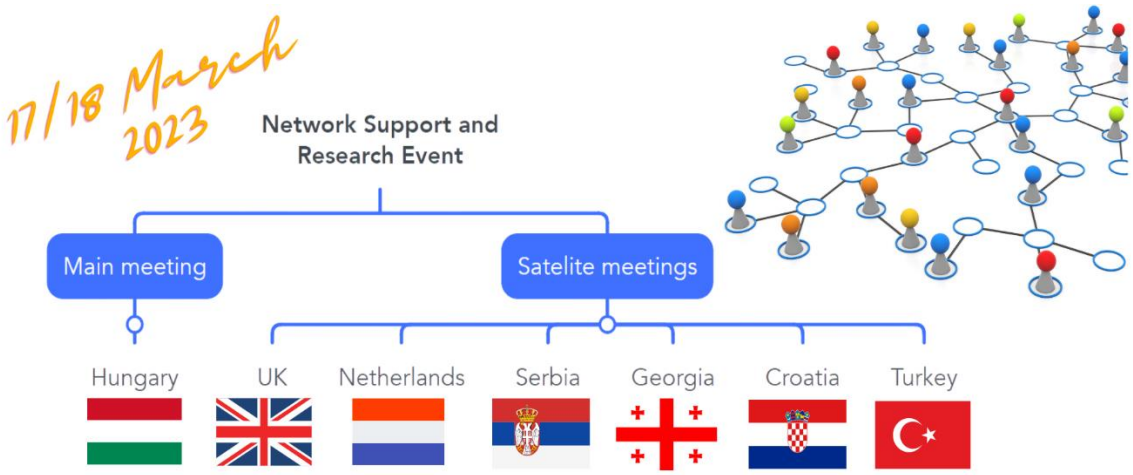
rett syndrome europe

Network Support and Research Event



ABSTRACT BOOK

Network Support and Research Event

Rett syndrome is a rare, genetic, neurological disorder affecting mainly females (1:10,000) and very few males. It is present from conception. After a period of 'near normal' development, the child experiences a regression in key skills, usually mobility, speech and hand use between the ages of one and two years. The child may appear very withdrawn during this stage and this often leads to a misdiagnosis of being on the autistic spectrum. Multiple comorbidities appear post regression including epilepsy, breathing disruption, scoliosis, gut and bowel problems. Whilst the disorder is genetic, in the majority of cases it is not inherited. Rett syndrome is usually caused by a fault on a gene called MECP2 which is found on the X chromosome. People with Rett syndrome have profound and multiple physical and learning disabilities. They are totally reliant on others for support throughout their lives, yet they are radiating love and enjoying their life to the fullest.

The idea of RSE was born in 1993, in the corridors of the Medical World Conference on Rett Syndrome, in Antwerp (Belgium). This was to create an organisation which would enable European national associations to meet each other on a regular basis.

Following this, in March 1994, representatives from 14 countries met for the first time officially in Luxemburg, where the EUROPEAN ASSOCIATION FOR RETT SYNDROME (E.A.R.S.) was founded. Our objectives were to exchange practical information relating to day- to-day management, therapies, care, equipment, etc; to improve communication between Rett families in Europe; to assess current and support new research projects.

In year 2000 during the meeting in Paris, in order to enhance our strength, it was decided to formalize the association, giving it a new name: RETT SYNDROME EUROPE (RSE). And a year later, in 2001 at the Budapest meeting, the statutes were approved and signed by representatives from Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Italy, Malta, Norway, Poland, Serbia, Slovakia, Sweden, Spain, Turkey and the United Kingdom.

rse rett syndrome europe

Network Support and Research Event

Aims of RSE

- To make Rett syndrome better known to the public, professionals, carers and those who are directly concerned in all European countries
- To improve communication within the European Rett community
- To promote, as a representative European organisation, the interests of people with Rett syndrome and their families
- To expand RSE to all European countries and to assist, if necessary, in the creation of national associations
- To promote research into Rett syndrome

RSE Network Support and Research Meeting

With funding from The European Joint Programme for Rare Diseases Network Support Scheme, Rett Syndrome Europe is delighted to present its first Network Support and Research meeting on Friday 17th and Saturday 18th March 2023.

The main meeting will be held in Budapest, Hungary, with satellite meetings held simultaneously in the UK, Netherlands, Serbia, Georgia, Croatia and Turkey.

Members of the Rett Syndrome Europe (RSE) Scientific Advisory Board (SAB) and others will be presenting to parent carers, clinicians, therapists and researchers, with opportunities for discussion and Q&As.

In addition, anyone with an interest in Rett syndrome can register to watch the talks from the comfort of their own home. Find out more below.

anavex LIFE SCIENCES Corp. NEUROGENE neuren pharmaceuticals TAYSHA GENE THERAPIES

RETT RETT SYNDROOM Rett UK Support today, hope for tomorrow Georgian Rett Syndrome Association

rett syndrome europe

Network Support and Research Event

Executive Board

Becky Jenner	UK	President
Stella Peckary	Austria	Treasurer
Laura Kanapieniene	Lithuania	Secretary
Sandrine Eifermann Soutarson	France	
Bojana Milanov	Serbia	

Member Associations

Albania	Armenia	Austria	Belarus
Belgium	Bosnia and Herzegovina	Bulgaria	Croatia
Cyprus	Czech Republic	Denmark	Estonia
Finland	France	Georgia	Germany
Greece	Hungary	Iceland	Ireland
Italy	Kazakhstan	Latvia	Lithuania
Luxemburg	Macedonia	Malta	Moldova
Montenegro	Netherlands	Norway	Poland
Portugal	Romania	Russia	Serbia
Slovakia	Slovenia	Spain	Sweden
Switzerland	Turkey	Ukraine	United Kingdom

rse rett syndrome europe

Network Support and Research Event

Day 1 Friday 17th March

rse rett syndrome europe

Network Support and Research Meeting

17th & 18th March 2023

EUROPEAN JOINT PROGRAMME RARE DISEASES

09:30 - 09:50	Introduction	Becky Jenner & Gillian Townend
09:50 - 10:20	Rett Syndrome: The road from recognition to treatment	Jeffrey Neul
10:20 - 10:35	Tea Break	
10:35 - 10:50	Basic research – animal models	Jean-Christophe Roux
10:50 - 11:05	Human stem cell-based models for the study of Rett syndrome: overview and perspectives	Sonja Guil
11:05 - 11:35	Discussion in Country Groups	
11:35 - 12:00	Q&A to Speaker Panel	
12:00 - 13:15	Lunch / Networking / Visit Virtual Booths	
13:15 - 13:35	Genetic Therapy Approaches in Rett syndrome - an update	Stuart Cobb
13:35 - 13:45	Neurogene Update: NGN-401: A Self-regulating Gene Therapy for Rett Syndrome	Stuart Cobb
13:45 - 13:55	Neuren Update	Nancy Jones
13:55 - 14:05	Taysha Update: An Investigational Approach to Gene Therapy for Rett Syndrome	Benit Maru
14:05 - 14:15	Burden of Illness in Rett Syndrome: Initial Evaluation of a Disorder-Specific Caregiver Survey	Walter Kaufmann
14:15 - 14:35	Clinical trial registries and databases	Daniela Tropea
14:35 - 14:50	Patients at the European Medicines Agency	Francois Houyez
14:50 - 15:05	Tea Break	
15:05 - 15:20	Parent associations & RSE	Becky Jenner
15:20 - 16:00	Discussion in Country Groups	
16:00 - 17:00	Q&A to Speaker Panel and Whole Group Discussion	
17:00 - 18:30	Drinks / Networking / Chat *	
18:30 - 21:00	Dinner *	

* organised for people at the in-person events

anavex LIFE SCIENCES Corp. · NEUROGENE · neuren pharmaceuticals · TAYSHA GENE THERAPIES · RETT · RETT SYNDROOM · Rett UK Support today, hope for tomorrow · Georgian Rett Syndrome and other rare diseases Association

anavex LIFE SCIENCES Corp. · NEUROGENE · neuren pharmaceuticals · TAYSHA GENE THERAPIES · RETT · RETT SYNDROOM · Rett UK Support today, hope for tomorrow · Georgian Rett Syndrome and other rare diseases Association

rett syndrome europe

Network Support and Research Event

Day 2 Saturday 18th March

rett syndrome europe

Network Support and Research Meeting

17th & 18th March 2023

EUROPEAN JOINT PROGRAMME RARE DISEASES

09:00 - 09:15	Re-cap and Introduction	Becky Jenner
09:15 - 09:30	I Can't Get No Sleep...	Karen Spruyt
09:30 - 09:45	Epilepsy in Rett Syndrome	Aglaia Vignoli
09:45 - 10:00	Control of Breathing	Ana Abdala
10:00 - 10:20	Tea Break	
10:20 - 10:40	Gastrointestinal Myths and Misconceptions in Rett Syndrome	Kathleen Motil
10:40 - 11:00	Identifying and Managing Emotional, Behavioural Autonomic Dysregulation in Rett Syndrome	Paramala Santosh
11:00 - 11:30	Discussion in Country Groups	
11:30 - 12:15	Q&A to Speaker Panel	
12:15 - 13:15	Lunch / Networking	
13:15 - 13:30	Physical Therapy Interventions in Rett Syndrome	Meir Lotan
13:30 - 13:40	Communication Research and the Rett Syndrome Communication Guidelines	Gillian Townend
13:40 - 13:50	Communication Continued: Well-being and Emotional Competence	Helena Wandin
13:50 - 14:00	Occupational Therapy: Role in Rett Syndrome	Pamela Diener
14:00 - 14:10	Music Therapy and People with Rett Syndrome	Linn Johnels
14:10 - 14:40	Discussion in Country Groups	
14:40 - 15:00	Tea Break	
15:00 - 15:30	Q&A to Speaker Panel	
15:30 - 16:00	Action Planning in Country Groups	
16:00 - 16:45	Feedback to Whole Group	
16:45 - 17:00	Summary, Thank You and Closing	Becky Jenner & Gillian Townend

anavex LIFE SCIENCES Corp. · NEUROGENE · neuren pharmaceuticals · TAYSHA GENE THERAPIES · RETT · RETT SYNDROOM · Rett UK Support today, hope for tomorrow · Georgian Rett Syndrome and other rare diseases Association

anavex LIFE SCIENCES Corp. · NEUROGENE · neuren pharmaceuticals · TAYSHA GENE THERAPIES · RETT · RETT SYNDROOM · Rett UK Support today, hope for tomorrow · Georgian Rett Syndrome and other rare diseases Association

Network Support and Research Event

Full Text Presentations in Turkey

1.Relationship Between Rett Syndrome and Microbiota	Asisst. Prof. *Özgen ÖZKAYA* Fenerbahce University, Faculty of Health Sciences, Istanbul/Turkey
2.Self-Care Management in Children With Rtt Syndrome	*Asisst. Prof. Nermin EROGLU* Fenerbahce University, Faculty of Health Sciences, Department of Nursing, Istanbul/Turkey
3.Education and Counseling for Parents of Children with Rett Syndrome About the Management of Epileptic Seizures	*Prof.Dr. Semiha AKIN* Health Science University, Hamidiye Faculty of Nursing, Istanbul/Turkey
4.Infection In Rett Syndrome	*Asisst. Prof. Gamze TEMİZ* Health Science University, Hamidiye Faculty of Nursing, Istanbul/Turkey

Rett Syndrome: The Road From Recognition to Treatment

Hello and welcome. My name is Jeffrey Neul, I'm the director of the Vanderbilt Kennedy Center at Vanderbilt University Medical Center in Nashville, Tennessee, and I'm glad to be here and thank you for the organizers for inviting me today. I'd like to talk about Rett syndrome, the road from recognition to treatment. I'd like to start with a brief history of Rett syndrome, which was originally described in 1966 by an Austrian pediatrician named Andreas Rett, who had observed a series of children in his office who had similar features. He published this in an Austrian weekly medical newsletter, but it wasn't really recognized widely until 1983, when Bank Hagberg and colleagues published his paper, which identified and clarified the clinical features of Rett syndrome and gave it the name by which we know it today.

So, for Rett syndrome in 2010, we created a revised diagnostic criteria that identified and reinforced concepts that were created by Andreas Rett and Bank Hagberg, which is this is a disease that has a pecific characteristic pattern in which children are born relatively normally and have an apparently initial normal development.

Although with careful evaluations you can find subtle abnormalities in retrospect, they undergo a developmental delay where they no longer obtain skills at quite the rate we would think they should. Notably then have a regression, specifically with a loss of acquired purposeful hand skills and a loss of acquired spoken language. And this loss may not be complete, it may be partial, so they may have retained function, but importantly, there's a stabilization. And so that's the important aspect of Rett syndrome, there's a regression with the stabilization. People with Rett syndrome also develop gait problems or fail to learn to how to walk or lose the ability to walk entirely. They also have characteristic repetitive hand stereotypies. And these are the core diagnostic criteria for Rett syndrome. We've learned that it's an excellent disorder that primarily affects girls and it occurs in about 1 in 10 thousand live female births. Now, in 1999, Huda

Zogby and colleagues identified the genetic cause for the majority of people who meet the clinical criteria of Red syndrome, which is mutations in the X gene methyl CPG binding protein two, or MECP2, which is a protein that binds to DNA and regulates the expression of other genes.

With this information, we know that 95% to 97% of people who have typical or classic Rett syndrome, meaning they meet all the diagnostic criteria, have mutations in MECP2. Most of these mutations are de novo, meaning they're not found in either mum nor father. And really they're a singular case in the family. But importantly, 3% to 5% of people who meet the clinical criteria for this syndrome do not have identified mutations in MECP2. And we've identified other mutations in other genes. And there are genes such as mutations in CDKL5 or FoxG1 that have similar and overlapping clinical features to Rett syndrome. And they were considered to be atypical Rett but we've recognized these as distinct disorders. Although this disorder primarily affects girls, there are boys who have these loss of function MECP2 mutations similar to those seen in girls with Rett syndrome. And classically, we've thought they have severe congenital encephalopathy meaning they're affected from birth. However, we've been recognizing there's a much broader phenotype clinical features in boys who have these loss of function mutations. And I think that we need to do more work in this area to better understand this.

Now, we've learned that you can also duplicate and have an extra copy. So again, a function of MECP2. And this also causes a severe neurodevelopmental disorder that primarily affects boys and they have autistic features and seizures, absent speech and infections. So there are some similarities with Rett, but it is also a distinct disorder. Now, with the identification of the genetic basis of most cases of Rett syndrome this allowed genetic engineering to engineer rodent models of eRtt syndrome and they remarkably reproduce many of the clinical features that we see in people with Rett syndrome.

Now, most work has been done in male animals which is not the exact best model for Rett syndrome which occurs in girls. But importantly, female animals also have similar phenotypes.

These are mostly done in mice, but rat models have been generated and they also show phenotypic abnormalities similar to those people seen in people with Rett syndrome.
Now, importantly, in 2007, Adrian Bird and colleagues did a genetic engineering of a Mouse in which the gene MECP2 was disabled. But they could turn the gene back on and they could turn it on in a temporal fashion using some genetic engineering tricks with a cree RECOMBINATE.
And when they did this, even after the animals were sick, they improved their survival improved a number of their behavioral features improved. And that originally was done in male animals but it's been subsequently reproduced in female animals and it also shows the ability to restore activity. So this really provided the basis for the hope that we could develop meaningfu clinical therapies that could improve the lives of people and modify the disease course.

And shortly thereafter, in 2009, Merganka Su and colleagues treated animals with a tri peptide. So three amino acids. It's derived from the amino terminus of insulin like growth factor one and that the three amino acids are starting in the middle.

It's called glyproglu or glypromate. And when they treated the mutant animals with this it improved their survival and improved their local motor activity and it improved their breathing and their heart rate features. So this was very exciting that this could be an avenue for treatment and this led to clinical development of drugs related to this insulin like growth factor one. And specifically, I'm going to talk about Trofinetide. So the Mergoncasers group treated with this IGF one, which is the structure shown on the left, Trofinetide is exactly that same compound except it has a methyl group added to it and this improves the drug qualities, meaning it can be taken orally and has a longer half life, so you can take it only twice a day. Now, two phase two clinical trials have been conducted with Trofinetide in Rett syndrome, in adults and in children and both of them showed that Trofinetide was safe, fairly well tolerated and showed signals of efficacy. This led to a phase three trial called the Lavender Trial run by Acadia Pharmaceuticals and the results of this have been released and it was successful. It met the primary endpoints that were specified. Specifically, the two primary endpoints were improvement on a caregiver reported scale called the Red Syndrome Behavior Questionnaire or the RSBQ and a clinician scale called the Clinical Global Impression Improvement or the CGI. And in both of these cases

over time, at the end of the study, people on Trofinetide showed improvement compared to the people who were on placebo. Now, that is very exciting. They successfully met the co primary endpoints. They also met the key secondary endpoint, which was a nonverbal communication scale.

There were adverse events, primarily diarrhea and vomiting observed more frequently in people on Trofinetide compared to placebo. The sponsor has submitted this a new drug application or an NDA to the US Federal Drug Administration or FDA in August of 2022 and the FDA has said they will provide a response to this in the March of 2023. Just mentioned briefly, concurrently, there's been development by a company called Anavex of Anavex 273, which is a sigma one receptor agonist, which showed benefit in an animal model of Rett syndrome.

And they are also conducting clinical trials and have reported some promising top level results and we'll have to hear more about that as time goes on.

Now I'd like to take a step back and introduce something that will be a basis of much of the talk, which is the Rett Syndrome and Related Disorders Natural History Study. This is a multisite study longitudinal study that was run by Alan Percy at the University of Alabama, Birmingham and I was the administrative head supported by the National Institutes of Health in the US. Ultimately we enrolled over 1800 people. We saw them longitudinally at over 8700 visits 14 sites across the US. And for classic or typical Rett we saw 1258 people and they were seen over 6800 times, with 83% of the people being seen more than once and 50% being seen more than four times. So an average of 5.4 visits.

So we have a lot of information and longitudinal information and I just want to point out how that relates to these other critical timeline of events in Rett syndrome. Research with the 1999 the discovery of the genetic basis allowed the development of animal models, and in 2007 showing reversibility in animal models showing there could be important treatments led to the development of treatment with the tri peptide by Mriganka Sur group in 2009 and now the successful phase three trials in Rett syndrome and importantly the natural history study began way back just shortly after the discovery of the genetic basis of Rett syndrome. And some of

the goals, really, of Rett Natural History Study is to develop clinical trial readiness for Rett syndrome. And what does that mean?

Well, one is to develop a network of clinical sites that are able to do the kind of clinical research you want to do in a clinical trial.ÖTwo is to characterize the clinical features and the natural history of the disorder And three is to develop clinical outcome measures and biomarkers.

