And in some studies, it's actually shown to be upwards of nine to 10% in mothers with epilepsy. And that compares to that of 1.7% in the general population. As a pediatric epileptologist, I see it every day that I see patients where we're looking for these genetic etiologies, but this does not mean that our adult colleagues are off the hook. Patients that present still under the age of 35 may still have a genetic contribution. And it's really only the older onset that where the yield of genetic investigations may be lower. But I think this is something that continues to evolve.

Dr. Joseph Sullivan: [00:13:20](#)

And you can't have a talk on genetics without a twin study. So these are very well done twin studies and again to beat on the epilepsy syndrome drum, we had 253 twin pairs in the Australian epilepsy twin study, about half of them actually had epilepsy. And most interestingly, of the monozygotic parents had epilepsy, but most interestingly is if those two twins had epilepsy, almost 100% of them actually had the same major epilepsy syndrome. So here already, back in 1998, we're building a very strong case that the epilepsy syndromes, if we can classify them with appropriate phenotyping that that is going to be the best path to arriving at these novel epilepsy genes.

- Dr. Joseph Sullivan: [00:14:07](#) And I just want to give one brief example of this. As Brandon said, in his opening, I'm part of the PCDH19 Scientific Advisory Board because I have a fair number of these girls in my clinic. And this is just a really exciting story. So there were 41 probands, who for all the world looked like they had Dravet syndrome, but they actually did not have any SCN1A mutation found on their workup. We know that that actually can still be the case in about 15 to 20% of patients with Dravet but all 41 with not a single SCN1A is a little bit unusual. But interestingly, there was a deletion found on the X chromosome in one male and the investigators went back and looked specifically at that chromosome and did sequencing in an additional 73 SCN1A-negative patients. And lo and behold, there were nine novel point mutations identified on this chromosome, and it was found to code for a gene called PCDH19.
- Dr. Joseph Sullivan: [00:15:11](#) So this was not a gene that was on the epilepsy radar at this time. And since then, there's been a lot of investigations by some very bright basic scientists that have really figured out that PCDH19 encodes for these protocadherins, which are involved in cellular communication, and therefore mutations in this gene can actually lead to cellular interference, and therefore, it was plausible that this was the underlying cause of the epilepsy.
- Dr. Joseph Sullivan: [00:15:41](#) And so why does that matter? Why does that matter? Well, now, those patients that were previously given a Dravet diagnosis are given a new diagnosis. And then we actually can go back and actually look at these PCDH19 patients and almost in reverse where I said that history, history, history is the first step, if we go back and know that now we have a single gene that is causing these patients' epilepsy, we can actually go out back and tease out some phenotypic features that may have escaped our eye at first glance. And that's essentially how this syndrome has evolved.
- Dr. Joseph Sullivan: [00:16:18](#) So now we have a syndrome that we can ideally suspect on clinical grounds, based on the fact that the gene was discovered. And most importantly, again, getting to the precision medicine approach. These same labs that were involved in discovering this gene are looking at what other pathways are involved and are realizing that these patients could have altered metabolism in their neurosteroid pathway that may actually explain why many of them present when they do and why some of them go into remission when they do. And while that's important, it's equally important to see, are there actually compounds that can be introduced into these patients to improve or alter those pathways involved in neurosteroid

metabolism that could potentially translate into novel treatments above and beyond the actual symptomatic treatment of the seizures themselves with our broad spectrum, anti-epileptic drugs?

Dr. Joseph Sullivan: [00:17:20](#) So this is the graph that I alluded to, you can see if we go back to 1999, 2000, when the SCN1A gene was discovered, and then there was just a long hiatus here in terms of really new gene discoveries. And then in the era of next generation sequencing, and this line, if you trust me, continues to have this meteoric rise, with new genes being discovered, not only in basic science labs, but with projects like the Epilepsy Genetics Initiative, literally every week to every month. And we hope this continues.

Dr. Joseph Sullivan: [00:17:54](#) So the ideal genetic testing scenario and I steal this from one of my mentors, Dan Lowenstein, where you could basically come into the clinic, you still are going to do your history and you're going to do your physical and maybe you'll do your EEG. But is it possible to order genetic testing at that first visit, and actually arrive at a specific genetic diagnosis that would not only help inform prognosis, but could it go one step further and help inform what drugs to use, and maybe even what dosage? That would truly transform the lives of many of the patients that we see with epilepsy so they don't have to continue to live with this epilepsy of unknown cause.

