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Celebrating the 50th anniversary of the first publication on Rett syndrome by Andreas Rett

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1 Extended abstracts of the invited speakers

1.01

Ten years of the Cardiff Rett Syndrome Clinic

Angus Clarke

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Abstract
The Cardiff Rett Syndrome clinic began with the support of Dr Alison Kerr from Glasgow more than ten years ago. Throughout its existence, the clinical team has included three consultants (clinical geneticist, paediatric neurologist and adult learning disability psychiatrist) and a physiotherapist; it is now also supported by an eye-gaze technology representative, a support worker from Rett UK and sometimes by a music therapist. The clinic is not able to see patients either frequently or regularly as it can only be assembled occasionally (x 2–3 per year): referrals are for once-off consultations.

The referrals fall into four main categories: (i) to address questions of diagnostic uncertainty, (ii) to discuss the questions that arise for families around the time of diagnosis, (iii) to consider care at the time of transition from paediatric to adult services and (iv) as a trouble-shooting assessment for difficult management problems.

This talk will review the range of questions and problems that have been seen in the clinic and the measures we have often been able to recommend.

I became involved with Rett syndrome while training simultaneously in both clinical genetics and paediatric neurology in Newcastle upon Tyne, England. My consultant paediatric neurologist was Dr David Gardner-Medwin, and in 1987 he introduced me to two sisters, who seemed both to have the same disorder, then becoming known as Rett syndrome. My interest in genetics meant that I was intrigued to see that sisters could be affected, and the family were very kind in allowing me to study them. I conducted metabolic studies that showed distinct abnormalities of intermediary metabolism and the urea cycle, in both girls and their mother. I also carried out some genetic linkage studies on this family and went on to gather samples from other families with affected sisters or half-sisters, to see if we could localise the gene in which mutations caused Rett syndrome.

The experience of becoming engaged in the research was formative for me, and led to my attending a meeting in Vienna in 1988, where I presented my work and also met Dr Rett and others studying this condition. I got to know Dr Alison Kerr, and that was also key in helping me to acquire a broader clinical experience of Rett syndrome and to appreciate the types of support and professional involvement that families wanted.

When the gene was identified in 1999, we were able to show that the two sisters and their healthy mother all carried a mutation in *MECP2*, but that the mother had complete skewing of X chromosome inactivation in her leucocytes (and presumably brain) and so was spared from being affected.

The experience of meeting that first family in 1987, of asking questions about the cause of the disorder and attempting to answer them, has shaped my approach to patients with this and other disorders ever since. I was privileged to meet Drs Andreas Rett, Alison Kerr, Alan Percy and Walter Kaufmann so early in my career and I owe them all a debt of gratitude for the example they set.

My feeling now is that we should be moving from laboratory work into clinical trials of novel treatments for Rett syndrome. This should start tentatively as we work to refine the outcome measures that are most useful in evaluating new treatments. My judgement is that we should begin with potentially symptomatic treatments, as with the modulation of neurotransmitter systems, in an attempt to stabilise the girls' physiology and gather information about the quality of life of the girls and women with the condition and also of their parents, siblings and the others around them.

It will take time to develop measures of cognitive function in Rett syndrome, most likely utilising eye-gaze technologies, to give us insight into the degree to which patients with RTT are 'locked-in'. I think this will be highly variable and so it will be important to be able to measure cognition before and after ther-

apeutic interventions, to use this an outcome of the treatments; we are simply unable to do so now.

The prospect of 'cure' is of great appeal, to families and professionals. This is strongest for those parents who have young, recently diagnosed children and tends to evolve over time into a wish for support, an appreciation of the gifts that affected girls bring, and a recognition of the ways in which affected girls can enjoy life and take pleasure despite all their various difficulties. 'Cures' may be found one day, perhaps through CRISPR or suppressors of nonsense mutations or sophisticated strategies of gene therapy. However, it will be vital to approach these technologies with caution because of the serious hazards of autonomic instability and/or raised intracranial pressure that may result from such interventions, especially when the intervention is irreversible. There is much to hope for and to work towards but it must be done responsibly and with a degree of humility.

Bio sketch

Angus (Fig. 1.01) studied Medical and Natural Sciences at Cambridge University, taking his BA Part II in Genetics, and then qualified in Medicine from Oxford University. After registration, he worked in General Medicine and then Paediatrics. He studied the clinical and molecular genetics of ectodermal dysplasia in Cardiff and then worked in clinical genetics and paediatric neurology in Newcastle upon Tyne, developing an interest in Rett syndrome and neuromuscular disorders. He returned to Cardiff in 1989 as Senior Lecturer in Clinical Genetics and is now Professor in Clinical Genetics.

Fig. 1.01 Angus Clarke

Angus has been custodian of the British Isles Rett Syndrome Survey since 2006 and leads the Cardiff Rett Syndrome Research Group and the Cardiff Rett Syndrome clinic, which was established more than ten years ago. Active projects include molecular research into CDKL5 and FOXG1 variants of Rett syndrome; in collaboration with colleagues from Bristol, the group also wishes to establish clinical trials for the treatment of the autonomic dysfunction in Rett syndrome and refine the outcomes measures for assessing interventions in Rett syndrome.

Angus has also maintained his interest in ectodermal dysplasia and has developed further interests in genetic screening, the genetic counselling process and the social and ethical issues around human genetics. He is engaged in a clinical trial of treatment for ectodermal dysplasia. He also represented the Chief Medical Officer for Wales on the Human Genetics Commission and contributes to discussions of policy and ethics in the UK (British Society for Genetic Medicine; Nuffield Council on Bioethics) and Europe (European Society of Human Genetics). He has (co)authored and edited seven books, including "Genetic Testing:" (2011 – jointly with Michael Arribas-Ayllon and Srikant Sarangi). He established and directs the Cardiff MSc course in Genetic Counselling and contributes to teaching of MB and BSc students and he has supervised several doctoral (PhD and MD) students.

1.02

Gross motor function and activity in Rett syndrome

Jenny Downs

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Abstract

Altered gross and fine motor skills have marked effects on daily function in Rett syndrome. During infancy, early development is largely normal although many parents describe their young baby as placid in nature or somewhat floppy. There may also be other concerns such as poor acquisition of developmental skills. During this early period, the majority of girls will learn to sit and approximately half will learn to walk. Regression is a fundamental feature of Rett syndrome and is characterized by loss of communication and/or hand skills, often coinciding with the development of hand stereotypies occurring on average at 19 months. Impaired gait is another core feature of Rett syndrome. This is characterised by ataxia if walking develops that is also accompanied by altered muscle tone and dyspraxia. Parents have described loss of balance during the regression period that can affect the child's ability to sit, stand, walk or engage in more complex gross motor tasks. When accompanied by other problems such as poor sleep and autistic type behaviours, this is a complex and distressing time for the child and family.

We have previously collected video data in 2004, 2007 and 2012 to observe functional abilities including gross motor skills in girls and women across Australia. These observations have allowed us to develop the 15-item Rett Syndrome Gross Motor Scale (RSGMS) and also to assess its measurement properties. A total score can be calculated, as can sitting, standing and walking, and challenging motor skills subscale scores, each with strong internal consistency. Repeatability in a clinical setting was also excellent. Overall across childhood and in the adult years, the distribution of skills broadly reflects those of the early years. The majority remain able to sit, slightly fewer than half walk independently and a smaller proportion can perform transition movements. Strong relationships between motor skills, mutation type have also been demonstrated that provide additional validation of the scale. For example, those with a p.Arg133Cys, p.Arg294* or p.Arg306Cys mutation generally score higher than those mutations such as the p.Arg270* or a large deletion. Compared with early childhood and taking into account the effects of mutation type, motor scores were substantially reduced in those older than 19 years. Some girls with Rett syndrome will maintain the ability to walk when adults, particularly with the protective effect of a milder mutation. However, some adults will have poorer skills, possibly related to the impairments such as bradykinesia and progressive scoliosis. We also observed that sitting scores declined significantly from the teenage years, possibly due to neurological or orthopaedic impairments but conceivably also because of longer time spent sitting with less opportunity for the practice of gross motor activities. The RSGMS potentially has a role to play in clinical monitoring and as an outcome measure in clinical trials.

Further to the evaluation of gross motor skills, it is also important to assess how these skills are used, eg, time spent active, number of steps walked. To this end, we have validated measures of physical activity in Rett syndrome finding that the StepWatch Activity Monitor (SAM) accelerometer was accurate in measuring steps. We recently used the SAM to assess whole day activity in 64 girls and women, three quarters of whom walked independently

and one quarter with assistance. The median average daily steps was 5093 steps (range 458–32,835) and the median percentage of sedentary time was 60 % (range 18–94 %) indicating that activity in Rett syndrome is generally reduced but that there is also marked variability. Not surprisingly, those who walked independently were more active than those who needed assistance, but walking was mostly at slow gentle cadences of less than 20 steps in a minute for both groups. Scoliosis and epilepsy managed with polytherapy were associated with less physical activity and more sedentary time. However, these effects were small in multivariate analyses where females aged ≥ 19 years took nearly 5000 steps fewer that girls younger than 13 years (coefficient -4997, 95 %CI -8024, -1970). A similar pattern was reflected for the duration of sedentary time, but sedentary time was markedly reduced for both teenagers and adults compared with the younger girls. Interestingly, the increase in sedentary time in the teenage years occurred prior to the marked deterioration in steps in adulthood and this could indicate that environmental deconditioning during the teenage years had a particular role to play.

Traditionally, a lifestyle with adequate physical activity and limited sedentary behaviours is associated with health benefits in the general population, and improved motor skills and fitness in youth with developmental disability. Of note, amongst those with a neurological condition and some walking skills, reducing time in sedentary behaviour, by increasing participation in light intensity physical activity would likely be a more realistic and achievable goal than aiming to increase participation in moderate to vigorous intensity physical activity. However, movement and physical activity is likely important for all girls and women with Rett syndrome. We recently interviewed parents of girls with Rett syndrome about what was pleasurable for their daughter and what challenged her, and we identified quality of life domains based on observable behaviours. Our data suggested that position changes assisted body comfort in those with severely impaired gross motor skills. For those with any level of motor skill, being able to sit, stand or move provided opportunity to achieve something independently. With or without assistance, many girls appeared to enjoy the rhythm of walking and expressed their motivation by walking towards something that they desired. Participation in a variety of physical activities such as swimming was appropriate for any level of gross motor skill and often provided satisfaction and pleasure. There are variable gross motor skills in Rett syndrome but use of gross motor skills has potential to contribute positively to health, wellbeing and quality of life in Rett syndrome.

Relation to the field of Rett syndrome

I have been deeply involved in research on Rett syndrome for more than 11 years investigating a range of health and wellbeing topics. My focus has been on outcome measures and evaluating how best to improve functional abilities and ameliorate adverse effects of comorbidities. My research experience has been markedly enhanced and enriched by meeting families and their daughter with Rett syndrome.

Where the field might move in the next years, what is needed, what can be expected

There is now more focus than ever on rare diseases and Rett syndrome is attracting particular interest. This is especially in the field of neurobiology where many potentially modifiable mechanisms to improve neurobiological function are being investigated and this is leading to a growing number of clinical trials. But there are many more needs. The clinical and therapy fields need to conduct more intervention studies to improve the evidence for treatments and allow for more precise informed clinical counselling. The clinical and therapy fields also need to disseminate this information effectively to the general clinical, therapy and educator communities in order to improve clinical care and life opportunities for all with Rett syndrome. With greater technological capacity, there is more awareness of Rett syndrome and increasing participation of affected families globally, in family associations, via social media and in the research databases. This provides more opportunities for families, clinicians and researchers to partner more efficiently and achieve the best outcomes for Rett syndrome.

Bio-sketch

Dr Jenny Downs (Fig. 1.02) is a physiotherapist and now researcher since completing her PhD in 2003. Always interested in research, she previously conducted two randomised controlled, one in her clinical career, prior to beginning work in 2005 with the Child Disability Group at the Telethon Kids Institute in Perth. Overall, she is working towards improving our understanding about disability in childhood and outcomes for affected children and their families. Firstly, she is investigating rare disorders including Rett syndrome, the CDKL5 Disorder, the MECP2 Duplication Syndrome, Duchenne muscular dystrophy and Early Onset Scoliosis, disorders that each has devastating impacts on affected children and their families. She is working to build and maintain disorder-specific databases that can provide the data to understand their natural history. Secondly, she is developing a quality of life measure for children with intellectual disability which will enable subsequent high quality studies on what factors contribute to the best quality of life for these children and how we can improve their quality of life. Thirdly, she is working to identify new intervention opportunities including environmental enrichment for young girls with Rett and a comprehensive investigation of clinical outcomes following gastrostomy for children with feeding difficulties.

Fig. 1.02 Jenny Downs

1.03

Behavioural biomarkers of typical Rett syndrome: moving towards early identification

Christa Einspieler[1], Peter B Marschik[1,2]

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[2]Center of Neurodevelopmental Disorders (KIND), Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

It was at the 8th International Congress on Child Neurology, organized by the International Child Neurology Association (ICNA), held in Ljubljana, Slovenia in 1998 that Dr. Einspieler gave an early morning lecture on the assessment of general

movements in young infants. In the following coffee break Dr. Alison Kerr asked her whether it would be possible to apply the same technique also to non-standardised home videos of individuals with Rett syndrome (RTT). After a few months, Dr. Kerr provided a collection of short video clips taken by parents of six typically developing infants and eight infants who later turned out to have RTT. Daily life sequences of the children were randomly assembled but with increasing age from birth to the first birthday, with 2 to 3 sequences per month. Together with Dr. Heinz Prechtl, Dr. Einspieler evaluated the recordings of movements and posture (without knowing the infants' history) as either conspicuous or inconspicuous. With an interscorer agreement of 96 %, 79 % of the clips were correctly classified. This pilot study encouraged them to carry out a detailed study on a larger sample. Focussing on the first 6 months of life, they found that general movements were abnormal in all 22 individuals later diagnosed with RTT. Among other behavioural deviances tongue protrusion (68 %), postural abnormalities (58 %), asymmetric opening of the eye lid after a blink (56 %), first stereotypies (42 %), or bizarre smiling (32 %) were the most frequently occurring atypical behaviours. Together with Dr. Marschik, who is also trained in neurolinguistics, these and some newly acquired family videos were re-analysed and a number of peculiarities in early verbal behaviour of infants and toddlers later diagnosed with RTT were found. These include an atypical verbal development (e. g., acoustical peculiarities objectified on signal level), deviations in socio-communicative behaviours and a restricted gestural repertoire, to name but a few. Our (i. e. research unit iDN together with an international network of colleagues from various scientific disciplines) research efforts point into the direction that we are on a promising way to define a set of behavioural parameters pinpointing RTT.

Bio-sketch
Christa Einspieler (Fig. 1.03) got her degree in Physiology and Psychology at the University of Graz, Austria. She has been working in behavioural analysis for over 30 years and received special training inter alia at the University of Groningen, the Netherlands (Heinz Prechtl). She is currently Professor in Physiology at the Medical University of Graz. Her main research topics are the ontogeny of behaviour, fetal movements and motor development in preterm, term and young infants. She is author or co-author of more than 120 scientific papers in indexed journals (SCI/SSCI/PUBMED), has contributed to more than 25 books from international publishers, and published two monographs on fetal and early motor behaviour (Clin Dev Med 167, Clin Dev Med 189).

Fig. 1.03 Christa Einspieler

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1.04

The rise and recognition of Rett syndrome, a personal view

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In September 1966 Dr. Andreas Rett, Primarius an der Kinderabteilung der Stadt Wien published a paper in the journal "Wiener Medizinische Wochenschrift" under the title "Über ein eigenartiges hirnatrophisches Syndrom bei Hyperammonämie im Kindesalter". His report was based on investigations of 22 girls. This is an almost-complete description of a disease now bearing his name occurring in approximately 1:10.000 girls.

The title of Rett's publication had two weaknesses: it was written in German, rather than English and it was based on a test that had gone wrong. The postulated hyperammonaemia found in these girls could not be confirmed by others. Rett's ingenious discovery was heading into the wrong direction. Patients who fitted the clinical diagnosis failed to meet the biochemical aspect. It was the time of metabolic screening. *Inborn errors of metabolism* had to be excluded by appropriate methods. "No metabolic defect, no new disease" was a doctrine of the time. Andreas Rett's discovery was moving in difficult terrain. Nevertheless Rett syndrome found its way into other German publications, particularly into the two-volume textbook "Die klinischen Syndrome" by Leiber-Olbrich. The 1972 edition shows under the key word 'hyperammonaemia' a photographic sequence of girls with typical Rett syndrome taken from Retts original documentation. Leiber and Rett had been friends for a long time. Leiber was head of the Department of Genetics at the University of Mainz in Germany. He was so impressed by his friend's clinical publication that he called it 'Rett syndrome' a special type of *inborn error of metabolism*, although the inborn metabolic error did not exist.

There it was, fully described and documented by photographs of affected patients, the Rett syndrome as we now know it. RTT was released in our world, printed documents find their way.

At this point I cannot avoid some biographical remarks referring to two people, from whom I learnt a lot and who became personal friends.

The first L. Crome, an eminent UK neuropathologist, published together with J. Stern, a biochemist (also UK based), the textbook *Pathology of Mental Retardation*. Morphology and biochemistry had joined forces. Both authors were able to read German. Crome became fascinated by Rett's clinical description, while Stern argued with the laboratory error of hyperammonaemia. Regardless of their actual arguments, key is that the RTT syndrome was already being discussed here in mainland Europe in 1972. As clinician I would comment at this point: "before starting research on an island you have first to discover it". Andreas Rett was an outstanding explorer.

The second is my very personal friend, the late Bengt Hagberg from Sweden. Starting in 1973 we held annual meetings between our departments in Göteborg and Berlin, later Göttingen, respectively, which continued for more than 20 years. During these meetings we discussed our active research and looked at patients with mainly unknown diseases, amongst them girls who presented all the symptoms of typical RTT syndrome. They are part of the 1983 publication by Hagberg and others describing the rediscovered RTT syndrome. I was invited to participate

in the Symposium on Rett Syndrome organized by Andreas Rett und Bengt Hagberg in Vienna in September 26–27, 1984, to present our results. At this symposium – historically significant to any scientist now working on the Rett syndrome – criteria for the inclusion and exclusion of a diagnosis of Rett syndrome were formulated. This has proven to be the most useful tool for securing of diagnosis of Rett syndrome over the years.

Going back to L. Crome and J. Stern, pathologist and biochemist, resp. from Queen Mary's Hospital for Children in Carshalton, Surrey, England. Between 1970–1972 I studied the brains of children with epilepsy stored at the institute under Crome's leadership.

Ten years previously another clinician, from Siena, Italy, the child psychiatrist Michele Zappella had also studied in Carshalton. I met Michele Zapella for the first time at the Baltimore meeting in 1985 when RETT Syndrome was for the first time discussed in the US.

At the end of this short review some lessons can be learned by looking at the history of Rett syndrome and I ask myself one question to which I have found no good answer.

First the lesson to be learned: for us clinicians the requests for help and research come from patients. It requires many different and advanced methods to search for an answer. The results of our scientific endeavor should always be returned to our patients in order to provide a better diagnosis, prevention or even cure.

The unanswered question is why the Rett syndrome remained unknown in the United States before 1985, even though it was already identified and well described by Andreas Rett in 1966 and Bengt Hagberg in 1983.

The discoveries of Andreas Rett and Bengt Hagberg were of great importance for the medical community and ignited research in different fields of science. A new era of relief and hope started for the affected patients and their families.

The parents' organizations became active all over the world, headed by Kathy Hunter. Their work proved to be of enormous importance.

The gene MECP2 was in 1999 identified as cause of RTT syndrome in more than 90 % of all affected patients by the group of Huda Y. Zoghbi working at the Baylor College of Medicine, Houston, USA.

Fig. 1.04 Folker A. Hanefeld

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1.05

Parents and parents associations: History of RTT

Kathy Hunter

International Rett Syndrome Association, Ohio, USA

Abstract
More than sixty years ago, in 1954, Dr. Andreas Rett of Vienna, Austria, noticed two girls sitting together with their mothers in his waiting room. The girls made the same unusual handwashing movements, and he discovered after examining them that their clinical and developmental histories were strikingly alike. After consulting with his nurse, Dr. Rett found six others like them in his practice and made a film of the girls, which he took all over Europe trying to find other cases. His findings were published in several obscure German language medical journals, which unfortunately never reached the attention of the worldwide medical community.

In 1960, Dr. Bengt Hagberg observed several girls who had similar histories and behaviors in his busy practice in Gothenburg, Sweden. He put these interesting cases in a special box under his desk, vowing to look further into the nature of this unusual and unknown disorder. In 1978, Dr. Ishikawa and colleagues from Japan described three girls in a brief note which also went unnoticed. Many years went by before Drs. Hagberg and Rett realized they were reporting the same disorder. Until his death in 1996, Dr. Rett worked ceaselessly to unite parents and professionals in a community of care to bring a better life to the girls whose disorder bore his name. Together, Drs. Rett and Hagberg held such enthusiasm and energy in the clinical and research arenas and these were only surpassed by their gentle spirits and compassion at the human level of the lives they worked so diligently to improve. They are no longer with us, but they remain always in our hearts.

Shortly before the first paper on RTT was published, my daughter, Stacie, who was ten years old at the time, was given the diagnosis of RTT by Dr. Mary Coleman of the Children's Brain Institute in Washington, DC. Dr. Coleman had attended a scientific meeting in Paris where the first English-language paper was presented before its publication. Stacie became the thirty-sixth documented case in the world and the first in the USA. She had developed normally until fifteen months of age, then began a regression that led to a loss of the few words she had developed, aloofness, withdrawal and irritability most of the time. She began mouthing and wringing her hands constantly. During the first ten years of her life, Stacie had a number of different diagnoses, including autism and Angelman (Happy Puppet) syndrome.

Two important events changed me forever. To this day, my spirit stays afire to keep the Rett movement alive–just so this will *never* happen to others. One is the young doctor who told me to just give up, saying that Stacie would never know what

it was like to be normal and that I had to accept that and try to keep my own life normal. He said I must give up. The other was a child psychologist who told me that it was possible I had *caused* the "autism," according to an outdated theory on autism that blamed "cold" mothers in the face of no other explanation for their child's regression. You can be sure that these were the first two people to receive information about RTT. Their insensitive comments launched me on a journey to prevent others from ever having to hear the same unfortunate advice. It was a broken road that led me to the diagnosis of RS, but the path forward from that news has been rewarding and hopeful.

