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Hello everyone. I'm Monica Coenraads the executive director of Rett Syndrome Research Trust. I'm joined today via Skype by Dr. Jeff Neul in Houston, Texas. We're going to be talking today about the definition of Rett Syndrome. Rett Syndrome is a clinical diagnosis which is confirmed with the MECP2 mutation but there are all sorts of situations out there of kids with MECP2 mutations that don't fit the criteria of Rett or kids with the Rett diagnosis who don't happen to have an MECP2 mutation. We hear about atypical Rett, we're now hearing about CDKL5 and FOXG1 mutations in individuals who are also called Rett. So there's a bit of confusion out there and I thought it would be helpful to have this conversation with Dr. Neul, whose in the unique position a being a physician scientist; so he's a pediatric neurologist but also very involved with the science behind Rett syndrome so he has a unique view into a lot of these issues and I thought it would be interesting to have a conversation with him today.

So why don't you start us off Dr. Neul by explaining why Rett Syndrome is still a clinical diagnosis given the fact that MECP2 testing been around for 15 years now?

JN: So the reason that we consider Rett Syndrome to be a clinical diagnosis is because it has become very clear that even people who clinically clearly have Rett Syndrome—they have all defining characteristics, they have lost hand skills, they lost language, they have difficulty walking, and they have a characteristic repetitive hand movement—not all those people have mutations in MECP2; five percent of people still don't have any identified mutations. So you can have the clinical features of the disease without having a mutation.

On the flip side we are increasingly recognizing people who have mutations in MECP2—mutations even that cause Rett Syndrome in many people—that do not have the clinical features Rett Syndrome. They never lost hand skills, they never lost language; they may have some intellectual impairment or they may have some learning problems but they don't have these characteristic features and in fact these people are potentially very hard to recognize as having a MECP2 mutation. So because of those two things—you can have a mutation but not have the clinical features, and because you can have a clinical features and not have a mutation—we think that is still needs to be strictly defined clinically.

One thing I think is an important concept to this is what is the reason to have a distinct diagnosis? Why do you we need a diagnosis ever? And really there are two related reasons to have a specific diagnosis. The first is for prognosis. If you have a diagnosis and it's in a disease that is well known, and I think in Rett Syndrome now we're having a increasingly clearer picture of the clinical problems, and of the progression and to be able to predict what will happen or what might happen, what kind of problems the child might experience, and also what we should be looking out for. And leads to the second major thing; once you know that prognosis you can start targeting treatments and therapies for those problems. Now if you start including a lot of

people into this diagnosis, let's say people who have MECP2 mutations but don't have Rett Syndrome, we don't know exactly if they're going to have the same problems and we don't know the course of the disease in those people. And therefore, in some ways, it is not very helpful to lump them into the same group because they are distinct and different from the people who have MECP2 mutations but have the clinical features of Rett Syndrome.

MC: Although from a treatment viewpoint, you know gene therapy, protein replacement activating the silent MECP2, regardless of what your symptoms are you should be helped by those approaches.

JN: It depends on the treatment. I think that the treatments you're mentioning, absolutely. If it's symptomatic treatments, maybe not.

MC: Right so these are some important points and it's important to get these points across to families because it gets them thinking.

You helped to develop a strict list of criteria which should be used to diagnose Rett Syndrome clinically and then the mecp2 positive result confirms that, but as mentioned is not required. Not everyone adheres to those or agrees with those criteria and I think oftentimes that's what leads to confusion. Can you speak to that a little bit?

JN: I think you're right. So just to backtrack a bit, I helped lead an international effort—this was an effort by investigators really around the world to try to come to a consensus about what would be the clinical features, the criteria that would define the disease. The impetus for that is that there have been multiple previous criteria that have been used but I felt, and a number of us felt, that the older criteria could be somewhat confusing and they maybe we're a little more complicated than they need to be. So we tried to come together and define
Rett Syndrome in as simple as possible terms and as clear as possible, so there wouldn't be confusion and also could be something that people could apply who are not experts that they really could be—they would be able to look at this and as a non-expert they will be able to apply the criteria and it would be meaningful if you apply it correctly and it really would have a high probability that yes you really do have Rett Syndrome. I think that bears out pretty well when we apply it strictly. We find about 95 percent of people have mutations, these clearly disease-causing mutations in MECP2.