Dr. Joseph Sullivan: [00:18:36](#) So unfortunately, we're not there yet. Hopefully all of you that are calling in are people that are working on this to help us get there. Does it help in determining treatment strategies? I think there are some scenarios where this does help. It does also help in certain specific conditions where it can help with prognosis. And it certainly helps in many cases, with the risk to siblings in programs, probands, sorry, depending on the nature of the underlying gene mutation.

Dr. Joseph Sullivan: [00:19:08](#) So where are we truly in 2017? Swaroop's going to go over this a little bit in his talk, because it really is a monopoly of different testing options. And while it may seem that it's just easiest to jump to whole exome or whole genome sequencing, hopefully there can be a few nuances in terms of how that is approached. And it's also important to acknowledge that not every single test is the same. You have to know based on your history, what type of epilepsy gene you are potentially considering, and you want to make sure that those lists of candidate genes based on the phenotype are well covered in these panels.

Dr. Joseph Sullivan: [00:19:52](#) But that being said, what if we take all the epilepsy or a handful of epilepsy centers in the country that are seeing these children and again, focusing on the younger children as a pediatric epileptologist, we have a feeling that it's the early onset epilepsies that really have a stronger underlying genetic cause. So what is the yield today? So this was a study that I was lucky enough to be part of, that was championed by Anne Berg and a handful of other pediatric epilepsy centers across the country that was really looking at just this. We wanted to see in patients with a new diagnosis of early life epilepsies, what was the diagnostic yield.

Dr. Joseph Sullivan: [00:20:34](#) And so this study recruited 775 patients over about a two and a half year period. You can see that about 80% of them did have neuro imaging, so that remains our go-to tool to try and get an underlying diagnosis of presentation. About 95 of these patients had brain injury, most commonly neonatal brain injury, but then out of the 680 that did not have brain injuries. So that's that 680 plus 95 equals to 775, about half underwent some type of genetic testing.

Dr. Joseph Sullivan: [00:21:09](#) And if we look at the different types of genetic testing, you can see that there still is value in some of the quote unquote, older genetic testing. Just genetic tests, including karyotype and chromosomal microarray. Now it's not going to come as a surprise that these are very likely patients that had a phenotype such as Down syndrome, or you wouldn't feel the need to go to the whole exome sequencing, you're going to make that genetic diagnosis based on the karyotype. But you can see, here that the yield as we march down the different types of genetic tests is anywhere between 20 and as high as 40%.

Dr. Joseph Sullivan: [00:21:49](#) And then if we actually look at this group, even a little bit more closely, and we look at those that actually had brain malformations, those that had the clinical dysmorphic syndromes, as I just mentioned, like Down syndrome, but most importantly, these ones down at the bottom. So the unexplained etiology. And those with both developmental delay, there's often been some lore that your yield is much higher if you have abnormal development and much lower if you have normal development. Well, hopefully you can see here that if you just look at this group that had no underlying cause, that a pathogenic variant was actually found in a significant number of patients. And it actually didn't matter whether or not you had normal development or developmental delay. The yield was a little bit lower. Hopefully, we would all agree that this is a significant amount of patients that gives them useful information for the family and the clinician.

- Dr. Joseph Sullivan: [00:22:42](#) And so if we look more closely at this, of those patients with unknown cause, there were still some that underwent chromosomal microarray, there were some that underwent epilepsy panel, and then there were some that underwent whole exome. And you can see that the yield here for those patients with a true unknown cause, their microarrays is still very low. So one could argue that we really shouldn't move into next generation sequencing either in the form of an epilepsy panel or with whole exome sequencing.
- Dr. Joseph Sullivan: [00:23:13](#) So to sum up the take home points, hopefully I've demonstrated to you that that clinical phenotyping still is a critical first step. We really can't approach the epilepsy gene discovery by just doing whole exome sequencing on thousands of patients without having the detailed phenotyping available so that we can tease that out.
- Dr. Joseph Sullivan: [00:23:35](#) Genetic panels that are growing in numbers, I remember when I first started training, the panels were between 10 and 20 genes now there's panels of upwards of 1000 genes, are very useful, especially when we feel that we do have a well-defined electro clinical syndrome. And as I mentioned in the beginning, that's really how we're going to be able to make a new epilepsy syndrome diagnosis with a clear genetic cause.
- Dr. Joseph Sullivan: [00:24:02](#) And then when the phenotype is not specific, and in my opinion, I do think that whole exome sequencing is more appropriate. And furthermore, even if you actually get a negative test, that we know that reanalysis can be beneficial. And this is really the point of EGI so that we can try and identify some of these new epilepsy genes. So with that, I'm going to turn it over to Swaroop and he will take this one step further. Thank you.
- Dr. Swaroop Aradhya: [00:24:35](#) Great. Thank you, Joe. And thank you, Brandon. And thank you all for joining us for this webinar series on epilepsy. What I'd like to do today is to present some of our data from epilepsy genetic testing, to give you a glimpse into the results that we see from multi-gene next generation sequencing panel. And I'm going to describe the spectrum of variants observed in the diagnostic yield and also show you the data related to disorders with treatment implications since I believe the next webinar in the series deals with that topic. And lastly, to address the often difficult question that clinical geneticists, child neurologists and genetic counselors often raise which is, the question of when to use a panel versus exome sequencing. I'm going to go through some data related to the technical differences between these two approaches and also summarize information from a major