So let's talk about having a network of clinical sites. Well, we had 14 sites across the US. They're shown here in the flags. We enrolled people throughout the entire US. And also people from 20 different countries across the world. And this has been fundamental sites for the industry sponsored trial. So I think that was very successful at creating this network of sites.

How about characterizing the clinical features and the natural history of this disorder? Well, we've published over 50 manuscripts with multiple additional manuscripts in submission and preparation. These manuscripts have covered many things such as looking at genotype phenotype relationships in Rett syndrome, looking at clinical symptoms such as epilepsy breathing abnormalities, hand stereotypies, sleep scoliosis, and developmental delay in Rett syndrome, looking at growth failure and gastrointestinal nutritional issues.

We developed Rett syndrome specific growth charts shown on the left We've looked at things like hand and other anthropometry growth, puberty development, and then GI features and biliary tract issues and bone mass issues. We also looked at the behavior and the quality of life, which has been fundamental, and also gaining additional information on Rett syndrome. And not to just end with Rett syndrome, because it did cover other disorders too. And so we published a series of papers talking about features in MECP2 Duplication syndrome, comparing across the disorders of Rett syndrome, MECP2 Duplication syndrome, CDKL5 deficiency disorder, and FoxG1 syndrome. And we've published a variety of papers on that. And there are many more papers that we prepared in this area. So let's talk about development of clinical outcome measures and biomarkers.

So first I'll start with biomarkers. So biomarkers are things that you can measure in someone that would allow you to correlate with, say, disease severity, to identify people who might respond to treatment.

And then, importantly, I think, is to show things that would show improvement during a clinical trial before you actually see clinical improvement. So that would help expedite the way clinical trials are conducted.

And I'm going to talk a little bit about one of the avenues that we did in the Natural History study, which was using neurophysiological features. So these are EEGs, these are noninvasive, and they're fairly well tolerated.

And I'm specifically going to mention things about doing what's called evoked potentials. And what this means is you provide some sort of stimulus and you record brainwave activities in response to the stimulus.

And this example in this cartoon is showing what's called the visual evoked potential, where you have a flashing grid of light that is seen and you record from the brain and you can look at you do this repeatedly and you can look at a characteristic brain wave response, electrical response in the brain. And that's shown on the bottom right, which shows the change in this activity where you have peaks that occur at a fixed set of times. So you have the time to the peak, which is called the latency, and the height of the peak, which is called the amplitude. And in the natural history study we looked at people with Rett compared to typically developing controls and we did both visual and auditory evoked potentials where it's clicks of sound and those graphs are shown on the left and we can see that they were different from people typically developing individuals primarily in the amplitude.

Their peaks are smaller and so they're different from people who are typically developing. Additionally, we could show that it was correlated with clinical severity that's shown in the middle. Now, my lab and other labs have göne on and looked in the animal models and we can reproduce very similarly these evoke potential responses and other neurophysiological features

in the mice and we've shown that they also correlate with disease severity and progress over the time.

And since we can do both things in both species, it offers the opportunity to translate the findings between the species and to accelerate both preclinical and clinical research. So how about the development of clinical outcome measures? Of course you can't do a clinical trial unless you have a good outcome measure. Now, we did work in the natural history study to help support the development of the clinical global impression scales and I'll talk a little bit more about that later. But to take a step back, I think importantly is to think about when you have an outcome measure.

You really like to measure what matters most to the people who are affected and to their caregivers.

And within the context of the natural history study we asked people, we asked caregivers at every visit what are their top three concerns and we provided them with a list of 21 options.

This list was generated through expert curation of the literature, discussion amongst expert clinicians and then feedback and input from stakeholders from the International Rett Syndrome Foundation and other caregiver stakeholders.

We also let people have the option of selecting other and entering a free text. And here are the results for the top concerns for classic or typical Rett syndrome and this is just so in the top ten concerns in ranked order. And I think importantly you can see that some of the top concerns like lack of effective communication, lack of hand skills, abnormal walking, repetitive hand movements, these are the clinical features that are really define Rett syndrome.
So it really makes sense that these are concerns that caregivers report. But importantly, you see symptoms such as seizures, number two, constipation, sleep, breathing problems, bloating, chewing and swallowing.
So there's more than just those cardinal features of Rett syndrome. There's these other symptoms that really matter to the caregivers, and it's important to think about when you develop treatments, they should really target these most important issues. Furthermore, if you have an

outcome measure, you really want to assess those issues to make sure that this is really making a meaningful impact to the affected individuals and their caregivers. So what do we have for clinical outcome measures?

Currently?

These are the ones that are being used in the clinical trials that have been completed or ongoing.

One is this caregiver scale, this Rett Syndrome Behavior questionnaire, the RSBQ, and this is a 45 item questionnaire that caregivers fill out. And it has these domains which is more than just behavior, although it's weighted more towards behavior. Now, the advantages of this is that it's relatively simple to complete, it doesn't take that long, and it's understood by the regulators, the FDA and the EMA.

The downside is that we haven't really established the relevant differences that would be meaningful on this measure. And while it does cover a variety of domains, it does not necessarily cover all the top concerns. Well, things like hand stereotypies and bloating are decently covered, but things like walking and hand use is not as well covered or fairly briefly covered, and other things aren't covered at all well.

How about the Caregiver Assessed scale, which is I mean, sorry, the clinician assess scale, which is the clinical global impression, improvement. And this is a fairly simple concept where you have a seven point scale where the clinician evaluates how things have changed for the person in the trial. And it's the global impression, with four being that there is no change, three, two, one being improvement, and five, six, seven being worsening. This is also understood and accepted by regulators, but it's important that you have disease specific anchors to help guide the regulators.

And we did develop those, and we also have to have a consistent training, which has also been developed and used in these clinical trials.

Now, one of the downsides is that while it talks about improvement or worsening, it doesn't provide specifics of what was improved or what worsened, and so it doesn't really give you an information on that level. So the question is, can we develop the Natural History?

Can we use the Natural History Study to develop additional outcome measures? And so we did this from the data from the Natural History Study, looking at a rating scale that's been filled out by the clinicians called the Motor Behavior Assessment Scale.

And this is a clinician rated scale that was used throughout the entire time of the Natural History Study that has 37 items. Each item had what's called a five point likert or ordinal scale with higher scores being worse, and then we can create a combined score for a total score. Now, the issue is that this was never really a value.

It wasn't developed as an outcome measure and it wasn't really evaluated as an outcome measure. So we applied formal psychometric techniques using people who had classic or typical Rett. We had 1258 people. We looked at their baseline visit and we split it into a two thirds development set and one third validation set. For the development set, we use exploratory factor analysis to look at the factor structure and the validation set would validate that.

And from this we developed the revised Motor Behavior Assessment or the RMBA. So this is 24 items that are clinician rated during the evaluation. These cluster into five factors, which is motor dysfunction, functional skills, social skills, behavior and breathing problems. Plus three important clinical features seizures rocks, hand stereotypies and body rock. And they were included because we felt that they were very important. So overall, this had very good fit statistics. It also showed pretty good chromebacks alpha or internal consistency.

Now, we looked at this, the validity of this by comparing to other clinician rated scales of severity, like the clinical global impression of severity, where higher scores meaning more severe. And you can see that for the total RMBA score, people who had the lowest CGIs three, they have a lower total RMBA than the people who are higher severity. So it climbs. We also compared it to another rating scale for severity and Rett syndrome called the Clinical Severity Scale CSS, and it's very well correlated.

We also showed that it had a genotype phenotype relationship that we've seen repeatedly with things like R 133 C having a lower score on the RMBA on average as a group compared to things like R 168 x, which is typically at the higher end of severity. So it's good consistent like that between the clinician scales.

How about with the caregiver? So we looked at this compared to a quality of life measure which is called the Child Health Questionnaire. And this gives two scores. One is Physical health, shown in orange and the other is Psychosocial health, shown in blue. And you can see that the RMBA, as the score goes up, the score on the physical health goes down, and that means worse quality of life. We did not see a relationship with the psychosocial health, which also is consistent with what we've seen in other measures. These RMBA subscales also correlated with caregiver reported items that would be represented on the subscale, such as Motor dysfunction, looking at things like ability to walk, Scoliosis, and there was a good correlation there.

And then on a small group of people, we looked at the RMBA subscales to the RSBQ, the caregiver ratings, and we compared things like that we think would be correlated, like functional skills to hand behaviors.

And you see good correlation social skills to anxiety, and you see good correlation breathing on the RMBA is the breathing measures on the RSPBQ, and again showing correlation.

So that would be convergent validity, where the two things you're looking to see, that something you think is measuring breathing that a clinician fills out is also what the caregiver reports. Now, look at divergent validity where you say, how does functional skills relate to fear and anxiety? Functional skills in the RMBA to fear and anxiety on the RSBQ, and you see they are not related. So that's also important because it goes along with you're measuring what you think you're measuring, you're not measuring something else. Now, we've been using the Longitudinal Data and Natural History Study to develop growth curves, the expected rate of change in different age groups of the RMBA.

And from this, this will allow us to make predictions on where things should be, and that will help us define what is minimally clinical change in an intervention trial. So how well does the

RMBA capture these important clinical issues? Well, pretty well better than the other things, but it's still not complete.

And the full range of functional skills on the RMBA, the range of options, is not really as broad as we might like to see. So again, can we use the Natural History, say, develop a better clinician rating scale? And then can we also use the information to develop a better caregiver rating scale? And so we've done this. We've followed exactly the same process that I outlined for the RMBA to develop a new clinician rated scale supported by Alcyone Therapeutics. We haven't named this yet, but it divided into six factors you see here with 31 items. So these represent many of the important things in Rett Syndrome. We did the same thing for a caregiver rated scale using caregiver reported forms that were completed. And we're calling this the Rett Syndrome caregiver assessment of severity and symptoms.

Or the RCAs. This was supported by the Rett Syndrome Research Trust. And this had four factors functional movement, communication, behavioral problems, Rett specific bbehaviors. 32 items, and the clinician fills this out. Now, we've shown that this correlates with the clinician rated severity, so the caregiver fills this out, and it correlated really well with the clinician score. At the same time, the RMBA, it also correlated with the Genotype, where, again, we see the higher scores, and on the left with R168X, and the lower scores on the right with R133C. So we're being consistent. We also showed that it increased with age, and I'm just not showing that here. So how did we do? Well, I think we did good in terms of developing them. I think we've covered the items, but we're still missing items that are in the top concerns, like some of the GI issues, like constipation, bloating and gas.

And we don't really have much about sleep in there. So the good thing, though, is that other groups have done things. We did it in the Natural History study to look at just using standard sleep evaluations in people with Rett syndrome. And we showed that that seems to be able to be worked. And the Baylor group in Houston has developed a gastrointestinal health questionnaire for Rett syndrome. So these combined with other things can help assess those clinical domains that are of top concerns. Additionally, we think about having functional

assessments like walking or hand use. And people like Jenny Downes and Helen Leonard have been working on this using video capture and scoring for hand function and gait.

People like Bernard Suter at Baylor College of Medicine have been looking at using formal gait analysis. And then for communication, the group at Duke has been looking at modifying the observer reported communication ability measure, the Orca, which was originally developed for Angelman's syndrome to be appropriate for Rett syndrome.

So I told you all this about the clinical trial readiness developing these outcome measures and the question is what's next? But I think it goes back to the work from Adrian Bird in 2007, the ability to show that you can reverse the disease if you turn the gene back on. And so this is something that has always led to the question, well, what about gene therapy? Why don't we do gene therapy? And I'm not going to go through all of the things Stuart Cobb is talking about later today and he's been instrumental in doing this work, but here's just a few examples. It's been done repeatedly in animal models and showing that it works. And now two companies, Taysha have started clinical trial, gene therapy in adults with Rett Syndrome in Canada and Neurogene, in late January 2023, got clearance from the FDA to start gene therapy in children with Rett syndrome. And they say they're going to start a trial in the US during 2023.

There are also a number of additional alternative ways that have been developed preclinically to reactivate MECP2, like X chromosome reactivation, read through therapy, DNA editing, RNA editing and even new things that are being developed.

So I think there's a lot of promise and a lot of hope that we can develop new therapies. And so really, the future, I think, is really bright. I mean, I think the success of the Phase Three lavender trial is really promising It shows that therapies can be developed for Rett and similar disorders and there are new opportunities and new avenues of treatment that I think will start moving to clinical trials.

And the natural history is fundamental in establishing the ability to do these clinical trials. And really, it's critical to understand what problems need to be addressed. And we can continue to use the Natural History study data to continue to develop these outcome measures.

Now this I just like to list and thank the people who were part of the Natural history study especially, Alan Percy, for his vision that we did this. And then, of course, I want to thank all the people who participated in the trials the International Rett Syndrome Foundation, who supported the Natural History Study and all the people that I really learned about Rett from which are all the patients, patients that I care for. Thank you for your time.

Human Stem Cell- Based Models fort he Study of Rett Syndrome: Overview and Perspectives

Good morning everybody. My name is Sonia Guil.My lab is based in Barcelona in Spain and I'm going to give you today a brief overview of how human cellular models have been used over the last decade or so to study Rett syndrome. In addition to the wealth of information that mouse models have provided and are still providing in the research of Rett syndrome, advances in stem cell biology have allowed the development of human cellular systems to model the disease. The idea is to be able to grow in a culture dish in the lab human neural progenitor cells and these can then be differentiated into the different neuronal and glial subpopulations that build up the brain. There are different sources of these neural progeneitor cells but I'm going to mention only the one most widely used and that is tissue from the patient, generally from a small skin biopsy which is converted in the lab through a reprogramming process into cells capable of now generating virtually every cell type in the body. These are called induced pluripotent stem cells or IPS cells and they can be driven towards neural progeneitor cells and then into mature neurons. This is the most valuable system since we can imagine that those neurons generated in vitro are similar to those in the person's brain and with the same genetic background of the patient including the mutation on MECP2 two that the girl is carrying. Therefore, these cells in vitro constitute an available platform to study mechanistic aspects of the disease as well as to search for biomarkers or even to use some therapeutic compounds or even to test gene therapy approaches. The obtention and generation of patient derived IPS cells and then from then the obtention of neural progeneitor cells and mature neuron is a lengthy process and very sophisticated procedure which with time has been now improved and

standardized. The end point is the obtention of these mature Rett neurons which generally show most of the defects that had been also observed in animal models or postmortem samples. Specifically, the defect in the electrical properties of the neurons. And here you can see some excitatory currents are shown as a consequence of alterations in the morphology and function of these cells. For example, see here in these Rett neurons the decrease in the green signal which reflects or detects the synaptic points where synaptic transmission is occurring. Indeed, these human neurons generated in vitro display a number of alterations at the molecular, morphological and functional level. To summarize, these cells show a poorer morphological complexity (poorer arborisation), a reduced number of spines where synapses take place and thus altered electrophysiological properties. All these features are characteristic of the disease and also in a whole organism. These cellular models are therefore useful to better understand Rett and have taken advantage of the general advances in the field of stem cells. In this timeline, I've highlighted some of the most relevant achievements in this area, including the obtention of the first patient-derived IPS cell line in 2009. not growing just on a petri dish, on a surface (in what we call two dimensional or 2d structures, (3D) called brain organoids that resembles much more ,the complex network of cells in our brains. Even brain region-specific organoids can be obtained and they can also be fused together to study their interrelations.

The first obtention of IPS cells from a Rett patient was described in 2010 and was followed by the establishment of protocols to prepare good isogenic controls, meaning that we can now obtain cells from the same patient where one cell line will be expressing the normal MECP2 and the other one will be expressing or using the mutated version. So these are like perfect controls for study. In the following years a number of labs have focused on the haracterization of these cells in 2D cultures and from 2016 3D cultures were developed and soon after rett organoids have been reported. More recently, some studies have achieved the fusion of organoids corresponding to different brain regions, and it is expected soon the vascularization and also the incorporation of blood-brain -barrier mimics will be implemented in these Rett cellular models. Altogether, the idea is to achieve the most realistic physiological environment. So let's look at some of the examples, that show the important information these systems provide. Here, I'm showing some results from the first study in Rett iPSC-derived neurons

showing reduced neuronal maturation, reduced calcium signaling and impaired firing activity, all indicative of functional defects. Let me highlight here the fact that in this study treatment with IGF1 (which as you know Trofinetide is an analog of IGF1), here too showed the ability to recover some of these defects. 2D cultures of neurons from iPS cells are also a good platform to test (or screen) a high number of compounds with potential therapeutical efficacy. In the study, for example, candidate drugs were first assayed in these human neurons in vitro and where the electrophysiological and morphological features were recovered by some of them, and then the good candidates were then tested in vivo, in animals, also showing some positive results. The test of potential therapeutic agents can be sometimes a consequence of the molecular data and studies that iPSC derived neurons can provide. In this study from Park lab an unknown aspect of MECP2 function when regulating other genes was uncovered, and this inspired the use of a particular compound which then showed therapeutic potential, since it can restore certain neuronal defects. For example, here we showed how calcium signaling is recovered, so now the cells treated resemble more the control the wild type cells. Also, very recently the Jaenisch lab has used similar 2D models to assay a strategy to recover MECP2 function based on the manipulation of certain DNA modifications, certain features and certain factors that bind to chromatin to reactivate the X chromosome that contains the normal MECP2 version of the gene. Here in this image, as a result of the study, you can see how the red colour in the control cells is absent in the Rett neurons. This colour detects MECP2, (the normal MECP2) and when the genetic intervention is implemented we recover the expression of MECP2 in these neurons. So while it is true that 2D models represent highly reproducible cultures with homogeneous distribution of nutrients and small molecules and are very handy for mechanistic studies and drug testing, 3D models allow the proper formation of distinct cell layers, synaptic connections and networks across a specialized neural cell types so that 3D organoids allow a closer mimic to the in vivo steps of human fetal neurodevelopment and maturation. So, let me mention a few works dealing with Rett organoid generation.

This first study from Jaenisch lab, for example, addressed molecular aspects of MECP2 function and linked them to structural defects observed in neurogenesis by analyzing different organoid zones, for example, they observed more expanded ventricular areas. As mentioned

before, the organoid field is quickly evolving and different labs have reported the methods to fuse organoids from different brain regions. For example, dorsal and ventral forebrain organoids can be fused vitro and their links and their interconnections can be studied. This was applied in a Rett study recently where ventral and dorsal, specific (or related) organoids were separately generated and then fused in vitro. And the migration of neurons from the ventral zone to the dorsal zone was then tracked by labeling the ventral neurons in green and here we can see how in the control situation, these neurons go more to the dorsal zone whereas the Rett neurons show defects in that regard. Lastly, I'm showing now some results from a work by Alysson Muotri's lab which I think illustrates very well the power of combining 2D and 3D models.