Fifty years ago, there was no place to turn for parents to find answers. We knew little about the child with Rett syndrome and her needs, much less the cause and treatment for the disorder. Through this last half-century, parents all over the world have shared their observations and experiences with researchers, inspiring and encouraging and even providing funding for their studies. Parents have contributed to publications and networks dedicated to help other parents cope and meet the challenges that will lead them to provide the best care for their children. Parent support organizations began with the International Rett Syndrome Association in the United States in 1983 (now known as rettsyndrome.org) and have spread all over the world. The Rett Syndrome Research Foundation (now known as the Rett Syndrome Research Trust) was launched in 1999. Eight World Congresses have been held over the years in Antwerp (Belgium), Gothenburg (Sweden), Edinburgh (Scotland), Karuizawa (Japan), Helsinki (Finland), Paris (France), New Orleans (USA), and Kazan (Russia). The wisdom and insight of parents and professionals who blazed the pioneer path combined with the cadre of more recently invested scientists and elevated technology, have cultivated a rich future for discovery in Rett syndrome. It is a goal we all share.

"I can only express my gratitude to all the parents for their love and the affection they give to their children, and my admiration for all their efforts and services they deliver to their girls. Don't lose heart in your work, keep your love for the children, and remember what I have always tried to say: watch the wonderful expression of the eyes of these girls, an expression which makes them so lovable." Professor Andreas Rett

Fig. 1.05 Kathy Hunter and Andreas Rett

Kathy Hunter (Fig. 1.05) attended Frostburg University and Graceland College.

Affiliation: International Rett Syndrome Association

Kathy Hunter founded the International Rett Syndrome Association after her daughter became the first child to be diagnosed with Rett syndrome in the USA in 1983. She led the IRSA for twenty-five years before retiring in 2008. Other work includes several years in special education, the Board of Directors of the National Organization of Rare Disorders, a Congressional appointment to the National Advisory Neurological Disorder and Stroke Council, an appointment to the Leadership Council of WE MOVE, as well as serving on the Child Neurology Foundation Advocacy Committee and the Rare Diseases Clinical Research Network. Mrs. Hunter testified before the U. S. Congress to increase funding for research on Rett syndrome for more than two decades, which resulted in allocations of more than $ 70 million. Her publications include *The Rett Syndrome Handbooks I and II, and Raindrops and Sunshine.* Mrs. Hunter's devotion to the Rett syndrome cause is deeply inspired by her daughter, Stacie, now 42, who lives at home and brings continuing love and joy to her family in Maryland, USA.

"It has been an honor and privilege to educate, inform and motivate others to work toward a richer life and a better future for families whose lives are changed forever by the diagnosis of Rett syndrome. At the heart of my commitment is the remarkable reward of coming to know and admire so many other families who demonstrate their extraordinary resilience in the face of adversity, turning tragedy to triumph as they overcome a multitude of daily challenges. This, of course, is made possible by the incredible courage, strength and determination of the Silent Angels in our lives whose inner voices lighten the burden and brighten our lives with their many blessings."

1.06

Synaptic abnormalities in RTT and their impact on brain plasticity

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Extended Abstract

A number of clinical and pathological abnormalities pointed to a disorder of synapse formation and regulation in RTT even before the importance of the mutations in the MeCP2 gene were discovered. Most babies with RTT have normal head circumferences at birth followed by declining brain growth in the first year of life at a time when the normal brain is proliferating synapses in large numbers that will overshoot the normal adult number of synapses by about 2 years of age (Naidu, *Ann Neurol*, 1997;42:3–10). This is consistent with postmortem neuropathological evidence that neurons in the cerebral cortex of girls with RTT are too close together, suggesting a postmortem defect in synapse formation (Jelliinger et al, *Acta Neuropathologica*,1988;76:142–153). Synaptic counts in postmortem tissue from girls with RTT have also been shown an approximately 50% reduction in synapse numbers and this abnormality has also been found in mice with RTT mutations. Nasal biopsies in girls with RTT and age-matched controls undergoing medically indicated surgical procedures showed that olfactory receptor neurons (ORN) replicated normally or in greater numbers in girls with RTT but then died after failing to form synapses with neurons in the olfactory bulb (Ronnett et al, *Ann Neurol*, 2003;54:06–218) Additional clinical evidence such as the prominent movement disorders seen in RTT, difficult to treat seizures and autonomic fluctuations also suggested abnormalities in synaptic neurotransmitters, especially in the excitatory amino acid glutamate and GABA (γ-aminobutryic acid) the major inhibitory neurotransmitter (Johnston, et al, *F1000 Research*, 2015, 4:1490). Two papers in the 1990's by Hamberger et al (*Neuropediatrics* 1992;23:212–3), and Lappalainen and Riikonen (*Ped Neurol* 1996;15:213–6) reported high levels of glutamate in the spinal fluid of girls with RT. In 2009 the group led by Sakku Naidu reported that brain levels of glutamate measured by magnetic resonance spectroscopy (MRS) showed that younger girls with RTT had higher levels compared to controls but older girls had lower levels of glutamate in girls with RTT compared to age-matched controls (Horska, *Ann Neurol* 2009;65:90–7) We reported the first data on the concentrations of glutamate and GABA receptors in postmortem brain tissue from girls with RTT in 1999, and we found elevated levels of NMDA type glutamate

receptors in frontal cortex for girls with RTT, but lower levels of NMDA receptors in girls older than 8 years of age (Blue et al, *Ann Neurol* 1999;45:541–5; Johnston, et al, *F1000 Research*, 2, 4:1490). Blue et al (*Anat Rec* 2011;294:1624–16340) also reported similar findings in the Bird Mecp2 deficiency model.

These data suggest a problem with homeostatic scaling in synapses, which is a process by which synapses reduce their post-synaptic receptors if levels of neurotransmitter are too high to maintain a balance between excitation and inhibition. Maezawa and Jin (*J Neurosci* 2010;30:5346–56) reported that microglia from the Bird model of RTT in mice showed toxic levels of glutamate, pointing to a defect in the uptake of glutamate by glia that normally control levels of glutamate in the synapse. Recently we monitored the 24 hour continuous sleep-wake cycle in the Bird model of mecp2 knockout mice in which we measured elevated levels of glutamate in cerebral cortex that were 40 % higher than normal controls (Johnston et al 2014; *Front Syst Neurosci* 8:118). These studies showed remarkably abnormal sleep wake cycles in the knockout mice, and long periods of sleeplessness were associated with very high levels of glutamate in the brain. These studies suggest that sleeplessness in girls with RTT might be associated with periods of high glutamate levels during wakefulness if the human sleep cycle is similar in mice and humans. Interestingly we found that cerebellar neurons from the Bird knockout model of Mecp2 deficiency are hypersensitive to glutamate mediated neurotoxicity in culture, consistent with the abnormal homeostatic scaling model we have proposed in RTT (Russell et al 2007;*Neuroscience* 150:563–74). Abnormal homeostatic scaling is expected to disrupt learning and memory and other forms of activity dependent plasticity because of the critical role of long term potentiation (LTP) in learning and memory. This may be an important mechanism for progressive impairment of brain function in older girls with RTT.

A number of papers have also shown alterations in GABAergic inhibitory neurons in postmortem brain tissue from girls with RTT and in animal models. Blue et al reported altered development of glutamate and GABA receptors in the basal ganglia of girls with RTT (Blue et al, 1999; *Exp Neurol* 156:345–52) and we also found alterations of GABA transporters in mice with Mecp2 deficiency (Kang et al, 2014; *J Central Nerv System Dis* 6:21–8)

Taken together, the deficiency of Mecp2 in these models is associated with enhanced excitotoxicity and with increased levels of glutamate action and reduced levels of GABA. These changes would be expected to disrupt activity dependent learning and plasticity and recent evidence supports this hypothesis. Coordinated function of NMDA glutamate and GABAergic receptor function appears to be particularly important for activity dependent reorganization of cerebral cortex, for example the re-assignment of visual cortex when one eye is covered in a young animal or human. Several recent papers indicate that these plasticity processes are disrupted in mice with Mecp2 deficiency (He et al, 2014;*Nature Commun* 5:5063) This is important information because it suggests that the cognitive disorder in RTT could be improved with interventions aimed at reducing NMDA mediated activity and/or enhancing GABAergic activity.

Based on this information, our group under the direction of Sakku Naidu has undertaken trials of dextromethorphan (DEX), an approved over-the-counter cough medicine that is a competitive inhibitor of the NMDA receptor (Naidu et al). We initially used this approach for the disorder non-ketotic hyperglycinemia (NKH) in which NMDA receptors appear to be overactivated at a glycine/serine site on the NMDA receptor complex. This therapy is commonly used for children with NKH to reduce seizures and try to improve outcome. We completed a preliminary prospective randomized, open blinded endpoint (PROBE) trial of DEX to evaluate safety and efficacy in girls with RTT and found that it is safe for girls with RTT and produced improvement in receptive language and clinical seizures. We are also completing a fully blinded, randomized clinical trial of DEX for RTT sponsored the Food and Drug Administration, and we expect to enter the final subject in the next month (Sakku Naidu, PI). In summary, our research approach to RTT has focused on synaptic neurotransmitter abnormalities, especially those involving glutamate and GABA, the major excitatory and inhibitory neurotransmitters in the brain, and ones which play a major role in synaptic plasticity. An important of the impact of MeCP2 mutations on brain function in RTT appears to be mediated by pathological glutamate and GABA synaptic dysfunction, which disrupt activity-dependent plasticity in multiple systems of the nervous system. Therapies that target these synaptic abnormalities in synaptic function might have some benefit if applied early in life.

My relation to Dr. Rett

I did not know Dr. Rett and although I trained at Johns Hopkins in pediatrics and pediatric neurology, I missed the meeting that Dr. Moser organized in Baltimore that was the first meeting on Rett syndrome in the US. At the time of the meeting I was on the faculty in pediatric neurology at the University of Michigan in Ann Arbor.

Where the field is going

I feel that the field is exploding with new information about RTT that will provide insights into treatment, and that new treatments will emerge that will provide some improvement in the CNS function along with progress in genetics. From my own perspective I am excited about the possibility that we may have some influence on brain development and plasticity in girls with RTT by focusing on ways to improve the function of synapses, especially when they are quite young.

Fig. 1.06 Michael V. Johnston

Short Biosketch

Mike Johnston (Fig. 1.06) is a Professor of Neurology, Pediatrics and Physical Medicine and Rehabilitation at the Johns Hopkins University School of Medicine in Baltimore and the Chief Medical Officer and Executive Vice President of the Kennedy Krieger Institute, where he also holds the Blum Moser Endowed Chair for Pediatric Neurology named for Hugo Moser. Mike attended medical school at the University of Pittsburgh and then came to Hopkins for residencies in pediatrics and pediatric neurology. His first faculty job was at the University of Michigan in Ann Arbor, and after 8 years he returned to his current post at Kennedy Krieger and Hopkins. His laboratory works on mechanisms of injury in the developing brain, especially the impact of excitotoxicity mediated by glutamate and his research includes both laboratory work and studies in the nursery on neuroprotection for hypoxic-ischemic brain injury. His work with Rett syndrome began through the influence of Professor Sakku Naidu who was beginning to study postmortem brain in RTT in the 1990's. He

became interested in Dr. Naidu's work with RTT through many discussions with her and observing patients. His interest grew as he and Dr. Naidu developed hypotheses about the apparent hyperactivity in the girls and the possibility that glutamate mediated excitotoxicity and a defect in synaptic plasticity might play a role in the pathogenesis of RTT. This collaboration has also included Dr. Mary Blue who is also very interested in the neurobiology of Rett syndrome.

1.07

Clinical trials in Rett syndrome: Opportunities and challenges

Walter E. Kaufmann

Center for Translational Research, Greenwood Genetic Center and Department of Neurology, Boston Children's Hospital, Boston, USA

Fig. 1.07 Walter E. Kaufmann

Drug trials have been performed in Rett syndrome (RTT) for the last 25 years. The identification of *MECP2* mutations as the basis for most cases of RTT in 1999 changed dramatically the foundation of clinical trials for the disorder. In the pre-*MECP2* era, knowledge of RTT pathophysiology relied on studies using biosamples from affected patients or animal models that tried to mimic the disorder. This limited understanding of RTT neurobiology led to targets of unknown role in the clinical and pathological evolution. Other factors that could explain the mainly negative or inconsistent results of this early era relate to trial design and outcome measures. Early drug trials' inclusion criteria were age-based, without taking into account the distinctive regression period that could complicate interpretation of changes over time, and endpoints that were not RTT-specific or had unknown measurement properties.

The *MECP2* era has brought invaluable mechanistic knowledge, and an expanded view of the disorder. Although RTT is unquestionably a neuronal disease, the role of astrocytes and microglia in its pathogenesis offered new and rather global targets. Despite this, evidence supporting the involvement of virtually every neurotransmitter system allows symptom- and neural pathway-specific focus. The fact that MeCP2 deficiency in GABAergic neurons may explain most clinical manifestations of RTT blurs the lines separating general and selective treatments. While most recent trials have used growth factor-like compounds targeting multiple neural systems (i. e., full length IGF-1 or its active peptide trofinetide), NMDA receptor antagonists (e. g., dextromethorphan) are probably the best example of time-dependent mechanism (i. e., increased NMDA receptor activity in early childhood). At present, there are many potential treatments, with variable degree of selectiveness. MeCP2-related experimental models of RTT can also assist with repurposing available drugs with known safety and pharmacological properties. This promising pipeline is, however, affected by the logistics of testing every candidate drug and the experience in other neurodevelopmental disorders; success in mice does not guarantee effectiveness in humans. Although animal models will continue to provide the proof of principle for potential treatments, the timing of transition to early phase trials (i. e., phases 1 and 2) is unclear. These trials are the only approach for determining for certain the safety and efficacy of drugs in a clinical population. Whether iPSC-based assays will add critical evidence is unknown; iPSC technology has progressed slower than expected, as is the case of many new methodologies.

The apparently inevitability of early transition from animal to human trials underscores the importance of "intelligent" trial design. Studies that collect data on safety and efficacy with a small number of participants and then adapt quickly to their findings, such as adaptive trials, have not been yet tried in RTT or other neurodevelopmental disorders. Considering the relatively low doses employed in most trials (confirmed in the adolescent/adult trofinetide trial in RTT) and the need for rapid adjustment, high sensitivity of outcome measures is essential. Although progress has been made in this area, and endpoints are beginning to be evaluated for measurement properties such as internal consistency, reliability, and different forms of validity (e. g., Motor Behavioral Assessment and the Anxiety, Depression, and Mood Scale), there is still a great need for outcome measure development and validation in RTT. Appropriate selection of cohorts, in terms of clinical severity and other features, has been helped by the application of Clinical Global Impression (CGI) ratings using RTT-specific anchors and other clinical severity measures (e. g., Clinical Severity Scale). Nonetheless, biomarkers or clinical indicators that can distinguish responders to a particular drug are not yet available in RTT.

The success of any intervention study depends on prompt completion of recruitment goals, which is influenced by a variety of factors. While acceptance of cross-over trials, which ensure drug administration, is greater than parallel design (i. e., drug vs. placebo), the longer duration of the former increases the chance of complications related to the severe nature of RTT that are difficult to differentiate from true adverse events. Social media could assist with recruitment and retention, if managed carefully. Application of stringent subject selection criteria to a rare disorder like RTT leads almost predictably to multi-site trials. Whether the recent emphasis by regulatory agencies on improvements in function and quality of life would result in increased interest in clinical trial participation in RTT is unknown; however, it will almost certainly increase the chance of meaningful drug studies.

Finally, two approaches that need to be explored in RTT and other neurodevelopmental disorders are [1] combining drug administration with cognitive or sensory stimulation paradigms that can enhance synaptic plasticity, particularly in early childhood, as a mean to optimize drug response; and [2] pragmatic trials that utilize clinical care as the setting for comparing the efficacy of common treatments.

Future of the clinical trial field in Rett syndrome

We are at the beginning of the second era of developing treatments specific for Rett syndrome (RTT); ones that are based on the advances in genetics and neurobiology of the disorder since the discovery of the link to *MECP2* in 1999. So far, progress has been modest and slow. The RTT field is experiencing the same challenges that drug development for other neurodevelopmental disorders has encountered. The future of clinical trials in RTT is closely linked to the success, among others, in learning the

proper transition from animal model to human trial, development of sensitive and informative outcome measures and biomarkers of response to treatment, and the implementation of dynamic trial strategies. There are many reasons to be optimistic, beginning with the suggestion of effectiveness of trofinetide in adults with RTT that raises the possibility of successful treatments throughout the life span of an individual with the disorder. New technology, including iPSC-based drug testing, comprehensive biometrics, and new analytical tools that include big data approaches and machine learning will play a central role in the future of RTT treatment. The potential impact of combining synaptic plasticity-inducing paradigms with drugs cannot be underestimated either, as well as the role of patient-customized treatment goals. With so many possibilities, the key to speedy and transformative trials will be organization and clever allocation of limited human and material resources. This will make the difference between breakthroughs and incremental improvements in the next half a century.

My pathway in the Rett syndrome field

I had the fortune to be introduced to the Rett syndrome field by Hugo Moser, when I was beginning to work on my NIH career award in the early 90s. It led to a nice transition from my basic science search for genes involved in intellectual disability to a more clinically oriented focus that has been central in my career. It also opened the door to a field of very committed clinicians and researchers, both at my institution at the time (the Kennedy Krieger Institute) and many others throughout the world, as well as wonderful families doing everything humanly possible to improve the lives of their daughters. Over time, my initial bench work on the neuropathology and neurobiology of Rett syndrome evolved into neuroimaging and behavior, leading eventually into clinical service and drug trials, particularly after my move to Boston Children's Hospital in 2012. An important event in this Rett pathway was my coordination role for the consortium RettSearch, which allowed me to have even more contact with my colleagues overseas. Fond memories of two pioneers and giants in the field, Bengt Hagberg and Masaya Segawa, will be always with me. There are too many to thank for their kindness and help in my attempts to improve the quality of life of girls and women with Rett syndrome.

1.08

Databases today and tomorrow: relevance for clinics and research

Helen Leonard

Telethon Kids Institute, University of Western Australia, Perth, Western Australia

Rare disorders make a significant contribution to childhood disability. Yet many aspects of rare disorders remain poorly understood. These include: diagnosis; natural history; life expectancy; clinical variation; appropriateness of interventions and possibility of cell based treatments.

The Australian Rett syndrome Database was established in 1993 and since that time has continued population-based ascertainment of Rett syndrome in Australia from multiple sources. As a consequence, by 2016 we have ascertained 432 cases, 79, 18 % of whom have died over these two decades. Over a period of 23 years we have administered up to seven waves of follow-up questionnaires allowing us to develop a comprehensive bank of information about the natural history and life expectancy of this disorder. We have also collected video data, bone density assessments and clinical information from hospital records.

We have been able to gauge how the incidence of diagnosis may have changed both as a result of increased awareness and availability of genetic testing. We have also been able to examine how age at diagnosis has been influenced by the specific mutation type and how individuals with specific mutations have been more or less likely to be diagnosed.

The Database has also provided insights into many aspects of the natural history of this disorder. These include early symptoms, timing of the regression period, gross motor and hand function and hand stereotypies and communication, the latter functional aspects being particularly enriched by the inclusion of longitudinal video data. The Database has also informed a better understanding of many of the comorbidities that occur in this disorder such as epilepsy, scoliosis, growth problems and osteoporosis. In addition to characterising these medical problems we have been able to identify specific risk factors, for example for scoliosis and for fractures. We have also been able to describe the range of treatments being used and, more recently, to evaluate the effectiveness of interventions such as scoliosis surgery and gastrostomy insertion.

The output and material provided by the Database stimulated the need to assemble together available information on the management of several of these comorbidities, scoliosis, growth and gastro-intestinal problems and compromised bone health. Using our own evidence and that accessible, often from literature on other developmental disorders we established series of draft recommendations for each of these areas. To do this we engaged expert panels of Rett syndrome and other specialists in virtual international collaborations and used a Delphi technique to incorporate panel members' opinions into final sets of guidelines for each condition. These guidelines have both been published in the academic literature and also in user friendly formats for both clinicians and families.

One of the greatest strengths of our longitudinal population-based cohort is that as well as the ability to use time to event analysis to measure the age of onset of a range of comorbidities and investigate factors affecting their occurrence risk it can also provide less biased estimates of mortality than possible using other methods and sources. This is because the oldest individuals in our database, who were in the paediatric range at the initiation of the database in 1993, are now aged 40 years and have been followed for 23 years. We previously demonstrated how much life expectancy in Rett syndrome had improved in comparison to Dr Andreas Rett's original cohort. Yet it is important, as we have done, to continue to revise our estimates as follow-up time is increased and more deaths accrue.

The Australian Rett Syndrome Database does not stand alone. It is complemented by data from the International Rett Syndrome Database, InterRett, established in 2002 to provide larger numbers than could be provided by a population database serving a population, at that time, of only 21 million people. The identification of the principal genetic cause of Rett syndrome in 1999 and the need to investigate the factors determining the clinical variability in Rett syndrome underpinned the requirement for an even larger repository of cases which could be used to investigate genotype phenotype relationships. This repository has certainly served this purpose well and has made a major contribution to understanding which point mutations are generally severe, which generally mild and which are associated with a more variable clinical pattern. It has also been used to describe the characteristics of mutation groups such as C terminal deletions and large deletions in *MECP2*. Unlike the Australian database which represents a population cohort, limited to full ascertainment over specific birth years, InterRett has no age limit and therefore has also provided an excellent

data source to describe the characteristics of adults with Rett syndrome. InterRett also provides the opportunity to involve families from majority world countries such as China to participate in research and for the rest of the world to understand the additional challenges that such families encounter in terms of access to diagnosis and interventions. However, such countries with very large populations can also contribute to research in unique ways, which are less feasible in more developed populations.

A final and very important contribution of InterRett has been the ability to identify cases, originally thought to have the early-onset seizure variant of Rett syndrome but now known to have mutations in the *CDKL5* gene and to constitute an independent disorder separate from Rett syndrome. Our bank of data allowed us to compare the clinical features of cases with a *CDKL5* mutation and compare them with their counterparts with a *MECP2* mutation. As a consequence of this the International CDKL5 Database was established in 2012 as a sister database to InterRett.