analysis that we did of recent publications on exome sequencing. And we hope this information will help you appreciate the types of results seen in a multi-gene panel and its utility in diagnosing children with epilepsy.

Dr. Swaroop Aradhya: [00:25:55](#)

So just to get started and to follow up from Dr. Sullivan's presentation, I'll go through a high level introduction to the molecular genetics of epilepsy. As you heard, previously, epilepsy is defined as the occurrence of two or more unprovoked seizures separated by at least 24 hours. And this is a definition provided by the International League Against Epilepsy. It's a common clinical disorder affecting more than three million Americans. And it's thought that more than 50% of epilepsy cases have some genetic basis and this is especially likely in those forms of epilepsy that affect children or have an early onset.

Dr. Swaroop Aradhya: [00:26:32](#)

And we've learned a great deal about the molecular genetics of epilepsy since those initial discoveries of mutations in ion channel genes. In large studies such as those done by CURE's Epilepsy Genetics Initiative, or the Epi4K collaborative consortium have really produced wonderful results of discoveries in novel genes implicated in epilepsy and illuminating novel biological pathways involved in its pathogenesis. So not only are more genes being discovered, but we're also beginning to understand the breadth of the clinical spectrum arising as a result of pathogenic variants in those genes.

Dr. Swaroop Aradhya: [00:27:15](#)

And genetic testing, I'm just going to go through what the utility of genetic testing is. Obviously it has various important uses. First of all, it can point to a molecular diagnosis as I mentioned earlier, identifying the pathogenic variant and the gene affected can help clinicians make clinical correlations and in some cases it can help define the clinical presentation, for example, to recognize whether syndromic and yet in other cases may be helped understand the prognosis. And in addition, genetic testing results can help determine recurrence risk in families and this is of course, an important part of counseling those families. And lastly, specific genetic test results can guide clinical management for instance, if a child has a metabolic disorder they can be treated with available diets or therapies.

Dr. Swaroop Aradhya: [00:28:02](#)

And genetic testing for epilepsies is being increasingly used to identify molecular causes and confirm clinical diagnosis. And we've come a long way in using genetic testing technologies from Sanger sequencing of single genes to now largely migrating over the last five to 10 years to next generation

sequencing of multi-gene panels, next-generation sequencing or what we just simply call NGS has largely become the standard today for diagnostic genetic testing. And multi-gene panels have been extremely useful for a really wide variety of disorders and are typically used when the clinical presentation is specific enough that a gene panel can be selected for genetic testing. But when the clinical presentation is not specific, or is syndromic, or a disorder is known to be genetically very heterogeneous, it's often best to use whole exome sequencing.

Dr. Swaroop Aradhya: [00:28:56](#)

And epilepsy in this regard presents an important challenge because it can be nonspecific, as such in some situations. But nevertheless panel testing has shown a high yield as you'll see shortly in the data I'm going to show you. But before we dive into that data, let me remind the audience that NGS has really become highly sophisticated and can accurately detect a pretty broad set of variants if it has been properly developed and validated in a clinical lab.

Dr. Swaroop Aradhya: [00:29:26](#)

At Invitae we've built our NGS processes to identify single nucleotide variants, both small and large, indels, as well as exonic deletions or duplications all from a single asset. So for this webinar, just as an outline here, I'll go through data from our epilepsy panel and highlight a few important themes. I'll describe results from a cohort of about 2000 children referred for genetic testing for epilepsy and these kids were diagnosed with various forms of epilepsy. So it's important to remember as we go through the data that this is an unselected cohort. And it's really typical of what a diagnostic lab sees. So in other words, the types of cohort seen by labs are not necessarily homogeneous in clinical presentation.