In this case, a battery of candidate compounds were tested in 2D neurons to then assay the ones that showed some effect, in more complex 3D organoids to do a more in-depth characterization of the therapeutical potential. With all this, the main take home message I would like to convey to you today is that the human neural models both 2D and 3D systems, (which are getting increasingly more complex and sophisticated as they incorporate even non-central nervous system-derived entities such as blood vessels or microglia...), are excellent platforms to recapitulate and to characterize Rett developmental features, and they also allow drug discovery and the analysis of drug responses. Thank you very much for your attention.

I'm leaving my email here in case you wish to contact me in the future for any questions.

Thank you.

Genetic Therapy Approaches in Rett Syndrome-an Update

Hello, my name is Stuart Cobb from the University of Edinburgh, and today I'm going to talk about genetic therapy approaches in Rett syndrome and give a general update on the field. So this first slide shows the key milestones in Rett syndrome translational research. From the initial description of Rett syndrome back in the 1960s to the discovery of MECP2 early in the 1990s, to then the link between MECP2 and Rett syndrome in the late 1990s.

And then since then, there has been the demonstration of reversibility of phenotypes in in animals modeling Rett syndrome. And then in the last ten years, there has been really a sort of explosion of research focusing on approaches to try and target the underlying cause of Rett syndrome. So in 2013, the first proof of concept papers for Rett syndrome were published. And ten years later, you can see from the list of companies on the right hand side here that there are multiple biopharmaceutical companies now very active in the Rett syndrome space in terms of developing disease modifying therapies.

So I'm going to start off talking about, what do we know now as a sort of prelude. So of course we know what Rett syndrome is, we know what causes Rtt syndrome is due to mutations in this protein within the nucleus of the cell called mecb two. And we now know what the main function of MECP2 is. So we know that for disease modifying therapies, the challenge is, can you put back functional MECP2 protein or correct the mutations that actually cause the loss of protein function. And this is what I'm really going to focus on today. So the next question is, what have we learned from animal studies to date? And the answer is a lot. So on the left hand side of this plot or this slide, this is showing that Rett syndrome is likely to be a treatable disorder. So we now know from studies going back 50 years now, that if you switch off the MECP2 gene in mice, the mice develop a range of phenotypes which are modeling those phenotypes seen in patients with Rett syndrome. But if one then switches the gene back on restores MECP2, not only do you halt the disease progression, but you actually improve multiple phenotypes of the disease, multiple features of the disease.

And basically this list here is a list of features that have been looked at. And the bottom line is that everything that's been looked at shows some potential for improvement, whether it's motor

function or things like autonomic function in the form of breathing or apneas, or whether it's things like eeg activity or seizures. So the next thing that animal studies have really sort of confirmed is that the brain is the main target system for looking for therapies, for Rett syndrome. So although the MECP2 protein is actually expressed throughout the body, it's in pretty much all cells throughout the body. It's really a lack of MECP2 within cells in the nervous system that is driving Rett syndrome. And therefore any therapeutic is really aimed at delivering MECP2 or correcting MECP2 in the nervous system.

And then finally, animal studies have also shown the importance of MECP2 levels. And this is really critical in thinking about therapeutics because too little MECP2 function drives disease in the form of Rett syndrome. But we also know that too much MECP2 can also lead to severe neurological disease. And this has been shown in patients with the duplication syndrome but it's also been studied systematically in experimental animals as well.

So again, it's showing that the correct levels of MECP2 are important. So translational efforts in terms of developing therapies for Rett syndrome, there has been a sort of shift from a lot of early work looking at small molecules of what's called more conventional pharmacology to therapies that are targeting really the root cause of the disease, which is mutations in the MECP2 gene.

So the left hand side really shows all the different pharmacological approaches and pharmacological agents that have been tested in Rett syndrome. And the bottom line is that the vast majority of these studies have been disappointing in terms of their outcome.

The one exception here is Trofinetide, which is likely to be an approved drug soon for Rett syndrome and an IGF1 tripetide. But really what I'm going to focus on is the right hand side here. So therapy is targeting the root cause of Rett syndrome.

So these are therapies that are either replacing or correcting the gene at the level of the DNA or correcting mutations at the level of the RNA or delivering MECP2 protein. I'm not going to talk about the last one. So conventional gene therapy, which is sometimes known as gene transfer or gene replacement therapy is really a fairly simple concept.

So where you have mutations that cause loss of function or loss of the presence of a gene, then you can deliver a therapeutic gene, what's known as a transgene, to replace the missing gene. And this is the case in Rett syndrome where you are basically restoring a working copy of the MECP2 gene. And of course, as I've mentioned, it's important to be able to replace that two cells in the nervous system. So a number of us have been now working on this for many years.

As I mentioned, the initial proof of concept studies were published exactly ten years ago now.

And this basically showed that if you took the MECP2 gene sequence and you packaged that into viral vector, which is basically the entity that allows you to deliver the gene to the body, and you use that, and you treat animals that are modeling Rett syndrome in this case, the most common mutation causing Rett syndrome, What you get is you get a reduction in the severity of the neurological features when you deliver this gene therapy product. And what we find from lots of studies and multiple groups is that basically you can see a therapeutic effect. But the reason it's taken a long time is that we've wanted to develop better gene therapy molecules with both improved effectiveness or efficacy but also better safety feature. Because what's been very clear is that there have been a number of reports showing that the safety window for Rett syndrome is very important and one needs to put back appropriate levels of MECP2. So this is just to give a couple of examples. For instance, this is a paper that was published a couple of years ago now showing that if you sort of overshoot and give too much MECP2 you can get a range of adverse effects. And similarly we had shown also that you need to manipulate the gene therapy cassette in order to get something that's safe and effective.

So I'm going to talk about more of this from a neurogene perspective shortly, but what I'd like to next go on to is talk about what's new or recent in terms of genetic therapies for Rett syndrome. So the first thing to say is that there's a lot of activity. So this slide is taken from the Rett Syndrome Research Trust who have really spearheaded genetic therapies in Rett syndrome and funded a lot of the foundational work in this area.

And what it's showing is that with conventional gene replacement gene therapy, there are multiple companies and academic groups working on this, the two most advanced are Taysha and neurogene which are going into clinical trials this year. There's also groups looking at

MECP2 reactivation. So that is switching back on MECP2 and what's known as the inactive X chromosome. I will come on to this shortly.

And then there are a bunch of labs and companies that are working on editing Rett causing mutations at the level of the RNA and further companies working at mutations at the level of the DNA. So I'll just give some examples of these shortly. So the sections, who's doing what in the field? So this is a bit tricky because the majority of genetic therapy efforts are really conducted by companies operating in stealth mode. So there's not actually all that much information out there in the public domain.

So the four companies that I've sort of highlighted here, the red circles are the companies that have actually shown publicly some data for the therapeutics that they are developing. But the majority of the companies are operating in stealth mode and have not shown it publicly. So the first example I give is the work of Gail Mandel's Lab and they've really been pushing the boundaries in terms of what's known as RNA editing and this is showing some promise.

So basically what they're doing is that they're attempting to correct Rett causing mutations at the level of the RNA. And this is very attractive because if you can correct the mutation, in theory you can correct specifically the cells that are expressing the faulty copy of MECP2. So this is a very attractive feature of editing approaches and the other attractive feature is you can't really overshoot because as you correct the mutation, the MECP2 would then be expressed at the normal levels in the correct cell types. So has she done this? This is a paper that was published very recently in which Gail's Lab has shown when they deliver their therapeutic molecules here they can achieve editing of a specific Rett causing mutation.

So they were looking at a very specific mutation and this is the model, this G311A model of Rett syndrome and the data showing here is that these mice develop apneas which is a characteristic phenotype, a cardinal phenotype seen in many Rett patients and this is seen in the mice as well. So this sort of gray, these pauses here are apnea events in the disease, untreated disease animals and you can see that following virüs treatment this respiratory pattern has been normalized and the number of apneas is greatly reduced. So this is the untreated animals here

you can see there's high number of apneas in many of the animals and in the treated animals this has gone back down to very, very low levels similar to normal healthy mice.

So this is very encouraging. Other data that's been presented in the public domain is this other approach, this what's known as X chromosome reactivation. So there's a company, Alcyone Therapeutics, who are pursuing this approach, and their drug, which is ACTX 101, is essentially an adeno associated virus. So it's a gene therapy virus, but instead of delivering a therapeutic gene, they're delivering what's known as a micro RNA sponge. And the idea of that is to really switch back on the healthy copy of MECP2 that is in the cells that basically normally lack MECP2 function in girls with Rett Syndrome. So they have presented mouse data at scientific meetings, it's not been published in papers yet and they've also shown safety data in large animal species.

So finally there's also a lot of progress being made on RNA editing and, in particular, there has been data presented on what's known as read through of premature stop codons. So as you're probably aware, many cases of Rett syndrome are caused by what are known as premature stop codons. So basically any of these that end in an X, any mutation that ends in an X has a prematüre stop codon and basically this therapy works by allowing you to read through this premature stop code on. So if you get a premature stop codon mutation what you end up with is a truncated, in other words, inactive or reduced activity form of MECP2. But using basically these read through molecules which is what's called a TRNA molecule, they can facilitate reading through to produce normal functional MECP2.

So there was a lot of work done on this a number of years ago with small molecules. But the results of those ended up being somewhat disappointing. And what the companies working in this area are claiming is that they get much more effective read through of these premature stop codons and there's been some data presented in this R255X model. So I would lastly just like to say that there's also very intensive efforts to develop better ways to actually deliver these genetic payloads in terms of these therapies. So one of the challenges is actually getting these genetic therapeutic agents into the nervous system. And most of the approaches to date are delivering directly to the nervous system.

So, for instance, the Neurogene studies delivering to the fluid field ventricles in the brain, the Taysha studies delivering to the what's known as the lumbar spinal cord. And there are other companies that are working using this what's called ICM delivery. But there is a definite requirement to get more effective delivery systems to the brain. And again, there are a lot of companies working on developing new viral vector capsids that can make it more effective to deliver to the nervous system. And ultimately this will with more benefit Rett syndrome because the brain is the key target. So, to conclude, loss of function of MECP2 is the root cause of Rett syndrome. And there are multiple animal studies, multiple labs that predict that Rett syndrome is a treatable disorder if you can actually restore MECP2 and the last few years have seen a great deal of activity in developing what are likely to be disease modifying therapies. And the three that sort of stand out are gene therapy, x chromosome reactivation and RNA level editing. And then my last point was really with all of these, delivery to the brain remains a challenge. And for a Rett syndrome therapeutic to be truly effective, we really need to maximize delivery to the nervous system. But with that I'll stop and thank you.

Neurogene Update: NGN-401: A Self-Regulating Gene Therapy for Rett Syndrome

Okay, so I'd like to switch gear now and I'm going to talk about NGN 401, which is the Neurogene gene therapy product. So this is a selfregulating gene therapy for Rett syndrome. So here are my disclosures. I'm chief scientific officer at Neurogene. So in terms of the science, as we've discussed, Rett syndrome is really driven by having too little MECP2 in cells. But the MECP2 gene is a dosage sensitive gene and we know from patients with duplication syndrome and also from animal studies that too much too is also a problem. And therefore when thinking about a gene therapy or treatment for Rett syndrome, one really requires a balanced treatment goal where you're delivering enough to be therapeutic but not too much to cause adverse effects. So this is really challenging in gene therapy because of the bi distribution, in other words, the way that the gene therapy distributes throughout the body. So when you deliver gene therapy, the AAV virus has the gene encapsulated within the virüs which generates what's known as a transcript or mRNA, which then makes the MECP2 protein. But different cells take up very different levels of the virus. So you get some cells that take up low levels of the gene therapy

product and therefore produce relatively small amounts of MECP2. But you have other cells that can take up a high quantity of virus and therefore produce too much MECP2 which is a problem. And therefore we have developed a technology to try and overcome this and this is using what we call our EXACT technology. Now this is what's known as a single gene circuit, that is a gene therapy that has an inbuilt thermostat, if you like. So that when it delivers the MECP2, it expresses that MECP2 at the appropriate level that's going to be therapeutic without driving too much MECP2. And the way it works is it delivers the MECP2 transgene which goes on to produce MECP2 protein within the cells. But if there's a lot of the product gets into a cell, then basically there's a safety valve such that the excess mRNA is broken down. So this means that in different cells that receive different amounts of the gene therapy product, there's a sort of thermostatic control if you like, in each cell. So we've shown that this works in cells and can also constrain expression in experimental animals. And if we move on to the Rett syndrome disease model, so these are mice that are modeling Rett syndrome so they're completely deficient of MECP2. And this is a very severe model. So these animals reach a human endpoint at around nine weeks of age. And these are the untreated animals in black here. But you can see if you treat with the gene therapy product, you're increasing the survival of these animals in a dose dependent fashion. So the light green is the low dose and as you increase in the dose of the gene therapy product, you get a larger therapeutic effect. And this is just survival. If one looks at the neurological phenotypes in this model, and this is a clinical score for Rett like phenotypes, things like abnormal breathing, locomotion and so on, or movement, then you can see that, again, the untreated animals in black here become more and more severe, but the treated animals in green you can see it's really pushing down the neurological score. So what we've shown is that NGN 401 produces fairly significant dose dependent increase in survival and a significant amelioration or reduction in Rett like neurological phenotypes or effects.

So, as well as effectiveness, one has to turn to safety as well. So in this study here, we tested the tolerability of the gene therapy product. And this is now in female mice that are what's known as heterozygous. So they express normal levels in some cells and not others.

So it's basically mirroring the likely clinical population. And what you can see is when we give NGN 401, it's completely tolerated. So all of the animals survive. Not only do they survive, but they don't go on to produce a toxicity score. So basically, at these doses which produce the profound therapeutic effect, we're not seeing any adverse effect here. So basically, this is well tolerated if you compare it to a conventional unregulated gene therapy. So this is the exact same gene therapy product, but without our EXACT technology, you can see that these mice drop out of the study because they're developing a severe neurological score. So this is basically overshooting the level of MECP2. So basically, the EXACT technology that's embedded in our gene therapy product confers both enhanced safety profile as well as being more efficacious. And we've also conducted further safety studies in other species to show that NGN 401 is well tolerated at doses well above the clinical dose that will be used in the clinical trial. So, in terms of the scientific conclusions, Rett syndrome is gene dosage sensitive disorder, and therefore a gene therapy is going to require appropriate regulation in order to be safe and effective.

I've shown that the EXACT technology that's embedded in NGN 401 enables autoregulation of the MECP2 protein expression and that this provides superior efficacy and safety relative to conventional gene therapy approaches. I've shown that NGN 401 is well tolerated in rodents and non human primates. And NGN 401 is also delivering the natural full length MECP2 to really maximize the potential for therapeutic benefit. And the route of administration is known as ICV. And this delivery approach is really selected to maximize the distribution of virus to the parts of the brain that we believe are most important for impacting the phenotypes of Rett syndrome. So in part two here, we have a planned clinical trial. So in January this year, the FDA gave approval for a first in human clinical trial. And this is going to be an open label trial. It's a single dose study and NGN 401 willÖbe administered by intracerebroventricular infusion so that's an injection into the fluid filled space within the brain. And the study population will be pediatric females with typical Rett syndrome. So. the objectives of the study are shown here. So this is primarily a safety study. So the primary objective of the study is to evaluate safety and tolerability of the single dose. And the assessments that will be looked at include an assessment of adverse events, neurological exams, clinical laboratory tests, and monitoring of potential immune response to the treatment.

And then as a secondary objective, is to obtain a polymer assessment of efficacy, in other words, effectiveness of NGN 401. And this will be monitored over the 60 month period, and this will be through both clinician and caregiver assessments. Okay, so with that, I'm very happy to take questions. Thank you.

Neuren Update

Greetings to you all. My name is Nancy Jones, and I am the vice president of clinical development at Neuron Pharmaceuticals. It's a great pleasure to join you remotely and give you this update. First of all, let me introduce you to Neuren. Neuren is a biopharmaceutical company specializing in the development of drugs related to neurodevelopmental disorders.

We are a small team and we're all dedicated to finding new treatments for patients. Patients who are affected by a rare genetic disease and have great medical needs but to date have no specific treatment. The table gives an overview of our clinical program. We have two new drugs in development. NNZ 2591 is in development for four genetic disorders in phase II clinical trials. The first molecule is called Trofinetide and is our most advanced program. We have completed a phase two study for Fragile X syndrome.

As you can see, we are evaluating Trofinetide as a potential treatment for Rett syndrome and a phase three clinical trial has been completed. A phase three clinical trial is a large study that's designed to provide the evidence for regulatory approval. It was our partner for North America, Arcadia Pharmaceuticals that conducted this phase three clinical study and submitted a new drug application or an NDA, which is the application for approval of a new drug in the United States and that was submitted to the Food and Drug Administration, the FDA. So what is Trofinetide and why might it be useful for the treatment of Rett syndrome? So before talking about Trofinetid specifically, it is useful to talk a bit about insulin like growth factor or IGF-1 and to understand how IGF-1 works in the brain normally. So, on the left is a picture of the cells in the brain. The orange cells are the neurons which are the main cells that you have for functioning of the brain. And there are other cells that support the neurons, notably the microglia that are shown here in brown and the astrocytes that are shown here in green. The microglia help with the growth and also the maintenance of the structures of the neurons and

also the function of the neurons. And this includes the dendritic spine. So those are those things that branch out from the neuron and they receive the messages from other cells. So, naturally in the brain, all of these cells produce IGF-1. An IGF-1 is a growth factor.

It's produced throughout the body and in the brain it's responsible for the growth and the regulation of neurons. It is also responsible for the communication or the connection between those neurons which are called the synapses. So here we see the IGF One molecule and when it does its job naturally it separates into smaller parts or what are called metabolites. And one of these small parts is GPE. And GPE is the terminal tripeptide of IGF-1. And what Trofinetide is, is a synthetic analog of GPE and it basically replicates the function of GPE. So what's an analog? An analog is something that has almost exactly the same structure, chemical structure, as the naturally occurring molecule, but has one small change. Here you can see at the bottom the chemical structure of GPE on the left and Trofinetide, they're exactly the same, except there's an addition of a methyl group, as you'll see on the right for Trofinetide. And one question people may ask is well, why wouldn't you then just use GPE as a drug, as medication?

And that's a good question. So, essentially, what are the benefits of this change? So what this change does is that gives Trofinetide better characteristics to be a drug or a medication. And in this case, for example, it stays longer in the body. So if you see on the graph on the bottom, this is an animal model. In a rat. GPE was found to last for five minutes, but Trofinetide lasted for 74 minutes, which is more advantageous because it would mean that you would be required to take the treatment fewer times per day because it lasts longer in the body. And that's just one example of sort of the properties that can be improved to make it better to use as a medication. Another important question, obviously is the relevance of Trofinetide for Rett syndrome.