In this presentation I will describe the establishment and history of the Australian population database, the international database, InterRett and its younger sister, the International CDKL5 Database. I will explain their differences, and relative strengths and shortcomings. More importantly I will describe the outcomes and the major contributions each has made to the understanding of Rett syndrome and related disorders and their management today and the opportunities these databases offer for the future.

Fig. 1.08 Helen Leonard

1.09

Dr Rett: a legacy of inspiring families

Yvonne Milne

Rett UK, European Association of Rett Syndrome (RSE)

Just after I founded Rett UK in 1985, I wrote a newsletter to our earliest members in which I related the "miracle" of Dr Rett accepting my invitation to come to the UK to meet us at our inaugural conference earlier that year. In my letter, I noted how he had flown over from Vienna at his own cost, and, as he put it, "with much love in his heart." This was typical of the affection and generosity of this kind, compassionate, charismatic man who has left us with a great legacy.

An inspiration

At our very first meeting it was as if he knew each and every one of our daughters, such was his insight into their lives and souls. If any of us had lacked the motivation to work for our newly formed charity, they could not fail to be inspired.

Two of the expressions he used at that landmark meeting, "The Rett girl is alive with the eyes" and "walking is more than just walking" not only endure in our memories, but are proved to be just as true today; new eye gaze technology is now bringing exciting opportunities for communication, and the enormous benefits of an active lifestyle to health and well being are now more clearly understood.

Our family has a special reason to be indebted to Dr Rett. When he examined our then 5 year old daughter Clare in 1985, she was unable to weight bear or move her legs to walk. Patiently he demonstrated his method of assisted walking. Inspired by this we enlisted the help of her school and therapists, working on this technique. Clare gradually gained in strength and confidence, and we were rewarded when she learnt to walk with support. This was something we had been told would not be possible. When I told Dr Rett the good news he was so delighted!

A European legacy

Dr Rett also pleaded with us to reach out to other countries in our work, to help families who were struggling with difficult social and economic circumstances.

We began this outreach work in 1994 with the inaugural meeting of the European Association of Rett Syndrome (RSE) which was held in Luxembourg, with 13 countries represented. This organisation has gone from strength to strength. At our last meeting in Rome in 2015 44 family associations or family contacts were reported as known to RSE, and the search for families or contacts in European countries where no Rett contact exists continues.

Dr Rett's legacy continues with RSE's plans for the future. These include drawing together resources on care and management of Rett people, which are held in centres of expertise or by parent associations throughout Europe; representing RSE at significant meetings and events, like the World Congress, Kazan and ECRD meeting in Edinburgh; and involvement in European calls for research projects.

We now also have representation as an ePAG member, for the proposed European Reference Network for the disease group "Rare Congenital Malformations and Intellectual Disability" thus ensuring Rett syndrome is well represented for the future landscape that is being drawn for rare diseases in Europe.

Emphasis on research

In the UK, Dr Rett's legacy is enshrined in Rett UK's vision as it looks to the future with renewed optimism. It has recently formed some important strategic alliances and identified new areas of collaborative work that will improve support to families in the UK, while also linking in with European colleagues to ensure work is not duplicated and valuable resources are not wasted.

In January 2015 Rett UK announced an alliance with the research charity Reverse Rett and later in July that year formed the Rett Disorders Alliance (RDA) bringing together several patient organisations, including CDKL5, foxG1 and Mecp2 Duplication.

Through the RDA there is now a group of experienced Rett clinicians and therapists who are working together to develop comprehensive clinical guidelines and a Rett reference network with 5/6 expert centres in the UK.

Practical support

In addition to its efforts to further research, Rett UK is also committed to improving practical support for Rett families.

It is doing this by developing Regional Hubs; bringing health, education and social service professionals to regional events to learn more about Rett syndrome; developing professional webinars on Rett topics; and new for 2016/17, the development of

Rett Education UK, an off-shoot of Rett UK providing educational support to both families and schools.

It was Dr Rett's dedication to "his girls" and to improving the quality of their lives that provided the vital initial spark that set Rett UK and RSE off on their very valuable journeys. Both organisations provide support to thousands of families and act as a conduit for research teams, to help them meet others and to find funding for their work.

Neither organisation stands still, and both are looking to the future, working out how best to continue the work that Dr Rett began all of those years ago. It seems fitting to me that we should try to honour his memory by carrying on this work with, as he put it, "love in our hearts."

Personal note of my link with Dr Rett

I got in touch with Dr Rett early in 1985, shortly after I started the Rett Syndrome Association in the UK. He responded immediately and with enthusiasm to the contact and my invitation for him to come to the UK to our very first meeting of families and professionals. In the years that followed, he was always willing to help and generously gave advice and support. He was a constant and enduring source of inspiration to families as they struggled to come to terms with the disability and deal with its day-to-day problems and challenges. Dr Rett said that it was very hard for him having such a serious disease bearing his name, with no known cure.

My thoughts on the direction work should move in the next few years

As the work to find a cure for RTT continues apace, I feel that we also need to keep focussed on the need for clinicians/families/carers to have clear agreed guidelines on the day-to-day care and management of the many and complex aspects of the condition. These would be especially welcomed in countries where the knowledge and understanding of RTT is less well advanced.

Fig. 1.09 Alison Kerr, Andres Rett and Yvonne Milne

Bio Sketch for Yvonne Milne MBE

My involvement with Rett syndrome began when my younger daughter Clare was diagnosed with the disease over 30 years ago. I founded the charity Rett UK in 1985.

My work in the UK led me to meet parents and professionals around Europe. I am one of the Founder members of Rett Syndrome Europe and am currently on the Board of this organisation.

I have served as a Non-Executive Director on the Board of two NHS (National Health Service) Trusts; for Primary Care and Mental Health/Learning Disabilities. I currently work as a lay member for the Health and Care NHS Trust.

In 1997 I was awarded the MBE (Membership of the Order of the British Empire) for Services to Health.

In May this year (2016) I was elected by EURORDIS (Rare Diseases Europe) members to be an ePAG (European Patient Advocacy group) representative to serve on the Board of the proposed ERN (European Reference Network) for the disease group "Rare Congenital Malformations and Intellectual Disability".

1.10

Clinical and genetic diagnostic criteria in Rett syndrome

Jeffrey Lorenz Neul

Department of Neurosciences, University of California, San Diego, USA

Rett syndrome (RTT, MIM 312750) is a severe neurodevelopmental disorder originally described in 1966 by Andreas Rett [1] and further recognized by Bengt Hagberg in 1983 [2]. The distinctive clinical features and progression of Rett syndrome led to the development and refinement of consensus clinical criteria for the diagnosis of "typical" or "classic" Rett syndrome. Over time, affected people with distinctive disease features or only presenting with a subset of typical Rett syndrome features have been recognized, leading the description of "atypical" variants of Rett syndrome.

Seminal work by Huda Zoghbi led to the discovery that mutations in the gene encoding Methyl-CpG-binding protein 2 (*MECP2*) are present in rare familial cases of Rett syndrome as well as associated with the common sporadic forms of Rett syndrome [3]. This discovery identified genetic mutation hot spots that cause over 65% of all cases of Rett syndrome [4]. Additionally, this discovery also allowed genotype-phenotype correlation studies that determined that specific mutations are associated with a propensity to either more or less severe clinical features [4]. Ultimately, the vast majority (95%) of people with typical Rett syndrome have disease-causing mutations in *MECP2*. These facts led some to propose that Rett syndrome should be entirely defined based on the presence of pathogenic *MECP2* mutations, however a number of important considerations led to the re-emphasis of the importance of a clinical definition of disease for Rett syndrome.

The first important issue that reinforces the importance of a clinically based definition is the fact that although the vast majority of people with Rett syndrome have mutations in *MECP2*, there remains some people who clearly have the clinical features of Rett syndrome but do not have any identified mutations in *MECP2*. In some of the atypical forms of Rett syndrome, such as the Early Seizure Variant or the Congenital Variant, mutations in different genetic have been identified. Specifically, mutations in the gene encoding Cyclin-dependent kinase-like 5 (*CDKL5*) have been associated with the Early Seizure Variant [5], and mutations in the gene encoding Forkhead Box G1 (*FOXG1*) have been associated with the Congenital Variant [6]. As additional information about the clinical features associated with mutations in these two genes develops, it is becoming increasingly clear that clinical disorders caused by these mutations have similarities but also a number of distinctive features compared with typical Rett syndrome, arguing that these likely should be considered unique disorders. Additional genetic studies on people with clinical Rett syndrome but lacking *MECP2* mutations have found mutations in a number of other genetic loci. Interestingly, the genes mutated in these people with Rett syndrome are also mutated in other neurodevelopmental disorders and in epilepsy syndrome [7, 8].

Furthermore, there is enrichment in genes encoding proteins involved in chromatin remodeling, excitatory, and inhibitory neurotransmission, arguing that these molecular functions are fundamental biological processes underlying the development of Rett-like clinical features. From this work we know that mutations in genes other than *MECP2* can cause Rett syndrome, indicating [8] that a mutation in *MECP2* is not necessary for the clinical diagnosis of Rett syndrome.

The second important issue that emphasized the importance of a clinical definition of Rett syndrome over a genetic definition is the identification of people with mutations in *MECP2* who do not have Rett syndrome. In rare familial cases of Rett syndrome, the *MECP2* mutation is inherited from an unaffected mother who has extreme skewing of X chromosome inactivation (XI), allowing for a normal clinical presentation [9]. Obviously the unaffected mothers with *MECP2* mutations should not be designated as having Rett syndrome. Additionally, boys with disease causing mutations in *MECP2* have been identified, but they are very severely affected with severe postnatal encephalopathy, early death, and absence of distinctive clinical features of Rett syndrome [9, 10] . Finally, a group of unique individuals with *MECP2* mutations and clinical problems such as autism or pervasive developmental disorder but lacking features such as regression that define Rett syndrome have been identified [11]. These unique individuals indicate that there are likely other biological factors such as genetic modifiers that allow milder clinical presentation. The fact that people can have mutations in *MECP2* and be unaffected, have a markedly more severe and distinct disease course, or not show characteristic features of Rett syndrome indicate that a *MECP2* mutation is not sufficient for the diagnosis of Rett syndrome.

With this in mind, in 2010 an international group, RettSearch Consortium, set out to create a revised clinical criteria for the diagnosis of typical and atypical Rett syndrome emphasizing the clinical nature of the diagnosis [12]. An important additional goal was to simplify and clarify features of the previous criteria in order to enhance acceptance and usability. The revised criteria emphasized the need for a typical disease progression in Rett syndrome, with a period of apparently normal early development, followed by regression and then leading to clinical stabilization. For major criteria were specified: 1) Partial or complete loss of acquired purposeful hand skills, 2) Partial or complete loss of acquired spoken language. 3) Impaired or absent gait, 4) Stereotypic hand movements. The diagnosis of typical or classic Rett syndrome required the disease progression, all four major criteria, and no exclusion criteria (evidence of significant brain injury or grossly abnormal psychomotor development in the first six months of life). The diagnosis of atypical Rett syndrome requires the typical disease progression, 2 of 4 major criteria, and 5 of 11 supportive criteria. Analysis of a large clinical cohort revealed that systematic utilization of these revised criteria for diagnosis was very consistent with previous diagnostic criteria [13]. This revised criteria has been widely adopted in the field and is utilized for clinical trials as well as clinical care. Moving forward, a major goal will be to further refine the criteria for atypical Rett syndrome and determine for-

Fig. 1.10.1 Jeffrey Neul eating dinner with Dr. Hagberg, Dr. Percy, Dr. Glaze, and Jane Lane (2004, Ottawa, Ontario, Canada)

mally the exact number of supportive criteria required to make a diagnosis of atypical Rett syndrome, and to refine the criteria for the diagnosis of specific atypical variants.

Personal note of relation to Andreas Rett or field in general

I never had the pleasure of meeting Dr. Rett in person as he died while I was still in medical school and I was not yet developed an interest in Rett syndrome. My tie to Dr. Rett is entirely through the mentorship I received in Rett syndrome from Dr. Daniel Glaze and especially from Dr. Alan Percy, both of who knew Dr. Rett. Dr. Percy was also very close to and fond of Dr. Bengt Hagberg, who in conjunction with Drs. Aicardi, Dias, and Ramos introduced the disorder widely and graciously named it after Dr. Rett who initially described this disorder. The picture I provide (Fig. 1.10.1) is a dear memory of mine from 2004 of the time I met Dr. Hagberg in Ottawa, Ontario, Canada. This picture shows me eating dinner with Dr. Hagberg, Dr. Percy, Dr. Glaze, and Jane Lane, Dr. Percy's longtime nurse manager who knows more about Rett syndrome than 99.9 % of physicians.

Thoughts on where the field will move in the next years

The discovery of the relationship between mutations in *MECP2* and Rett syndrome by Huda Zoghbi opened up a new era in Rett syndrome, allowing the field to develop a number of highly useful disease models to dissect the pathophysiology of the disease and identify and test novel therapeutic approaches. The critical work by Drs. Adrian Bird and Jacky Guy surprised the world by showing that the disease may be significantly modified if not reversed-thus now we know where we want to go. The challenge is figuring out how to get there and then systematically testing exciting therapies in people. The field has now embarked on clinical trials, and I think the future will be an exciting time in which we work to rapidly move innovations from the lab into the clinic. Nonetheless, major challenges remain. The molecular biology of MeCP2 remains challenging and we have yet to have a firm understanding of how this important protein works. In the clinical arena, we have a great need to develop well-validated quantitative measures of key clinical features such as hand use, communication, and gait. These challenges acknowledged, I think that the future for Rett syndrome is very bright and I feel that we will make substantial improvements in function in Rett syndrome that will lead to greatly improved quality of life for affected individuals and their caregivers.

Short bio and picture

Jeffrey Neul MD (Fig 1.10.2), Ph.D. is the Division Chief of Child Neurology, Professor and Vice Chair in the Department of Neurosciences at the University of California, San Diego and Rady Children's Hospital-San Diego. After receiving his undergraduate degree from the University of Illinois and his medical degree and Ph.D. in Developmental Biology from the University of Chicago, Dr. Neul completed a residency in pediatric neurology at Baylor College of Medicine, Houston, TX. During clinical training, he nurtured a long-standing interest in neurodevelopmental disorders and became interested in Rett syndrome (RTT), ultimately doing a post-doctoral fellowship in the laboratory of Dr. Huda Zoghbi at Baylor. Currently he is deeply involved in both clinical as well as basic science research understanding the nature of RTT and developing therapeutic strategies to treat this disorder. Dr. Neul currently holds research funding from the U. S. National Institutes of Health and the International Rett Syndrome Foundation, and was the principal investigator on the first of its kind industry sponsored clinical trial of a potential disease modifying treatment in Rett syndrome, sponsored by Neuren Pharmaceuticals and the International Rett Syndrome Foundation. Dr. Neul serves on the medical advisory committee for the International Rett Syndrome Foundation, the International FoxG1 Foundation, and is on the executive committee for RettSearch.

Fig. 1.10.2 Jeffrey Neul

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1.11

Rett syndrome, pathophysiology; from observation to insight

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The initial observation

Rett syndrome was first identified and described by Prof. Andreas Rett in1966.

Dr. Rett organized the first symposium on Rett syndrome on April 8, 1983, in Vienna, Austria. A rather small group of people from Europe and Japan attended. In that meeting, Dr. Rett described girls and women of various ages with Rett syndrome. The similarities and dissimilarities of characteristics symptoms according to the ages of the patient were obvious and striking. During this symposium, I presented a short report of our data and suggested the possible catecholamine involvement in Rett syndrome. Prof. Walter Birkmayer immediately commented on the similarity of the akinesia of Parkinson disease and the kinesie paradoxale, and the symptoms of Rett syndrome including the gait.

The syndrome was initially assumed to be a progressive disorder with the regression period around late infancy. The clinical symptoms of the patients become apparent around late infancy after seemingly normal period for the first 6 to 18months. Because of this clinical process the degenerative changes in the central nervous system was initially suspected.

In the second symposium organized by Prof. Rett in 1984 in Vienna we (Dr. Segawa and I) were the first to propose that Rett syndrome is the unique developmental disorder, and probable involvement of the monoamine and catecholamine systems.

In this presentation on the occasion of the 50th anniversary of the first paper by Prof. Rett, I review our proposed pathophysiology of Rett syndrome based on clinical observation and neurophysiological findings on sleep.

Our clinical and neurophysiological observation

The most frequent presenting complaints of the patients of Rett syndrome visiting Segawa Neurological Clinic for Children were abnormalities of early development. The abnormalities most frequently perceived by the parents were very subtle that something was not right with the child. The baby was placid, and often described as very good, does not cry much, and slept most of the day.

The decreased muscles tone of the trunk and extremities were noted in early and mid-infancy. The delay in motor milestones became apparent regarding the crawling. The gait is characterized by lack of the coordinated movement of upper extremities. The girl move forward by rocking the body from side to side with wide based posture. This walking pattern was called as apraxia, but we analyzed this is not the apraxia of gait but it is the failure in locomotion. The development of the skillful hand use was delayed from mid-infancy.

Around one year old the regression takes place, in that girls begin to show the characteristic clinical features involving motor and behavioral symptoms. Loss of purposeful hand use, pathognomonic stereotyped hand movement, and change of behavior occur. Then, the symptoms, such as the increasing muscle tone (dystonia), epilepsy, and abnormal breathing pattern may become manifested. As the age advances scoliosis may appear and progress. As to the stereotyped movements of the hands it starts by very simple movements changing to the complexed movements with increasing dystonic posture. The head circumstances show the deceleration starting after mid-infancy, resulting in acquired microcephalus. These characteristic features appear age dependently, and show the age-related processes.

We suggested the dysfunction of higher cortical function correlated with the grade of deceleration of head growth and grade of failure in locomotion.

In infancy the Rett girls tend to sleep longer during the day, with the delay in the formation of day-night rhythm. Slower physiological decline in daytime sleep and longer total day sleep time comparing to that of normal peers are observed. The former indicates development of abnormal sleep amplitude after 4 months of age. The circadian sleep-wake rhythm developed.

Polysomnography revealed that rapid eye movement (REM) sleep parameters are present by 36 weeks' gestational age. Abnormalities included the development of the phasic inhibition index, that is, inhibition of the twitch movement appearing in the period of occurrence of REMs burst in REM stage, and leakage of atonia of REM stage into nonREM stage. These findings indicate that the abnormality of Rett syndrome is taking place between 36 or 38 weeks' gestational age and 4 months' postnatal age. The body movement during sleep showed the both gross and twitch movements revealed different patterns between the patients younger than 6 year-old and older than that.

Pathophysiology

The natural history of Rett syndrome and the age-dependent appearance of the specific clinical features reflect the changes occurring along the maturation of the responsible neuron or neuronal systems.

The earliest clinical features of decreased postural tone and failure in locomotion suggest the hypofunction of the brainstem monoaminergic (noradrenergic and serotonergic) neurons. Hypofunction of these brainstem monoaminergic neurons also cause poor response to environmental stimulation, and the abnormalities observed in sleep rhythms.

Locomotion is controlled by propriospinal locomotion systems. The tonic innervation of the brainstem monoaminergic system is necessary for the system to be activated.

The brainstem monoaminergic system involved in postural tone and locomotion influence the function of the pedunculopontine tegmental nucleus, and then to dysfunction of the nigrostriatal and ventro-tegmental dopaminergic and Meynert nuclei. The dysfunction could finally cause the failure in synaptogenesis of the frontal cortex.

In summary

Based on these observations we proposed that the disorder starts from early infancy, with dysfunction of brainstem monoaminergic neurons. However, it took some time before the disorder was widely recognized as a developmental disorder, and the onset is much earlier than initially identified.

Evaluating the natural history of Rett syndrome is important because initial symptoms indicate primarily affected neurons, and alterations of symptoms with age implicate the spreading of the involved neurons or neuronal systems influenced by the neurons initially affected. In Rett syndrome the initially involved brainstem and midbrain monoaminergic neurons

were suspected to cause malfunction of hierarchically arranged neurons or neuronal systems of various levels.

The discovery of the causative gene, *MECP2*, was very intriguing. The role of *MECP2* in the developmental processes at specific neurons or neuronal systems involved in the pathophysiology of Rett syndrome are important key points to understand this disorder.

A paragraph of my relation to Prof. Rett

It was a day in May 1982, when I received international telephone from Dr. Masaya Segawa, who was in the meeting in *Göteborg* after attending Copenhagen international congress of child neurology. The words by Dr. Segawa are still vivid to my ear, that in the *Göteborg* meeting Dr. Hagberg reported the new syndrome which had been already reported by Andreas Rett in Handbook of Neurology. We had been following four cases till then with the identical symptoms thinking this was a specific disease. I immediately opened the Handbook and what I saw there was exactly the same as our experience in the Segawa Neurological Clinic for Children.

In the autumn of that year there was international neuropathology congress held in Vienna under Prof. Seitelberger. I decided to go to the congress and to visit Prof. Rett (Fig. 1.11.1). On arriving Vienna, I phoned Prof. Rett. He instructed me to take taxi and to come to his institution, Ludwig Boltzmann Institute for Research on Infantile Brain Damage, Hospital for Neurological Diseases, Rosenhügel. It was an unforgettable day. I showed the precise data of the five (initial four and additional one) patients. Prof. Rett excited by saying that "Yes, these patients have my disease! There are patients of my disease also in Japan!" He showed me his patients and gave me the photo of two girls of his first patients, and told me how he first noticed the disease. We had an interesting discussion on that day. I remember I said to him the characteristic gait by Rett patient is not the apraxia but the deficit of locomotion. He said that "We discussed that among famous Viennese neurologists and their opinion was that it may be best described as apraxia of gait, from adult neurologists' view". When I returned to the hotel I received a call from Prof. Rett. He said "I am crying that I could not discuss with you more about my disease. I will organize the first meeting next year. Do you come?"

On April 8, 1983, he organized the first symposium in Vienna.

There were famous neuroscientists including Prof. Franz Seitelberger and Prof. Walter Birkmayer, and other many important persons of the government of City of Vienna and Country of Austria. That was the very impressive and informative day.