Dr. Swaroop Aradhya: [00:30:16](#)

And so in this cohort, I'll describe to you what we found in terms of the variant types and diagnostic yield. I'll show you some examples of exonic copy number variants that we detected since we performed this deletion duplication testing within our NGS assay. And also show you the cases that had treatment implications. So you can appreciate the proportion of those types of cases solved by panel testing. And lastly, I'll go over the technical comparison between panel testing and whole exome sequencing and hopefully, shed some light on the advantages and limitations so you can make perhaps informed choices for your own patients when deciding between panels and exomes.

Dr. Swaroop Aradhya: [00:30:59](#)

So at Invitae, we currently have a multi-gene panel for epilepsy that consists of 189 genes, a previous version had about 105 genes and patients referred to us were tested for all these

genes. And in a few cases for only a subset of the genes based on specific clinical presentations. For instance, we had a few instances as shown in this table, we had a few instances in which patients were tested only for genes for infantile epileptic encephalopathies or for Rett/Angelman disorder spectrum.

Dr. Swaroop Aradhya: [00:31:30](#)

And I should note that at Invitae clinicians can select a sub panel but can later re-requisition for the full panel at no additional charge. So many clinicians opt to take this route in doing the testing. And our epilepsy panel includes genes for infantile epileptic encephalopathies, Rett/Angelman syndrome disorder genes, the progressive myoclonic epilepsy, such as the Neuronal Ceroid Lipofuscinosis, or Lafora, tuberous sclerosis and a range of other disorders.

Dr. Swaroop Aradhya: [00:32:00](#)

And with this approach, our intention was really to offer a panel intended to capture all the major known genetic causes of epilepsy in a single test that identifies sequence and copy number variants simultaneously, and produces a high diagnostic yield. And so all positive findings from our panel, which is to say that all pathogenic or likely pathogen variants are confirmed with orthogonal methods, like Sanger sequencing or custom designed array CGH. And the table shown here is a summary of the results. And so as I said, nearly 2000 children tested on our panel, most were tested for the full panel and 20% of individuals had a pathogenic or likely pathogenic variant. 16% of all these patients received a definitive molecular diagnosis meaning an answer to their clinical diagnosis, which means that the genetic cause, really for the epilepsy was identified.

Dr. Swaroop Aradhya: [00:33:02](#)

And this number increased to 19% in children tested only for genes for early infantile epileptic encephalopathy, and then further to 25% in children suspected to have a syndromic disorder such as Rett/Angelman and other related conditions. And this range of positives release is quite consistent with published data from epilepsy panel testing done by other groups.

Dr. Swaroop Aradhya: [00:33:32](#)

When we look a little deeper into the diagnostic yield in terms of which genes were affected, we see some familiar genes and some perhaps less expected ones. The sodium channel gene, SCN1A is really well known as Dr. Sullivan just described in Dravet syndrome. And it's a classic gene associated with epilepsy, and it's not surprising that we see many of the positive results in this gene. It accounted for more than 15% of all positive molecular diagnosis in our panel. Similarly MECP2 for Rett Syndrome, KCNQ2 for infantile epileptic encephalopathy, CDKL5 for Rett syndrome and STXBP1 for Ohtahara syndrome.

These are all common contributors to the pathogenesis of genetic epilepsies.

Dr. Swaroop Aradhya: [00:34:19](#)

And what's perhaps particularly interesting in our data is that we have a couple of biochemical disorders represented among genes with the highest positive yield, even though these are typically considered to be ultra-rare conditions. But this is likely because the biochemical defects in these patients pointed directly at which genes needed to be tested. And a good example of this is ALDH7A1, which is associated with paradox independent epilepsy. We also saw an appreciable frequency of positive diagnosis genes for disorders that were thought to be rare or somewhat more recently discovered. And I'll tell you a little bit about it in the next couple of slides. These include PCDH19, SYNGAP1, DEPDC5.

Dr. Swaroop Aradhya: [00:35:01](#)

And the chart on the right shows the proportion of positive diagnosis. If you split up genes into different categories, 28% of positive variants were in genes associated with the infantile epileptic encephalopathy, 17% in genes for syndromic disorders, and 22% were in genes for various metabolic disorders and other types of treatment implications and many were scattered among other types of genes.

Dr. Swaroop Aradhya: [00:35:30](#)

Now, if we look at the types of variants we saw, just as a reminder, our NGS panels again, our NGS panels have designed to detect single nucleotide variants, small indels, large indels as well as exonic, deletions or duplications or what we also call copy number variants. These are shown in various colors in the chart here. In our epilepsy panel we have also optimized detection of the trinucleotide expansion in the X-linked gene called ARX which is associated with an early infantile epileptic encephalopathy and other allelic disorders.