And we do know that has been observed, that there are insufficient formation of the new synapses by neuron those connections and then excessive pruning of the synapses by those overactive microglia, those supporting cells. So, Trofinetide is thought to improve synaptic function and restore synaptic structure and it inhibits overactivation of inflammatory microglia and astrocytes of supporting cells, and then also to increase the amount of In preclinical studies, we have also specific evidence for Rett syndrome. So I'm showing here the results, a mouse

model. And in that mouse model, we found that GPE treatment improved the effects of Rett syndrome. Specifically in this study, it increased lifespan, brain weight, and also the density of those dendritic spines.

And on the right hand side you see in a cell based study. So, this is looking at the effect on cells and we found that GPE and IGF-1 can correct neuronal deficits caused by MECP2 mutant astroscytes. So, supported by this information, two phase two studies were conducted. And phase two studies are studies to assess the safety and provide the indications of efficacy. So, on the left here is the findings from the Rett study. One was a double blind, placebo controlled study with 56 participants, 60 to 45 years old. And participants were randomly assigned to receive one of two doses of Trofinetide, or placebo, for 28 days. Trofinetide was well tolerated and there were encouraging trends for efficacy. The second study was a double blind, placebo controlled study with 82 participants between the ages five and 15 years old. Trofinetide was also well tolerated in the study and for the highest dose, we observed improvement in two assessments.

That was in a questionnaire for the Caregiver, which assessed key Rett symptoms and also in the Clinical Global Impression of Improvement, which is a measure of overall improvement. Daffodil is another study, a phase Two study which is an open label study looking at young children aged two to five years old. The goal for this study is to evaluate safety and pharmacokinetics and pharmacokinetics means learning how the drug is used and absorbed by the body. And this study is ongoing but no longer enrolling. The phase three program consists of three studies Lavender, and that is the controlled doubleblind study Lilac, which is the Open Label treatment study and then Lilac 2 is a study for participants who complete the Lilac 1 study but could continue on the drug until the new drug application is reviewed. So Lavender was randomized, double blind placebo clinical study. The duration of treatment was twelve weeks. The study included 187 girls and women aged 5 to 20 years old. The co primary efficacy assessments were the RSBQ and the CGI. Secondary efficacy assessment was the Communication and Symbolic Behavior Skills Developmental Profile. Specifically the Social composite score.

That's the CSBS. The CSBS is an assessment of early communication behaviors. This includes emotion and gaze, gestures, communication of needs and requests. And the study was conducted in the United States. And so a little bit more about the co primary efficacy measures. And so the Rett Symptom Behavior Questionnaire is a validated 45 item rating scale. It's completed by the Caregiver and the valuation covers eight areas specific to Rett. It's scored from zero to two and higher scores indicate more impairment. And this measure has been correlated with functioning and quality of life in Rett. The Clinical Global Impression Scale of Improvement, or CGI, is a global assessment of a change completed by the clinician. The scores range from one to seven with scores of one to three indicating improvement, four indicating no change and five to six are scores that indicate worsening.

The overall findings for the Lavender study are presented here. There were typically significant separation from placebo on the two primary endpoints and there was also statistically significant separation from placebo on the key secondary endpoint. I'll talk now in more detail about the Lavender study. Here we see the summary of the participant characteristics. So the treatment groups, the different treatment groups, placebo and trofinetide were similar for age and also the baseline severity on the CGI, which is a global impression of severity. And just over half the girls were between five to ten years old, just over a quarter were between eleven to fifteen years old and about 20% were between sixteen and twenty years old. And based on the CGIs, the majority of participants were moderately or markedly ill and very few were considered extremely ill. Here are the results for the RSBQ and the CGI in detail.

We observed a statistically significant improvement in RSBQ compared to placebo. And we observed a statistically significant improvement of CGI in the Trofinetide group compared to placebo. For the secondary endpoint on CSBS, there was a statistically significant separation from placebo. So what types of symptoms changed?

This can be seen on the RSBQ, which has subdomains in addition to the total score. And here, what you're seeing on the graph is a measure of the magnitude of effect or the difference in magnitude between the placebo group and the Trofinetide group in each of these domains. The direction on the left indicates a change in favor of Trofinetide. As you can see, all the subscales

were directionally in favor of Trofinetide. And another important point is to note that the effect was not actually due just to a signal substance. Here we see the findings for safety. The most common adverse events were diarrhea, with an 81% in the Trofinetide group versus 19% in the placebo group.

In the Trofinetide arm, of those that had the diarrhea, 97% were characterized as having a case that was mild to moderate. The next most common adverse event was vomiting, 27% in the Trofinetide group and 10% in the placebo group. And in the Trofinetide arm, 96% were characterized as having cases that were mild moderate. Serious adverse events were observed in 3% of the study participants in both the Trofinetide and the placebo group. So, what is the status of the new drug application? The Acadia submitted the new drug application in July of 2022 for the treatment of Rett syndrome in patients two years of age and older.

The FDA accepted the NDA for a priority review. So the decision date set for the 12 March in 2023. The decision we based review of the pivotal efficacy, which is the positive phase three Lavender study supportive efficacy, which is from the phase two trial in Rett syndrome and then also the safety data from the completed and ongoing studies. As of the date of the recording of the presentation, the decision date had not yet been reached and an update on the outcome of the review on March 12 would be announced on our website. And what is the current situation for the European market? Neuren retains ownership of Trofinetide outside North America, including Europe, and Neuren is currently in discussions with potential commercial partners for Europe. We intend to apply for marketing authorization for Rett syndrome for the European Medicines Agency, the EMA, using the results from the US program. We have received scientific advice from several national regulatory agencies which indicated the steps to follow to increase the probability of a positive approval. So I'd like to end by acknowledging the efforts of the clinicians and the study teams that participated in the studies I presented here. And of course, very very special thanks to the girls, the women and their families who participated and are participating in the studies. I thank you for your attention and I hope you enjoy the rest of the conference.

Taysha Update: An Investigational Approach to Gene Therapy for Rett Syndrome

Hi there. I am Benit Maru. I am an adult neurologist and currently the chief medical officer and head of clinical development at Taysha Gene Therapy. Before proceeding further into this talk, I need to make sure everyone is aware that what we are discussing here is an investigational gene therapy product. Until clinical trials are completed and regulatory agencies such as the European Medicines Agency and the US FDA potentially approve a product, we cannot make any claims about the potential safety and efficacy of an investigational product. Our goal here is to educate about our investigational clinical trial. Taysha is focused on the development of gene therapies for central nervous system disorders. The strategy is building upon one of the most well studied delivery vehicles, the delivery vehicle, the AAV nine vector adeno associated virus. The therapies Taysha are developing target the underlying biology of a very specific condition. And Taysha utilizes an intrathecal delivery, which is an injection into the lower part of the back into the cerebral spinal fluid. At Taysha, we work with advocacy groups and seek input on materials and trial protocols. We wanted to understand the journey of diagnosis to Rett syndrome. What are some of the most challenging symptoms? What's the significant burden and unmet needs? We also wanted to understand the variation in Rett syndrome experience over time. We also wanted to understand and gather input from the community regarding just clinical trials in general and overall expectations. Now, this work here was a two pronged approach. There was a quantitative survey, multiple choice questionnaires participants from four countries.

The second piece was slightly more in depth. There was a daily survey, there was a guided discussion. There was a focus group as well, and it was driven by 20 caregivers in the US. Now, what you can see here is a brief overview of some of the impacts that we garnered from the work within the community. There's lots of symptoms here which do cause impact, loss of speech being one of them.

This was specifically centered around caring for an adult with Rett, and this has obviously led to us implementing certain endpoints within our current adult trial as well. So now, moving on to gene therapy, what is it and how could it work? Every gene in the body makes a specific

protein, and these proteins ensure our body works appropriately and properly. Unfortunately, there are lots of diseases that can occur when genes are impacted. Here we are specifically speaking about monogenic. So one gene causing diseases. There are a multitude of ways that a change in a gene can actually cause a problem.

For example, a gene change can result in a non functioning or even missing protein, and this occurs in diseases such as in certain neuromuscular conditions. There is also the potential when a gene can go wrong, that it can produce too much protein, which can in itself then be harmful. For example, there are genetic eye diseases which cause blindness due to too much protein which becomes toxic and unfortunately, people lose their sight and also fibrosis of the lung as another example where there is too much protein. Now, some gene therapies work by delivering new copies of the gene or by helping to silence the protein that is being overproduced. These genes are delivered to the cell inside the body by various delivery routes with Taysha's primary choice route of administration being the intracecal mode of delivery. So a quick overview on Rett. As I'm sure you're all aware, MECP2 is the gene that tells cells how much Mecp2 protein should be made. Now, in Rett, a reduction of Mecp2 reduces normal protein in the brain and nerve cells. Unfortunately, on the other side of it, there is another condition which is MECP2 Duplication syndrome where when you have too much Mecp2, this can cause signs and symptoms of a disease as well.

And this is known as the Goldilocks phenomenon where too much is harmful and too little causes Rett syndrome. So this is that balance between too much protein being expressed and not enough protein being expressed as talked about in the slide before when something happens or something goes wrong with a gene. So Tayshas Gene Therapy delivers a working copy of the MECP2 gene to the affected areas. And now we know that there is a concern of too much MECP2 which could cause a Duplication syndrome and it can cause challenges in itself.

Now, our extremely talented collaborators develop technology which I personally think is quite cool, which can help control the expression of protein and minimise the harm from overexpression, but also by helping control the cells which do not have enough.

So it can help increase Mecp2 protein in the cells that do not have enough and it can stop cells from producing too much where it could go wrong. Potentially, the gene is put in a delivery vehicle. The delivery vehicle of choice for Taysha is the AAV9.

Now, the AAV9 is the packaging, think of it as an envelope that has a certain size, due to that size restriction and because of the additional control that we're putting in with the mirror technology, which helps us control for any overexpression that with the MECP2 gene, it's not all going to fit within the AAV9 envelope.

Because of that, certain parts of the MECP2 gene were removed and the most important parts to give you a functional working Mecp2 protein were kept which allows us all to fit into this one envelope. Now, based on all of our non clinical data packages, there have been no adverse events that have been seen. And overall, we can surmise that the product does change outcomes.

In the mouse model, the therapy will be delivered via an injection in the lower back and once the product has been injected into the cerebral spinal fluid, it will travel up the spinal cord and it will be transferred to the cells ultimately that need it. So we do currently have adult study ongoing. It is an open label phase one two dose escalation study, primarily a safety and tolerability study with an early efficacy signals to be monitored during over two dose levels. This will be the first time any person with Rett will have been treated with a gene therapy. And our first site is located in Canada. What are our plans for the broader Rett syndrome community?

So firstly, as we mentioned, we have an active site in Canada. The dosing of the first adult patient is expected to be the first half of this year 2023.

Initial data from the study is expected to be shared from the first half of 23 and quarterly updates on clinical data which will primarily repeat safety given thereafter. With regard to our pediatric plan, we plan to go to the United Kingdom, we plan to go to the MHRA and submit a clinical trial application by the middle of this year to allow us to start a study in pediatric females with Rett syndrome. With regard to the US, we plan to submit an IND (Investigational New Drug) in the second half of this year. For males with Rett currently, at this time, Taysha is primarily

focused on running preliminary clinical trials in adult and pediatric females, with the hope to demonstrate potential safety and efficacy of this investigational product.

We do have plans for global clinical trials, and if there are any questions, please do email me info@tasiagtx.com now.

Finally, a great big thank you. Thank you to Rett Syndrome Europe and to all other advocacy groups that have supported us, work with us, partnered with us. Massive thanks to Sarah Harrison at Stephen Gray and Burj Manassian at UT Southwestern. These are the extremely smart collaborators who I think have created a rather nuanced, novel and intriguing piece of technology. And thank you to the entire community to help partner with us, work with us, and help us bring this therapy forward. Thank you.

Burden of Illness in Rett Syndrome: Intial Evaluation of a Disorder- Spesific Caregiver Survey

Rett syndrome is a disorder characterised by unique, diverse and complex clinical manifestations. For that reason in order to know the impact on affected individuals and their families it's important to do focused research in this area to see to what extent the clinical manifestations of Rett syndrome have on quality of life, of individuals and their families.

And this is the project I'm presenting now. The first analysis is labeled as a study of burden of illness of Rett syndrome which is essentially about the impact on quality of life. It was a community effort, a collaborative Project involving multiple stakeholders beginning with caregivers, advocacy organisations in the US, United Kingdom, Italy, Germany and Australia, also clinicians and investigators in those countries and industry partners. The data was collected with support from Newron Pharmaceutical and with assistance from Parexel and the data has been maintained, managed and analysed with support from Anavex Life Sciences Corporation. In addition to thanking the participating families and clinicians, without them this could not have been possible. A special thanks to Steve Kaminsky, the chief scientific officer of IRSF for launching the project.

The objective of the project as I mentioned before was to evaluate the impact of the symptoms and clinical manifestation of Rett syndrome on affected individuals and their caregivers. In particular, to identify a symptom which had a high impact and determine whether they impacted the same the individuals, the patients and their caregivers in the same way. Then also to examine the relationship between the severity of the problem and its impact. The way that this was carried out it was mainly by a caregiver survey aligned with that was also health care provider survey and these were developed using conventional methodology literature and also input from parents of children and adults with Rett syndrome. The survey focused on 21 major clinical manifestations of Rett syndrome which were grouped in 15 domains or categories and they were mainly scored in a scale of one to five, with one being the lowest level of the severity or impact and five the highest. The healthcare providers evaluated only severity while the caregivers severity and impact. This was the largest study involving 756 caregivers mainly parents of children with classic Rett syndrome and MECP2 mutations who are the majority of the cohort and 112 clinicians from the countries that were mentioned previously.

The ethical methodology is conventional for this and includes, in addition to descriptive the chi square test, which examined the relative proportion of answers, each one of these levels of severity to impact and how they relate to each other. This graph is to show you an example of how diverse the impact of symptoms is, in this case on caregivers. These are three common symptoms of Rett syndrome. In blue, hyperventilation, in red dystonia and in yellow or orange nonverbal communication. And you can see they are distributed differently. You see much more blue on the left from the centre to the left meaning that it has less impact, hyperventilation is in the middle and you have the reddish of dystonia.

Most of the answers are centered around 'somewhat' (in terms of impact) which is the mid level of impact and in contrast with this, is the nonverbal communication which is skewed or distributed more toward the right, meaning it has a higher impact on the caregivers. So which problems or which clinical manifestation were the most impactful, had the greatest impact on affected in individuals and caregivers were those related to the core features of the disorder.

By core features we refer to those symptoms that are the ones used to make the diagnosis of Rett syndrome, that have distinctive characteristics. These are communication impairments, fine and gross motor impairments such as ambulation and hand function. This is where you see the greater concentration of four. This table presented data in a different way than the graph. The percentages that you saw before are grouped in four quartiles, meaning if the percentage was zero to 25 it's a one, if the percentage was 75% to 100%, were a four and in between two and three. So when you see a lot of fours and threes it is because they have high severity or high impact and that's what this red frame is highlighting here. Now, the second question is who is most affected? And even though the impact on affected individuals and caregivers was high for many symptoms, for a group of them that was greater on caregivers. And those symptoms that have that profile are highlighted in the larger rectangle and they tend to be symptoms that have episodic characteristic and those include seizures, abnormal behavior, sleep problems and pain.

In addition to that, we examine what is the relationship is between problem severity and impact. You can see particularly in the lower part of the table, there are many severities, that are one or two and they have higher numbers in terms of impact. And this is both for individual and caregivers. And this is what we identify as the main finding. That even mild to moderate problems have high impact. And this is one of the main outcomes of this analysis.

So we can conclude that even mild severity impairment can place a disproportionate burden. In other words, they can have high impact, and can affect the quality of life of both affected individuals and in some cases even more caregivers. Why this information is important is because this information will help to develop and evaluate existing treatments. These treatments and care for patients with Rett syndrome should be guided not only by the clinician impression and severity of the symptom, but also by the impact that this symptom has on the patients and their families. What really matters in terms of affecting the individuals and their families.

This was the first analysis of this data and therefore it's a big picture view, an overview of the data. We cover the entire age range so we need to examine in the future whether this relationship, these findings are the same in younger individuals or older individuals. Whether the clinical type,e.g. classic Rett syndrome or atypical has an impact, and particularly, whether

health care services have a role in these profiles and the impact of these symptoms on individuals and their families. It has been a particularly gratifying project for me to participate because it represented a community effort in obtaining this very important data that can help to improve the quality of life of affected individuals, patients with Rett syndrome and their families. Thank you.

Parent Assosiations & RSE

Good afternoon. I'm Becky Jenner, president of Rett Syndrome Europe, a voluntary role, but I also work for Rett UK, and I'm mum to Rosie, who's and has Rett syndrome. It's great to be here. I hope you are finding the conference really helpful. You heard before the break from Francois about how patient advocates can be involved in the regulatory organizations, which is going to become an increasingly important piece of work as gene therapies and drug treatments advance. But you need to have families and strong advocates to engage in this process. So I'll talk a little now about that aspect. The time is now in respect of getting our houses in order. With new therapies and treatments on the horizon, we need to be ready to engage with the EMA and the funders in each country so they know about Rett syndrome. And as the treatments are approved elsewhere, we will then be well placed to make our case about the need, the impact they will have and how many people are likely to benefit. Knowing how many people with Rett are in your community is important for several reasons. For the families, first and foremost, of course, it's about connection, information, advice and support. Then ensuring all those that can benefit from any treatment are going to be contactable. But for the pharma and gene therapy companies it's about the viability of running a clinical trial are there Rett centres with experienced clinicians who can run a trial? Is there an infrastructure, a patient organization that can assist in disseminating information, recruiting patients? Are they in contact with enough people to make the trial feasible? All these things are important for the EMA and funders. They will want to understand what the impact of Rett is, how a treatment could make a difference, how much of a difference, and for how many people. It's also important to have a confirmed MECP genetic test to know the particular mutation the person has. You need to know this for any trial, but it's very possible that there will be treatments that target particular mutations.

We also need to be able to show the full spectrum of Rett and find more males. It's highly likely that males with Rett remain the most undiagnosed group and therefore could be missing out on potential treatments, therapies and support. All of these families though, have powerful stories to share and the regulatory authorities need to hear them. So how can you find more families? Social media is an obvious one, and using hashtags helps. But not everyone, of course, is on social media, especially families of older people with Rett, who are also potentially amongst some of the numbers of the undiagnosed people in some countries where information about Rett syndrome is only more recently being shared.