It was already obvious that Rett syndrome was a particular disease. My presentation about the developmental dopamine and serotonin disorder as the pathophysiology prompted the Prof. Rett and Wien group, which resulted in the catecholamine and monoamine analysis of the autopsy brain presented by Dr. P. Riederar at the next year conference in 1984.

Since then there were meetings almost each year, where we discussed about this disorder. Prof. Rett was always the leader and his extraordinary warm and kind personality made the each congress so exciting and fruitful. We were all like a family. Prof. Rett called me as his Rett sister.

In March 1988, he visited Segawa Neurological Clinic for Children. Dr. Segawa and I organized a symposium (Fig. 1.11.2) inviting many Japanese clinicians and researchers. Prof. Rett examined the patients at Segawa Clinic.

On the occasion of the Joint Meeting of the 5th International Child Neurology Congress and the 3rd Asian & Oceanian Congress of Child Neurology, Tokyo, on November 3–4, 1990, Dr. Segawa and I organized the International symposium in Aoyama, Tokyo. Prof Rett was the guest of honor (Fig. 1.11.4–6). The Japanese parents association of Rett syndrome was established on that occasion. It was the exciting time for the parents, families and all of us.

Fig. 1.11.1 My first visit to Prof. Rett. The photo of two girls was given to me from Prof. Rett (1982 autumn)

Fig. 1.11.2 From left to right: Ms Anna Benis, secretary, Yoshiko Nomura, Prof. Rett, Dr. Segawa (1988, March 11, Tokyo Symposium)

Fig. 1.11.3 Rett congress (1988, November, Wien)

Fig. 1.11.4 From left to right: Dr. Segawa, Ms Barbara Rett, Prof. Rett, Yoshiko Nomura (1990, November 1, Tokyo)

Fig. 1.11.5 Establishment of Japanese Rett syndrome parents' association. Panelist, from left to right: Prof. Rett, Prof. Hagberg, Dr. Ingegerd Witt Engerström, Dr. Alan Percy, Dr. Alison Kerr, Mrs Karhy Hunter, Dr. Sakkubai Naidu, Dr, Asayo Ishigaki, Dr. Masaya Segawa (1990, November 4, Tokyo)

His last appearance in the international meeting was in 1993, international meeting in Antwerp.

In June 1996, I visited Prof. Rett at his house with Dr. Segawa. Prof. Rett had been ill, but his passion for the sciences and love for the patients had not changed. It was another unforgettable time in my life. What I learned from Dr. Rett still remains alive in myself.

Where the "field" might move

Although the pathophysiology of Rett syndrome have been discussed and explored, and causative gene was identified, we are still behind in regard to the treatment or managements.

Collaborative study by the clinical and basic sciences are mandatory.

A short bio-sketch

Yoshiko Nomura (Fig. 1.11.7)

Completing my post graduate training in pediatrics (Mayo Clinic, Rochester Minnesota), child neurology (Washington DC Children's Hospital, Washington DC) and neurology (Georgetown University, Washington DC) in the U. S. A., I was given the opportunity to work as the assistant director at the Segawa Neurological Clinic for Children, Tokyo Japan in 1975 two years after its establishment by Dr. Masaya Segawa.

The clinic work covered the wide range of child neurology. Among those the care and clinical research on Rett syndrome was one of the main theme.

What Dr. Segawa aimed was to understand the diseases at neuron level and find the way of treatment. I learned very much from him. We also practiced not only child neurology, but also life-long neurology.

Dr. Segawa passed away in December 2014, and the clinic automatically closed because it was his private clinic.

On August 1, 2015, I opened my clinic, Yoshiko Nomura Neurological Clinic for Children. I will continue my work and mission as a medical professional.

Fig. 1.11.7 Yoshiko Nomura

Fig. 1.11.6 Establishment of Japanese Rett syndrome parents' association. Prof. Rett, Yoshiko Nomura (1990, November 4, Tokyo)

1.12

Progress in Rett syndrome: First steps to natural history study

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Abstract

Rett syndrome (RTT) was essentially unknown in the US until the 1980s. Dr. Mary Coleman, a child neurologist in Washington, DC, had learned of this disorder at a meeting in Paris and Dr. Vanja Holm, a developmental pediatrician from Seattle, Washington, had attended a meeting in Sweden in the early 1980s. In 1983, shortly after the publication of the first widely-read English language paper by Bengt Hagberg and colleagues, I was asked to see a young girl in Houston, Texas whose pediatrician suspected that RTT could explain the clinical features displayed by this girl. After reading the paper of Hagberg et al., I confirmed this diagnosis and together with colleagues in Houston identified five additional girls with this disorder within the next few months. Following this introduction to RTT, I contacted Bengt Hagberg and Andreas Rett regarding their expertise with this disorder and was invited to the meeting organized by Dr. Rett in Vienna in 1984. Thereafter, a clinic was established at Baylor for individuals meeting the diagnostic criteria promulgated at the 1984 meeting. Daniel Glaze and Huda Zoghbi joined in this clinic. A genetic cause was suspected by the evident preponderance of this disorder in females and by the suggestion that an X-linked disorder affecting girls only would be X-linked dominant. Huda, who was in genetics training, pursued studies that, based on unusual chromosome findings in our first girl, focused attention to the distal portion of the long arm of the X chromosome. Additional studies by Carolyn Schanen further limited the area of interest to Xq27-28. This very gene rich region with a number of other X-linked disorders proved to be the locus of the causal mutation in the methyl-CpG-binding protein gene (*MECP2*) at Xq28 (Amir et al.). Identification of *MECP2* mutations in the Zoghbi laboratory was subsequently confirmed in several laboratories. The *MECP2* gene had been studied for more than a decade, particularly in relation to tumor biology, but this association with RTT led to robust basic science investigations in the central nervous system and beyond as well as to enhanced interest in gathering natural history data in prepara-

tion for possible clinical trials. As such, the Rare Disease Clinical Research Network had been established at the NIH based on a federal mandate in the late 1990s. We were fortunate to be awarded grant funding to initiate a RTT natural history study. Presently, we are in the thirteenth year of such studies, now addressing not only RTT, but also *MECP2* duplication disorder, individuals, both females and males, with mutations in *MECP2* but not fulfilling diagnostic criteria for RTT, and individuals with mutations in *CDKL5* and *FOXG1*. Over this period, we have gathered longitudinal data on more than 1300 individuals and have provided important information on RTT including developmental characteristics, age at diagnosis, growth, epilepsy, gastrointestinal features, genotype-phenotype correlations, scoliosis, quality of life for both the affected participants and their caregivers, and survival with a second study on the changes in survival over time. These results will be presented and discussed in relation to current progress in basic and translational studies that have paved the way for recent and on-going clinical trials with disease-modifying agents. It is a distinct honor and privilege to provide this report on the 50th anniversary of the first publication on RTT by Andreas Rett.

A personal note on Andreas Rett

I was fortunate to attend three meetings that Andreas (Andy) Rett organized in Vienna in 1984, 1986, and 1988 and to meet him on many occasions in the US. I witnessed both the concern and the compassion that he expressed for girls and women with Rett (RTT) syndrome and for their families. After all, this is a very debilitating disorder that raises many issues beyond the physical and emotional effects of RTT. Andy welcomed new ideas and different perspectives. Above all, he evidenced the passion to address these issues and greeted new physicians and researchers openly. I regret that he did not live to see the identification of the gene mutations in RTT, and I can only imagine that he would marvel at the advances over the past twenty years. Still, he would continue to focus on fundamental treatment approaches to erase the difficulties that these girls and women and their families must face. He was warm and welcoming, intense and imaginative, always searching for progress.

Thoughts on the field

In the more than 30 years that I have been involved with Rett syndrome (RTT), remarkable progress has been accomplished both clinically and in the laboratory. We have clearly improved the management of individuals with RTT and have provided guidance not only in terms of medical problems, but also with regard to proper nutrition, therapeutic interventions, and the new and emerging field of augmentative communication using computer-assisted strategies. We have witnessed the potential for fundamental reversal of RTT with genetic strategies. Taking the broader perspective of progress in RTT, we need to harness the energies of the international community at all levels of research from basic through translational to clinical studies. We see many disease-modifying strategies advancing through translational research and reaching the level of clinical trials. These follow important knowledge at all levels of investigation and represent a remarkable surge in therapeutic agents geared for disease modification. At the same time, strategies to reverse the underlying genetic defect must continue apace whether it is to replace the defective gene or reverse X-chromosome inactivation. While these approaches are being addressed aggressively, they do offer fundamental challenges that must be overcome. The expansion of clinical trials will require efforts of all in moving these forward. These efforts will require the commitment of funding agencies, pharmaceutical companies, researchers, and families at the very least. New and more investigators must be recruited. Parent advocacy groups must continue to press for continued progress. Clinical trials are neces-

Fig. 1.12.1 Original Natural History Study group; back row (left to right): Heather O'Leary, Daniel Glaze, Fran Annese, Steve Skinner, Walter Kaufmann, Katherine Barnes, Lauren Baggett, Susie Geerts, Jane Lane, Kay Motil; seated (left to right): Judy Barrish, Alan Percy, Jeff Neul

sary, but they are also labor intensive, demand careful conduct, and require direct participation of families and other caregivers. They require patience and courage, focus and understanding that this is a step-wise process. Above all, one must recognize the necessity of human involvement. Without involvement of everyone, we cannot advance to the desired goals of effective treatment and, ultimately, a cure.

Alan Percy, MD (Fig 1.12.2) is a pediatric neurologist at the University of Alabama at Birmingham in Birmingham, AL, USA. Following medical school at Stanford University in Stanford, California, he trained in Pediatrics there and in Child Neurology at Johns Hopkins in Baltimore, Maryland. Although engaged ath the time in laboratory studies of complex lipid biochemistry in the CNS, in 1983 he along with Dr. Mary Coleman in Washington, DC and Dr. Vanja Holm in Seattle, Washington were the first physicians to recognize Rett syndrome (RTT) in the United States. In 1984, Dr. Hugo Moser, Dr. Holm, and Dr. Percy attended the RTT conference in Vienna organized by Dr. Andreas Rett. Thereafter, Dr. Percy actively pursued clinical and laboratory studies in RTT, establishing centers at Baylor College of Medicine and later at the University of Alabama at Birmingham where he is the principal investigator of the Rett syndrome and Rett-related disorders Rare Disease Clinical Research Consortium. This Natural History Study of RTT now includes more than 1300 participants. He encouraged Dr. Huda Zoghbi to pursue genetic studies in RTT leading to identification of mutations in *MECP2*. He currently collaborates actively with Dr. Michelle Olsen and Dr. Lucas Pozzo-Miller in basic approaches to RTT at UAB. Since 1983, he has authored more than 120 scientific papers, chapters, and reviews on RTT. He remains committed to finding meaningful approaches to effective treatment for this unique neurodevelopmental disorder.

Fig. 1.12.2 Alan Percy

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1.13

The challenging Rett syndrome: to watch, to measure and to compare

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Extended Abstract

All clinical expertise is the result of a journey through many special encounters with girls and women with Rett syndrome (RTT). We have seen more than 400 individual cases with about 60 cases in follow up. Even after so many years one is still challenged by every new girl in trying to understand the on-going pathology. Hagberg's description of RTT as "a continuing dissociation between diminishing motor performance and a relatively better preserved personality and ability to communicate mainly through the eyes", reflects the many facets of this unique neurological disorder and its many challenges for clinical and basic research. The development of the modern techniques in molecular genetics and its overall availability has shown us a lot of detectable mutations and rearrangements in the MECP2-gene confirming the clinical diagnosis in the vast majority of cases. Diagnosis is now made at an earlier age and parents shouldn't be left long in uncertainty anymore. More and more female and male phenotypes with MECP2 mutation are described. And nowadays a growing number of MECP2 mutations and still unclear intron variations are detected by whole exome sequencing. Whole exome sequencing leads also to a number of atypical MECP2 related phenotypes in girls (and boys) with learning disabilities, that were not suspected clinically and to newly discovered pathogenic mutations in other genes (CDKL5, FOXG1, TCF4, PURA, i. e.). Smaller and larger cohorts of genotype phenotype correlation studies delineated those with better prognosis and better outcome. The CTS deletions and the R133C as manifest examples. Nevertheless variation of clinical severity in RTT is large and the search for epigenetic regulatory factors is on-going in this era of genomic analysis. The rare atypical MECP2 related and milder phenotype seem to manifest only the cortical features and not or lesser the extrapyramidal and brainstem features and represent a good model for studying behaviour and learning in the more severe RTT that cannot walk or speak and has dyspraxia. The focus on brainstem involvement in RTT is a longstanding issue. Breathing irregularities are a consequence of brainstem immaturity characteristic for RTT and discriminating epileptic from non-epileptic spells may require cortical-bulbar neurophysiologic assessment. In a recent multicentre study we used objective and robust data of cardiorespiratory variables in the investigation of genotype-phenotype correlation in RTT. All females with RTT had dysautonomia, and this was not restricted to nor influenced by one specific group or single recurrent mutation. We believe that objective information obtained from non-invasive neurophysiological evaluation of the brainstem autonomic functions will contribute to the understanding of the ongoing pathology in RTT and its life-long management. The focus on communication is an area where substantial progress is being made, using eye gaze technology in developmental testing, neurocognitive profiling, training in communicative ability in general and emerging literacy in the younger girls. The focus is also on aging in RTT. Longitudinal studies are ongoing and must be contin-

ued. Of special interest is the age group above 35 years and the smaller survivor group above 50 years.

MeCP2 has different levels of influence in biological pathways leading to clinical phenotypes. It regulates gene expression of many other genes and it up and down regulates metabolites in RTT. For the clinical and basic researchers it becomes a difficult task to keep up with all this growing knowledge and trying to fill in the gaps that still remain. Finding the right target genes/proteins/metabolites may build a bridge to known drugs which interfere with exactly that pathway leading to improvement of symptoms. Bioinformatics help us in this.

Personal notes

As a young paediatrician I attended the Prof. Hagberg's clinical "Rett-weeks" Eastern Hospital in Gothenburg, Sweden and stayed in contact with him during his active engagement in this syndrome. He presented me to Andreas Rett during the World Rett Conference in Antwerp in October 1993. Both clinicians were great examples for me. They not only teached me but also learned me to network with colleagues and parental associations of the first hour.

Families, clinicians and basic researchers are clearly committed to Rett syndrome. The quality of life today is improving and one is reaching out for a cure in the future. This joined efforts must be continued and new fields of cooperation, e. g. bioinformatics, genomics, metabolomics, must be developed in order to support the research and to find a cure.

Bio-sketch

Eric E. J. Smeets (Fig. 1.13), obtained his medical degree at the Catholic University of Leuven, Belgium. Trained as a paediatrician in developmental neurology and rehabilitation at the University Hospital in Leuven, he worked in the Centre for Human Genetics, University Hospital Gasthuisberg in Leuven, Belgium and in the Department of Clinical Genetics of the Maastricht University Medical Centre in Maastricht, the Netherlands. He has a long standing clinical experience with developmental disorders, genetic syndromes and intellectual disabilities in children and adults. He obtained his PhD at the University of Maastricht in 2005 on the subject of Rett syndrome and is the author of several articles on Rett syndrome and *MECP2* related disorders. In 2012 the multidisciplinary Rett Expertise Centre at the Maastricht University Medical Centre became operational under his impulse and leadership.

Fig. 1.13 Eric E. J. Smeets

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1.14

Communication in individuals with Rett syndrome: What we know now and what we have yet to find out

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Background: Individuals with Rett Syndrome (RTT) demonstrate severe limitations in their ability to communicate through conventional channels such as speech and hand signs/gestures, due in (some) part to the influence of apraxia, an inability to control voluntary purposeful movement. Understanding of RTT, its characteristics and the underlying neuropa-

thology, has moved a long way since Andreas Rett first described the syndrome in 1966, especially in the last 17 years since the discovery of mutations in the MECP2 gene [1]. Yet, whilst much is now known about RTT, many questions still remain. Disruption in communication skills has a fundamental and significant impact on quality of life for individuals with RTT and their families. To what extent apraxia is to blame, as opposed to any deeper language and cognitive impairments, is one area open to ongoing debate.

Objectives: This paper presents an overview of communication research to date, relating to later (post-regression) communicative forms and functions and intervention strategies, and suggests areas for future development and research.

Methods: A systematic review of research papers published over the last 20 years forms the basis for this presentation.

Results: A number of studies seek, through parental reports via structured interviews or questionnaires, behavioural or observational checklists and/or various experimental paradigms, to describe and categorise the communicative behaviours of individuals with RTT, the forms and functions expressed and levels of underlying intentionality [2–9]. Many studies conclude that individuals with RTT are often operating at pre-linguistic, pre-intentional levels of communication with meaning attributed to their behaviours by caregivers and communication partners. These behaviours include stereotyped hand movements, facial expressions, body movements, (undifferentiated) vocalisations, eye gaze, and in some cases hyperventilation. Where intentionality can be identified, reasons for communicating include seeking attention, protesting, requesting and making choices. Several studies point to seemingly low levels of language comprehension and cognitive functioning [10], especially when standardised receptive language, IQ or adaptive behaviour tests are applied. Reviews by Demeter [11] and Sigafoos et al. [12] point to the difficulties associated with reliably assessing individuals with RTT and highlight the need for functional tests which balance observational and standardised measures and which set a "gold standard" for assessment [12, p. 698]. In studies where parents are interviewed they frequently express the opinion that their children know more than they are able to express or demonstrate on assessment [2, 9]. Alongside these studies, research profiling genotype-phenotype relationships has also delineated more specific speech-language profiles according to variant and MECP2 mutation type [13–16]. In particular, individuals diagnosed with the Preserved Speech Variant offer a milder presentation, retaining or recovering an ability to speak, using single words or simple sentences, and having more control over hand function and mobility [17–19]. One common feature of the studies into communicative ability is the reported use of eye gaze as the most frequent form of expressive communication. Some authors observe that eye gaze may only equate to fixating on an object, indicating need or preference at a pre-intentional level, and raising the question of whether individuals with RTT use true referential gaze where communicative intent is signalled by switching gaze between a desired object and a partner [2, 20]. A study by Hetzroni & Rubin [6] demonstrated that this behaviour (at least in their cohort) can be trained. Hetzroni and colleagues [21] were also responsible for publishing one of the first studies linking the use of eye gaze with a computer screen to identify (meaningful) graphic symbols to verbal command. Other studies exploring cognitive performance through more advanced eye tracking technologies yield mixed results. One study by Baptista [22] demonstrates that children with RTT can follow key word instructions, recognise and match picture pairs, and categorise pictures. However, a later study by de Lima Vellosa et al. [23] is unable to substantiate recognition of concepts. More recent studies [24–29] which fuel interest in the potential benefits that eye tracking technologies can offer to individuals with RTT have led to calls for the development of more objective eye tracking-based cognitive and receptive language assessments which can be used to validate data gathered from parental reports [9, 30]. Alongside these assessment-based studies, findings from a small number of experimental design studies have recently been published which report on attempts to introduce various forms of augmentative and alternative communication (AAC) for functional communication [31–36], while surveys have been used to document professionals' [37] and families' experiences [38] of using AAC (including eye gaze technology) as forms of communication intervention. Although the cohorts reported so far are few in number and the results are mixed, the overall conclusions suggest that AAC strategies are worth pursuing with individuals with RTT. Studies exploring the development of reading and writing skills which can be utilised for communication purposes are also beginning to be published [39].

Conclusions: Although growing, the number of published studies relating to post-regression communicative forms and functions and intervention strategies employed with individuals with RTT remains limited. Eye gaze continues to be regarded as offering the best and most reliable form of access for both assessment and intervention/functional communication purposes, with more findings reported anecdotally than published as research paradigms. "Presume competence" is the watchword for many but for other practitioners the predominating view may be one of underlying severe intellectual impairment, which can be at odds with parental opinion. This leads to hugely differing expectations and wide variation in clinical practices with regard to assessment, intervention and management of communication. Practitioners should be encouraged to report and publish their work in order to build a stronger evidence base. In addition, the project currently funded by Rett-syndrome.org to develop international guidelines for the management of communication in individuals with RTT, founded on a combination of available evidence and expert consensus, should help to raise awareness and knowledge and to promote consistency of practice across the field[1].

Relationship to the field

Throughout my career I have been committed to working to improve quality of life for individuals with severe communication impairments and their families through the application and utilization of augmentative and alternative communication (AAC) strategies and through the use of technology. My work within the Rett Expertise Centre Netherlands and with international colleagues offers exciting opportunities to see developments which can make a real impact on lives. By working together to raise awareness and to develop best practices in relation to communication we can make a difference to individuals with Rett syndrome and their families.

Where the field might move

Developments in technology (especially eye gaze technology) will open up new ways to assess with greater certainty the levels of cognition and language understanding in individuals with RTT, to facilitate the development of expressive communication skills and to enable access to literacy. All of this, in combination with the development of guidelines for good clinical practice, will make a significant impact on the participation in society of

1 „Development of clinical guidelines for the management of communication in individuals with Rett syndrome", a HeART Grant-funded project which began in February 2016 led by the Rett Expertise Centre Netherlands. The international consortium includes: Prof. dr. Leopold Curfs and Gill Townend (Netherlands), Dr. Theresa Bartolotta (USA), Sally-Ann Garrett (UK and Ireland), Helena Wandin (Sweden) and Anna Urbanowicz (Australia).

individuals with RTT and will contribute to an improved quality of life.

Short Biosketch: Gillian Townend, B. Med. Sci. (Speech) (Hons), M. Phil., CertMRCSLT

Gill Townend is a researcher at the Rett Expertise Centre Netherlands in Maastricht which is recognised as the national reference centre for Rett syndrome for the Netherlands. Prior to this she worked as a Speech-Language Pathologist in the UK, specialising in augmentative and alternative communication and speech generating devices. In October 2013, she organised the communication track of the 3rd European Rett Syndrome Conference Maastricht and is currently joint lead with prof. dr. Leopold Curfs of an international project funded by Rettsyndrome.org to develop clinical guidelines for the management of communication in individuals with Rett syndrome (RTT). Her research interests include: early (pre-diagnosis) communication development in typical and atypical RTT (in collaboration with Dr. Peter Marschik, MU Graz, Austria and Karolinska Institutet, Stockholm, Sweden); eye tracking and the functional use of eye gaze for communication; European policy in relation to rare diseases and its implications for RTT; and the establishment of clinical and research-based networks and collaborations both within and outside of Europe.

