PEDIAPOD FEBRUARY 2022 TRANSCRIPT

Geoff Marsh
Hello and welcome to Pediapod for February 2022. This month identifying gene variants that contribute to the development of epilepsy after acute symptomatic neonatal seizures.

25% of children who survive acute symptomatic seizures as neonates will go on to develop epilepsy. Whilst there are several known risk factors, currently not enough is known about the mechanisms behind the development of epilepsy following neonatal brain injury, and thus, it's not yet possible to reliably predict individual risk of developing this disease in this group of patients. In this episode, I spoke to Adam Numis from the University of California San Francisco. He and his team used whole exome sequencing with targeted gene analysis to look for genetic risk factors for developing epilepsy following acute neonatal seizures, and to identify potential biological processes behind this epileptogenesis.

Adam Numis
My name is Adam Numis and I'm a pediatric epilepsy specialist at the University of California, San Francisco.

Geoff Marsh
And you've been on the podcast before, and last time we were speaking about using cytokines as a predictor of epilepsy after neonatal brain injury. You're here again, this time we're talking about the potential contribution of pathogenic variants. So you've changed tack slightly…

Adam Numis
Slightly. What we're really trying to understand is how epilepsy develops in those kids who are at most risk. So whether that be kids with brain injury from trauma, from birth related injuries, hypoxic ischemic insults. We're trying to understand why some of those children develop epilepsy and others don't. And it's likely going to be a multifaceted reasoning. It's probably not going to be just one thing like elevated cytokines or genetic variants, but really a combination of all these things.

Geoff Marsh
And if you could just give us a quick reminder about neonatal seizures resulting from brain injury and how that relates to epilepsy?

Adam Numis
Yeah, neonatal seizures happen in about one in 1000 children and we know that about 25% of those kids will develop epilepsy later on in life. And right now, we still don't have great prediction paradigms to understand those who are at greatest risk.

Geoff Marsh
So there are well established risk factors for epilepsy but you're saying that they don't explain all the data, we don't yet have a full picture about the pathophysiology of how epilepsy sometimes pops up after neonatal brain injury?

Adam Numis
Exactly. And so with this particular study, we were evaluating genetic risk factors. So, within epilepsy we know that there are certain genes that will result in a severe type of epileptic encephalopathy like Dravet syndrome, KCNQ2 related encephalopathies… we weren't hypothesizing that those genes would really influence the development of epilepsy in these children with brain injury and neonatal seizures, but rather, there would be risk factor genes. So we're not looking for one gene that's going to cause epilepsy in all these kids, we really think it's probably an accumulation of different pathogenic variants and epilepsy-associated genes that cumulatively can increase the risk of developing epilepsy after neonatal seizures. And what we did was we did a whole exome sequencing, but then looked at 200 genes that we know are associated with epilepsy to some degree, so there's some evidence that there's an association. This includes some of the genes that have been expertly curated as being definitively associated with epilepsy but that's a very small amount. The Epilepsy Gene Curation Expert Panel, or for short- ClinVar, is currently working on expanding that number. But we wanted to reach more than that, right? Because we know in a few years, the ClinVar dataset is going to be much larger and so we looked at other genes that are available in commercial panels, added them in and used that as our sort of dataset. So genes that had some type of evidence for an epilepsy association.

Geoff Marsh
In terms of the design of the study, you used family trios. Was the rationale behind that so that you could tease apart which variants are popping up new in a generation and which had been passed on from the parents?

Adam Numis
Exactly. So we did limit it to families where we knew that the child had biological parents who could provide specimens exactly for that reason. We wanted to know whether these variants were *de novo*, or whether they were inherited from one or other of the parents. And when we're classifying variants as ‘pathogenic’, ‘likely pathogenic’, ‘benign’, ‘likely benign’, or a ‘variant of uncertain significance’, that data is important to help us with processing and interpreting that data. So it was really important for us to have that.