MC: So I'm hearing a lot about kids that have an atypical diagnosis of Rett and I wonder how much of that has to do with the non-Rett clinician, your local neurologist at home who has seen maybe a couple a Rett kids or a handful of Rett kids in their practice over the years, giving an atypical diagnosis. How often do you give an atypical diagnosis?

JN: I've given an atypical diagnosis fairly infrequently but I do give it and a lot of times the people that I've given an atypical Rett diagnosis—we also addressed this in the criteria in 2010, and really what we said for there is that to have an atypical Rett diagnosis you still have to have one of the cardinal features of Rett Syndrome, which is regression, and then you have to either lose hand skills or language and then you have the repetitive hand movements and the walking. So you don't have to everything, but you have to have some. In my experience the bulk of the people who I say are atypical have either lost hand skills or language, they did not loose both, and usually those people are generally higher functioning children. Or they never really had a normal period of development; really the family and the clinicians all knew something was amiss really from birth and they're usually much more severely affected in the long run. Not always, though, but usually. So those are really the two situations that I give the diagnosis of atypical Rett. Even within those situations, in my experience my personal clinical experience, most of those people end up having mutations in MECC2. Again not all, but most have mutations.

MC: Okay, so early onset, or no regression but problems from birth, you could—of course you have to look at all the other symptoms and all the other history—but there are kids that could be diagnosed as atypical Rett even though they didn't have a regression?

JN: No, I would not diagnosis them as atypical Rett without regression. They still could be affected early on, but I still put a lot of weight, and the criteria puts a lot of weight, on having some degree of regression and I think the reason we've been emphasizing that is that it [regression] is very distinctive in Rett. There are a few clinical conditions that have true regression; some forms of autism actually have some amount of regression, but there are a lot of other conditions that just have severe developmental delay. And the group of conditions that cause severe developmental delay are very broad and variable in terms of why that happens, whereas in Rett Syndrome, the bulk of people have MECP2 mutations and most of them have this regression, and so it's very important to you bring that together. Some of the very mild people, who are so mild they don't even meet the criteria for Rett Syndrome, never have regression and those are very distinctive individuals and I think it's important to consider them separately for both scientific and just for clinical practice purposes.

MC: Ok now what about CDKL5 and FOXG1? You know there are a number of papers that call these individuals Rett Syndrome or Rett-like. In the Australian database that lists all the mutations CDKL5 and FOXG1 are listed there as well. What are your thoughts on that; are they Rett or are they not Rett?

JN: So my personal experience with people with CDKL5 and FOXG1 mutations, there are Rett like features, "Rett-oid" features, oftentimes in the breathing dysrhythmia, some of the repetitive—some abnormal movements. But the clinical course of these diseases is distinctive, and even the clinical characteristics are different. What do I mean by that? Well girls with Rett

Syndrome usually use intense eye gaze and make intense eye contact whereas in my experience a lot of people with CDKL5 and FOXG1 have an eye-avoidance and it has a different feeling when you interact with the child. So I think to me that they're distinctive and in most cases, I have had some children that have CDKL5 who it does seem like they did regress and may have lost language or speech so by the strict criteria we might say yes these people do fall into this group. I sort of believe strongly that it benefits the community, the scientific and the clinical community and the parents, if we don't overly lump diseases but we actually have splitting and do deeper characterization.

This goes back to my initial comment which is that ~~it~~ I don't think if people who have CDKL5 mutations have distinctive seizure issues, have distinctive GI issues, and they have a distinctive disease course, it's not helpful if we group them just with Rett, and try to make them into that same group which has a very different overall clinical course. What is very helpful is that we actually try to understand what the clinical course is for CDKL5, what the clinical course is for FOXG1 so we can best understand them and really develop treatments. I don't want that to make it seem like there aren't things, there isn't cross-fertilization, and there isn't anything in common. I think there are a distinct number of things in common between these disorders and I think that has led to people grouping them together, but I do think that we can both take things but that we do have to separate them out to be distinctive. Moving forward with what we've been doing for the last ten years with the natural history study for Rett Syndrome, we are proposing to move forward and have FOXG1 and CDKL5 but be distinctive elements within the natural history study and be characterized distinctively.