Think about having an awareness raising campaign. But you don't need to start from scratch. There are resources out there that can be either translated or adapted, and Rett Syndrome Europe will help in these endeavors and I know other organizations are happy to share resources and support any awareness raising activities you do, so please reach out to them too. Think about all the places people with Rett are likely to be target doctors, health visitors, special schools, day centers, etc. also genetic testing centers.

Here are just a couple of examples and campaigns other organizations have done. This one from Rett UK was aimed at GPS, health visitors, etc. and was drawimg attention to one of the most distinguishing features about Rett, the stereotypical hand movements. And they also include the diagnostic criteria emphasizing that an early diagnosis leads to better longer term outcomes for both the child and the family, but also now it is about having Access to therapies and specific treatments.

Our colleagues at rettsyndrome.org have an awareness raising toolkit. Lots of great ideas and resources in there, definitely worth having a look at that. They've included these lovely awareness raising cards which can be personalized and you can have information about Rett syndrome on the back. So obviously they're visually appealing and can catch people's eye. So hopefully you'll be finding more families and be ready to engage with them. Have some resources ready that you can share with them. There's lots out there already, but you may need to get some key ones translated. Having more families involved helps spread the work load and brings more skills to your group. Maybe start a WhatsApp group, to connect people. You don't

need to go all out to set up a registry, but a simple spreadsheet to record contact details, name and age, of the person with Rett, MECP mutation, if known would be sufficient. You will need consent protocols of course to store the information.

You can start with some simple activities like online forums on Zoom or Teams, connecting people, sharing tips and advice, etc. Then we would suggest engaging with Daniela Tropea and the Data Rett platform encouraging healthcare professionals involved with the families to get on board. You don't need any more information than you've collected in your spreadsheet to contact families and get them involved with this initiative. Starting another patient registry is not necessary and may well be beyond the resources of most organizations. Some other resources out there - there is a Patient Journey for Rett syndrome, which is on the Eurordis website and has been developed by Patient Reps from ITHACA, which is the European Reference Network for rare diseases. This is a great tool to share with healthcare professionals as well as families as it gives a snapshot from that early development when things first start to go wrong, to the comorbidities that are likely to occur later and what's needed to support the child and the family. And then I will just mention the Rett Resource which is on the Rett Syndrome Europe website. It is available in some languages and can be translated to others. Please get in contact should you wish to do that. We're also offering the opportunity for other countries to have access to the platform that we've used to do the translated subtitles on all the presentations in this conference. So again, please contact me if you're interested in finding out more about that. And there's just a few key organizations I would suggest are worth engaging with that can help you develop resources, but also provide training and support for patient advocates. So the Global Rett Leaders Forum meets quarterly online amd that's organized by Paige Nues at rettsyndrome.org. There are very many people in that, and everyone's happy to help share resources and support whatever you're trying to do to develop your organization. Then there's ITHACA, which is the ERN, European Reference Network for rare diseases. They've done a lot of work specifically around Rett Syndrome. There's lots of resources on their website and then also on Eurordis. We heard from Francois earlier training and support for patient advocates. So please do look up those organizations. Thinking about next steps then, if you've got a few willing, amd able parents to galvanize your Rett community, develop an action

plan but keep it simple - think about who can help, who has time and skills to do certain tasks, and decide if you want to formalize as an organization. This is recommended, as you're more likely to be able to attract funding to support your work that way.

And here's a few funding opportunities that are worth looking at. I will just highlight this middle one, as it does have a March deadline, and it's particularly aimed at rare disease advocacy groups. It's a fairly simple application for up to €. The other two sites provide information on sources of European funding and a funding toolkit you might find useful.

Finally, I'd like to share this slide with you. It's a good mantra when things seem overwhelming, and remember, Rome was never built in a day. So thank you for listening. Please do contact us at Rett Syndrome Europe for further support and I hope you enjoy the rest of the presentations.

I can't get no Sleep

Hello everybody, my name is Karen Spruyt and today I will talk to you about sleep. I would like to thank the organizing committee for the invitation to talk. And so my talk will be divided into three sections. So what do we know about sleep, what are the most common issues and what can be done and who to consult? So sleep is defined based on our brain waves and also on our eye movements, the muscle tone as well as cardio respiratory function. So based on these brain waves and these functions we can divide sleep into stage N stage N and stage N or what we call the non REM sleep, so the non rapid eye movement sleep which is characterized by slow eye movements, reduced muscle tone and slow and regular cardiovascular body function.

We also have the REM sleep or the rapid eye movement sleep which is a paradoxical sleep or a paradoxical stage because we have rapid and or fast random eye movements, we have an absent muscle tone and we have fast and irregular cardio respiratory function. But this structure is creating or is maturing, is developing throughout our childhood. And so when we are adults, when we go to bed we fall asleep and that is we spend some time in stage N. Now, we will go to slightly deeper sleep which is spent in stage N and then we will spend some time in the deepest sleep, stage N. Sometimes the sleep becomes a little bit lighter and we might turn and then as you can see, our first REM sleep starts. So this is called sleep cycle. So the cycle from non REM all the way becoming a little bit lighter sleep and then our first REM period or REM episode, depending on our sleep duration, we will have four to six of these sleep cycles because in adults a sleep cycle lasts about to minutes.

Now when we are born, this sleep cycling is much faster, it's around to minutes. And also the characteristics of stage N stage N stage N are not as developed or not as characterized as we would see it in adults. So we talk about the quiet sleep which will develop later on, will become the non REM sleep.

We'll show these characteristics of stage N two and three and we will have as a baby, active sleep which will develop, which will mature into the rapid eye movement sleep. Also, as you can see here. So this baby falls asleep in REM and then spends some time into the lighter non REM sleep as well as in the deeper sleep. So at around three months, this sleep structure starts to have more of the characteristicsthat we see it in adults. Some of the characteristics, the physiological characteristics at the age of six or seven months, really clear on the polysomnography or on our sleep study. So as a child, what is also interesting to see is that it spends a lot of its first period of its sleep in this deeper non REM sleep, whereas towards the end of its sleep the child will spend more into the REM sleep. So proportionary will spend more time in REM sleep but also at all ages the sleep structure will still change. So this structure of sleep or these characteristics of sleep through our lifespan change. As an adult, we spend about % of our total sleep duration in the REM sleep and around % in the non REM sleep. But as I just said, this is not the case when we are born. So when we are born, as a newborn, you spend about % of your sleep time in this REM sleep and % into this non REM sleep. And in this figure it is showing, this proportion will change throughout your life, and so it will determine your sleep, your quality of sleep. And then there's another element that comes into play and that is what we call the two process model of sleep which explains that throughout the day the sleep pressure will increase. So the homeostatic, the drive, the need to sleep will increase and at the same time the circadian rhythm. The biorhythm will try to keep us and push us to remain awake up to a certain point in the evening where actually they will start to the two processes towards sleep and they will make sure that we can actually fall asleep and remain sleep and be repaired and refreshed for the next morning. So it is a distribution of the REM and the non REM sleep, the characteristics of the non REM sleep as well as the homeostatic pressure, the sleep pressure and the circadian REM all combined as a cocktail will make up our sleep, our quality, our duration and our timing of our sleep. So that is sleep in a nutshell. So what are the most common issues of sleep in individuals with Rett syndrome? So first you need to know in terms of typically developing children if they come to the sleep clinic they will sleep about hours and they will spend about minutes to fall asleep. During the period that they are asleep they spend around half an hour, minutes being awake spread out over that sleep period. So, if you look at

this distribution of the sleep stages across the sleep duration it's around % of their time spent in stage % in stage % in stage deep sleep as well as % in the REM sleep.

Now, if we combine all the polysomnography or all the sleep studies done in children or people with Rett syndrome that are published in literatüre and who also come to a sleep clinic, you see that they sleep about hours, that the sleep onset latency, so the time to fall asleep, is about minutes and that the period that they stay awake throughout this sleep duration is around minutes. So you can also see here that the percentage in stage N is % and stage is %, then in the non REM stage so the deep sleep, it's %. In the REM sleep it's about %. If we group data in the literature in a different way, the literature of cases, so only one child being reported in the scientific paper, or two or three or a couple of children reported in the literature, if we combine all those data, they also come to a sleep clinic, they sleep about hours. The sleep onset latency to the time to fall asleep is about minutes. And you can see in these cases, the time wake after sleep onset is much longer. It's almost minutes. And what you can see again in the distribution of the sleep. proportional sleep stages, it's % in stage and %, almost % in the REM sleep. Then we go into those only affected by the MECP a characteristic or group based on this genotype, so here you see the sleep duration is . hours in a sleep clinic, the time to fall asleep around minutes, the time awake after sleep onset is about minutes. And then you see again the distribution for the stage N and also for the REM sleep. Now, in the literature we also see that the cases or the reports are on children or on adults that come to the sleep clinic because of sleep disordered breathing. So we split the results into those without sleep disordered breathing, because sleep disordered breathing is affecting the structure of sleep, the quality of sleep. And so these individuals slept around hours, took about minutes to fall asleep and minutes awake after sleep onset. You can see here that the sleep proportion again of stage N stage N stage N and then of the REM sleep. Now, what is interesting, as I jus said, a large proportion of individuals with Rett syndrome have sleep disorder breathing. You can see the severe sleep disorder breathing in childhood is almost % of them suffer sleep disorder breathing. So if you look at the sleep structure of these individuals, they sleep around, hours, minutes to fall asleep. They had a huge sleep need, the pressure was clearly there. And then you have minutes of awake during the sleep period. And you can see that the stage N also the lighter non REM sleep,

is %, as well as the stage N is .%. And then you have % in REM sleep. So, if we compare that statistically to these typically developing children, then we can conclude that individuals with Rett syndrome spend much more time awake during this sleep period. So fragmented awakenings during the night, during sleep period, that the spending time in the stage N is slightly longer than is typically developing, that they spend much more time in the deep sleep, and that this REM sleep is also significantly shorter. So if I now show those structures, those hypograms of individuals with Rett syndrome, you actually see here that for this girl it took a long time to fall asleep. And you can see immediately here that she spent a lot of time awake during this sleep recording during the sleep period, and not that many hours, or not that much time in the non REM sleep. The second one has much more in the non REM deep sleep and some periods of REM sleep, whereas the other one had a lot of fragmented sleep, a lot of short awakenings, in contrast to the top one. So what we can see, and as a complaint, you might come with, okay, my child, my girl with Rett syndrome has difficulties falling asleep, has difficulties maintaining her sleep, and as a consequence of this poor sleep, may show more sleepiness, sombalance during the daytime. So what can be done and who to consult?

So, if there is a sleep complaint, all sleep experts will first try to figure out what is the sleep duration. So how much time does this person spend to sleep? What is the sleep quality, are there certain sleep behaviors, are there certain sleep elementsm issues, that we need to know about? Like is there snoring, is there difficulties with breathing, is there teeth grinding?

And then we will also ask apart from what the sleep is like, how does this relate to daytime function? So how sleepy are they during the daytime, are they more moody, are they more irritable? And what we also will look for is when the sleep falls. So the timing of sleep in our hour rhythm, because all combined makes that cocktail, as I just showed on one of the previous slides.

So when you come with a sleep complaint, it is ideal that you first have kept a little bit of a diary for around two weeks, where you make a note of the bedtime, the getting up time, the number of wakings, how long the wakings were, certain behaviors or certain things that you notice or you observe. And you can write it down in that diary. We will also ask you to talk to

your primary care physician about it, because often with individuals with a neurodevelopmental disability, that they will think that the sleep issue is a comorbid element, as a comorbid symptom. But first of all, if that is the case, we need to consider and discuss this and talk about potential management.

Sometimes, like sleep disorder breathing, it could be that treating the sleep disorder breathing and the quality of sleep will improve and also the daytime symptoms will disappear. So first talk with your primary care physician, talk to the treating physician, talk to the multidisciplinary team and discuss the current pharmacological treatment, discuss the medications the child is currently on.

What we will also recommend is that there is a medical checkup and a medical checkup, for example, iron deficiency, because it can be associated to certain sleep disorders. Now, the first line treatment for any sleep disorders and primarily for those with insomnia with difficulties falling asleep, difficulties maintaining asleep, and difficulties with waking up too early. Our first line approach is sleep hygiene and behavioral treatments or behavioral approaches. So having a regular bedtime, making sure that the bedroom is quiet, is dark, is relaxing, comfortable temperature, comfortable bedding, positive routines to go to bed, and then daytime activities that promote optimal sleep. Keep the bedroom for sleep.

Now, pharmacological management could be and it's reported in the literature, a few cases are reported with respect to Rett syndrome that is, melatonin or a dopamine agonist or GABA agonist or polytherapy. But I would really like to stress that there's too little or too few studies on pharmacological management in Rett syndrome to make any firm recommendations.

So I would highly recommend you to talk with your treating physician, to talk with the multidisciplinary team, and to check the current pharmacological treatment and to implement all these behavioral approaches to the little issues that might surface and then take it step by step to work towards an optimal sleep of your child.

So with this, I'd like to thank you for your attention and I'm looking forward to your questions.

Thank you very much.

Epilepsy in Rett Syndrome

Good morning and thank you for inviting me to this international Rett syndrome meeting. Today I'm going to talk about epilepsy in Rett syndrome. Epilepsy is one of the major issues for clinicians and also for families of people with Rett syndrome. What is epilepsy? Epilepsy is a chronic noncommunicable disease of the brain and it affects around 50 million people worldwide. Epilepsy is characterized by the recurrence of seizures which can involve part of the brain and in this case we talk about focal seizures or the entire brain and we talk about generalized seizures. The seizures can be associated with loss of consciousness and different clinical science. Indeed, epilepsies comprise a broad group of disorders with different sociologies electroclinical presentations and also marked variability in their clinical outcomes. In patients with intellectual disability the prevalence of epilepsy is around 20% compared to 1% in the general population. So epilepsy is the most common health need in this population. But especially in intellectual disability not everything that shakes is epilepsy. And so the differential diagnosis of paroxysmal events may be particularly challenging. And this is the case in children and individuals with intellectual disability. And this is particularly the case of people with Rett syndrome. We know from this historical paper by Dr Glaze that when we monitor patients with Rett syndrome with video EEG recordings, a high number of typical seizures, 42% of typical seizures were not associated with a clear, EEG discharge. So what we think to be an epileptic seizure as a matter of fact is another event especially when motor signs are present or where there is a behavioral arrest, stareing or breath holding spells which can be misinterpreted as epileptic seizures.

So first of all, we should be aware that people with Rett may show different paroxysmal events and not all of these events are seizures.

When we look at epilepsy in classic Rett syndrome such as in this paper dealing with more than 1000 female patients, we found that around 70% of patients with Rett may show epilepsy and around 30% of these patients have a drug resistant epilepsy which is in line with the general population with epilepsy.

In this longitudinal study by American authors the prevalence of epilepsy during the lifetime of people with Rett approached 19% and as a matter of fact, only a small percentage of women with syndrome 30% were seizure free and of anti seizure medication. So, epilepsy can occur throughout the lifetime of people with Rett syndrome and only in a small number of cases we are able to get them off medications without a recurrence of seizures. Now to talk about the management of seizures in patients with Rett we usually use conventional anti seizure medications especially carbemazepine, valporic acid or lamotrigene depending on the type of seizures. In this study conducted on Italian patients with Rett syndrome we found that lamotrigene could be particularly effective in patients in whom epilepsy started later than typical age. And also we found that the association between valporic acid and lamotrigene could be a good option when seizures are drug resistant.

We also know that in classic Rett, epilepsy has different characteristics depending on the age of the patients. And so in younger girls, seizures can be treated with a monotherapy, in most cases with valporic acid. Then, between five and 15 years of age, seizures are more frequently drug resistant and we need to use more than one anti seizure medication.

But after 15- 20 years of age, seizures are usually better control and the most used anti seizure medications are carbemazepine and lamotrigene. What do you do when seizures are drug resistant? Indeed, in the last years the introduction of new anti seizure medications has enabled us to improve seizure control. Also in subjects with Rett syndrome, we have data about the use of levetiracetam, zonisamide, topiramate lacosamide, perampanel or rufinamide as add on medications in patients with Rett syndrome and drug resistance seizures. We all know that anti seizure medication may have side effects. The spectrum and the rate of side effects of anti seizure medications in people with Rett is comparable to what we see in other patients with epilepsy. But people with Rett syndrome may show the presence of comorbidities that can cause some difficulties in the choice of the drug in order to not worsen the quality of life of girls with Rett syndrome.

So we know that valporic acid may increase the risk of fractures, that it may increase their irritability, or benzodiazepines, may worsen respiratory function and increased somnolence during the day, thus reducing alertness and participation in daily activities.

So we should be aware of these side effects and take them in to account in order to decide the best treatment for each patient. In very recent years, the introduction of cannabidiol and its derivative has brought new hope for the treatment of drug resistant seizures and specifically in girls with Rett syndrome. We have some initial information regarding the use of cannabidivarin which is a derivative of cannabidiol in a small number of patients, only five children up to now. But this drug reduced the frequency of seizure and showed only mild side effects, an increase in somnbolence and in drooling. So in the future, the possibility of using cannabidiol in patients with Rett will also considered in patients who have drug resistant seizures.

We can also use an alternative treatment for epilepsy, such as the Ketogenic diet, which is a high fat, low carbohydrate, adequate protein diet that can be prescribed in patients with drug resistant seizures.

The Ketogenic Diet is compliance demanding and requires a high degree of medical and dietetic monitoring, so it can be used only in a small number of patients. Another therapeutic option is vagus nerve simulation, which requires a surgical implantation of electrodes around the cervical vagus nerve connected to a stimulating device implanted under the anterial chest wall. Vagus nerve stimulation has been used in patients with Rett and it was safe and effective. It also improved their quality of life. So this is the surgical option that can in drug resistant seizures in Rett. I have a few words about epilepsy in CDKL5 deficiency disorder. In this disorder, epilepsy is often severe with early onset seizures. Seizures are typically polymorphic. That means that a patient can show different types of seizures, epileptic spasms, generalized tonic seizures, focal seizures, myclonic seizures etc. So the treatment depends on seizures type and almost all anti seizure medications have been used in CDKL5 deficiency disorders. And we know that ketogenic diet may be effective in these patients. And also cannabidiol can be particularly effective in CDKL5 disorders. So cannabidiol should be offered for epilepsy according to the regulatory requirements in each country for CDKL5 disorders.

We also know that subjects may show honeymoon periods during which time patients are seizure free for weeks, months or even years.