Dr. Swaroop Aradhya: [00:36:05](#)

And our data show that most variants that we classified as pathogenic, which is the first column in the chart, were not missense changes, but rather other types of single nucleotide variants such as nonsense or frameshift variants. Interestingly, you can see that we observed a high frequency of indels and exonic deletions or duplications. And in this cohort 16% of pathogenic variants fell into this category, which is really a high proportion. We also identified a few instances of mosaic pathogenic variants in CDKL5, FRRS1L and the two tuberous sclerosis genes. And these mosaic variants tend to be rare but can be found in panels with high depth of covered sequencing, such as the one that we used. And this chart also shows that in contrast to pathogenic variants, those that were classified as variants on certain significance, were largely and not

surprisingly missense variants. And this is shown in the middle column. I'm going to show some more detail about this whole event in a second.

Dr. Swaroop Aradhya: [00:37:08](#)

And the last column here shows variants in genes that we curated as having emerging evidence for association with epilepsy disorders and variants and these genes also mostly missense and classified as having uncertain significance. Before I jump further into the details about those variants, let me quickly show you some examples of the exonic deletions or duplications or copy number variants we'll find on our NGS since this approach is still not broadly utilized in our community.

Dr. Swaroop Aradhya: [00:37:42](#)

NGS based copy number detection, it really is quite sensitive and has high resolution and you can learn... We have some more information on our website that you can check out. The examples that I've shown here show partial gene duplications in CDKL5 in the panel on top. CDKL5 is a X-linked gene associated with Rett syndrome. And the panel on the bottom shows a partial gene duplication in STXBP1, which is associated with autosomal dominant Ohtahara syndrome, the red dots in each of those panels, the dots that have deviated from the horizontal baseline indicate the location of the duplication.

Dr. Swaroop Aradhya: [00:38:24](#)

Now looking at the number of variants we found and the classification categories they fell into we find that most individuals had anywhere from zero to two variants that went through clinical interpretation and there were some individuals who had three or more variants but in aggregate, accounted for about a third of all individuals tested. And 14% of all variants reported were classified as pathogenic or likely pathogenic, meaning they were clinically significant mutations, the rest were classified as variants of uncertain significance. And of course, the likely benign and benign variants are typically high frequency polymorphisms in the general populations and are not shown here.

Dr. Swaroop Aradhya: [00:39:00](#)

The 14% pathogenic or likely pathogenic variants together provided the 20% diagnostic yield overall in this cohort that I mentioned over the last slides ago. However, some of the results classified as variants of uncertain significance can also be resolved with further testing and contribute to that positive diagnostic yield. And this rate of variants of uncertain significance can be... It really can be frustrating for clinicians just as much as it is for the labs that report them. And these are invariably classified as such because of limited available evidence. But I'm going to show you a little bit more data we can take a deeper dive into these variants of uncertain

significance to better understand their sources and distribution hopefully, they'll provide some insight into what can be done about them and in which situations.

Dr. Swaroop Aradhya: [00:39:52](#)

So this slide here shows a chart illustrating the different categories of variants of uncertain significance. We plotted the percent of all variants of uncertain significance on the y-axis separated by which types of genes they were in, in terms of the mode of inheritance of those associated disorders. For instance, the first column here shows all variants of uncertain significance that appeared as single alleles genes associated with autism and recessive disorders. The second column is for genes associated with dominant disorders with reduced penetrance. The middle column, I guess, is the third column is for dominant genes with high penetrance. And the last two columns on the right show variants of uncertain significance that occurred as two alleles in genes for recessive disorders. And the hatched boxes in each column represent variants of uncertain significance that occurred, along with another variant that was classified as a clinically significant positive variant in some of the genes. Suggesting that the one with uncertain significance had less relative importance.

Dr. Swaroop Aradhya: [00:40:51](#)

So what's clear from this chart, is that most variants of uncertain significance are actually found as single alleles in genes for autosomal recessive disorders. So there's not much utility in trying to resolve the significance of these since we did not see a second variant in the same gene. The variants of uncertain significance in dominant disorders reduced-penetrance, which are shown in the second column, are also difficult to resolve unless there are many affected individuals in their family or their functional studies or the types of evidence available. But those sorts are often difficult to resolve.

Dr. Swaroop Aradhya: [00:41:23](#)

And the middle column and the last three columns, however, are different. Because these variants of uncertain significance in these categories actually have a decent chance of being resolved through family testing to determine perhaps if there de novo events in dominant disorders or by apparently inherited alleles in recessive disorders and this can contribute to classifying those variants along with any other evidence that may be relevant.