Fig. 1.14 Leopold Curfs, Gillian Townend, Peter Marschik, Eric Smeets

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1.15

On the origins and rise of Rett syndrome

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Abstract

There is a sound international documentation on the rise of Rett syndrome since the middle of the 1980 years [1, 2, 3]. However, what is nowadays known as Rett syndrome had been described as early as 1965 by Andreas Rett with the original publication in 1966 [4] followed in the same year by a short contribution in a Viennese medical journal [5]. This early description offers almost the complete clinical symptoms nowadays recognized as diagnostic criteria for Rett syndrome: A progressive encephalopathy, characterized by cessation of normal development at age 6–18 months, loss of purposeful hand use, acquired deceleration of head growth, autistic symptoms and stereotypic hand movements ("hand-washing movements"). Yet, not seldom in research, there is an insignificant but mostly signifying story prior to this rather long incubation period between 1965, the single author publication of 1966 [4] and the agreement on diagnostic criteria of an international panel of respected experts in neuropediatrics during a Vienna based symposium in 1984, with proceedings published in 1985 [1]. During the first half of the 1980 years more than 1,200 female patients were detected in many regions over the world, all of them having a consistent set of clinical manifestations which conform to the complex phenotype of Rett syndrome. Even despite the presence of a specific diagnostic marker, this fact alone, the clinical behavioral phenotype, has led at that time to a general acceptance of Rett syndrome representing a distinct nosological entity and being included since the first edition in ICD-10. Nowadays Rett syndrome is known as a genetic disorder (X-chromosome, MeCP2) that strikes roughly 1:10,000 girls just as they are beginning to walk and talk. They end up with severe intellectual and physical disabilities and need extensive support and full-time care.

With regard to the twenty years of incubation two facts might be addressed: First, Rett's original publication of 1966 written in German language, a 68 pages volume, produced by a local Viennese publisher with modest visibility. Thus it can be assumed that this publication did hit only rare reads outside Austria. Rett produced a couple of additional German-language publications, with hyperammonemia being the first characteristic of the syndrome. Rett reflected on the neurotoxic effect of a constant high level of ammonia, thus seen as a causal agent for this highly devastating developmental disease. Indeed, his data revealed extreme high concentration of ammonia in the serum, with the blood analysis of the investigated population being processed in the labs of colleagues at the University of Veterinary Medicine in Vienna. Second, shortly after the original publication the hyperammonemia hypotheses turned out to be the effect of a systematic bias while determining the ammonia level. The access of ammonia found in the girls' serum was a result of a sloppy lab work: Rett's samples had not been tested immediately, and uric acid converts to ammonia over time. This flaw was not without any consequences: Rett's scientific reputation was strongly contested within the Viennese medical research community. At least since that experience Rett turned more to a lone-fighter going on with his cause. And in 1972 he saw the syndrome included in a German-language publication with the first time being recorded as Rett syndrome [5]. And continuing to be persistent, with finally recognizing the importance to reach out for an international research community, Rett himself provided in 1977 an extensive English-language report on Rett syndrome, sticking to his early metabolic pathogenetic view, with references not going beyond those used in the 1966 publication [6]. Yet, this article should get the attention of Bengt Hagberg, a leading Swedish researcher in the field of neuropediatrics. Hagberg obviously had studied analogue cases and on their way to publish these in 1982, he met Rett during a Toronto conference. Hagberg lectured on the cases he had studied, brought up his theories, outlined the yet unpublished paper in which he referred to the hand-washing syndrome. Thereupon Rett addressed him in his typical vivid way and invited Hagberg to Vienna to evaluate his cases. In the meantime the Hagberg team recalled their manuscript, reviewed it, to be finally published in 1983 and reported the cases with the label Rett syndrome [7]. Hagberg's acknowledgement of Rett's pioneering first description of a new nosological entity is a stunnig gesture and an expression of a noble character showing deep academic appreciation of Rett's early contribution. This was the beginning of a series of international symposia and conferences on Rett syndrome staged in Vienna, the United States and Japan. Besides the rising commitment of many researches from various fields to contribute to the enigma of Rett syndrome the evolution on the syndrome's knowledge was strongly fueled by the activities of the International Rett Syndrome Association, a parent's driven association.

In the end, now on the real origin of Rett syndrome, the story prior to the long incubation period and the popping as well as thrilling years in the 1980ties. According to Rett's personal communication it occurred in 1964 when one day he walked into the crowded waiting room in his hospital and saw two little girls side by side on their mothers' laps, rocking slightly with a distant look in otherwise impressive yes. However what was striking him most was the way they entwined their hands in a complex, frantic movement that resembled a rigorous hand-

washing. The mothers hold the hands of their daughters when they felt observed by Rett and he asked the mothers to let go of the hands, and again, the handwashing movement came up. He called his secretary, Martha, a nurse, mentioning that their might be other cases like this. And she answered, *yes* and named all at once six of them! They brought them all in, lined them up on a bench and they all behaved exactly as one another. He searched the medical books and found no description of this. He made a film and began an unusual odyssey to bring to the world's attention what he had discovered. After all, the "prodromal" phase triggering Rett's 1966 publication was very much triggered by his secretary, Martha, a nurse, who had a broad knowledge and memory on the many of his young patients, and it was Andreas Rett who put the crucial question to the right person at the right time[2]!

Personal note relation to Andreas Rett

It was in December 1983, just a few days before Christmas, when I first had the opportunity to meet with Andreas Rett. As my post-doc Fulbright position at the State University of New York at Stony Brook came to an end I searched and reflected on research centers in the area of clinical developmental neuropsychology to join as next for widening my experience and knowledge. One of the few was Andreas Rett, medical direcor of the Children's Department of the Neurological Hospital of the City of Vienna, Rosenhügel and Director of the Ludwig Boltzmann Institute for Research on Brain Damage in Children. Unexpectedly, Rett responded to my unsolicited application with an invitation for an interview. My expectation was to contribute to research in the area of children with neurodevelopmental disabilities. On that morning Andreas Rett, foresightful as he was, however, suggested research in the area of aging and disability. As a pediatrics, with a deep commitment to the life of the children he had come along with at that time during more than 30 years, he saw them growing up to late adulthood. Indeed life-expectancy showed since some decades a rapid and stunning increase for people with intellectual disability. His analysis on the bio-psycho-social situation and opportunities for older people with intellectual disabilities was so convincingly that we agreed on that morning to start a major research project on aging in people with intellectual disability! Aging, a new area and according to him the future in disability research was to be sponsored through resources of the Ludwig Boltzmann Institute accredited for research in children! This was just Rett: offbeat and unorthodox! For me the years with Rett showed to be highly inspiring and they were early career years with a weighty mentor. Further, these were the years where I learned to add to my early neuropsychological view, perspectives that show to affect the lives of people with intellectual disability at least as much as our biological foundation: The social and societal issues with challenges mostly being men-made! When I signed my contract on that very day, I was not yet aware that in the very next months I would experience a unique moment of international front-line research: the rise of Rett syndrome. Indeed, the international agreement on the diagnostic criteria was established during the symposium on Rett Syndrome in September 26–27, 1984 in Vienna.

Short note or some thoughts on "where the field" might move in the next years, what is needed, what can be expected

Definitely research on Rett syndrome will move on, especially in the area of microbiology and gene technology up to gene therapy. Further our experience of Rett syndrome might again get attention when delineating subtypes in the autism spectrum disorders. Further, as in Rett syndrome care still is leading before cure, the many areas of needs of support for girls and women with Rett syndrome will have to be revisited within the framework of the UN-CRPD especially with regard to social inclusion and participation.

Short bio-sketch

Germain Weber (Fig. 1.15), Ph.D., born in Luxembourg, is professor of Psychology at the University of Vienna, Faculty of Psychology, Department of Health, Development and Intervention. He is acting as dean of the Faculty since October 2008. Professor Weber holds an associate professorship at the Université de Luxembourg. Further, Dr. Weber is since 2004 president of „Lebenshilfe Austria", the major Austrian NGO organization offering systems of support for over 10.000 persons with intellectual and developmental disabilities and advocating for and with them on a national and European level. Dr. Weber's research is focusing on health and mental health issues both in people with intellectual and developmental disability and on older people. In 2000 Dr. Weber was awarded with the „International Award for Significant Contribution to Research, Policy and Practice in the Field of Intellectual Disability" offered by the „American Association on Intellectual and Developmental Disabilities". Since 2008 Dr. Weber is serving for the „International Association on Scientific Studies in Intellectual and Developmental Disabilities" (IASSIDD), actually as a member of the Executive Board with the function of Vice-President Europe.

Fig. 1.15 Germain Weber

2 Anecdote based on personal communications with Andreas Rett and Martha, the nurse in early 1990, as well on Irene Wielawski's cover-story report in „The Providence Sunday Journal", of December 7th, 1986 „Rett syndrome – A medical odyssey"

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1.16

Rett Syndrome: A winding path from clinic to bench and back to the clinic

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Rett syndrome is a postnatal childhood neurological disorder that causes a broad range of severe neurological and behavioral disabilities. It is unusual in that its symptoms appear after a period of normal development and point to disturbances in most brain cells and regions. In 1999, the Zoghbi lab discovered the gene (*MECP2*) that causes Rett syndrome and before long it became clear that mutations in this same gene can also cause autism, bipolar disorder, and juvenile onset schizophrenia. The path from gene discovery to therapy, however, is not a straightforward one and requires deep understanding of the role of MeCP2 in the brain, and knowledge of the brain cells and networks that cause specific symptoms. Through the use of genetically-engineered mice, the Zoghbi lab learned that the brain is acutely sensitive to the levels of MeCP2 and that both decreases and increases in MeCP2 levels (or function) can lead to neurological and beahvioral features that are also observed in humans. They showed that the protein is needed throughout life to maintain normal neurological functions and that normalizing its levels can reverse disease-like features in a mouse model of the human *MECP2* duplication syndome. Zoghbi and collaborators have been gradually pinpointing the neurons that mediate various neurological and psychiatric symptoms, and more recently uncovered circuit abnormalities in both the Rett and duplication mouse models. Building on this understanding of neural substrate-phenotype relationships, they showed that deep brain stimulation of a specific neural network improved learning and memory in a Rett syndrome mouse model.

Personal note on my relationship with Andreas Rett and the field

My relationship with Rett syndrome goes back to 1983 when I encountered a beautiful five-year-old girl with Rett syndrome at Texas Children's Hospital. I was struck by her clinical history and the fact she had excellent early development up to two years of age when she then started to regress and lose communication skills. Her symptoms and disease course matched those of the individuals described in an article by Hagberg and colleagues (Annals of Neurology, 1983). I was a neurology resident at the time and saw the young girl with two faculty members, Dr. Vincent Riccardi (a geneticist) and Dr. Alan Percy (a child neurologist). The girl's course of normal development followed by regression in the absence of neurodegeneration, together with the constant hand-wringing, left an indelible impression on me. A week later I was in the Blue Bird Circle Clinic where, as residents, we get to choose the type of patient we wish to see, so I chose a girl with the diagnosis of cerebral palsy. When that 12-year-old girl walked into my clinic office wringing her hands, I immediately recognized the other Rett sydnrome features. Seeing two girls with Rett in one week (when no U. S. physician had yet published on the syndrome) struck me as unusual and convinced me that there must be more. Indeed, working with the clinic volunteers, I canvassed medical records and found a handful of additional patients varying in age from two years to teenagers. To this day, I am thankful for the serendipidity of seeing two girls in one week as it was this, followed by discovering more within a short period of time, that imprinted the clinical picture of Rett syndrome in my head and inspired me to work on this disorder. Having seen and studied these patients together with the attending faculty and collaborators (Drs. Riccardi, Percy, Glaze, and Butler) I had the fortune to be invited to the International Rett conferences where I first met Dr. Andreas Rett. Perhaps the most treasured experience I had was watching him examine dozens of girls at the first U. S. conference in Baltimore. He was attending to every girl, carefully documenting her history, and evaluating her activities and behavior. He was gentle, caring, and curious. Following that we had additional encounters both in Vienna and in the U. S., and I was always touched by his gentleness approaching the girls and his dedication sitting on the floor to patiently watch them and learn from them. As for me, I continued to see the girls while completing my residency, but I felt compelled to determine how such a disorder comes about in the hope that one day we could do something to help the girls. I was driven to pursue Rett through research because I was intrigued by three facts: 1) that the syndrome appeared after a period of apparently normal development; 2) that the symptoms developed in a certain sequence and involved most brain functions; and 3) that affected individuals were predominantly girls. The latter was a clue that a genetic defect was likely the root cause of this sporadic, yet clinically distinct, disorder. Throughout the years while searching for the gene, I would meet Andreas Rett at conferences and we would have a chat about progress and the nuances of the clincal features of the disorder. He was extremely kind and encouraging, despite the fact that my quest for the gene was not yet bearing fruit.

Thoughts on where the field might be moving

We have learned a lot since the discovery of the Rett sydrome gene 15 years ago. Many of the research findings give me hope that the future will bring effective therapies to those affected with Rett syndrome (loss of MeCP2 function) and with the *MECP2* duplication syndrome (gain of MeCP2 function). While learning that MeCP2 itself is the best target to restore brain function given its broad effects and discovering that its levels have to be just right to have normal neurological functions poses some challenges, I do believe many discoveries provide opportunities for developing and testing therapies. First, learning that *MECP2* disorders are reversible in animal models provides hope that when effective therapies are developed many or at least some of the symptoms might be subdued or supressed. Second, discovering that alterations in various neurotransmitter systems might provide a pathway for therapeutics is promising, even if combination therapy has to be implemented to boost multiple systems. Third, the fact that the Rett brain is receptive to neuromodulation (at least in mouse models) opens up the possibility of targeting various symptoms using such approaches. Fourth, genetic screens to identify suppressors of Rett-like phenotypes or modulators of MeCP2 levels and activities provide opportunities for potential new therapeutics. Lastly, approaches specifically targeting MeCP2 through gene therapy, genome editing, or regulation of expression of the wild-type allele, will become more viable with advances in technology and safety.

Huda Zoghbi, MD (Fig. 1.16), is Professor of Pediatrics, Neurology, Neuroscience, and Molecular and Human Genetics at Baylor College of Medicine and serves as an Investigator with the Howard Hughes Medical Institute. She is also the founding Director of the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital.

Dr. Zoghbi's interests range from neurodevelopment to neurodegeneration. Her discovery that Spinocerebellar Ataxia type 1 is caused by expansion of a polyglutamine tract and that such expansion leads to accumulation of the mutant protein in neurons has had profound ramifications since many late-onset neurological disorders involve similar accumulations of disease-driving proteins. Zoghbi's work in neurodevelopment led

to the discovery of the gene *Math1/Atoh1* and to showing that it governs the development of several components of the proprioceptive, balance, hearing, vestibular, and breathing pathways. Zoghbi's group also discovered that mutations in *MECP2* cause the neurological disorder Rett syndrome. We now know that mutations in this gene are responsible for a broad spectrum of disorders ranging from mild cognitive disabilities to autism. Her lab is focused on understanding how loss of MeCP2 alters neuronal function to cause behavioral abnormalities. Zoghbi trained many scientists and physician-scientists and is a member of several professional organizations and boards. Among Dr. Zoghbi's honors are the Gruber Prize in Neuroscience, the Pearl Meister Greengard Prize from Rockefeller University, the Scolnick Prize from MIT, and the March of Dimes Prize in Developmental Biology. In 2000 she was elected to the Institute of Medicine, and in 2004 she was elected to the National Academy of Sciences.

Fig. 1.16 Huda Zoghbi, MD

2 Oral presentations

2.01

Development and characterization of a human Rett syndrome cell model using a non-integrating reprogramming strategy

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Background: Classical Rett Syndrome is caused by mutations in the gene coding for MeCP2, a transcriptional modulator, highly abundant in the mammalian brain and crucial for normal development of the central nervous system. The purpose of this study is to establish a human cell model for Rett syndrome via a non-integrating direct reprogramming method, to accurately characterize it and to evaluate *in vitro* protein replacement therapy using a TAT-MeCP2 fusion protein.

Methods: Fibroblasts of a male Rett patient and from a healthy control were transfected with two episomal plasmids coding for Pax6 and Sox2 transcription factors. Cells were kept under specific reprogramming culture conditions for 30 days post transfection followed by a weekly replating protocol for additional 3 weeks. Morphological changes and expression of neural progenitor specific genes were monitored during this reprogramming process. The induced neural progenitor cells (iNPs) were used for neural and astrocytic differentiation. Differentiated cells were stained for neuron-specific markers Tuj1, MAP2 and Synapsin1 by immunofluorescence staining. Furthermore, we used S100b as a marker for astrocytes.

Results: Morphological changes were observed in bright field microscopy throughout the reprogramming process and RT-qPCR analysis revealed elevated mRNA expression levels of the neural markers HOXB9, NCAM1 and Nestin together with SOX2, PAX6, FOXG1 and OCT3/4 pluripotency and progenitor cell-associated genes. The iNPs from a male Rett patient with known MeCP2 mutation and iNPs from a healthy control were differentiated into neuronal cells, showing typical neuronal morphology and expressing Tuj1 and MAP2 neuronal markers as well as the synaptic protein Synapsin1.

Conclusion: We demonstrated the successful reprogramming of fibroblasts of a male Rett patient and from a healthy control into iNPs using a transient non-viral transfection strategy. These iNPs were able to differentiate into the neural lineage expressing defined neural and synaptic markers. To further characterize our *in vitro* Rett syndrome cell model, electrophysiological and RNA-Seq analyses are planned.

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2.02

Reduced inhibition and excitation underlies circuit-wide changes in vivo in a mouse model of RTT

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Background: Balance of excitation and inhibition (E/I) plays a key role in refining neural circuit development and plasticity and is disrupted in many neurodevelopmental disorders. Rett syndrome (RTT) arises from loss of function mutations in Mecp2 in the brain.

Objectives: The functional effects of MeCP2 on synaptic E/I and circuit-level computations, and the role of MeCP2 in inhibitory neuronal subtypes, especially Parvalbumin (PV+) and Somatostatin (SOM+)-expressing interneurons that play an important role in shaping circuit plasticity and E/I balance, are unresolved.

Methods: We used *in vivo* two-photon guided cell-attached and whole-cell patch-clamp recordings and awake Ca^{2+} imaging from cell-type specific conditional and global MeCP2 mutant mice. Age-matched MeCP2 wild-type littermate animals and Floxed-MeCP2 mice served as control.

Results: By analysing visual cortical responses *in vivo*, we show that visually-evoked excitatory and inhibitory conductances are both reduced in pyramidal neurons. Deletion of MeCP2 from PV+ and SOM+ expressing inhibitory interneurons reduces their responses and selectivity. PV-specific deletion substantially recapitulates effects of global MeCP2 deletion, by differentially reducing response levels, reliability and selectivity of pyramidal neurons. Interestingly, MeCP2 deletion also results in defective KCC expression leading to chloride (Cl^-) imbalance, further impacting the effectiveness of GABAergic inhibition. Administration of human recombinant IGF1 (rhIGF1) cell-type specifically restores PV+ responses and increases KCC2 expression to correct the polarity of GABAergic inhibition.

Discussion: Loss of MeCP2 from specific interneuron types, and especially PV+ neurons, contributes crucially to the cell-specific and circuit-wide deficits of RTT, suggesting that such neurons have a pivotal role in the functional deficits that characterize the disorder. It also demonstrates a cell-type specific and mechanism-based therapeutic role for rhIGF1 in treating RTT.

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2.03

Swedish national Rett center

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Background: People with Rett syndrome (RTT) need support to be as active and healthy as possible. They also have the right to influence their own lives and to develop throughout life. Rett Center is the Swedish national center for RTT. As RTT causes symptoms in many different areas, a trans-disciplinary teamwork is necessary.

Objectives:

- Equal and optimal care for individuals with RTT across the country.
- To promote the rights and interests of individuals with RTT in Sweden.

Methods:

- To cooperate with other stakeholders such as Swedish National Agency for Rare Diseases (NFSD), the Swedish interest association (RSIS), regional expert teams and local habilitation centers.
- To perform research and disseminate knowledge and information.
- To see patients from all over the country in cooperation with the county councils.
- To base assessments, recommendations, research and support to families and professionals on a trans-disciplinary approach. In addition, each professional assess and provide support within their specific area of knowledge (music therapy, neurology, occupational therapy, orthopedics, physiotherapy, and speech and language pathology).

Results: The majority of children and adults with RTT have been assessed at the Swedish national Rett Center or met with the team at consultations in their home town. A number of parents, carers, educators and medical staff contact the center by e-mail or telephone regarding individuals with RTT or general information on the diagnosis. Basic courses on RTT, or courses with a specific topic, are held at a yearly basis in cooperation with the Swedish parent organization, RSIS. Rett Center also hold courses in various forms and information on RTT is disseminated at conferences internationally and nationally. Information is spread through the website, via facebook and through a Youtube channel. Currently two PhD-projects and two other studies regarding RTT are conducted at the center and during 2013–2015, five studies on RTT have been published.

Discussion: The collected resources at a national Rett Center, likely contributes to raising awareness and disseminate information about RTT. It is also likely that being a national Center encourages research and thus the knowledge of RTT increases. Importantly, a national center for RTT, together with other stakeholders, increases the chances of equal care across the country.

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2.04

The need for life-long medical follow-up of individuals with Rett syndrome

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Background: Rett syndrome (RTT) is a neurodevelopmental disorder characterized by multiple disabilities and a risk of several comorbidities. There is an increased mortality; however, many individuals live long into adulthood. In order to treat and prevent comorbidities, knowledge and experience in RTT is important.

Objectives: It is our hypothesis that individuals with RTT receive medical treatment aimed at comorbidities or the risk of these throughout life but that the pattern of treatment changes. We therefore aimed to investigate the extent of medication in all age groups of a Danish cohort of females with RTT known to the Danish Centre for Rett syndrome (CRS).

Methods: We included 88 females with RTT and a MECP2 mutation from the Danish cohort of individuals with a clinical diagnosis of RTT. They were divided into three age groups; group 1: 2–14 years, group 2: 15–29 years and group 3: 30–60 years. Medical files from their last follow-up visit at CRS were reviewed according to current types of and indications for medication. The percentage of treated females was calculated for each comorbidity and was divided into the three age groups.