And this may be initially related to a specific anti seizure drug, but this is characteristic of the disorder. So second message is that epilepsy is a prominent symptom in people with classic Rett and also in CDKL5 disorder. The majority of patients usually reach seizure control with conventional anti seizure medications, but up to 30% of patients are drug resistant. And in patients with drug resistant seizures, new anti seizure medication could be potential aids as well as ketogenic and vagus nerve simulation. At the moment, regarding cannabidiol, we can only rely on preliminary findings in girls with Rett, but this drug may offer a new treatment option for people with Rett. Thank you for your attention.

Control of Breathing

Hi, I'm Ana Abdala Sheikh and I'm a senior lecturer at the University of Bristol. My research focuses on how the brain generates breathing and controls it. And I'm interested in a number of conditions where breathing is disrupted, one of them including Rett syndrome. Over the years, my team has used Mouse models to try and understand the breathing disturbances and try and find treatments for them. But today I will be focusing on the more practical aspects regarding the breathing dysfunction. The most described breathing problem in people with Rett syndrome are apnea. So, apneas are defined as a cessation in breathing when the person with the syndrome is awake. Central apneas are the most common. So, these happen when the brain stops generating the effort to breathe that contracts the diaphragm, and therefore the breath is absent. Central apneas can also happen during sleep, but in people with Rett syndrome, they are usually a lot less frequent than when they are awake during sleep. Obstructive apnea and hypopneas are more common. Obstructive apneas occur when muscles and the soft tissue on the back of the throat collapse during sleep, causing a blockage in the upper airway. This means that air doesn't move into the lungs, even though the chest muscles in the diaphragm may still be contracting.

If the blockage is only partial and stops airflow by about half, this is considered a hypopnea.

So how common is it for people with Rett syndrome to experience apnea when they are awake? The Natural History study tried to establish that, they followed 778 females with classic Rett syndrome for up to nine years. They found that over the lifetime, it was estimated that about

nine out of ten people with typical Rett syndrome would develop a breathing problem at some point in their lives. The plot on the screen shows on the vertical axis the percentage of participants suffering from apneas. And on the horizontal bars, you can see the percentages in different age groups. So this data suggests that the prevalence of apnea in adults may be lower than in younger ages. So we know that apneas are very common in people with Rett syndrome, but are they actually harmful?

Transient abnormal breathing is not necessarily bad for the health as long as it does not change blood gasses and the heart rate. However, early data shared by the STARS Clinical Trial revealed that people with Rett syndrome can experience periods of abnormal blood gases. So this study used an at home recording device to record people for 6 hours over a period of three days. They were able to do these recordings in 102 study participants. So this plot is some of the early data that they shared. And you can see here on the vertical axis the number of episodes per hour. And the column on the left are the apnea. So, those are periods where breathing stops for longer than 10 seconds.

And the column on the right are the blood oxygen saturation. So these are periods where it was below 90%. So this is considered clinically worrying, clinically relevant. The dots here on those columns indicate the maximum, the minimum and the medium, with the medium being a point where 50% of the participants were either above or below it.

So you can see for oxygen saturation, for low oxygen saturation, actually not a large number of participants showed worrying levels of low oxygen saturation, so the episodes were very infrequent. So, you can see that there have been a number of large studies investigating the breathing dysfunction that happens when people with Rett syndrome are awake. However, studies looking at the sleep breathing disturbance are fewer and they also have enrolled a smaller number of participants. But recently this group here on the screen published a meta analysis where they took information from all these different smaller studies that were performed looking at the breathing disturbance at night and they performed a new statistical analysis based on the data combining all these different studies. So, we have information then that is grouped for a larger number of participants. And what they found was that sleep apneas are also very prevalent, very common in people with Rett syndrome. And quite often they can

be of a severity that warrants clinical intervention. So this table here on the screen shows you the apnea hypopnea index. So these are periods where breathing stops or is insufficient and they compared, many of these studies had comparisons with age matched neurotypical controls. So you can see here on the middle column the expected values for somebody who's neurotypical and the values that they found through this new analysis using a larger group of patients with Rett syndrome. So, we have covered the different types of breathing dysfunction in Rett syndrome and how common they are and their severity.

But what causes them? The breathing dysfunction, especially the one that happens during wakefulness, is caused mainly by the abnormal development of the brain stem due to the lack of MECP2 protein activity. So, the brain stem is the part of the brain that controls the breathing muscles in the body. So, this includes the muscles in the chest and also in the upper airways. Although this abnormal brain stem development will also contribute to the breathing dysfunction during sleep, there are other factors that can contribute to this that are independent of having Rett syndrome. So, there are also risk factors for obstructive sleep apnea in the neurotypical population and this includes being over the age of 40. So, we know that as we age, the risk of having obstructive sleep apnea increases directly with age. And it's almost ubiquitous in people over 90 having a narrow airway such as large tonsils adenoids or a tongue, having nasal congestion or a small lower jaw. So these are things that can affect children who are neurotypical and can also cause sleep disordered breathing during sleep in neurotypical children having a severe scoliosis. So, that is another thing we know is very common in Rett syndrome, especially those very severe scoliosis angles. They compromise the functioning of the breathing muscles in the chest and the volume of the lung and they can make disordered breathing during sleep more common to you being overweight or having too much fat around the neck. So, this is not necessarily a common problem found in Rett syndrome, but it's something to watch out for that can increase the risk and taking certain medicines. So, particularly those that have a sedative effect that depress the brain, such as sleeping tablets and anti anxiety medications and some of the anti epilpesy medications. So again, those types of medications can be common in the population with Rett syndrome and it is a risk factor to watch out for. Of course, the best treatment for the breathing disorder in Rett syndrome would be gene therapy to establish the

function of the MECP2 protein in the brain stem. But while this is not available, there are other ways where breathing symptoms can be managed either with devices or not using drugs or through drugs. The treatment will always be prescribed by a specialist only after a diagnostic assessment. This sleep breathing disorder is probably the easier one to manage compared to your weak breathing dysfunction, although these devices have been used to help both. So there are various types of intervention listed here on your screen, as you can see, and they have all been used to manage the breathing disorders in Rett syndrome.

And you can see the references on the right there. However, most of these studies were done in small groups of participants and they weren't really designed to evaluate efficacy. However, common sense indicates that most of these interventions are likely to be useful for managing the breathing disorder in Rett syndrome, as they are indeed used to manage sleep breathing disorder in people who are neurotypical with excellence efficacy. So, some of the interventions you can see here include continuous positive airway pressure or CPAP. So this is used to keep the upper airway open in those cases where the airway collapses during sleep.

We also have non invasive assisted ventilation or NIV. And this is used when there is no drive to breathe so when patients experience central apneas and these devices are able to generate breaths for the individual. In some cases where the upper airways may be narrow or in cases where there is very severe scoliosis, then surgery may be recommended to try and improve breathing on the individuals who hyperventilate. So that's a slightly different problem. They often suffer from negative symptoms of having high CO2 in their blood. So as I mentioned before, either not breathing enough or having hyperventilation can shift your blood gases in directions that are harmful. So in the case of hyperventilation, the individuals can experience very low levels of CO2 in their blood, which can cause some symptoms like dizziness, but also muscle spasms, which could be confused with seizures. So in those cases, again, a handful of cases, CO2 has been successfully supplemented via inhalation canula or sometimes via rebreathing masks. And they can help abate those symptoms from low CO2. Although those devices are potentially very efficacious and devoid of side effects, not everyone tolerates them.

So, the drugs that have been shown in the literature to have some benefit for some individuals with Rett syndrome include drugs from a family called carbonic anhydrase inhibitors. So, what those drugs are doing is mimicking the presence of normal levels of CO2 in the blood.

It's actually tricking the brain into believing that there is more CO2 around increasing the drive to breathe. So, the other class of drugs that have been used are those drugs that mimic a brain chemical called serotonin. So serotonin is important for several brain functions, including the control of breathing. And there are studies in the literature both in people with Rett syndrome, but also using the Mouse models, indicating that drugs that act like 5HT1A agonists, so those are drugs that bind and activate a certain receptor in the brain that's called 5HT1A have been shown to improve very severe, very acute episodes of breathing disturbance. Another class of drugs in that family are the selective serotonin reuptake inhibitors. So, they increase the availability of natural serotonin that the brain is producing. They make it hang around for a bit longer so that it can act on its receptors. So this class of drugs have shown some benefit in the literature. It's also worth remembering that some drugs, not just the ones I've listed here, can have a negative impact on the heart rhythm. For neurotypical individuals, that's usually not really a problem. It's a very small effect and very unlikely to cause problems. But the heart rhythm of people with Rett syndrome shows already some baseline abnormalities in some cases. And people with Rett syndrome may be particularly vulnerable to the cardiac effect of these drugs. So, this would be an adverse effect of these drugs. One example is fluoxetine has a high propensity to cause those problems.

So, when those drugs are being prescribed in people with Rett syndrome, it's really, really important to have a baseline assessment of the heart rhythm to make sure that individual already doesn't have a severe problem. And then again, a reassessment once the drug treatment is started, to make sure that the drug is not tipping that vulnerability over the edge into a level that where it could be problematic. Those types of heart abnormalities of heart rhythm caused by those drugs have been associated with certain cardiac deaths, so it's not something to be taken lightly. So today we learned that the incidence of breathing problems is high, but they can come and go during life. The severity of the breathing problems can also change with age, and

particularly during key developmental stages like puberty and during menopause. So reassessments may be required and a rethinking of the treatment strategies with it.
The awake. But particularly these sleep breathing dysfunctions are both manageable and treating them may well improve other neurological symptoms. Although we still have don't have direct evidence of this in the literature, it is sensible and reasonable to hypothesize that.
However, when prescribing drugs, one should always mind the cardiac risks are not just drugs to manage breathing, certain antibiotics also come with a cardiac risk. So, this should always be taken into consideration when prescribing Rett syndrome and assessments of cardiac risk before prescription and after are highly recommended. Finally, I would just like to highlight that other issues are often underestimated when it comes to the severity of the breathing disturbances. And through experience we have found that a very sudden severe exacerbation of the breathing disturbance can always, always be tracked back to either pain or a behavioral or an emotional issue. So we shouldn't really underestimate the effect of mood and emotion on the breathing disturbance in Rett syndrome. To finalize, I would like to acknowledge the funders and the collaborators who contribute to the research in my lab over the years. And also I would like to thank you for your attention.

Gastrointestinal Myths and Misconceptions in Rett Syndrome

Good morning, and thank you for the invitation to speak to you today. My name is Dr. Kathleen Motil. I'm from Baylor College of Medicine in Houston, Texas. The title of my presentation is Gastrointestinal Myths and Misconceptions in Rett Syndrome. I want to disclose that I'm a consultant for Acadia, the company that sponsored Trofinetide. I have no financial relationships with manufacturers of commercial products, I will not discuss the unapproved use of commercial products, and the International Rett Syndrome Foundation has provided funding for my rRett related research activities.

The objectives of my talk are one, to highlight the clinical features of two common gastrointestinal problems in Rett feeding difficulty and constipation, and the second, to describe treatment approaches to feeding difficulty and constipation. Parents often have misconceptions about feeding problems and bowel habits in their children with Rett. My daughter has a great

appetite. She eats all the time, but she doesn't gain weight. My daughter won't go to the bathroom unless I give her something to make her go. I don't want her to become addicted. These are often two common concerns that I hear about the girls with Rett syndrome. It's important to know that gastrointestinal symptoms complicate the clinical course and quality of life of individuals with Rett throughout the Rett lifespan. Gastrointestinal problems pose substantial medical burdens for the caregivers of individuals with threat, and the gastrointestinal manifestations may be more debilitating than the underlying neurological features of Rett. Parents often say, my daughter has a good appetite. She eats a lot. However, girls with Rett syndrome have feeding difficulty. Girls with Rett have a prolonged feeding time, taking approximately 30 to 60 minutes to finish a meal. They have decreased oral efficiency due to chewing and swallowing dysfunction. Oral dysfunction leads to decreased dietary energy and nutrient intakes, and decreased dietary intake leads to poor weight gain. We can evaluate feeding difficulty by a visual feeding study. We ask about coughing or choking when drinking fluids or eating food, and we ask about increased drooling, both of which indicate difficulty swallowing. We can evaluate feeding difficulty with a swallow function study, which is a radiographic study shown here in this slide. We will evaluate poor jaw movement, poor tongue lateralization, poor food propulsion, pulling of fluids or food in the throat, delayed initiation of the swallow reflex or laryngeal penetration, and tracheal aspiration of fluids and food therapy to improve oral feeding skills is limited. We focus on postural alignment, oral desensitization, jaw stabilization, and tongue organization. No loss of function is good progress. We can thicken liquids to facilitate swallowing. We use finely chopped or pureed foods to facilitate chewing. Appetite stimulants are generally not useful. There is no apparent benefit with Vital stem, a device that is used to stimulate swallowing, and we encourage parents to learn cardiopulmonary resuscitation CPR in the event of aspiration. Abnormal growth, shown in the blue lines on this slide, is a pervasive problem in Rett. Weight deviation begins as early as six months of age, length deviation occurs by 17 months of age.

The pivotal increase in height and weight is absent in Rett. Poor growth is associated with greater developmental delay and higher disease severity. The growth abnormalities are due in part to poor nutrition. The body mass index, or the BMI, is the standard method to assess

nutritional status in Rett syndrome. Body mass index is the ratio of weight to height or length. The BMI for pre pubertile RET girls when normal, is between the 25th and 50th percentile and for the post pupertal Rett girls normal is between the fifth and 25th percentile. We consider alternative feeding methods if the BMI is less than the fifth percentile or off the graph as shown in this particular slide. Energy imbalance accounts in part for growth abnormalities in Rett. Dietary energy consumption is approximately 30% lower in RET than in unaffected girls. Total daily energy expenditure and sleeping metabolic rates are approximately 15% lower in Rett than in unaffected individuals. Energy expenditure associated with physical activity is reduced in Rett and there is no evidence of energy losses by malabsorption in Rett. Treatment approaches to feeding difficulty include oral supplements of blenderized, food and formulas or gastrostomy button placement. Dietary energy needs for catch up growth are approximately 130% to 140% of the resting metabolic rate. The indications for gastrostomy button placement include a BMI persistently following less than the fifth percentile for more than six months significant chewing and swallowing dysfunction with or without aspiration or parental request for freedom in feeding. Refusal constipation is defined as difficult defecation with straining during stool elimination.

Bowel supplements bowel movements are usually less than two times per week and stool consistency is hard. Small pebbles or large bowl size, blood may be seen on the stool. The cause of constipation is probably multifactorial and includes abnormal nerve muscle function and dysbiosis of the gut microbiome. Complications of constipation include anal fissures and fecal impactions. Adverse outcomes of constipation include abdominal pain, feeding refusal, urinary tract infections and increased frequency of seizures. This slide illustrates the differences in intestinal neurotransmitters in the interior nervous system in Rett syndrome. Intestinal neurotransmitters, such as nitric oxide is expressed in greater amounts in Rett shown in the top slide than in control cases shown in the bottom slide. This observation supports the concept that there is dysregular regulation of nerve function in the girls with Rett syndrome. This slide illustrates differences in the gut microbiome in Rett based on clinical severity.

Mild to moderate clinical severity is shown on the left panel and severe clinical severity is shown on the right panel. The relative abundance of bifidobacterium, a type of bacteria, is higher than the relative abundance of FICO bacterium more severely affected than in less severely affected girls. This slide and this difference supports the concept that dysbiosis of the gut microbiome contributes to intestinal dysfunction in Rett syndrome. This slide illustrates the differences in the gut bacterial metabolome in Rett shown in red on the slide and unaffected girls shown in blue on the slide. The fecal concentration of two neurotransmitters, gamma aminobutyric acid and glutamate are lower in red than in unaffected girls. The fecal concentrations of two neurotransmitter precursors, kerosene and tryptophan, are lower in Rett than in unaffected girls. This observation supports the concept that alterations in the gut metabolome contribute to intestinal dysfunction in Rett.

The assessment of constipation includes an abdominal and rectal exam. We may obtain an abdominal X ray to look for large amounts of impact stool in the rectal vault shown here in this slide. We may obtain a barium enema to look for a redundant colon. Parents often say I want something natural to treat constipation, but natural options usually are not enough in Rett syndrome, dietary fibre in fruits, vegetables and cereals may be helpful. Fruits that contain sorbitol, such as prunes or pears may also be helpful. Prebiotics such as psyllium or flax seed may be helpful. Probiotics that contain lactobacillus, rhamnosus, lactobacillus, L.reuterii or bifidobacterium may be helpful. But parents need to beware of herbals that contain licorice because licorice may aggravate the prolonged QTc abnormality in Rett syndrome, medications that soften, push and pull individually or in combination are used to treat constipation.

However, softeners such as polyethylene, glycol, lactulose, and mineral oil are slow to act, require frequent dosing, and may produce gas. Pushers such as milk of magnesia and sennosides have dosing limits to avoid side effects. Pullers such as glycerin, bisacodyl or docusate suppositories are useful and used with increasing frequency as age increases.

The surgical approach to constipation includes psychosomy, button placement or colonic resection for colonic redundancy or atony. And finally, physical activities such as walking, standing or physical therapy should be encouraged. In summary, feeding difficulty and

constipation are the two most common gastrointestinal problems in Rett. Despite parental fears and frustrations, treatment strategies for feeding and constipation improve improve the quality of life of individuals with Rett. Thank you.

Physical Therapy Interventions in Rett Syndrome

Hi there. My name is Meir Lotan and I'm a physiotherapist from Israel. And today's talk is about how can physical therapy enhance your child's abilities. So physical therapy is all about function and physical activity is actually health. So for your child with Rett syndrome, physical activity is basically medicine. So if you keep on doing physical activity, you keep your child as healthy as possible.

So what can your child with Rett syndrome gain from physical therapy? So, first of all, scoliosis. This is an article that we published last year talking about an intensive postural and motor activity program which was found to reduce scoliosis progression in girls and women with Rett syndrome. So an intensive program of a few months can do that. Improve core activity. If we can improve core activity, then actually we can improve the stability and postural control of the child. And here you see a child who is not able to sit by herself outside the wheelchair, but here on a moving surface on a vestibular plate, she is able to maintain stability and actually work with her core muscles. And this can also be done with girls who are much more high functioning. As you can see in this video, this girl is standing on a stool on a vestibular plate while movin and at the same time is able to reach dow and grab her favorite doll at her feet. During physiotherapy, we can improve mobility. In this case, we are looking at the Upsee and you can see that with the Upsee, the mother can go around the house with the child. She can introduce different parts of the house. The child can dance with her baby brother and also can go outside of the house to the playground and like any other child, go out to the playground and extend her world to other places that she couldn't do before. Physical fitness is another thing you can do. Here you see a girl walking on a treadmill. And in the program that we did, girls with Rett who walked for one month on a treadmill for 30 minutes a day were able to improve their physical fitness and at the same time improve their functional ability. Fear of movement is something that is typical for many with girls with Rett syndrome. And on the video you can

see this girl walking by herself on the beach. But when we got to her house at the beginning, this girl had to be held by her parents for 20 minutes every time she woke up because she was so afraid of the movement.