Geoff Marsh
You were casting your net really quite wide and seeing what you pick up. But another focus of the study was targeted genetic analysis…

Adam Numis
Yeah, so we casted a wide net with the whole exome sequencing, but the first part of our study was really doing this targeted analysis of these genes that had an epilepsy association. But when you do a

whole exome you're right, you get a plethora of data, many many more genes. Thousands an thousands of additional genes. And so we leverage that data set to help us do some sensitivity analysis. So we found this association where children with postneonatal epilepsy had a higher likelihood of having a pathogenic variant in an epilepsy-associated gene compared to those who do not develop epilepsy. As a sensitivity analysis, we want to make sure that this looks good and make sense so we looked at genes associated with coronary artery disease. You wouldn't expect children with epilepsy or their parents to have an increased enrichment of pathogenic variants in coronary disease genes compared to those kids who didn't develop epilepsy. So we looked at 85 genes associated with heart disease and compared those two groups and found no difference between them, which is what we would expect. If this association in epilepsy genes is real, you would hope that you would not see any signal in other types of disease. Those associations shouldn't be there. But what was reassuring for us was that we saw a signal in the disease process that we were looking for and we did not see any signal for an association in a disease process that we thought was unrelated.

Geoff Marsh
So what kind of variants did you pick up then from the analysis?

Geoff Marsh Adam Numis
We found six pathogenic variants: five of those in the children who developed postneonatal epilepsy, one in a child who did not develop postneonatal epilepsy, and these were in different genes. So we can kind of hypothesize that we wouldn't find variants in genes that are associated with the very severe types of epilepsy that would independently cause an epilepsy syndrome like SCN1A, SCN8A, KCNQ2, but rather, we would find these variants in genes that have an association with epilepsy where the penetrance may not be 100%. So a parent might have a variant and be unaffected, though the child may have it with the same type of variant. What we found was there were these epilepsy-associated genes. So that's where we found these variants hiding, within these epilepsy-associated genes. So in their own right they can cause epilepsy in some children. Our hypothesis is that in the correct setting like a child with brain injuries resulting in neonatal seizures, having this sort of second hit just increases their likelihood of seizures to develop again later on. And then we looked at the entire data set. With an exome analysis, you have a huge plethora of data. And what we then did was we looked in an exploratory fashion at different pathways that might be changed between these two groups, in terms of the children who develop epilepsy and those who don't develop epilepsy, and what we were looking for was an understanding of the mechanisms that might be related to epileptogenesis in these children.

Geoff Marsh
And so were there any neat stories in terms of trying to unpick any potential mechanisms for how these variants are increasing risk?

Adam Numis
Yes. So in the whole exome analysis, we remove the epilepsy association of genes. We know that those are already associated in our study, so we take all of those out. And then we look at all of the other genes in the exome. And what we found was there was a relative enrichment in genes associated with synaptic transmission in those kids who develop epilepsy and there is an enrichment in ubiquitin,

or cell death related genes in those who didn't develop epilepsy. And so this is something we're still trying to understand, but certainly could help give insights into mechanisms. And certainly, this is something we're looking at doing in a much larger cohort. But it kind of makes sense, right? Epilepsy is inherently an imbalance between excitation and inhibition in the brain. So kids who develop epilepsy you expect may have differences in the way their synapses are transmitting and the way their neurons are talking to each other.

Geoff Marsh
I know you want to do a large study and get more robust numbers. But in terms of some of those ideas you're having about pathophysiology, do you think that these results might lead the way toward a more personalized therapeutic approach to childhood epilepsy?

Adam Numis
Exactly. Some of the genes we found in the group of children who develop postneonatal epilepsy, there have been some antiseizure medications that may be better for kids with that particular gene change. So we've been trying those medications in kids within our cohort and we have seen benefit. So certainly, I think that's our goal, to develop this personalized type of approach to epilepsy management.

Geoff Marsh
And you started this conversation by reiterating that epilepsy isn't one thing is it. Do these results shed light on the etiology of particular forms of this disease more than others?

Adam Numis
I think it gives us a lot more questions then answers just yet. But again, going back to the work that we published before and other works that we have in the review process, I think genetics is going to help us understand why some kids have altered inflammatory response, or have altered histone modifications as a result of these acute symptomatic seizures or brain injuries. So you may have a genetic change that alters the way your body reacts to a particular injury or seizures early on and that can help us understand that better. So there's a lot of things we need to try to understand and that's where we have we have grant proposals in right now that hopefully will get funded and will help us answer some of those outstanding questions.

Geoff Marsh
In your opinion is the paradigm with regards to epilepsy shifting from something that we need to work out how to predict but then deal with, to something that we might one day understand to the degree that we could prevent it happening in the first place?

Adam Numis
That's my hope. That's the whole goal of what I do. I think we are making a lot of progress. As these sorts of technologies become more accessible to us in terms of sequencing, small RNA sequencing- we're finding out a lot and really we will be able to do some pretty cool stuff actually preventing epilepsy and not just managing it.