Results: Group 1 included 28 females aged 2–12 years (y) (mean 6.8 y), group 2: 29 females aged 15–27 y (mean 19.6 y), group 3: 31 females aged 30–60 y (mean 40.9 y). All females except two in the youngest age group were treated for medical comorbidities or the risk of these. The number of treated comorbidities was: 0-7 (mean 2.89) in total; 0-4 (mean 1.89) in group 1; 1-7 (mean 3.14) in group 2; 1-6 (mean 3.55) in group 3.

A total of 77.3 % of the individuals received D-vitamin/calcium (group 1: 20.6 %; group 2: 36.8 %; group 3: 42.6 %); 76 % were treated for constipation (28.4; 35.8; 35.8 %); 56.8 % for epilepsy (22; 38; 40 %); 16 % for gastroesophageal reflux (14.3; 50; 35.7 %); 9 % for sleep disorder (50; 12.5; 37.5 %); 8 % with painkillers on a daily basis (0;14.3; 85.7 %); 6.8 % for osteoporosis (0; 16.7; 83.3 %); 6.8 % for muscle stiffness/dystonia (0; 33.3; 66.7 %); 6.8 % for psychosis/behavioural problems (0; 16.7; 83.3 %); 6.8 % for asthma (50; 16.7; 33.3 %); 5.7 % for menstrual disturbances (0; 100; 0 %); 4.5 % for depression (0; 0; 100 %); 4.5 % for risk of urinary tract infections (0; 25; 75 %); 2.3 % for respiratory disturbances (0; 100; 0 %); one girl in group 2 was treated for hypothyroidism.

Discussion: The current study showed that almost all individuals with RTT are medically treated for comorbidities in childhood, adolescence and adult life. However, the amount and pattern of treated comorbidities changes and is not equally distributed in these three phases of life. It is for instance mainly adults who are in treatment for pain, psychosis, behavioural problems and depression. We conclude that it is essential for individuals with RTT to be followed lifelong by RTT experienced professionals for comorbidities and the risk of these. It will provide greater safety in appropriate medical treatment which could be an important factor for the quality of life in these individuals.

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2.05

Lack of Mecp2 interferes with mechanisms of cortical progenitors proliferation and differentiation

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Background: Classical RTT cases are linked to mutations in the X-linked Methyl-CpG-Binding Protein 2 (MECP2) gene encoding for MeCP2, a multifunctional protein ubiquitously expressed from development to adulthood. RTT symptoms become overt after an apparently normal early post-natal development. However, many evidences now demonstrate that early signs of the pathology can be observed in both girls and RTT animal models even before the onset of overt symptoms.

Objectives: To verify whether lack of Mecp2 affects brain functions even during early stages of life, we investigated the role of Mecp2 during the embryonic development of the cerebral cortex, a brain area affected by most of RTT neurological features.

Methods: Our studies started by profiling gene expression in the embryonic cortex of Mecp2 null mice. Based on these transcriptional data, we studied proliferation, differentiation and functionality of Mecp2 null cultured neuroprogenitors and the derived neurons. Through in vitro imaging approaches and in vivo histology, we studied the dynamics of cell cycle progression and exit of neuroprogenitors and the subsequent developmental steps leading to mature newborn neurons. Neuronal functionality was assayed through calcium transients imaging.

Results: The transcriptional profile of the Mecp2 null embryonic cortex suggests that lack of Mecp2 affects mechanisms of early corticogenesis, as newborn postmitotic null neurons express markers that are typical of neuronal progenitors. This implies that in null samples the refinement of postmitotic identity is impaired. In line with this, null neurons express reduced levels of transcripts encoding for mediators of responsiveness to external stimuli (such as glutamatergic and GABAergic receptors subunits and ionic channels components), clearly suggesting a delay in the acquirement of proper neuronal functions. Moreover, we produced data showing that part of the morphological defects typically displayed by null brains in adulthood are already detectable during embryonic and early postnatal stages, suggesting that some of these features originate during early development and persist through adulthood. Discussion: Altogether, our data suggest that already during embryonic and early post-natal life lack of Mecp2 affects the acquirement of features that are necessary for proper cerebral cortex functions later in life. In fact, delayed maturation of Mecp2 null neurons is likely causative of the defects in neuronal networks establishment thoroughly described in Mecp2 null adult tissues. This suggests the importance of the role Mecp2 plays during embryonic development and implies that the impairments displayed by RTT animal models can be considered the worsening of a condition that, at least in part, is generated during early stages of life.

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2.06

Application of Telehealth principles to deliver therapy services for Rett syndrome

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Background: Physical therapy for Rett syndrome within an enriched environment has been associated with maintained or improved skills. However, Rett syndrome occurs rarely and local clinicians and therapists typically see few cases. This limits their capacity to develop specific expertise. Telehealth is being increasingly used to counter poor access to health services and has been used for rehabilitation services. There is a need to develop mechanisms by which a clinician with expertise in a rare disorder can provide support to families or carers over a long distance who do not have access to services or expertise.

Objectives: This pilot study assessed the feasibility of providing therapy support for females with Rett syndrome using a Telehealth model over large geographical distances.

Methods: ML was invited to review therapy supports for girls and women with Rett syndrome who attended a family support day organised by the Rett Syndrome Association of Ireland. Nine families were invited to participate in an ongoing therapy support program using Skype for communication with ML in Israel and four of those agreed. A Participatory Action Research model was then implemented. Following initial assessment, goals and strategies were identified together with the family, the family then embedded the strategies in daily life to enhance functioning, and monthly meetings over six months were held on Skype to review progress and activities. Parent feedback on the feasibility and suitability of the program was sought after six months.

Results: Each of the four girls who had not received previous therapy services gained gross motor skills. A four-year-old girl who could walk independently achieved ability to climb stairs; a five-year-old girl who was unable to sit independently achieved this and took steps with assistance; a seven-year-old girl who was able to walk and run achieved skills of standing on one leg and jumping; and an 18-year-old woman who needed assistance to walk achieved some independent steps. Parents reported that the program enabled them to encourage their daughter's abilities and was suitable for integrating into their daily lives.

Discussion: We have demonstrated the feasibility of bringing specialist expertise over long distances to families with a child with Rett syndrome in their own environment. Our findings will be of interest to other specialities involved in the management of Rett syndrome such as neurology, speech therapy and gastroenterology which could replicate this model.

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2.07

Novel insights in biological pathways of Rett syndrome data

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Background: Pathway analysis combines experimental (omics) data with structured existing knowledge. A typical repository of structured biological knowledge is WikiPathways.org, a curated database of biological pathways. One of the strengths of this approach is that it provides an overview of how the biological network is affected without being biased by hypothesis. Moreover, the analysis and visualization methods allow to explain and to prove whether and how a certain pathway is altered. This approach is especially useful for investigation of Rett syndrome where the genetic cause and the clinical phenotypes are well known but there are still gaps in understanding how the biological mechanism is modified leading to those phenotypes.

Objectives: The objective of this study is to reveal the biological pathways which point from a MECP2 mutation to the various phenotypes like breathing abnormality or seizures. In this study we investigated the differentially expressed genes and affected pathways in neuronal cells using published (but not to that extend investigated) data from a Mecp2- mouse model that was originally published by Sugino et al. (2014) [1].

Methods: This dataset is available on ArrayExpress under the accession number E-GEOD-8720 and provides gene expression data of four different neuronal cell types of a Rett syndrome mouse model and control using an Affy-45 microarray. We performed quality control, data preprocessing (normalization according to GC-RMA) and statistics using ArrayAnalysis.org [2]. We extracted a list of significantly changed genes for each cell type using a cutoff of ± 0.5 log2 fold change and $p \leq 0.05$. For visualization, analysis and interpretation of this data we used PathVisio [3].

Results: Using these criteria, we obtained for each cell type between 74 and 259 differently expressed genes which is a rather low number. Nevertheless, there are different sets of pathways affected in each cell type, using a z-score ≥ 1.96, minimum number of changed genes of 3, and $p \leq 0.05$. E. g. the locus coeruleus cells showed an impaired glutathione and amino acid metabolism while the fatty acid metabolism was the dominant changed pathway in fast spiking interneurons in the motor cortex.

Discussion: We expect that the application of these tools and methods is going to improve not only the research on Rett syndrome or other neurological diseases, but it will contribute to a better understanding of neurological physiology in general.

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2.08

Digital transformation of healthcare a chance for rare diseases

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Background: Overview of market forces regarding personalized medicine; the impact of technology, big data and cost in the healthcare industry; the relevance of smart data in research and clinical application; the value of in-memory solutions; example of SAP Medical Research Insights and SAP HANA; outlook of possible application in the field of rare diseases

Objectives: to give an overview why digitalization is so important for healthcare industry and how new technologies like in-memory solutions can be applied in order to improve research and clinical processes also in context with rare diseases

Discussion: what can digitalization add in the future? Espec. In RTT? Disadvantages?

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2.09

The characterization of a novel RTT mouse model provides new information on MeCP2 regulation

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Background: Rett syndrome (RTT) is primarily caused by sporadic mutations occurring in the *MECP2* gene. In the last decade MeCP2 has appeared as a multifunctional protein involved in many biological processes. This multifunctionality could be regulated by post-translational modifications (PTMs) of MeCP2 and by its interactions with different binding partners. Despite the efforts put on RTT research, our comprehension of MeCP2 functions and their association with the complex clinical symptoms is still far from being complete. In particular, the regulation of MeCP2 roles during neuronal maturation and in mature brain needs to be clarified.

Mouse models have been instrumental for the study of RTT pathogenesis and of Mecp2 properties. Notably, although only few girls carry large deletions of *MECP2, Mecp2*-null male mice are the most commonly used models. However, the complete absence of the protein together with the associated severe phenotypes probably lead to compensatory processes that might differ between animals and mask direct pathogenic mechanisms.

Objectives: We believe that new mouse models mimicking patients' mutations offer the possibility of a better comprehension of RTT molecular origin. Thus, we have generated a novel knock-in (KI) mouse carrying a human missense mutation in the MBD of Mecp2. Since the mutated residue is subjected to phosphorylation, we believe that this model will also provide important information about the regulation of MeCP2 through its PTMs.

Methods: The generated KI mouse model has been phenotypically characterized exploiting a previously published scoring system and a battery of behavioural tests. Brains and neuronal cells obtained from the mutated mice have been biochemically and morphologically analyzed at different time points. Moreover, the effects of the mutation on the DNA/chromatin binding properties of MeCP2 have been studied by EMSA and salt extraction assays.

Results: Our KI mouse manifests severe RTT-like phenotypes and a reduced lifespan. Behavioral tests evidenced motor impairments together with alterations in the animals' working memory. In accordance with its localization in the MBD, the studied mutation impairs the DNA binding properties of Mecp2 *in vitro. In vivo*, the mutated protein appears less tightly bound to chromatin in adult brains, but not in immature ones. Notably, this molecular alteration correlates with the reduction of Mecp2 abundance observed in the adult, but not in the immature, KI neurons. In brain, the mentioned molecular defects lead to evident transcriptional impairments that diverge from those observed in the null mouse model, although leading to similar functional alterations in cultured neurons.

Discussion: Our results indicate that the comprehension of the pathogenic mechanisms of RTT require the study of animal mutants carrying *MECP2* pathogenic mutations affecting the most relevant functions of the protein. Furthermore, they clearly indicate the importance of performing the analyses at different developmental stages.

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2.10

RNA sequencing analysis in iPSCs derived Rett neurons to identify shared molecular alterations

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Background: Rett syndrome is a severe neurodevelopmental disorder ranging from the classic *MECP2*-related form to the *FOXG1*-related congenital variant, which represents the most severe extreme of the spectrum with a shorter or absent normal perinatal period and more severe microcephaly than the classical Rett syndrome. Both *MECP2* and *FOXG1* encode transcriptional regulators playing fundamental roles during brain development. Given the presence of shared signs/symptoms and the overlapping function of the two genes, we hypothesized the presence of common molecular mechanisms behind Rett phenotypic spectrum.

Objectives: Considering the role of both genes in transcriptional regulation, we aimed at identifying common alterations in the transcriptome of *MECP2* and *FOXG1* mutated human neurons compared to controls.

Methods: We took advantage of the breakthrough technology of genetic reprogramming to establish patient-specific iPSCs as an innovative human cellular model. We characterized by RNA-seq the transcriptome of iPSC-derived neurons from 2 patients with *MECP2* missense mutational hotspots (p.Arg306Cys and p.Thr158Met), 2 patients mutated in *FOXG1* (p.Trp255* and p.Glu154Glyfs*301) and 2 controls, using the Ion Proton Sequencer platform (Life Technologies).

Results: About 43.000 transcripts were significantly expressed (>1 FPKM) both in controls and *FOXG1* and *MECP2*-mutated samples. Using the Cuffdiff tool from Cufflinks, we identified 252 overlapping genes differentially expressed in both *MECP2* and *FOXG1* mutated neurons (2 fold change; $p < 0.05$): 159 upregulated and 93 downregulated transcripts. Gene Ontology (GO) enrichment analysis on these common genes identified relevant dysregulated biological processes/pathways, including an upregulation of transcripts involved in extracellular matrix and collagen fibril organization, cell adhesion and microtubule assembly. We also found a dysregulation of several genes implicated in axonal outgrowth. These results confirm the existence of disrupted biological processes/pathways common to both *MECP2* and *FOXG1* mutated patients.

Discussion: These findings strongly support the involvement of common patho-mechanisms in the onset of Rett spectrum disorders and suggest potential relevant targets for therapeutic intervention.

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2.11

Diagnosis age, classification and severity scales in a large cohort of Rett patients

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Background: Rett Syndrome is a neuro-developmental disorder almost exclusively affecting females, characterized by a broad clinical spectrum of signs and symptoms and peculiar stages. In the Tuscany Rett Center at Versilia Hospital and in the Genetics Unit in Ferrara University, we collected data from 179 subjects with a clinical diagnosis of Classical or variant RTT syndrome.

Objectives: For each subject, we assessed the severity of the condition with clinical-rating scales (ISS, PBZ), and we collected clinical and genetic data to classify the classical and variant Rett and establish the diagnosis age and severity in single forms.

Methods: We used multivariate statistical analysis of the data to evaluate the relation between the different clinical RTT forms, the different genetic mutations and the severity of the clinical presentation. Patients were classified according to the following categories: Classical RTT and three atypical RTT: Z-RTT, Hanefeld, Congenital. Also, we considered an additional group among the atypical variants: ARTT-NOS (Atypical RTT-Not Otherwise Specified), which includes all the cases with a clinical presentation for atypical RTT, but can not be classified in any of the above atypical categories.

Results: In our cohort (179 subjects) we have patients with Classical and Atypical Rett distributed as follows: 120 cases with the Classical RTT (67 % and 59 with the atypical RTT (33 %). In the atypical RTT cases 22 patients (12.3 %) present with Z-RTT variant; 13 (7.3 %) with Hanefeld variant; 3 (1.7 %) with Congenital variant, and 21 cases with ARTT-NOS variant (11.7 %).

In our cohort the mean age at assessment is 12.3 (range 1–49), for Classical Rett: age 12.8; Z-RTT age12.4; Hanefeld age 6.1; Congenital age 2.3; ARTT-NOS age 13.9.

In the whole cohort the mean age of the diagnosis is 8 years. Specifically: 6.7 years Classical Rett; 7.4 years Z-RTT; 4.6 years Hanefeld; 1.3 years Congenital and 8.4 years ARTT-NOS.

For all the patients the clinical severity was measured with ISS (range 0–42) and PBZ (range 0–104) severity scales, and we calculated the average scoring for each presentation as follows: ARTT-NOS: ISS = 21.6 and PBZ = 38.6; Congenital forms: ISS = 21 and PBZ = 64; Hanefeld ISS = 20.1 and PBZ = 54.6; Classical Rett ISS = 19.5 and PBZ = 39. The less severe presentations measured with both scoring systems are those for Z-RTT ISS = 21.2 and PBZ = 12.4.

Discussion: Considering the seriousness of the clinical form and the age of diagnosis we find that the milder forms are the most difficult to identify and have a late diagnosis (due to milder manifestations, the presence of language and characteristics typical of autism disorders). In contrast the Hanefeld and congenital forms present more severe phenotype and have an earlier clinical diagnosis, generally confirmed by genetic diagnosis. The ARTT-NOS present on average with severe symptoms, with a high prevalence of absence of known mutations, and have a delay in diagnosis, representing unusual clinical patterns where the diagnosis is reached for the exclusion criterion.

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2.12

Intelligent pre-linguistic vocalisation analysis: a promising novel approach for the earlier identification of Rett syndrome

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Background: For many years, an apparently normal early development has been regarded as a main characteristic of Rett syndrome (RTT). The speech-language domain represents a key domain for the clinical diagnosis of RTT, which is often still made beyond toddlerhood. Recent studies have built upon the assumption that speech-language and socio-communicative development are already affected in the pre-regression period.

Objectives: In this study, we aimed to apply machine learning methodology based on acoustic signal parameters in prelinguistic vocalisations for an automatic identification of RTT in the first year of life.

Methods: We analysed more than 16 hours of home video recordings of 4 girls later diagnosed with RTT (3 girls with typical RTT, 1 girl with the preserved speech variant of RTT) and 4 typically developing girls aged 6 to 12 months. We manually segmented a total of 4,678 pre-linguistic vocalisations. A comprehensive standardised set (ComParE) of acoustic features (e. g., fundamental frequency, harmonics-to-noise ratio, Mel-frequency cepstral coefficients, jitter, shimmer) was extracted from the vocalisations as basis for the binary classification paradigm RTT versus typical development. We applied linear kernel support vector machines as classifier.

Results: A promising mean unweighted recognition accuracy of 76.5 % was achieved using a kernel complexity of C = 1.0E-4 and best-possibly-vocalisation-number-balanced 4-fold leave-one-speaker-pair-out cross-validation.

Discussion: To the best of our knowledge, this is the first approach to automatically identify infants later diagnosed with RTT based on acoustic characteristics of pre-linguistic vocalisations. Our findings may build the basis for facilitating earlier identification and thus an avenue for an earlier entry into intervention.

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2.13

Using eye gaze to access an augmentative communication device for persons with Rett syndrome

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Background: Most individuals with Rett syndrome (RTT) are nonverbal and the evidence to support symbolic communication ability in the population is limited (Bartolotta et al., 2011). Because of their complex communication needs and limited physical abilities most individuals with RTT have difficulty utilizing AAC devices using direct selection or scanning methods (Bartolotta, 2013). Eye gaze for communication is frequently observed in RTT (Neul et al., 2010) however documenting eye gaze patterns in a clinical setting is challenging (Bartolotta & Remshifski, 2012). Eye tracking technology has been demonstrated to be a reliable tool to measure cognitive abilities, including attention and perception, in RTT (Baptista et al., 2006; Djukic & McDermott, 2012; Djukic et al., 2012). However there is little research to support use of eye gaze to access augmentative and alternative communication (AAC) systems for persons with RTT (Simacek, Reichle & McComas, 2015). There is a pressing need for controlled assessment of this tool to determine the clinical relevance for persons with RTT.

Objectives: The purpose of this study is to demonstrate that eye gaze can be used as an effective strategy to access AAC devices for persons with RTT. We studied the use of pictures to request favored objects, which is recommended as an initial target for communication intervention in RTT (Sigafoos et al., 2009). The following hypothesis was developed: Eye gaze is an effective strategy to access an AAC device to communicate basic needs in RTT.

Methods: This single-subject multiple baseline study investigated use of eye gaze for communication using the Tobii PCEye Go. Caregivers completed an informed consent, history forms, the Inventory of Potential Communicative Acts, and a Quality of Life scale. Caregivers provided a list of preferred and nonpreferred items for use as stimuli. Initial baselines were obtained using a field of single and multiple (two) pictures. Participants were trained to use single pictures to request favored items, followed by training using one distractor. Training continued until each participant was successful in activating the AAC system using eye gaze without prompts in 60 % of trials across three training sessions for both conditions. Post-training baseline data was collected using a field of two pictures (one preferred item and one nonpreferred item).

Results: This study demonstrated that persons with RTT can be trained to successfully use eye gaze to access an augmentative communication system to request preferred items.

Discussion: These outcomes are consisent with previous findings of Simacek, Reichle & McComas (2015) and provides preliminary evidence of the effectiveness of eye gaze to convey basic needs. Interestingly some participants used additional modalities, including pointing, finger touching, vocalizations, and body movements to convey requests. The presenters will discuss strategies for expanding use of AAC to include other communicative functions, contexts, and additional communication partners.

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2.14

Males with MECP2-related syndromes and their affected mothers: clinical, genetic and family environment interface

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Background: Phenotypes of males with *MECP2* and related gene mutations range from lethal congenital and neonatal encephalopathies to PPM-X syndrome, non-specific intellectual impairment and other phenotypes referred to as Rett variant. Only a minority of phenotypic males resemble the classical RTT.

Objective: To examine the expanding phenotypes of the *MECP2*-related syndromes in males and their affected mothers.

Methods and results: We report 3 males with normal karyotypes who presented with evolving complex neurodevelopmental encephalopathies onsetting before 1 year of age with multifaceted presentations of epilepsy, ataxia, spasticity, movement disorders, behavioural issues and severe intellectual impairment. The phenotypes did not prompt us to identify Rett syndrome variants early in childhood. Two were brothers with *MECP2* a 2-bp deletion near the 3' end causing a frameshift and premature truncation. The third boy had a clinical picture that in retrospect could fit into three Rett-syndrome staging. He was found to have a missense mutation C to T change at nucleotide 925 in exon 4 (c.925C>T). The mutations in these males were inherited from their mothers, both of whom had modest intel-

lectual, mental-health, social and GI impairments. Neither was independently able to care properly for her son(s).

Discussion: We propose a practical clinical and genetic classification for age related clinical presentations of males with MECP2, CDKL5 and FOXG1 mutations in the table 2.14.