Dr. Swaroop Aradhya: [00:41:58](#)

Now, because parental testing results can influence variant classification, we were able to do that testing for at least 60 or so eligible variants and found that about half of those patients saw a reclassification of a variant because it turned out to be de novo which is an observation that contributes weight, along

with other pieces of evidence towards a classification as a disease causing change. In some cases, the clinical information itself can also be useful.

Dr. Swaroop Aradhya: [00:42:28](#)

Epilepsy, as you all probably know, by itself has clinical features, can be nonspecific, but additional information, particularly observations of biochemical defects, or identification of characteristic pattern mnemonic features in some syndromic disorders, these can be very helpful. And that type of information in very specific instances can be taken into account in the course of classifying variants, because it addresses the prior probability of some variants being pathogenic. And I've added a table here showing some examples of situations where specific phenotypic information mainly by chemical defects in this case was helpful in some of the cases that we looked at.

Dr. Swaroop Aradhya: [00:43:14](#)

So in the next couple of slides I want to show you some interesting results from our panel. This slide lists a few cases in which we found more than one diagnostic result. In the first case, we found pathogenic variants in STXBP1 for Ohtahara syndrome and in SYNGAP1 which is associated with an intellectual disability syndrome. This was in an infant with seizures and normal brain morphology by MRI. In a second case, we found a large deletion that include both the SCN1A and SCN9A genes. This is not necessarily the same as finding two separate results but it did affect two known epilepsy genes. And the third case was a four year old with intractable epilepsy and developmental delay and had a pathogenic variant in CDKL5 associated Rett syndrome, but also a second pathogenic variant in the PRRT2 gene.

Dr. Swaroop Aradhya: [00:44:08](#)

And note that most of these genes that I'm describing here are associated with disorders that show high penetrance. And it's unclear if, in this case, both variants in each of these individuals contributed to the phenotype or if it was actually a blended phenotype. But these examples, highlight the more comprehensive answers when we get from using a large multi-gene panel. Although admittedly, those answers may not always be immediately clear in their significance.

Dr. Swaroop Aradhya: [00:44:38](#)

Interestingly, we also identified positive results in a number of genes associated with what were typically considered rare forms of epilepsy or in genes that have been more recently described. We have positive results in at least four such genes including PRRT2, DEPDC5, PCDH19 and SYNGAP1 as shown in this table. And it's now evident that pathogenic variants in DEPDC5 account for an appreciable proportion of focal epilepsy cases. And similarly PRRT2 appears to be involved commonly

and self-limiting infantile epilepsies. And epilepsy related to PCDH19 which is sex-limited form seen in females as Dr. Sullivan mentioned as well. And an epilepsy related to SYNGAP1, I actually considered rare but we have seen close to 20 individuals with pathogenic variants in these genes in this cohort about 2000 children suggesting that perhaps they may not be that rare after all.

Dr. Swaroop Aradhya: [00:45:45](#)

Lastly, I'd like to take a moment here to highlight the positive diagnostic yield of genes that are associated with disorders for which there are treatment implications. And by implications I mean that there may be known or established therapies for these disorders or there may be contraindications in our cohort, 22% of all definitive diagnosis were in genes associated with these types of disorders. And this is a significant finding because multi-gene panel testing can be relatively quick and provide results to manage treatment in these cases. And the table here shows some examples of genes and disorders that fall in this category. And as you can see, many of these are biochemical disorders for which there are various types of interventions that can ameliorate the clinical presentation. SCN1A is listed here because individuals with pathogenic variants in this gene should avoid certain types of drugs that can further aggravate their epilepsy.

Dr. Swaroop Aradhya: [00:46:54](#)

So finally here in the last couple of minutes, I'd like to switch gears and address that important question of when to consider using multi-gene panels versus all exome sequencing. This is not an easy question to answer and it depends on the specificity of the clinical presentation and the genetic heterogeneity and also on some practical things like test reimbursement, etc.

Dr. Swaroop Aradhya: [00:47:54](#)

But what I'd like to attempt perhaps, is provide some perspective in terms of technical sensitivity and where panels may fit in the process of genetic testing so that you can maybe make more informed choice. In doing this, we did two types of analysis. The first was an assessment of the technical sensitivity to detect variants, we looked specifically at all known epilepsy related variants in our database and in HGMD and mapped them across three different whole exome methods. And in general, what we found was that about 1.6 to 2% of variants would be at low coverage, which is below 20X coverage and so were at risk of getting missed. And this is not really surprising given that exomes are known to have gaps and at least one other recent publication has corroborated our findings. So it's about 2% risk of missing variants by exome.