And after three months of an intensive program of dancing and movement, you can see this girl who was actually wheelchair bound, who now is able to walk by herself on the beach without any problems.

Another problem that is typical for some with Rett syndrome is hip subluxation. And here you can see that this girl at the beginning of the program, she visited an orthopedic surgeon who told her that she actually needs to have very an intensive hip repositioning surgery. And by intervening with an intensive program for a year and a half, we were able to reduce the subluxation up to very small measure. So the surgery was postponed and she doesn't have to do the surgery. Another thing we can do in physiotherapy is regain walking ability. When I first met this girl, she was sitting in the wheelchair for five years, and after, again, an intensive program. This is a video describing her first steps out of the wheelchair. So this girl was sitting five years in a wheelchair, and with an intensive program of walking and stretching, she was able to regain her walking abilities. And all the things I described to you just now are things that a few years ago, you needed the physiotherapist to be there, to be present. And now with technology, all those things can be achieved by remote rehabilitation. So I can be in Israel and supervise a program that can go on in any other country in the world. So what other areas can PT be useful for your child with Rett, feeding, sitting, osteoporosis, adjusting technology, aging, apraxia, ataxia, and much more. So my message to you today from here is stay as active as possible so you can walk up and downstairs, you can be active in the Snoezelen, which is a multisensory environment.

You can be active in the swimming pool. It could be during hydrotherapy, but it also can be just taking your child to a pool near your house. And a child who cannot walk on land can maybe be able to walk in the water and maybe learn how to swim and stay active in the water. Hippotherapy is another way to keep your child more active and even skiing. Here you see a child with Rett syndrome skiing with a ski instructor. And this is actually an amazing video of

a child with Rett syndrome doing skiing. Take walks with your child with Rett syndrome in the woods, on the beach, everywhere. Go to the mall, go to a playground near your house to visit, family members and neighbors. Thank you for listening and stay as active as possible.

Thank you.

Communication Continued: Well-being and Emotional Competence

Hello, I'm Helena Wandiin, speech and language therapist at the National Center for Rett Syndrome and Related Disorders, and I'm also an associate researcher at Utsala University. I would like to thank the organizing group for the opportunity to present on this busy event and Dr. Gill Townend who has provided an overview of research on communication in Rett syndrome and I will continue with a narrower scope and talk about wellbeing and emotional competence and how they relate to communication and AAC. And I chose this subject because I think this is an important subject for people with Rett syndrome and their families and there is very little research in this area. So this is an attempt to encourage conversations among families and researchers and encourage research on this subject.

So this is the first key message. Psychological well being needs to be addressed. As Dr. Santosh has already talked about, anxiety and low mood is an issue in Rett syndrome. And Buchanan and colleagues found that 77% of the participants were reported to show or had shown anxiety or anxiety like behaviors. It's common. And these experienced colleagues found that 38% of adult women had been or were treated with antidepressant or antipsychotic medication. So it is an issue that we need to address and to support psychological wellbeing. Different approaches can be taken and pharmacological treatment is one, behavior activation is another. And this means that the individual has Access to activities that are meaningful to the individual and that the individual also feels that he or she are in control during the activity.

And a third approach is to support emotional competence, which is what this presentation is about. And the second key message is that emotional competence develops through interactions and experiences as many other things. And emotional competence is to be aware of one's own and others emotional state and also to know the label of emotions, the names of emotions, for

example, sad, proud, nervous. Emotional competence also involves being able to talk about emotions and the reasons behind the emotions and how to manage them.

What can I do? What do I need?

And it also includes to be aware that other people can have other emotional experiences than oneself. And the researchers you can see below on the slide belongs to two research groups that are currently evaluating and developing intervention to support emotional competence. The third key message is that most effective learning takes place when the individual is in a calm state. So it's important to find and creative opportunities where the individual is not agitated, because talking about emotions when someone is agitated may not facilitate learning. So suggested activities may be when reading books, watching videos, family dinner, conversations, etc. And nine colleagues developed an systematic interview Early Development of Emotional Competence, an interview that can be used when planning interventions. And the interview includes questions about temperamental behaviors, the individual's reactions and how they express emotions and how they recognize emotions in others and also how communication partners respond to the individual's emotional expressions, but also their input, how and what they talk about regarding emotional competence or emotions. And the other research group, Rangel Rodriguez and colleagues, developed a profile Early Development of Emotional Competence profile and it's a template for collecting information and presenting the information from the interview.

And in the template there's a section about me about the individual, how they communicate, what emotions they express and how and what their communication partners talk about and how they do it, and also goals and strategies to develop emotional competence. And nine colleagues developed Steps strategies for talking about emotions as partners. It is a programme to encourage communication partners to talk about emotions and they encourage support partners to provide and model labels for a variety of emotions and to validate, that is, to accept and discuss emotions and also communicate about the research and appropriate responses to emotions.

And both research groups have evaluated this programme and both groups have evaluated using steps while reading books, while in shared book reading and to the right of the slide you can see an overview. So they encourage partners to talk about the label to emotions, the reasons to emotions and the solutions. And while doing this, they also have this chain of strategies to ask or comment and models or a copy of the individual system communication system to model the emotions and then wait, provide wait time and then respond to the individual's signals. Either if the individual say something themselves, Express something, or they either respond by providing their own suggestions and both parents and children talked more about emotions after the intervention. I would like to really encourage anyone that is interested to read these articles because they are very practical, concrete examples of how to support emotional competence.

I would like to finish with this case study by Bhattacharya and colleagues because it describes very nicely how a young girl with Rett syndrome used her emotional competence and her AAC system to talk about emotions and advocate for herself. Before the regression, she had 80 spoken words and she received her eye gaze device by the age of three and then it was used daily. And the case study presents data from her communication software and care giver diaries and she was able to take active part in medical examinations, describe symptoms and ask for information. And this is a really good summary of what emotional competence can do in everyday life. And with this I would like to say thank you for listening.

Occupational Therapy: Role in Rett Syndrome

I am Pam Deiner, an OT in Pediatrics, a developmental neuroscientist studying Rett syndrome, and a sibling to a 57 year old woman diagnosed with with Rett. Today I'll wear my OT hat to discuss the role of occupational therapy in Rett syndrome, focusing on how therapy uses functional tasks to minimize hands stereotypies, to encourage arm movements and their associated postural adjustments in order to to boost the client's attention for and participation in functional tasks in other words, activities of daily living. I'll also provide some examples of adaptive equipment that can be incorporated at home and at school. At its core, OT promotes health and wellbeing through occupation, which changes depending on your phase of life, but generally encompasses whatever the needs are for someone to be prepared for and involved in

their daily activities of selfcare or activities at play or within school. While individuals with Rett have both similarities and unique differences, most are generally dependent in their every day living skills. So it's the OT's role to determine how and to what extent each individual's deficit impacts function, and then craft a customized treatment plan centered upon improving motor and cognitive skills, providing positioning and when and where needed, adaptive equipment to increase participation in their Adapted Daily Living. ADLs are used because they are meaningful functional tasks that naturally train movement and cognition, as opposed to using rote exercises.

Naturally learning occurs because ADL performance relies on developing many of the building blocks of motor and cognitive skills such as sensory awareness, postural control, reaching, grasping, eye, hand coordination, sequencing, choice making, etc.

All areas of deficiency in Rett syndrome movement is then trained by breaking down ADL tasks into their component parts using a backward chaining approach. Tasks are taught incrementally, where the last step of the task, the rewarding step, is taught first. As you can see in this video clip, when an individual with Rett syndrome is handed a loaded fork near her mouth, she's practicing, only taking the fork to her mouth the last rewarding step.

We then progress to holding the fork closer to the plate to encourage reaching farther to the fork to complete more of the task independently until the whole task is learned.

This can take months, which is why goals are incremental so achievement can be celebrated more often, building confidence. This approach also enhances one's motivation and internal competition to do better, which in turn influences attention span, because each person now remains focused, trying harder, staying with the task longer as it no longer seems hopeless or unattainable. Finally, motor memory, response time and precision improve due to continued rehearsal and repetition of each step of the task. In this video clip, you may also observe that by working on functional tasks, postural skills such as sitting, erect or strengthened. This is because when we move our arms away from our body, core muscles become active to resist the shift in gravity as the weight of our arm moves.

In other words, our core stops us from falling in the direction of the reach and by training, visual and arm hand reaching and grasping. Using varied stimuli in various settings, such as at the dining table and maybe in the bath with bubbles, one demonstrates generalization of learning to any situation rather than just using reaching in one particular condition. Many of you may be thinking if the individual's hands are occupied, they can't reach your grasp. But OTS aim to combat these competing factors, such as stereotypies, loss of purposeful, arm use, dyspraxia and postural deficits, and even reduced attention spans because they all affect function and limit exploration of one's environment, which is how typically developing individuals should be learning. So to ensure best treatment outcomes, OTs work closely with families, educators and the rehabilitation teams to incorporate and integrate multidisciplinary goals related to all of the factors I just mentioned. We begin treatment by first meeting clients where they are and then enhancing their skills to promote independence and improve quality of life. Each session must be meaningful and motivating, so OTs refer to the parents and caregivers who know their child's interest and behavior best. We then observe and evaluate each individual to determine what sensory cues and motor training will be needed to advance their current abilities an minimize their compulsion to perform stereotypies.

Attention span can be addressed by adjusting the length of the sessions and the number of breaks given. We can incorporate music, which activates many cortical regions with its inherent rhythmicity. It encourages body sway, rhythmical muscle activity and vocalization. Keeping the individual engaged and alert and attending. Providing verbal or nonverbal instructions about every 10 seconds gives the individual time to successfully plan and execute a response. This accommodates dyspraxia but also improves attention as abilities to perform increase and to promote participation, independence, ease daily care and enhance quality of life at home caregivers participate in training to learn about techniques and adaptive equipment that were used in therapy. While therapeutic sessions are structured similarly as I've just described, they must be customized for each individual and carried out several times per week to provide a lot of practice. So let's look at some examples of how an OT incorporates these ideas in a treatment session to meet the individual where they are. That's the first step. We start with regulating or calming techniques to minimize anxiety often caused by the unfamiliar clinic or simply

transitioning to it. Some methods that we use include slow rocking on a therapy ball or hammock, providing calming through deep pressure by rolling a ball with moderate pressure over the body or bundling in a suspended swing where stretchy material adds extra deep pressure as it resists any spontaneous movement or massaging lotion into the skin of the arm, hands and legs. We can also stimulate the vestibular system that's located in the inner ear.

It's responsible for receiving sensory input about how we're moving through space and then guides our automatic responses to the direction, orientation and speed of those movements so that we achieve good postural control to develop the stable base upon which we can move our extremities. The OT facilitates strengthening of balance and core stability by eliciting these spontaneous or automatic reactions after stimulating or when stimulating the vestibular system. To do this, the client is placed on a therapy ball that we move in multiple directions. As you can see here, both core and arm responses help maintain balance on the ball, but you also see that the postural or protective arm responses interrupt midline stereotypies and facilitate the same movements that we need to reach in these multiple directions. Adding strong citrus scents is a technique that additionally activates proper postural head movements in the direction of the odor to further stimulate postural responses.

So repetition of this one activity stimulates the core, minimizes stereotypes, and produces movements that will be needed for reach training in ADLs. Varied sensory input can also be directed at stimulating the specific body parts needed to reach and grasp. We begin by applying deep pressure to the shoulder joints to activate the muscles needed to stabilize these proximal joints so that the shoulder supports the weight of the arm moving away from the body, we apply sensory cues to the skin directly to signal the arm and hand mitts to be used.

Large feather brushes or placing the client in ball pits can stimulate large surface areas, while soft prickly hand mitts that are placed on either hand can be guided to brush over the face or the arm and hands. So therefore, specific or precise body parts to grip the hand must recognize what's been placed in it. But often the item is in the same general temperature of the hand, so sensing its presence is hard. However, we can change the temperature of the palm by using warm or cold packs to heighten recognition of the object in the hand. Or we can place the item

in a warming blanket or in the freezer before placing it into the hand so you can see that stimulating the senses of touch, sound, vision and smell under various conditions in each session activates and integrates neural circuitry for receiving, processing and integrating sensory input needed to plan and execute motor responses. With the body, arm and hand ready, the OT can then begin facilitating active involvement in the functional ADL task. Specific ADL training begins with the individual's trunk and feet supported for stability when seat. After being seated in an appropriate chair, then stereotypies must be stopped in order to reach for the target. While many recommend the use of passive elbow extension, splints preference is towards and evidence from my research in this area indicates, that the use of less restrictive and more active methods leads to independent cessation of stereotypies and promotion of purposeful arm use. So we use verbal cues, demonstration, hand over hand assistance and gentle holding of the non active hand to promote concentration on the active hand or arm. As you can see here with the gentle hand holding that the arrows are indicating the OT senses. When the nonactive arm initiates movement to assist in accomplishing the task, we then can remove our hand to allow for follow through of this movement, or we can lightly assist in guiding that movement so that even the smallest of efforts are rewarded. This training occurs for both the right and the left arm. The self feeding clip and the image of switch use show that after many months of ADL sessions, independent cessation of hand wringing and grasping becomes a reality for some, even after strengthening the core, light trunk and pelvic supports are helpful, as you can see by this corner chair and its strap, or through the use of a wheelchair and lateral supports. These are helpful because they allow one to concentrate solely on arm use because the postüral supports are helping with the stable seating. During ADL training, adaptive devices may be used such as grip assistors or built up handles and wash mitts to help with self care needs when the individual's grip is poor or weak. Cut out cups can assist with oral motor skills to facilitate safe drinking by eliminating the need for head tilt, which can cause coughing and choking. Larger pieces of adaptive equipment for safety in the bathroom and for transfers between surfaces in the home and community can be trialled and discussed with Family and Caregivers, and your OT will first make recommendations and then demonstrate and train the use of the equipment to minimize anxiety that may be increased.

As new devices are trialled and trained, calming music can be incorporated. Finally, each OT session should end similarly to how it began with a check in with the Family and Caregivers in order to report on the day's progress, demonstrate and train activities on the home programme and discuss recommendations and parental concerns and answer any questions. So like we end in a therapy session, please feel free to share any of your thoughts or questions by sending an email to the address on the slide. Also listed is the URL for clinicaltrials gov. If you would like to learn more about my current research project, thank you so much for your attention this afternoon.

Music Therapy and People with Rett Syndrome

My name is Linn Johnels and I'm a music therapist at the Swedish National Center for Rett Syndrome and Related Disorders. I'm also a PhD student at the Department of Special Education. I would like to thank the organising group for the opportunity to present at this interesting event. In this presentation, I will use examples from recent research to describe the potential of using music therapy with people with Rett syndrome, and I will present two key messages. I would like to begin with a quote from Clive Robbins, pioneer within music therapy.

As the citation tells, there is something in us as human beings that is open to music, that needs music, and that is fulfilled by music. So what is music therapy? Here is the definition from the World Federation of Music Therapy and as you can see, it's a broad and inclusive definition in order to cover all the different aspects of music therapy. Music therapy is the professional use of music and its element as an intervention in medical, educational and everyday environments with individuals, groups, families or communities who seek to optimise their quality of life and improve their physical, social, communicative, emotional, intellectual and spiritual health and wellbeing. Key message one music is a big interest and motivates many people with Rett syndrome. Hence, it's a meaningful activity in and of itself, as well as a means for showing capacities and for development.

And already Andreas Rett recommended the use of music with people with Rett syndrome back in the 1960s, and many clinicians and music therapists continue to describe the large music interests in people with Rett syndrome. And as stressed in several studies, including the tool

listed here, music therapy can sometimes uncover hidden resources that may not be seen in standard educational or clinical assessments. And in particular, studies have shown that capacities relating to communication, cognition, learning and motor abilities can potentially be enhanced or retained through music therapy. The second key message I would like to raise is music therapy creates connection for people with Rett syndrome as well as for the social network. In a study from 2019, eleven families with a child or youth with Rett syndrome received music therapy in addition to standard treatments. During a period of 24 weeks, a comparison group of families only received the standard treatment.

This study showed that participants who received music therapy were more active in social interaction, had better results on communicative skills and hand functioning than the comparison group. And importantly, the parents in the intervention group also reported less stress. And I think that this is a crucial point since we know that parents of children with Rett syndrome and related disorders carry a heavy burden when it comes to care, constant meetings, etc. If we can find meaningful and inclusive activities that can be used in everyday settings, this is very worthwhile.

My co authors and I performed a scoping review regarding musical interaction therapy, together with children and youths with severe or profound intellectual and multiple disabilities. Where we targeted all peer reviewed research published in the last 20 years. People with Rett syndrome was the largest subgroup. One of the most interesting research questions concerned what was described or showed as promising components of musical interaction. The identified categories helped in answering the question what is it in music therapy that is especially helpful?

The first categorywas responsivity of interaction partner. This meant that the interaction partner or music therapist listened attentively, tuned in and followed the person's initiatives. It was emphasized that the music therapist's energy and vitality could motivate the child to be more actively engaged in the interaction. Moreover, the amount of energy and stimulation needed to be adjusted to the child's arousal and day to day variation of performance. The second category was singing songs. To sing the participants name and to sing instructions was raised as

motivating and supporting participation. The repetition in songs was reported to contribute to opportunities for learning and making choices.

Pauses in songs were also raised as important since they function as cues, giving the children possibilities to be active and take initiatives. The third category concerned structure and predictability in music activities. To have a flexibility within a set structure was described to be supportive for the participants comprehension, to enhance possibilities, to anticipate what was going to happen and hence allowing reciprocity in the interaction. The fourth category concerned the benefits of long term interventions. For many individuals, it takes a long time to adapt to and to appreciate something new. For the interaction partner, long term intervention can provide opportunities to learn how to identify subtle signs of change and development in the participant. The fifth category concerned the benefits of using multisensory and technology mediated music activities to motivate participation. This was reported to give participants new ways to express themselves, since many of these children had limited possibilities to master more traditional music instruments due to their motor disabilities. And the 6th category concerned the importance of a therapeutic alliance for the quality of the musical interaction. A secure and trusting relation was stressed as vital in order to support development in participants altogether. These insights contribute to the knowledge base and provide practical strategies for the use of music therapy with people with Rett syndrome.

Finally, I would like to show you a short video clip of a young woman named Emma, her assistant and myself, which I think illustrates the points I have tried to make. We are jamming a blue song called Emma's Song and Emma is playing the violin with her eyegaze control device, so let's see if it works.