Table 2.14 classification for age related clinical presentations of males with MECP2, CDKL5 and FOXG1 mutations

Phenotype	Karyotype	Associated Gene mutation
Profound congenital, neonatal, or early infantile encephalopathy ± epileptic spasms (West syndrome)	46 XY	*MECP2* same mutations as female RTT
	46 XY	*CDKL5*
	47 XXY	
	46 XY somatic mosaicism	
	46 XY	*FOXG1*
Later onset severe encephalopathy, often with epileptic spasms	46 XY	*MECP2* duplication/ triplication
Rett-like syndrome	47 XXY	*MECP2*
	46 XY somatic mosaicism	
	46 XY missense mutation	
Non-specific intellectual impairment- (XLMR)	46 XY	*MECP2*, often with A140V, mutation not typical for female RTT mutation; additional symptoms like tremor may aid in diagnosis
Psychosis/severe behavioural impairment, ± pyramidal signs, parkinsonism, macro-orchidism	46 XY	*MECP2*-often with A140V mutation
Intellectual impairment ± epilepsy, progressive spasticity, ataxia, distal atrophy, movement disorder (RTT variant)	46XY	*MECP2*
Angelman-like syndrome	46XY	*MECP2*
Prader-Willi like syndrome	46XY	*MECP2*

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2.15

Rett syndrome – early signs of literacy enhancement in girls with Rett syndrome

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Background: Over the last 2–4 years the number of girls with Rett syndrome (RTT) that got access to Eye Gaze computers increased in the Netherlands (Townend et al., 2015). Parents and professionals from all over the world share their experiences on the use of Augmentative and Alternative Communication (AAC) and RTT through groups on Facebook. In special education and at home (Home Literacy Environment) specific applications on Eye Gaze-computers for literacy enhancement became popular to support goals for intervention. Parents and professionals are beginning to realize that the girls can profit from AAC to further develop communicative and literacy skills. Becoming literate provides individuals with RTT the opportunity to participate more easily in our literate society by applying alphabetical AAC-systems instead of graphic symbols preselected for them.

Research interest in literacy studies with AAC for children with intellectual and developmental disabilities (IDD) is growing (National Reading Platform, 2012). Language, phonological awareness and letter knowledge are among the most important precursors for early literacy in children with IDD (Van Tilborg, Segers, van Balkom & Verhoeven, 2014).

Objectives: Most children with RTT are non-vocal and experience severe motor challenges. Regular methods to measure cognitive skills aren't reliable due to limited hand function and the extra challenge to execute actions on demand due to the severe apraxia. The use of eye-point may help them to indicate graphic symbols, letters and words on their AAC-device. We used this set-up in a case-study to explore signs of letter knowledge, phonological awareness (sequential arrangement of letters in accordance with conventional spelling rules) and other linguistic skills in girls with RTT as a first explorary step to study AAC and (early) literacy in RTT.

Method: Video analysis was used to observe the early literacy skills of a 12-year-old Rett girl. Since 3.5 years her parents and caregivers focused on communicative skills and she has been introduced to symbol based AAC and eye gaze technology. She has been exposed to literacy education for 4 to 6 months prior to the data collection using a literacy program that is used in her special education school in the Netherlands.

Results: We found signs of initial letter and phonemic recognition and parents reported a remarkable increase in her focused, attention span (1–1.5 hours) while being engaged in reading picture books and writing letters on an iPad. Further analysis will be done prior to the conference and results will be used in the presentation.

Discussion: Varying degrees of proficiency in literacy skills in individuals with RTT would be expected but should be separated from their capacity to learn literacy based skills. We would like to find out which precursors are essential in training literacy through AAC for those with RTT. What kind of methods could best be used to help these girls to become literate? How can parents and professionals contribute to future research i. a. data collection via Facebook groups?

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2.16

Diagnostics & eyegaze learning curve

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"You are not able to do it!" Many children and adults with severe motor and cognitive limitations and unclear consciousness have to live with this prejudice.

We want to show how getting even clients with no or hardly existing unique body's communication channels (among others ICP, condition after coma and more) the opportunity to show their skills. Likewise, solutions will be shown how the use

of eye control can be just introduced in a playful way; no matter whether for AAC or for a job.

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2.17

Brain phosphorylation of MeCP2 at serine 164 is developmentally regulated and globally alters its chromatin association

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Background: MeCP2 is a transcriptional regulator whose functional alterations are responsible for several autism spectrum and mental disorders. Although the protein was originally described as a repressor capable of inhibiting gene expression through chromatin compaction, nowadays MeCP2 is recognized as a multifunctional protein that modulates its activity depending on its protein partners and post-translational modifications (PTMs). A number of MeCP2 residues have been found differentially phosphorylated in brain and most studies have characterized residues located within the methyl-binding domain or the transcriptional repression domain of the protein. However, no data have so far been able to demonstrate an effect of MeCP2 phosphorylation on its general intracellular distribution or chromatin association. We found that the intervening domain is a frequent target of PTMs; among these, Serine (S) 164 was found phosphorylated in normal and epileptic rodent brains but its functional role remained uncharacterized.

Objectives: Although S164 is conserved in evolution, it has never been found mutated in MECP2-related disorders. However, this residue is proximal to a region of MeCP2 that is often mutated in patients; furthermore, a close by pathological mutation, R167 W, has recently been linked to intellectual disability and autism in males. Considering all this, we decided to analyze the molecular consequences of MeCP2 phosphorylation at S164 (P-S164) and its regulation along brain development.

Methods: The spatio-temporal distribution of P-S164 was investigated through a custom made phospho-specific antibody. In silico modeling, electrophoretic mobility assays, fluorescence recovery after photobleaching, chromatin solubility and fractionation were used to describe the impact of this specific event of phosphorylation on MeCP2 binding to methylated DNA and chromatin. The biological relevance of P-S164 was assayed by investigating differentiation of neurons expressing wt MeCP2 or the corresponding phosphodefective mutants of S164.

Results: We show that phosphorylation of S164 in brain is dynamically regulated during neuronal maturation. S164 phosphorylation highly impairs MeCP2 binding to DNA in vitro and largely affects its nucleosome binding and chromatin affinity in vivo. Strikingly, the chromatin-binding properties of the global MeCP2 appear also extensively altered during the course of brain maturation. Functional assays reveal that proper temporal regulation of S164 phosphorylation controls the ability of MeCP2 to regulate neuronal morphology.

Discussion: Our results highlight S164 phosphorylation as a developmentally regulated PTM of MeCP2 with a relevant impact on its DNA/chromatin binding properties. Further, they provide support to the hypothesis that MeCP2 PTMs might integrate different signals, which finally lead to adaptive structural and/or transcriptional outputs.

The current lack of pathologic mutation at this site can possibly be explained assuming that additional PTMs synergistically occur with S164 phosphorylation to develop a full functional outcome. It is also possible that by extending the cohorts of patients screened in molecular testing to include mild neurological symptoms, pathological mutations at S164 will be identified.

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2.18

How do females with Rett syndrome communicate and what factors influence successful communication?

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Background: Rett syndrome is a neurodevelopmental disorder generally associated with severe language and physical impairments, each of which contributes to difficulties with communication. Eye gaze is considered a common mode of communication and conventional methods, such as talking and gestures, less common. However, the literature is limited by small sample sizes, the inclusion of cases that have not been genetically confirmed and a paucity of studies investigating the numerous barriers and facilitators to successful communication.

Objectives: This presentation will describe the communication abilities of females with Rett syndrome and investigate factors that are positively and negatively associated with communication outcomes.

Methods: Mixed methods were used to explore the communication abilities of females with Rett syndrome and the impairments of body function and structure, activity limitations and contextual factors that influence these. Seventeen Australian parents were interviewed about their daughter's communication and the factors that act as barriers and facilitators to successful communication. Questionnaire data from the Australian Rett Syndrome Database and the International Rett Syndrome Phenotype database ($n=766$) were used to describe speech-language abilities and explore relationships with genotype.

Australian questionnaire data were used to describe the use of eye gaze and gestures, and the ability to make requests, and relationships with genotype, gross motor abilities and age in 151 females. Australian video data were used to describe the choice making abilities of 64 females.

Results: During interviews all parents reported their daughters were able to express discomfort and pleasure, and make requests and choices using a variety of modalities including body movements and eye gaze. They also reported level of functional abilities and environmental factors influenced communication. Questionnaire data on speech-language abilities showed 89 % (685/766) acquired speech-language abilities in the form of babble or words at some point in time. Of those who acquired babble or words, 85 % (581/685) experienced a regression in these abilities. Those with a p.Arg133Cys mutation were the most likely to use one or more words, prior to (RRR = 3.45; 95 % CI 1.15–10.41) and after (RRR = 5.99; 95 % CI 2.00–17.92), speech-language regression. Australian questionnaire data (n = 151) found women aged 19 years or older had the lowest scores for eye gaze. Females with better gross motor abilities had higher scores for the use of eye gaze and gestures. The use of eye gaze did not vary across mutation groups, but those with a C-terminal deletion had the highest scores for use of gestures. The video study found 82.8 % (53/64) made a choice, most using eye gaze. Of those who made a choice, 50 % did so within 8 seconds.

Discussion: Females with Rett syndrome share communicative strengths including the use of eye gaze and the ability to make choices. Interventions should target communicative strengths, such as the use of eye gaze, and factors showing to impact communication, including the skills of communication partners. Reporting and accounting for genetic information in future research would help improve our understanding of the relationship between *MECP2* and communication abilities, which may in turn improve our knowledge of the role *MECP2* plays in neurodevelopment.

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2.19

Brain SRB1 modulation as a possible player in Rett syndrome pathogenesis

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Background: Rett (RTT) syndrome is a severe neurological disorder that affects almost exclusively females. Several detectable mutations in the X-linked methyl-CpG-binding protein 2 gene (MECP2) are responsible for the onset of the disease. MeCP2 is a key transcription regulator involved in gene silencing via methylation-dependent remodeling of chromatin. Recent data highlight that lipid metabolism is perturbed in brain and liver of MECP2-null mice. In addition, altered plasma lipid profile in RTT patients has been observed. Furthermore redox modulation of the HDL receptor scavenger receptor B1 (SRB1) was observed in skin fibroblasts isolated from patients with Rett syndrome.

Aim: Based on the mentioned work, the aim of our study was to evaluate the presence of SRB1 in brain of MECP2-null mice at different stages of the diseases (pre symptomatic, 5 weeks old and symptomatic 9 and 17 weeks old).

Results: The data showed that SRB1 expression decreases significantly during the progression of the disease in MECP2-null mice and almost disappears in 17 weeks old MECP2-null mice. These data correlated with the levels of OS markers (Isoprostane and 4HNE), that are higher in the symptomatic animals respect to the control.

Thanks to the IF we were able to show that SRB1 co-localized with tubulin III, suggesting its presence in the neurons.

As a proof of concept the levels of SRB1 was also analyzed in the human cortex of healthy subjects and also in this case there was a clear co-localization with the neuron.

It is possible to sustain that the lost of SRB1 it is a consequence of increased OS levels that can affect SRB1 post-translational modification as previously shown in RTT fibroblasts.

Conclusion: These results, together with our previous studies, demonstrate that SRB1, which is one of the main protein belonging to cholesterol regulatory network is altered in RTT animal model, providing the proof of principle that cholesterol metabolism may be taken into account as a new target for the treatment of specific features of RTT pathology.

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3 Poster Presentations

3.01

The socio-communicative domain: Can we observe peculiarities in individuals with typical Rett syndrome already in the first year of life?

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Background: Some individuals with Rett syndrome (RTT) were reported to achieve certain speech-language and communicative milestones before regression. However, there is still a lack of knowledge about pre-regressional pragmalinguistic capabilities and development.

Objectives: We aimed to compare socio-communicative abilities in individuals with typical RTT and typically developing (TD) infants at the end of the first year of life.

Methods: Five females with typical RTT and five TD females were included in this study. In order to identify socio-communicative forms and functions between 9 and 12 months of age we analysed 759 minutes of family audio-video recordings using the Inventory of Potential Communicative Acts (IPCA). According to the IPCA we assigned communicative forms (e. g., body movements, eye gaze, vocalisations) to 10 different communicative functions: (i) attention to self, (ii) answer, (iii) hoice making, (iv) request information, (v) social convention, (vi) reject/protest, (vii) request object, (viii) request action, (ix) comment, (x) imitate.

Results: The total amount of socio-communicative forms per participant ranged from 3 to 12 in the RTT group and from 7 to 17 in the TD group. In the RTT group, non-verbal forms (e. g., reaching, eye contact) were more common than non-linguistic verbal forms (e. g., pleasure vocalisations, crying). (Pre-)linguistic verbal forms (e. g., babbling, proto-words) were not observed in communicative settings. In comparison, more TD females used verbal forms for communicative purposes.

The communicative forms were assigned to 3 to 6 communicative functions in the RTT group and to 4 to 8 functions in the TD group. All females with RTT used at least one communicative form to (i) gain attention and to (ii) answer. In addition to (i) gain attention and (ii) answer, all TD females were observed to (vi) reject/protest and (ix) comment. No participant was observed to (iii) make choices or (iv) request information.

The communicative milestones of babbling and proto-words were not met by any female with RTT between 9 and 12 months of age, but were observed for all TD females. Only one participant with RTT was observed to use a gesture, but four TD participants used one or more gestures.

Discussion: Our results indicated a limited repertoire of socio-communicative abilities and a predominance of non-verbal communication strategies in the participants with RTT. The findings are in line with previous studies showing pre-regressional speech-language and communicative deficits in individuals with typical RTT. However, the samples we analysed might not be representative and our results could depend on the opportunities provided during the observational samples. Further studies with larger sample sizes and structured opportunities during interactions across a wider age range are needed to draw a more concise picture of the early socio-communicative abilities of individuals with RTT.

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3.02

Rett syndrome is associated with altered Gut Microbiota Community

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Background: Girls and women with Rett Syndrome (RTT) are characterized by an altered gastrointestinal homeostasis that results in gastrointestinal discomfort, posing a significant burden for their caregivers. Changes in bacteria (microbiota) composition, as observed in other neurological disorders such as autism, may account for several typical symptoms associated to RTT. Indeed, it is shown that a dysbiotic microbiota in gastrointestinal tract may affect the function of the nervous system. The gut-brain-axis is a communication system that integrates neural, hormonal and immunological signaling between the gut and the brain, and provides the intestinal microbiota and its metabolites with a potential route to access the brain.

Objectives: The main goal of this preliminary study was to characterize gut microbiota in RTT patients, and to compare it with a control group of female students of the same age.

Methods: Eight RTT patients were enrolled at the Child Neuropsychiatry Department of San Paolo Hospital of Milan, Italy. Age and sex-matched healthy women (N), studying/working at the University of Milan, were recruited. From all subjects we collected stool samples, anthropometric data and dietary habits. Microbiota characterization was achieved by amplicon sequencing using 16S rRNA regions (V3-V4) genomic region with a Next Generation Sequencing approach on Illumina platform. Concentration of SCFAs was determined by gas-chromatography analysis.

Results: Body mass index (BMI, kg/m2) was 17.4±3.9 (± SD) mean in RTT patients, and 20.9±2.2 in control group ($p = 0.0284$). Individual dietary habits were recorded by food diary report, and processed by a dietitian. We did not observe differences in the mean value of Kcal/die ($p = 0.43$). Macronutrients analysis showed an increase in protein content ($p = 0.029$) and a lower intake of sugars ($p = 0.0035$) in RTT diets. Microbiota analysis showed a significant lower alpha-diversity in RTT samples compared with control group (chao index and species richness $p < 0.0001$; shannon index $p = 0.0015$; and inverse simpson index $p = 0.013$). The predominant bacterial taxa in both RTT

and N subjects were *Firmicutes* and *Bacteroidetes*. Although the *Firmicutes/Bacteroidetes* ratio was similar in the two groups, at family level *Bacteroidaceae* were significantly higher in RTT samples (P = 0.0009), whereas *Clostridiaceae, Ruminococcaceae* and *Christensenellaceae* were strongly reduced.

Discussion: We demonstrated in a small sample of patients that RTT gut microbiota is significantly different from the control group. Our hypothesis is that a dysbiotic gut in RTT patients could result in alterations of SCFAs that can worsen clinical symptoms by interacting a various levels (gut, brain, liver). Understanding critical changes could offer new tools for a diet intervention or probiotics supplementation to improve RTT associated symptoms and, ultimately, psycho-physical wellness.

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3.03

The association between bruxism, Rett syndrome and epigentic deregulation

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Background: Bruxism is defined as a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth, and/or bracing or thrusting of the mandible. There are two distinct circadian phenotypes of bruxism: sleep bruxism (SB) and awake bruxism. Both types of bruxism are associated with Rett syndrome (RTT) and other methyl-CpG-binding protein 2 (MECP 2)-related disorders, Prader-Willi syndrome (PWS), and Angelman syndrome (AS). The detailed etiology of bruxism so far remains unknown.

Objectives: The present manuscript reviews the possible role of epigenetic mechanisms in the etiology of both types of bruxism based on the epigenetic pathways involved in the pathophysiology of RTT, PWS, and AS, and on other epigenetic disruptions associated with risk factors for bruxism, including sleep disorders, altered stress response, and psychopathology.

Methods: The method of our investigation is a bibliographic analysis of relevant scientific literature on etiology of bruxism published in National Library of Medicine's (NLM) Medical Subject Headings (Mesh) Database, PubMed, Science Direct, Sage Databases and internet browser Google published in the last 10 years, with particular focus on search of possible associations between epigenetic deregulation and bruxism.

Results: RTT, PWS and AS, in which both types of bruxism occur as main oral manifestations, are caused by disruptions in the epigenetic DNA expression regulation, and these associations suggest a mechanistic link may exist between epigenetic deregulation and bruxism. MECP2 mutation may be a potential mechanism underlying these associations as its presence seems highly indicative of both RTT and bruxism. The pathophysiology of sleep, with which SB is strongly associated and which is also a constant finding in RTT, AS, and PWS, is additionally highly associated with MECP2 modulations.

Discussion: Bruxism is a complex disorder with a controversial etiology. Evidence from genetic studies indicates that bruxism is caused by a mix of genetic and environmental (GxE) factors. No studies have yet been conducted to investigate the association of bruxism with epigenetics, even though epigenetics specifically focuses on research modalities that investigate GxE interactions. Further, both types of bruxism are associated with neurodevelopmental disorders that are caused by epigenetic disruptions, including RTT, PWS and AS, and these associations suggest that a direct link may exist between epigenetic deregulation and bruxism. There have also been no studies of the association between sleep pathology, bruxism and epigenetics; although SB is closely associated with sleep disturbances and the latter have been linked to RTT, PWS and AS. Future research focusing on the genes and mechanisms involved in the epigenetic pathophysiology of RTT, AS, PWS and other risk factors for bruxism is warranted.

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3.04

Functional imaging confirms a redox imbalance in cytosol and mitochondria of MeCP2-deficient neurons

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Background: Rett syndrome (RTT) is a neurodevelopmental disorder which occurs almost exclusively in girls. Growing evidence indicates that RTT is associated with mitochondriopathy and cellular redox imbalance. Mitochondria of MeCP2-deficient (*Mecp2*$^{-/y}$) mouse brain are partly uncoupled and show increased respiratory rates.

Objectives: Earlier we found exaggerated responses of *Mecp2*$^{-/y}$ hippocampus to redox challenge and mitochondrial inhibition. These analyzes were now intensified focusing specifically on neurons and in particular at their cytosolic and mitochondrial compartments, to unveil the molecular causes of subcellular redox impairment.

Methods: Real-time imaging of subcellular redox dynamics was performed with the genetically-encoded redox sensor roGFP1. In dissociated cell cultures and organotypic slices of mouse hippocampus, optimized expression was achieved by viral transduction. Response calibration of roGFP1 sensors enabled quantitative redox imaging in cytosol and mitochondrial matrix.

Results: Detailed excitation ratiometric fluorescence microscopy and 2-photon imaging confirmed that in *Mecp2*$^{-/y}$ hippocampal neurons the redox imbalance affects the cytosolic and the mitochondrial compartment. These changes were especially obvious for more complex organotypic hippocampal slices. Redox challenge by H_2O_2 and severe hypoxia elicited intensified oxidizing and reducing transients in *Mecp2*$^{-/y}$ neurons, respectively. Inhibition of superoxide dismutase elicited only a dampened oxidation in *Mecp2*$^{-/y}$ cytosol and mitochondria, suggesting a decreased efficiency of this scavenging enzyme in Rett mice. More importantly, stimulation by glutamate, dopamine, serotonin, and norepinephrine consistently evoked intensified oxidizing shifts in the cytosol of *Mecp2*$^{-/y}$ neurons. Taking advantage of recently generated transgenic redox-indicator mouse lines, the cytosolic redox impairment could also be confirmed for hippocampal neurons of adult, symptomatic *Mecp2*$^{-/y}$ mice.

Discussion: Genetically-encoded roGFP1 sensors enable semi-quantitative recordings of subcellular redox dynamics. The redox imbalance associated with RTT clearly affects cytosol and mitochondria of central neurons. Even physiological events such as neurotransmitter stimulation are sufficient to provoke overshooting redox responses in *Mecp2*$^{-/y}$ neurons. As these

changes are already evident in presymptomatic mice, they may promote the progression of RTT. By generating roGFP1 transgenic mice, we successfully extended quantitative redox imaging to all postnatal stages and more complex preparations. This revealed for the very first time a functional readout of subcellular redox conditions in living brain tissue of adult, symptomatic Rett mice. Hence, this will now enable a detailed correlation of disease progression and redox homeostasis throughout the brain of maturing Rett mice.

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3.05

NLX-101, a novel 5-HT1A agonist, for the treatment of respiratory arrhythmias in Rett syndrome

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Background: Dysrhythmic breathing and dysautonomia, as well as neurological and cognitive deficits, are highly prevalent components of the Rett syndrome (RTT) phenotype. Dysrhythmic breathing, presenting as apnoeas and an irregular interbreath pattern, leads to significant falls in arterial blood oxygen saturation that may contribute to cognitive decline, gastrointestinal difficulties and propensity to seizures in RTT. Although previous studies demonstrated that serotonin 5-HT_{1A} receptor agonists reduced apnoeas in different mouse models of RTT, their poor selectivity is associated with off-target effects. In contrast, NLX-101, a novel potential therapeutic for respiratory dysrhythmia in RTT, is a highly potent, selective and efficacious biased agonist that targets 5-HT_{1A} hetero-receptors, which, unlike auto-receptors, are associated with positive effects on respiratory drive.

Objective: Here, we aimed to characterize the effects of NLX-101 on respiratory dysrhythmias in two genetic mouse models of RTT.