Dr. Swaroop Aradhya: [00:48:51](#)

We also do the second analysis in which we looked at recent publications of large studies of whole exome sequencing and diagnostic testing and we asked the question, of all the positive results described in these studies, how many were in individuals with any mention of epilepsy? And in what genes were those results in? And this is shown in the chart. But it turns out that about half or more of the positive results from whole exome sequencing were in genes that were already present on existing multi-gene panels. And this number, of course, increases significantly if the cohort tested by exome sequencing is really enriched for epilepsy cases, which was the case in at least one of these studies shown on the left.

Dr. Swaroop Aradhya: [00:49:37](#)

So based on this, and given the lower cost, the faster turnaround for results and the fact that the major known genes for epilepsy are technically covered very well, on the panel suggests that panel testing may be a good first tier test for epilepsy followed by reflex exome sequencing if the panel is negative.

Dr. Swaroop Aradhya: [00:50:01](#)

So just to summarize some key takeaways, multi-gene panels have a high yield for epilepsy and cover the most important genes. Well, we found on our panel, we found a diagnostic yield of roughly 20% overall, and this increased to about 22% if we looked at diagnosis that had immediate treatment implications, and this is an observation that's important in the context of the faster turnaround time panel testing. And we've found that simultaneous detection of single nucleotide variants and copy number variants is useful. In our cohort 16% of positive variants, were not sequence variants that was an important finding.

Dr. Swaroop Aradhya: [00:50:36](#)

The high rate of variants of uncertain significance observed in large multi-gene panels is in large part due to single variants in genes for recessive disorders, or for dominant disorders with reduced penetrance. And parental testing can help resolve variants of uncertain significance depending on which genes they're in and phenotype information can also be helpful in specific instances. Lastly, exome sequencing yields positive results for epilepsy in gene mostly found on panels already, and exome already also has technical limitations compared to panels. But it's important to know that because exome sequencing allows us to cast a wider net to search for the diagnostic cause and even maybe identify novel genes, it's very important in many cases, particularly where the search for the molecular cause threatens to turn into a long diagnostic odyssey.

- Dr. Swaroop Aradhya: [00:51:29](#) So with that, I hope you found it useful to go with these data from our multi-gene panel for epilepsy, I hope you were able to appreciate the details of the high diagnostic yield on this panel, the spectrum of variants we found, the sources of the variants of uncertain significance, and the relative use of panels versus exome for epilepsy. Now, I'd like to end by acknowledging our epilepsy team on this next slide and take any questions.
- Brandon Laughlin: [00:52:01](#) Thank you very much, Dr. Swaroop and Dr. Sullivan as well. We can actually begin the Q&A slide or the Q&A portion of our meeting. Again, if you have any questions for our speakers, go ahead and submit them in the questions pane of the GoToWebinar control panel, and then click send.
- Karen Leydiker: [00:52:27](#) Okay, great. Thank you, Brandon. So this is Karen Leydiker from Invitae and I'll go ahead and start asking the questions that have been submitted. So just one second here. The first question we have is for Dr. Sullivan. And they asked, would you wait until the patient has had at least two seizures before considering genetic testing? Or would you consider genetic testing after a single seizure?
- Dr. Joseph Sullivan: [00:52:55](#) That's a great question. I think for all intensive purposes, I think you really do need to wait for that second seizure, specifically in Dravet syndrome, we're really trying to arrive at a very early diagnosis and we've actually looked to see if genetic testing should be sent after a young child under the age of one has had their first episode of prolonged convulsive status. That may be an isolated situation where I think a single seizure could be argued, but other than that, I think you really need to wait for that second seizure.
- Karen Leydiker: [00:53:33](#) Okay, thank you. And the second question here is for Dr. Aradhya and it says the table showed a relatively high yield of TPP1, can you speak to this?
- Dr. Swaroop Aradhya: [00:53:49](#) Yeah, absolutely. And this was surprising to us as well and a couple of them were actually... It turned out a couple of them were actually because of signatures that are associated with TPP1 related Neuronal Ceroid Lipofuscinosis. So there was a prior probability that we would discover it. But in several of those other ones, we actually didn't expect those. And so it may be that as rare as this disorder is, and perhaps it is under diagnosed. And some of you may know that there's a program that BioMarin has that is looking at these individuals with mutations in TPP1 and seeing who may be eligible for a drug therapy. And I think it appears that this disorder may not be as rare as we've thought before. But really, I mean, even in the

quarter of 2000 children we're seeing a number but I would really be interested in seeing a much larger cohort and seeing if this number really holds true.