Thank you very much for your attention and for listening to this presentation.

FULL TEXT PAPERS

RELATIONSHIP BETWEEN RETT SYNDROME AND MICROBIOTA

Asisst. Prof. Şebnem *Özgen ÖZKAYA*
Fenerbahce University, Faculty of Health Sciences, Istanbul/Turkey

Rett Syndrome (RS) is a serious and progressive neurological problem caused by mutations in the Methyl CpG Binding Protein 2 (MECP2) Gene, occurring in 1 in 10,000 live female births. Gait disturbance, microcephaly, autistic features, epilepsy, seizures, dystonia, respiratory problems and movement disorders are seen frequently in these patients. In addition, chewing and swallowing dysfunction, gastrointestinal problems (gastroesophageal reflux, gastroparesis, biliary tract disorders, gas bloating, constipation) and growth retardation are commonly reported symptoms in RS. Although growth retardation is multifactorial in these patients, insufficient food intake due to nutritional difficulties and excessive energy expenditure due to involuntary and frequent movements are the most important factors.

Intestinal microbiota is a complex ecosystem that includes all commensal, symbiotic and pathogenic microorganisms such as anaerobic bacteria, viruses, fungi, and protozoa and functions like an organ. Gut microbiota plays an important role in the function and integrity of the gastrointestinal tract, maintenance of immune homeostasis, energy metabolism and neurophysiological functions. As it known gut microbiota alter in a variety of neurological disorders, such as Autism Spectrum Disorder and Parkinson's Disease. Microbiota influences central nervous system function and the central nervous system influences the microbiota composition through its effects on the gastrointestinal tract. Studies has showed that microbial dysbiosis can initiate the formation of neurological diseases and affect the progression of the disease. Gamma-aminobutyric acid (GABA) is synthesized from "Lactobacillus and

Bifidobacteria", norepinephrine from "Escherichia, Bacillus and Sacromices", serotonin from "Escherichia and Enterococci" and dopamine from "Bacillus and Cerracials". *Lactobacillus acidophilus* increases the expression of cannabinoid receptors in the brain stem.

Nutritional problems, gastrointestinal dysmotility and antibiotic use also contribute to the development of dysbiosis in individuals with Rett Syndrome. In a study conducted in 50 individuals with RS in Italy, it was determined that microbial diversity decreased and *Bifidobacterium, Clostridia, Erysipelotrichaceae, Actinomyces, Lactobacillus, Enterococcus, Eggerthella, Escherichia/Shigella and Candida* increased compared to healthy individuals. It has been reported that Bacteroidetes and Firmicutes become dominant in the gut microbiota of female with RS compared to the control group in the USA. This indicates that the gut microbiota has become inflammatory and dysbiotic in RS. In addition, metabolomes such as GABA, tyrosine and glutamate were also found to be lower in individuals with RS. However, the mechanism of the gut microbiota in the pathophysiology of RS is not clear yet.

As a result, the decrease in the diversity of the microorganism community in the intestine or the increase in pathogenic microorganisms affect the central nervous system. Disease-specific problems in individuals with RS can cause dysbiosis. In addition, the central nervous system may influence microbiota composition via the brain-intestinal axis. This may lead to an increase in the severity of neurological problems in individuals with RS. Future studies should focus on the relationship between increased gut microbial diversity and neurological symptoms in individuals with RS.

References

Dhami, M., Raj, K., Singh, S. (2023). Relevance of Gut Microbiota to Alzheimer's Disease (AD): Potential Effects of Probiotic in Management of AD. Aging and Health Research, 100128.

Borghi, E., & Vignoli, A. (2019). Rett Syndrome And Other Neurodevelopmental Disorders Share Common Changes İn Gut Microbial Community: A Descriptive Review. International Journal of Molecular Sciences, 20(17), 4160.

Strati, F., Cavalieri, D., Albanese, D., De Felice, C., Donati, C., Hayek, J., De Filippo, C. (2016). Altered Gut Microbiota in Rett Syndrome. Microbiome, 4(1), 1-15.

Gallucci, A., Patterson, K. C., Weit, A. R., Van Der Pol, W. J., Dubois, L. G., Percy, A. K., Morrow, C. D., Campbell, S. L., & Olsen, M. L. (2021). Microbial Community Changes in A Female Rat Model Of Rett Syndrome. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 109, 110259.

Thapa S, Venkatachalam A, Khan N, Naqvi M, Balderas M, Runge JK, et al. (2021) Assessment Of The Gut Bacterial Microbiome And Metabolome Of Girls And Women With Rett Syndrome. PLoS ONE 16(5): e0251231.

Wong LC, Chen Y-T, Tsai S-M, et al. (2021). Dietary İntake And Growth Deficits İn Rett Syndrome—A Cross-Section Study. Autism Research.1–10.

Şahin, İN. (2022). Gut-Beyin Ekseni, Nörodejeneratif Hastalıklar Ve Mikrobiyatanın Etkileri. İstanbul Sabahattin Zaim Üniversitesi Fen Bilimleri Enstitüsü Dergisi,4(2),80-84.

SELF-CARE MANAGEMENT IN CHILDREN WITH RTT SYNDROME

Asisst. Prof. Nermin EROGLU

Fenerbahce University, Faculty of Health Sciences, Department of Nursing, Istanbul/Turkey

Children with Rett syndrome need the help of adults, especially mothers, to carry out their daily life functions. In addition to developmental delay in children, the inadequacies such as not speaking and adaptation difficulties hinder the services to be provided to these children.

Although normal children acquire skills that include daily life functions such as self-care skills such as washing and drying hands and face, brushing teeth, combing hair, eating and drinking water, dressing, undressing and going to the toilet, children with Rett syndrome do not fulfill these functions. they need to be trained to do so. The insufficient number of institutions providing education to children with Rett syndrome in our country, the insufficient number and quality of educators and assistants working in these institutions have made it necessary for families, especially the mother, to contribute to the children's acquisition of these skills. With an appropriate education program, mothers can be provided with desired behavioral changes in their children.

The most frequently used method in the education of children with Rett syndrome in the family environment is "family guidance based on behavioral approach". Points to consider before starting the training; daily education period, selection of appropriate time periods, characteristics of the home environment, child's age, intelligence level, communication style, ordering skills from simple to complex, dividing them into small steps, repeating each function several times, and rewarding the child. The skills to be taught within the scope of self-care skills are first applied to the child by the educator and shown to the mother.

Necessary conditions for effective family education are that families change their own behavioral habits, that the necessary changes are made together with the child, and that the changes in behavior or the skills gained are maintained.

In the 6th article of the Law on the Disabled, it is stated that "Disabled people should primarily lead their lives in health, peace and security in the environment they are in, care and rehabilitation in a way that they can manage themselves and become productive in the society, temporary or permanent care of those who need them, or to them. It is essential to provide home care services." statement is included. Providing counseling and guidance services especially for mothers in the education of children with Rett syndrome in the family environment will relieve the family economically, socially and psychologically.

References

1. Edanur, T.A.R. (2021). Orem öz-bakım teorisine göre otizm spektrum bozukluğu olan çocuk ve ailesini tanılama süreci: olgu sunumu. Güncel Hemşirelik Araştırmaları Dergisi, 1(3), 126-134.
2. Kaba, D., &Aysev, A. S. (2020). DSM-5 tanı ölçütlerine göre erken çocukluk döneminde otizm spektrum bozukluğunun değerlendirilmesi. Türk Psikiyatri Dergisi, 31(2), 106-12.
3. Karıcı, A. (2020). Fiziksel ve motor engelli çocukların öz bakım problemlerinin derin sinir ağları ile sınıflandırılması. Politeknik Dergisi, 23(2), 333-341.
4. Zarchi M.S., Bushehri S.M.M. F. & Dehghanizadeh M. (2018). SCADI: A standard dataset for self-care problems classification of children with physical and motor disability. International Journal of Medical Informatics, 114, 81-87.
5. Öztürk, Y. E., Şentürk, Ş., & Macit, Y. (2017). Evde bakım hizmeti alan engelli bireye sahip ailelerin bakım verme yüklerinin belirlenmesi: Amasya örneği. Türkiye Sosyal Hizmet Araştırmaları Dergisi, 1(1), 48-67.
6. Yusuf, G. (2016). Engellilerin sosyal sorunları ve beklentileri. Sosyal Politika Çalışmaları Dergisi, 35(2), 65-92.

EDUCATION AND COUNSELING FOR PARENTS OF CHILDREN WITH RETT SYNDROME ABOUT THE MANAGEMENT OF EPILEPTIC SEIZURES

Prof.Dr. Semiha AKIN

Health Science University, Hamidiye Faculty of Nursing, Istanbul/Turkey

Rett syndrome is an X-linked dominantly inherited, progressive neurodevelopmental disorder. It is a disorder characterized by a decrease in growth rate after a short-term normal development (6-12 months after birth), impaired hand movements, repetitive hand movements, slowing of brain and head development, walking problems, seizures, respiratory arrest while awake, communication and intellectual skills being adversely affected. The frequency of seizures is highest in the 7-12 age group. This review aims to explain the education about the management of epileptic seizures so that parents of children with Rett syndrome can effectively cope with the effects of the disease. Especially apraxia, motor problems and seizures are among the common problems in these children. It has been reported that 60-90% of children with Rett syndrome have epileptic seizures. In some cases, some motor behaviors associated with Rett syndrome can be misinterpreted as seizures. Video-EEG testing is needed to determine the appropriate treatment method for seizures. Although there is no definitive option for Rett syndrome, treatment is based on multidisciplinary team approach (such as pediatric neurologist, pediatrician, nurse, dietitian, physiotherapist, physical therapy and rehabilitation specialist) and focuses on symptom management. Medication may be needed to cope with respiratory (respiratory irregularities) and motor functions (rigidity, dystonia). Anticonvulsive (monotherapy or multiple therapy) drugs are used to control epileptic seizures. In resistant cases, methods such as ketogenic diet and vagal nerve stimulation may be preferred in the management of seizures. A ketogenic diet (a diet high in fat, low in carbohydrates and protein) is recommended for children with epileptic seizures. It has been reported that feeding a ketogenic diet in drug-resistant epileptic children reduces the number of seizures and complete seizure control is achieved in approximately 15% of the cases. The ketogenic diet is especially

beneficial in myoclonic epilepsy. Parents should be taught about the main differences between seizures with motor behaviors specific to Rett syndrome. Children fed a ketogenic diet and their families should be closely monitored in terms of the amount and type of foods and the level of adherence to the diet. Parents and siblings of the child are needed to be informed about first aid principles during the seizure (staying calm, protection from traumas, not restricting the movements of the twitching extremities, loosening tight clothing, not giving food or drinks by mouth during the seizures, laying the child down and laying the child on one side to provide an open airway until the seizure is complete, not leaving the child alone until the end of the seizure, observing the duration and movements during the seizure, reassuring and calming the child following the seizure, informing the parents, school administrator and teachers). Parents were taught about the conditions that require medical attention (seizure lasting longer than 5 minutes, suffer from frequent seizures, not waking up following the seizure, shortness of breath or breathing difficulties despite the seizure completion). The family is asked to record seizures (date, time of onset, aura, extremities affected during seizure, seizure pattern). Video taken during the seizure provides important data for diagnosis and determination of appropriate treatment. Children are prevented from being exposed to bright lights and loud noises, as they will trigger seizures and the development of anxiety, and arrangements are planned for a stress-free life and a comfortable sleep. The psychological reactions of the family and siblings and the need for support in treatment expenditures are evaluated to cope with the seizures and effects of the disease. Parents should be trained by a team of physicians, nurses and pharmacists about the safety and proper use of anticonvulsive drugs, side effects (rashes, weight loss, sleep problems, failure to respond to treatment, mood changes, tremor, loss of appetite). Due to the psychological, social and physiological effects and burden of the disease the individual, family and society, it is recommended that couples with a daughter with Rett syndrome be counseled about prenatal screening tests and social support resources when they want to have a child again.

References

- Jian L, Nagarajan L, de Klerk N, Ravine D, Christodoulou J, Leonard H. Seizures in Rett syndrome: an overview from a one-year calendar study. Eur J Paediatr Neurol. 2007 Sep;11(5):310-7. doi: 10.1016/j.ejpn.2007.02.008
- Krajnc N. Management of epilepsy in patients with Rett syndrome: perspectives and considerations. Ther Clin Risk Manag. 2015 Jun 10;11:925-32. doi: 10.2147/TCRM.S55896
- Managing Children with Epilepsy. School Nurse Guide. Retrieved from: https://www.choc.org/userfiles/file/EpilepsyHandbook.pdf, date: February 25, 2023.
- National Institutes of Health (NIH). https://www.ninds.nih.gov/health-information/disorders/rett-syndrome date: February 25, 2023.
- Operto FF, Mazza R, Pastorino GMG, Verrotti A, Coppola G. Epilepsy and genetic in Rett syndrome: A review. Brain Behav. 2019 May;9(5):e01250. doi: 10.1002/brb3.1250
- Rett Syndrome: Primary Care Guidelines. International Rett Syndrome Foundation. www.rettsyndorme.org. date: February 25, 2023.
- Tatlı B, Cebeci AN, Ekici B. Çocukluk çağı epilepsilerinde diyet tedavisi. Türk Ped Arş 2013; 275-80.
- Zengin Akkuş P, Utine GA. Rett sendromu. Çocuk Sağlığı ve Hastalıkları Dergisi 2016; 59: 76-85.

Network Support and Research Event

INFECTION IN RETT SYNDROME

Asisst. Prof. Gamze TEMİZ
Health Science University, Hamidiye Faculty of Nursing, Istanbul/Turkey

Rett Syndrome is a unique postnatal neurological disorder that can be diagnosed in infancy and is almost always seen in girls. Rett Syndrome is a postnatal neurological disorder. It is not a degenerative disorder. Rett Syndrome causes problems in brain function responsible for cognitive, sensory, emotional, motor, and autonomic functions. These problems can include learning, speech, sensory senses, mood, movement, breathing, heart function, and even one or more chewing, swallowing, and digestive issues.

Children with Rett syndrome face many problems from birth or from the moment they are diagnosed. In children with Rett syndrome,

• Due to neuromuscular diseases; Aspiration pneumonia and recurrent aspiration as a result of gastroesophageal reflux or aspiration of food during feeding, difficulty in swallowing, difficulty in excretion of secretions, bronchopulmonary dysplasia, inability to cough effectively due to abdominal muscle weakness, decreased flexibility in the lung and chest wall due to immobility, contracture and scoliosis, and insufficient functioning of respiratory muscles. respiratory tract infections,

• Pharyngitis, sinusitis, bronchitis, bronchiolitis, middle ear infections due to constantly open mouth breathing,
• Secondary infections due to maceration as a result of uncontrolled drooling,
• Pressure sores, ulcerations, serious tissue damage, and wound infections in these areas due to limitation of movement and circulatory disorders,
• Urinary tract infections as a result of urinary incontinence and vesicourethral reflux,
• Due to insufficient self-care and poor hygiene

- parasitic infections such as intestinal parasites and head lice
- gastrointestinal infections
- dental caries and periodontal infections
• Infections after trauma, injury, or bites,
• Sexually transmitted infections due to sexual abuse
• Infectious diseases due to incomplete or irregular vaccination
• With gastroenteritis and vaginitis due to overuse of antibiotics
• Nosocomial infections due to a long stay in the hospital are seen.

What to do to Prevent Infection

Frequently touched surfaces such as door handles, toilet sinks, whiteboards, dining tables, sinks, and computer keyboards in nurseries and schools can be a source of infection. Therefore, it is the most important step for parents to wash their children's hands frequently to prevent infections. Wiping hands with wet wipes does not provide hand hygiene and may even cause the microbe to be transmitted from one hand to the other. Wet wipes should only be used to clean the dirt, then hands should be washed with soap and water.

One of the most important points in preventing the transmission of infections is hand cleaning. For this reason;

• Before and after the meal,
• Before and after taking medicine or syrup,
• Before and after joint water activities,
• Before and after the toilet,
• After contact with body fluids (blood, mucus, vomit, sputum),
• After contact with animals, after contact with animal coops or cages,
• After playing in the playground or field,
• After contact with a dirty surface, hands should be washed with soap and water.
• In addition, children's teeth should be brushed regularly.
• Attention should be paid to their healthy and balanced diet.

• Children should be provided with exercise, and for this, they should regularly continue their favorite sports activities.

• It should be ensured that they sleep regularly

• It should be ensured that children are fully vaccinated, and annual flu vaccinations should be paid attention to.

• Mothers and fathers smoke inside the house and on the balcony. Even parents should not have any tobacco habits.

• It should be ensured that the house environment is at the appropriate temperature and humidity and the house should be ventilated regularly.

Efforts to prevent infections in children with Rett syndrome should be supported by the provision of adequate education and health services. Studies have shown that infections prolong hospital stays and increase morbidity and mortality. Therefore, early recognition of infections is vital. On the other hand, it should be kept in mind that the unnecessary use of antibiotics will increase the cost and cause the emergence of resistant strains.

References

1 Neul JL, Kaufmann WE, Glaze DG, et al. Rett syndrome: revised diagnostic criteria and nomenclature. Ann Neurol 2010;68: 944-950

2 Bienvenu T, Philippe C, De Roux N, et al. The incidence of Rett Syndrome in France. Pediatr Neurol 2006;34: 372-375

3 Diagnostic criteria for Rett syndrome. The Rett Syndrome Diagnostic Criteria Work Group. Ann Neurol 1988;23(4):425–8

4 Hagberg B, Hanefeld F, Percy A, Skjeldal O. An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett Syndrome Clinical Criteria Consensus Panel Satellite to European Paediatric Neurology Society Meeting, Baden Baden, Germany, 11 September 2001. Eur J Paediatr Neurol 2002;6(5):293–7

5 Nissenkorn A, Gak E, Vecsler M, Reznik H, Menascu S, Ben Zeev B. Epilepsy in Rett Syndrome-the experience of a National Rett Center. Epilepsia 2010;51(7):1252–8

6. d'Orsi G, Trivisano M, Luisi C, Demaio V, Di Claudio MT, Pascarella MG, et al. Epileptic seizures, movement disorders, and breathing disturbances in Rett Syndrome: diagnostic relevance of video-polygraphy. Epilepsy Behav 2012;25(3):401–7

7. Cuddapah VA, Pillai RB, Shekar KV, et al. Methyl- CpG-binding protein 2 (MECP2) mutation type is associated with disease severity in Rett Syndrome. J Med Genet 2014;51:52-158

8. Ellaway C, Christodoulou J. Rett Syndrome: clinical update and review of recent genetic advances. J Paediatr Child Health 1999;35:419-426