MC: Right, that makes sense and I think from a scientific viewpoint we also need to cultivate people studying CDKL5. For example, some of the symptomatic treatment approaches may be the same but the comment I made earlier about gene therapy and some of the approaches that we're taking, may not apply to CDKL5. So those families are going to want to really cultivate a rich research group that will be looking at ways to treat their disorder as well.

JN: Absolutely, what I would say is that on one hand you have the symptomatic treatments you rightly say could overlap and on the other hand you have the extraordinary disease specific treatments, like gene therapy, which aren't going to help things. But in the middle you might have disease-modifying therapies that may benefit both.

MC: Ok makes sense. Now what about the five percent that don't have a mutation? One thing I want to mention for families who may be listening whose children have not tested positive for MECP2 mutation, especially if they're test was done a long time ago, do make sure you get tested again. There are two types of tests; there is Tier 1 Tier 2 sequencing and if that comes back negative there's a specific task sometimes called MLPA or large exonic deletion test that looks for large chunks of the gene missing—which is undetected, the sequencing can't detect

that. So if your child is had the sequencing but hasn't had that second test, make sure that your child gets it, because it's not an automatic thing. You have to order it and you have to pay for it. It's separate. But for children who have had both of those done and FOXG1 and CDKL5 testing, and everything has come back negative, obviously it's tough for those parents. They feel left out, in limbo. What do you suggest?
I think you have a project ongoing for those kids?

JN: I just want to first reiterate what you said on how important it is to have that Tier 2 testing done. Ten percent of people who have Rett Syndrome and have a specific type of mutation in MECP2 don't get detected with standard sequencing. I will say this that if the sequencing is done and is sent to Baylor for the diagnostic purposes, it is actually a reflex response to do the second-tier test and so sometimes it has already been done so you need to pursue that a little deeper. So when you don't have a mutation in MECP2 but clinically seem have Rett Syndrome, there are a couple different options. One is that I am doing clinical research collecting DNA and doing sequencing on all the genes in the body for people who clinically meet the criteria of Rett Syndrome but don't have a mutation in MECP2 with the hope of discovering new genes.

Now in that process, we may actually find people who have mutations in some of these other genes MEF2C, CDKL5, and a variety of other genes, and that's great if we can find that and we also want to find new genes.

Outside of research realm, one thing that you people ask me all the time is should I just go through CDKL5, FOXG1, MEF2C, etc. and the list keeps growing all the time. It's a lot of testing and I think it's helpful if you have a clinician who can help guide that a little bit so it isn't just shotgun testing. I would say that if it is shotgun testing, there are options. Greenwood Genetics has a large panel for what they call *Syndromic Autism* that includes all these genes and will sequence all of them. It is more expensive than having one gene sequence but it's far cheaper than having all the individual tests done.

MC: Should parents pursue exome sequencing or whole genome sequencing?

JN: Clinically or on a research basis?

MC: Both.

JN: Obviously, I'm interested in it from a research basis. I think the way this field is going there's more and more clinical exome sequencing being done and that might be revealing. Eventually, things haven't really ramped up in a large way into genomic sequencing and I think that right now probably exome sequencing is still a better bet. Ultimately though that is probably the way you would want to go. I'm interested in doing this on research basis and I would be happy to

discuss this with people if they want to contact me. But doing it clinically just through your pediatrician's office or neurologist's office is an option.

MC: So there are two pieces to that; the sequencing piece is the easy part, the trying to figure out what it all means is the more difficult part. On the other hand, the more people that have it done, the more information out there, the more you'll be able to determine what all the different variations mean, right?

JN: That's right.

MC: Okay I hope that families found this helpful. If you have questions feel free to contact me. I'll do my best to answer them or seek out answers if I don't have them. We wish you luck Dr. Neul on all of your various projects. Thank you for taking the time to speak with us.

JN: No thank you for talking with me.