Karen Leydiker: [00:55:02](#) Great. Okay. And now for Dr. Sullivan, how difficult has it been for you to get genetic testing approved for your patients with epilepsy?

Dr. Joseph Sullivan: [00:55:11](#) Oh, great question. So it really does depend on the type of insurance coverage. But it also depends on the attitude of the person on the other end of the line when you're calling in to get the prior authorization. I would say that in my practice, because we still serve about 50% of our patient population is state-insured or under-insured, Medicaid Medical, that it is still very challenging, but I know that there are a lot of companies that are working with us to try and come up with an arrangement so that those patients who could benefit the most can get testing but that's a process in evolution right now.

Karen Leydiker: [00:55:58](#) Okay, great. Thank you. And then for Dr. Aradhya, is it best to order a panel that includes deletion duplication studies performed simultaneously with the sequencing? Or does it make more sense to see what the findings of the sequencing are and then only order deletion duplication for the genes indicated based on the sequencing results?

Dr. Swaroop Aradhya: [00:56:21](#) Yeah. I think that's a good question for a couple of reasons. One, I think it's fair to say that we really didn't appreciate how prevalent these exonic copy number variants are in disease genes, aside from maybe a handful like Duchenne muscular dystrophy, or some of the other ones. But where we're really seeing this evolve to is being able to detect a much broader spectrum of variants, including single nucleotide variants, indels, exonic, copy number variants, etc, all within a single test.

Dr. Swaroop Aradhya: [00:56:58](#) And so it should be, we should all be moving towards being able to get all those pieces of information together from a single test. And we're finding through internal analysis of our data, that the prevalence and frequency of these exonic copy number variants is actually pretty high that it accounts for almost 10% of all the pathogenic variants that we've ever reported from Invitae. So it's clearly a high proportion of clinically important findings. So my opinion, is that these tests should be looking at exonic copy number variants at the same time as sequence variants so we have the most optimal clinical sensitivity of testing.

- Karen Leydiker: [00:57:46](#) Great, okay. And then this will be the last question since we're running out of time, and it's going to be for Dr. Sullivan. It says, when would you recommend testing an adult patient and is that something that you need to consider with a higher concern if they have relapsed? And what would your recommendations be for them?
- Dr. Joseph Sullivan: [00:58:10](#) Yeah. Well, this is a great question, I'm glad that this is being asked. I think that because I commented that a lot of the early onset epilepsies have a very high diagnostic yield, if an adult being seen in an adult epilepsy practice, had an infantile onset epilepsy, and there still is not a known cause, I would say that those group of patients actually represent a very rich group where the yield could be exceedingly high. Especially if the clinician has the time to go back and take that early childhood history, I'm convinced that there are hundreds of patients sitting in an adult epilepsy clinic with SCN1A mutations that had Dravet syndrome but then when they turned into adults they have a little bit more of a nondescript phenotype and our adult epilepsy colleagues may not have that index of suspicion. But I still think that those group of patients just to summarize, are those with infantile or childhood onset who continue to have seizures and have no known cause.
- Karen Leydiker: [00:59:23](#) Great, thank you. So at this point, we'll hand it back to Brandon for closing statements.
- Brandon Laughlin: [00:59:30](#) Great. Thank you, Karen. Now that we've come to the end of our hour, I do want to thank all the attendees for their participation. Also extending a special thank you to Dr. Joseph Sullivan, and Dr. Swaroop Aradhya for sharing this valuable information with us. Again, this webinar was recorded and it will be available to watch on both the CURE and Invitae websites. You will receive an email notification of this in the next coming days.
- Brandon Laughlin: [00:59:59](#) To close, we did want to share one interesting fact about epilepsy testing from Invitae. Knowing the specific genotype underlying an individual's seizures can inform treatment by allowing the physician to choose the best anti-epileptic medication for their patient's condition, while avoiding those that are contraindicated.
- Brandon Laughlin: [01:00:20](#) Our next webinar in the series will be on December 13th, and that is titled Targeting treatments for the genetic epilepsies: Clinical cases with Lacey Smith from Boston Children's Hospital, and Katie Angione from Children's Hospital Colorado. You can

register for this webinar at [invitae.com/webinars](https://www.invitae.com/webinars). I want to thank everybody again and have a great day.