Methods: All procedures conformed to the UK Home Office guidelines on animals (Scientific Procedures) Act 1986 and were approved by the University of Bristol's Animal Ethic Committee. Using unrestrained whole-body plethysmography, we measured the frequency and duration of apnoeas in Mecp2tm1.1Bird ($n=18$) and Mecp2tm1.1Coyle ($n=22$) heterozygous female mice (>6 months of age) during 1 hour pre-treatment and 1 hour post-treatment with randomly-assigned multiple ascending doses of NLX-101 (0.04, 0.16, 0.63, 2.5 mg/kg, 1 mL/kg) or vehicle. In a separate group of Coyle ($n=24$) heterozygous females, we measured respiratory parameters before and 7, 10 and 14 days after continuous infusion (subcutaneous mini-pump) of NLX-101 (5 mg/kg/day) or vehicle (randomly assigned). At the end of experimental protocols, plasma and brain exposure levels of drug were measured.

Results: NLX-101 dose-dependently and significantly reduced both the frequency and the length of apnoeas in both strains of mice. In Bird females, NLX-101 (2.5 mg/kg) reduced apnoeas profoundly to 12±6 apnoeas/hour compared to 142±12 apnoeas/hour in vehicle treated females (mean±SEM, ANOVA, $p<0.05$). The length of the remaining apnoeas was shorter, 0.712±0.144 s in NLX-101 treated group compared to 1.415±0.107 s in vehicle-treated females. The drug was equally effective in Coyle females, reducing frequency to 12±4 apnoeas/hour and length to 0.862±0.057 s, compared to 153±32 apnoeas/hour and 1.534±0.1 s in duration in vehicle treated controls. Likewise, continuous infusions of NLX-101 effectively reduced the frequency and length of apnoeas in both strains of mice by about half.

Discussion: The results demonstrated that NLX-101 reduced apnoea frequency and duration, in a dose-dependent manner, in heterozygous females from both Bird and Coyle strains. The Coyle model mimics one of the most common mutations (R168X) in human patients with RTT, which makes it highly relevant for translation to human studies. The present results will guide selection of the dosing schedule of NLX101 for future clinical trials in patients with RTT.

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3.06

Gut microbiota analysis in Rett syndrome mouse models

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Background: Recent findings pointing on the existence of a microbiota-gut-brain axis suggest that these "organs" can communicate each with other and that gut bacteria can influence the brain functions and vice versa. In addition, a variety of gastrointestinal symptoms, such as constipation and gastroesophageal reflux, can occur commonly in patients with Rett syndrome (RTT), but the etiology of these dysfunctions is not well understood.

Objectives: To better understand the gastrointestinal state and to verify a possible implication of gut microbiota (GM) in RTT, we investigated the GM composition of two different RTT

mouse models with altered methyl-CpG-binding protein 2 gene (MeCP2 gene).

Methods: Next generation sequencing using Illumina MiSeq System was performed to analyze GM composition of MeCP2-null and MeCP2-308 female mice and their wild type (wt) littermates. The sequences of V3-V4 16rRNA gene were filtered and processed with QIIME to obtain Operational Taxonomic Units (OTUs) and their taxonomic classification against Greengenes Database. We determined the species richness and diversity within communities using alpha diversity metrics; while diversity shared across microbial communities (beta-diversity) was assessed by applying unweighted and weighted Unifrac distances. To identify key OTUs, that discriminated between mutant and control groups, Metastats comparison and Galaxy platform-based LDA Effect Size analysis were used.

Results: Data showed a different composition of GM between MeCP2 mouse models and their wt counterparts. We found variation in abundance of some key OTUs, in particular a reduction of S24-7 family of phylum Bacteroidetes and an increase of Erysipelotrichaceae family of phylum Firmicutes in MeCP2 mutant mice compared to controls. Interestingly, alterations in these taxa are in common with other independent induced (non-genetic) and spontaneous mouse models of autism (MIA, VPA and BTBR). These results prompted us to investigate microbiota composition in Rett patients in order to find commonalities and differences between human and mice microbiota alterations. This topic is currently under study.

Discussion: Our results support the potential usefulness of RTT mouse models to study the gut-brain axis dysregulation and the effects of microbiota modulation and transplantation on neurological and gastrointestinal symptoms.

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3.07

FOXG1 syndrome in two patients with severe developmental delay and microcephaly identified by array-CGH

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Background: The FOXG1 congenital variant of Rett syndrome has been described as a clinically identifiable phenotype, called the „FOXG1 syndrome“, an epileptic-dyskinetic developmental encephalopathy with features of classic Rett syndrome, but earlier onset since the first months of life. The main phenotype comprises microcephaly, severe developmental delay and dyskinesia. Other features include hypotonia, strabismus, feeding difficulties, bruxism, seizures and corpus callosum dysgenesis.

Objectives: We present two novel cases with *FOXG1* microdeletion identified by array-Comparative Genomic Hybridization (array-CGH).

Patient 1: A 6-month-old female infant, born at 38 weeks of gestation after in vitro fertilization, presented with microcephaly, developmental delay, irritability and feeding difficulties. A small forehead with bitemporal narrowing, dyspraxia, poor eye contact and strabismus were also noted. At 10 months the proband exhibited focal seizures and required valproic acid treatment. Sleep deprivation EEG showed signs of focal cerebral dysfunction. The brain MRI revealed a septum pellucidum cyst and brain asymmetry.

Patient 2: A 3-year-old male who was born after a 33 weeks twin pregnancy, following in vitro fertilization, presented with microcephaly, severe intellectual disability, central hypotonia, strabismus and minor dysmorphic features. At the age of 12 months the proband had seizures. His EEG was normal while the brain MRI showed small and thin corpus callosum.

Methods: In both patients, G-banding karyotype was normal, (46, XX) and (46, XY) respectively. In patient 1, the *MECP2* gene DNA analysis was normal and further investigation by array-CGH (Agilent Technologies, resolution 200 Kb) was performed.

In patient 2, high resolution 4X180K CGH+SNP Agilent Human Genome microarrays with average spatial resolution of 7.9–8.9 Kb were used.

Results: In patient 1, array-CGH revealed a 14q12 microdeletion, 4.09 Mb in size. The breakpoints were mapped between genomic coordinates chr14:25,843,560-29,938,629 (GRCh37/hg19) and included *FOXG1* and *NOVA1* genes.

In patient 2, array-CGH analysis revealed two small chromosome aberrations, a 6q22.32-q22.33 microdeletion, 2.1Mb in size, between nucleotides 126665820-128744012 (hg19/build 37) containing 9 genes, and a 1.13 Mb microdeletion at 14q12, between nucleotides 28625754-29758147 (hg19/build 37) encompassing *FOXG1 and C14orf23* genes.

Discussion: Patient 1 presented with similar features like patients carrying 14q12 deletions except for corpus callosum dysgenesis. *NOVA1* gene possibly plays a significant role in the regulation of neuronal development and synaptic plasticity. Despite the significant role of *NOVA1* gene, defects of this have not been linked to any human phenotype yet. As our patient's phenotype is in line with the FOXG1 syndrome, it is difficult to estimate the impact of the *NOVA1* haploinsufficiency. *NOVA1* haploinsufficiency may act synergistically with *FOXG1* deletion or it may cause no difference in the final clinical outcome.

In patient 2, the 14q12 microdeletion encompasses the *FOXG1* gene and the proband's phenotype is also in accordance with the FOXG1 syndrome. The impact of the 6q22.32-q22.33 microdeletion in the patient's phenotype remains unclear. Such deletions are very rare and they have been linked to mild intellectual disability, microcephaly, heart defects and cleft lip/palate.

In conclusion, FOXG1 syndrome should always be considered in patients with early onset severe developmental delay, microcephaly and minor or absent dysmorphic features.

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3.08

The blood-brain barrier as an important factor for drug delivery of „RETT-Therapeutics“ to the brain

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Background: The Rett syndrome (RTT) is an X-chromosome-linked progressive neurodevelopmental disorder that affects girls almost exclusively, with an incidence of about 1 out of 10,000. RTT is known to be caused in 95 % of the cases by sporadic de novo loss-of-function mutations in the MECP2 gene. MeCP2 is a basic nuclear protein that is highly expressed in the brain, especially in neurons. A main goal in the development of a suitable therapy for RETT patients would be to achieve physiologically levels of normally functional MeCP2 protein in the brain. Drug delivery to the central nervous system (CNS) remains a major pharmaceutical challenge due to the blood-brain-barrier (BBB). The BBB is responsible for the homeostasis of the CNS and to prevent entrance of pathogens such as viruses or bacteria, but also of xenobiotics. In order to deliver functional MeCP2 into the CNS it has to overcome the BBB, which could be enhanced by the modification of the protein with a cell penetration improving strategy such as the addition of a TAT sequence. In the preclinical phase BBB in-vitro studies are a valuable tool for drug development and optimization of therapeutics.

Objectives: The aim of this study was to establish an in-vitro model of the mouse BBB to investigate the transport properties of TAT-MeCP2 preparations.

Methods: Paracellular barrier properties of a Transwell in-vitro model based on mouse brain endothelial cells were characterized by transendothelial electrical resistance (TEER) and permeability of marker FITC-dextran 40. This model was used to investigate the transport of TAT-MeCP2 preparations across the endothelial cell layers, and permeability coefficients were calculated following the clearance principle. Data were compared to the transport properties of TAT-eGFP. TAT-MeCP2 was quantified by means of a newly developed ECLIA assay, TAT-eGFP and FITC-dextran 40 were measured via their fluorescent properties in a plate reader.

Results: An in-vitro model of the mouse blood-brain barrier was successfully established and characterized. Transport data with this model revealed a distinct barrier by brain endothelial cell layers for the transport of the investigated TAT-proteins, but also showed that significant amounts of TAT-protein preparations were able to cross the BBB in-vitro. Moreover, concentration dependent increase of permeability coefficients indicated a possible active transport process mediated by the TAT-modification. In addition, the combination with TEER and FITC-dextran 40 data allowed to monitor the influence of TAT-proteins on BBB integrity.

Discussion: An in-vitro model of the mouse BBB was successfully established to study the transport of potentially therapeutic TAT-MeCP2 proteins. This model enables to investigate transport mechanisms across the BBB in-vitro and could be used as a screening tool to select for the best preparations. In conclusion, this model could serve as a valuable tool in the development of “RETT therapeutics”.

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3.09

MECP2-expression in GlyT2-neurons does not improve survival in a mouse model of Rett syndrome

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Background: Mutations in the gene for the methyl-CpG-binding protein 2 (MeCP2) are known to cause the neurodevelopmental disorder RETT syndrome. A typical problem that occurs in patients is a disturbance of breathing.

Objectives: Here we addressed the role of inhibitory neurons in the development of this breathing impairment in a mouse model of Rett syndrome.

Methods: We therefore generated a mouse line that restores MeCP2 in inhibitory neurons in the brainstem by crossbreeding a mouse line that expresses the cre-recombinase in neurons under the control of glycine transporter 2 promoter (GlyT2-cre) with a mouseline that has a floxed-stop mutation of the *Mecp2* gene (*Mecp2*$^{stop/y}$).

Results: Unrestrained whole-body-plethysmography at postnatal day P60, revealed a low respiratory rate and prolonged respiratory pauses in *Mecp2*$^{stop/y}$ mice but not in GlyT2-cre positive *Mecp2*$^{stop/y}$ mice. Both, respiratory frequency and rate of ventilatory pauses, were improved as compared to GlyT2-cre negative *Mecp2*$^{stop/y}$ mice.

Discussion: The data support the concept that alterations in inhibitory neurons are important for the development of the respiratory phenotype in Rett syndrome. However, although we observed this striking improvement of breathing, the overall survival of the mice was not rescued, indicating that an early impairment of breathing cannot be considered as causal for the progression of the major pathophysiological changes in Mecp2 deficient mice.

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3.10

A study of Methyl-CpG Binding Protein 2 mutations and locomotion ability: Consideration from japanese Rett syndrome database

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Background: Rett syndrome (RTT) is a major neurodevelopmental disorder, having various characteristic features. We reported 0.009 % of Japanese RTT patients prevalence in under 20 year-old females. Thereafter, we established Japanese RTT database (JRDB) at 2013, and have managed it since then.

Objectives: JRDB includes 102 patients clinical data. In order to clarify Japanese RTT patients' characteristics, we analyzed them.

Methods: We selected developmental history, symptoms and causative mutations from the database and investigated their correlations. Here, we focus on locomotion ability and distribution of MECP2 mutations.

Results: The JRDB was registered 102 RTT females (the median age was 11 year-old). Among them, 44 patients were able to walk alone (43 %). The averaged age of initial walking was 21.6 month-old. MECP2 mutation analysis was received 88 % patients (82 of 93). Genetic abnormalities of MECP2 is recognized 96 % (79 of 82) of patients. The most frequent mutation is R168X (11.8 %, 11 of 79). The next is T158M (8.6 %, 8 of 79), and the third is R255X (6.4 %, 6 of 79). Furthermore, patients with R168X were relatively severe locomotion ability, showing 3 patients walking alone and 5 rolling over. On the other hand, patients with R133C, R294X or R306C were relatively mild, 80 % patients (8 of 10) walking alone.

Discussion: The JRDB has registered clinical data of over 100 patients. From the JRDB, we could obtain various interesting features of Japanese RTT patients. The distribution of MECP2 mutations in JRDB is similar to those of other countries, such as Australian database. We revealed MECP mutations-depended RTT phenotypes of Japanese patients. To advance the next stage, we would like to collaborate with world-wide database to clarify clinical characteristics and seek advanced therapies.

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3.11

Systemic free-radical scavenger treatment of Rett Mice: Merits and limitations of trolox

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Background: Rett syndrome is a severe neurodevelopmental disorder typically arising from spontaneous mutations in the X-chromosomal methyl-CpG binding protein 2 (MECP2) gene. The almost exclusively female Rett patients show an apparently normal development during their first 6–18 months of life. Subsequently, cognitive and motor impairment, epilepsy, and highly irregular breathing manifest. Early mitochondrial alterations and oxidative stress are considered to contribute to disease progression. Accordingly, supplementation with antioxidants and/or radical scavengers seems of potential merit.

Objectives: Since the vitamin E derivative Trolox improved synaptic plasticity, neuronal function and hypoxia tolerance in isolated hippocampi of symptomatic, male MeCP2-deficient mice, we now performed a full preclinical trial to define the therapeutic potential of a chronic systemic Trolox treatment of Rett mice.

Methods: Blinded, placebo-controlled treatment of male mice started at presymptomatic stages (postnatal day 10–11) and was continued for ~40 days. Mice were randomly assigned to a treatment group and either vehicle (PBS), 10 mg/kg Trolox or 40 mg/kg Trolox were injected intraperitoneally every 48 h.

Results: The intraperitoneal injections and the chronic Trolox treatment were tolerated well. Detailed phenotyping revealed that in *Mecp2*$^{-/y}$ mice the blood glucose content, lipid peroxidation, synaptic short-term plasticity, hypoxia tolerance, and certain types of environmental exploration were improved by Trolox. Yet, the reduced body weight and size, smaller brain volume and thinner cortical layers, impaired motor function, and the regularity of breathing were not rescued. Neither was the increased hematocrit of MeCP2-deficient mice ameliorated upon Trolox treatment. It became apparent though, that the frequent animal handling and intraperitoneal injections decreased the phenotypic differences among placebo treated wildtype and MeCP2-deficient mice.

Discussion: Chronic systemic *in vivo* Trolox treatment was shown to be capable of improving certain aspects of the complex Rett phenotype. In general, this indicates a potential merit of a free-radical scavenger based pharmacotherapy. Hence, in combination with other therapeutic approaches, antioxidants and/or free-radical scavengers might serve to improve at least some features associated with this severe neurodevelopmental disorder. In view of further trials, frequent animal handling and the very route of drug administration are critical parameters to be optimized.

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3.12

Social counselling at the danish center for Rett syndrome

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Background: Rett syndrome (RTT) is a complex disorder including multiple disabilities and several comorbidities. Individuals with RTT have a lifelong maximal assistance need in all activities of daily living. Thus, qualified social counselling in the cooperation with the social authorities is needed. In Denmark this counselling is provided by the social worker at the Danish Center for Rett Syndrome (CRS).

The main focus for the CRS is to optimize the quality of life for the persons with RTT and their families, which is done by clinical follow-ups, multidisciplinary counselling, and advice about the management of persons with RTT for local health care and social authorities.

Objectives: To describe the scope and complexity of the problems encountered by families (and others related) with RTT in relation to the cooperation with the social authorities.

Methods: All records from 2015 of individuals with RTT followed at the CRS, were reviewed to describe the content of any counselling provided by the social worker. The inquiries were divided in four main social work categories with several subcategories: 1) Aids (Housing modifications, vehicle, wheelchair/seating system, mobility aids, small aids, communication aids and medical aids); 2) Care (Accommodation, respite care, home care, personal assistance care, social assistance care); 3) Legal rights (Counselling about legislation, transition to adulthood, legal guardian, employment and support allowance, high-school special education, home habilitation); 4) Economic compensation (Additional costs, lost working hours).

Results: In Denmark 110 individuals are diagnosed with RTT and of those 98 individuals are followed at the CRS. During 2015, the social worker provided social counselling to 32 families and residential social workers who have a child with RTT or who have a resident with RTT (~33 %). The majority of involved individuals with RTT were younger than 18 years ($n = 22$, ~69 %) and lived at home with their families. Overall in the 32 cases, 14 had inquiries in one main social work category, 4 had in two categories, 5 had in three categories and 9 had in all four categories. In category 1) Aids, 23 inquiries were made and in 19 cases counselling including a written appeal were provided by the social worker. In category 2) Care, 3) Legal rights and 4) Economic compensation the numbers were 17 (inquiries)/11 (counselling/appeals), 17 (inquiries)/8 (counselling/appeals) and 12 (inquiries)/10 (counselling/appeals), respectively.

Discussion: Approximately one third of the families and residential social workers needed counselling before or during contact with the social authorities. The categorization of social work emphasized the scope and complexity of the social counselling at CRS. The results especially highlight the family's need of having a person, with whom they can discuss the complexity of having a child with RTT. It is undesirable that this guidance is provided by a person with a dual role as both counselor and as authority with the executive power.

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3.13

Phenotyping the first two years of life in monozygotic twins with RTT – a case study

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Background: Fifteen twin pairs with RTT have been reported in the literature, a few of them with concordant phenotype, while others had noticeable differences in their development. So far, none of these reports included a detailed description of the first year(s) of life.

Objectives: To identify possible early indicators of RTT by characterising the early motor and communicative development.

Methods: We report on a pair of female monozygotic twins born at 34 weeks' gestation. Typical RTT was diagnosed at 24 months for both girls. We retrospectively analysed 38 video clips of Twin 1 and 36 video clips of Twin 2, parental diaries and medical history data. Videos were recorded before parents were aware that their daughters had RTT although first concerns about developmental delays, especially in Twin 1, arose during the 7th month post term.

Results: Both twins missed most developmental milestones with the exception of head control and head centred in the midline at 3 months. Spontaneous general movements were already abnormal during the first month after term. Further abnormalities in motor, socio-communicative, and speech-language domains were present before the children exhibited any obvious signs of regression.

Discussion: This exploratory study supports the long-held idea that the early development of females later diagnosed with RTT reveals certain abnormalities even several months before the parents had concerns about their daughters' development.

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Comparison of communication level in a young and older cohort of females with Rett syndrome in DK

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Background: Communication in females with Rett syndrome (RTT) is complex. How they can express themselves somewhat depends on the people around them.

During 2015 the Danish Center for Rett syndrome (CRS) examined and observed two cohorts of females with RTT and a MECP2-mutation who participated in two separate studies of participation and functional abilities [1], respectively. The communication was observed in the two cohorts and the general impression was that the older women were harder to interpret and they only used few communication tools. Thus, we hypothesized that the communication skill level was different in the two cohorts.

Objectives: A retrospective comparison of communication skills in a young and older cohort of females with RTT.

Methods: The WeeFIM communication subscale was used to assess comprehension and expression in the two cohorts. The number of AAC tools was recorded. It was noted if choice-making and using/developing a yes/no was part of the AAC strategy. The Hoffer Ambulation Scale and Clinical Severity Scale were also administered.

Comparisons were performed with independent t-test and Mann-Whitney U test for numerical and categorical variables, respectively.

Results: The young cohort consisted of 22 girls aged 3–19 years (median 8.7 years) and the older cohort [1] of 27 women aged 30–60 years (median 41 years). No significant differences were seen in ambulation level ($p=0.22$) or clinical severity ($p=0.06$). Comparison of the WeeFIM communication subscale scores showed a significant difference ($p=0.001$) in favor of the young cohort (young cohort mean = 5.1; older cohort mean = 3.7). Many of the females in the young cohort had high- and/or a low-tech AAC tool (high-tech = 54.5 %, low-tech = 22.7 %), whereas it was rarely the case in the older cohort (high-tech = 0 %, low-tech = 14.8 %).

As part of the AAC strategy, 86.4 % in the young cohort participated in choice-making. In the older cohort, this applied for 29.3 %. Furthermore 50 % of the females in the young cohort and 29.3 % of the females in the older cohort were either able to or in the process of developing a yes/no.

Discussion: The results confirm the hypothesis as the young females have significantly better communication skills as measured by the WeeFIM. However, the WeeFIM has apparent floor effects in these females with RTT who have severe communication difficulties. Due to the retrospective nature of this study we are not able to perform further analysis of the possible variables explaining the observed differences. Further studies of communication skills should be prospective and longitudinal and use a more sensitive measure. We speculate that the paradigm shift on the development of communication in females with RTT can explain the differences seen in the two cohorts e. g. better understanding of communicative competencies, comprehensive initiatives during school years, available high-tech solutions like eye-gaze computers.

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Expression of nuclear Mecp2 is dependant on induction of neuronal plasticity and application of IGF1

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Methyl-CpG binding protein 2 (MECP2) is a chromosome-binding protein that regulates the development and maintenance of brain circuits. Altered function of the protein product of MECP2 plays an important role in the etiology of many neurodevelopmental disorders. Mutations involving a loss of function are implicated in the etiology of Rett syndrome, intellectual disability, psychosis and severe encephalopathy. Conversely, MECP2 duplications have been identified in autism and intellectual disability. MECP2 action is dependent on neuronal function, as the DNA binding is modulated by activity, and it is phosphorylated in response to stimulation. Although MECP2 is considered a major risk factor for neurodevelopmental disorders, and it is a mediator of activity-dependent mechanisms, the expression levels in response to neuronal activity have never been measured. We studied the expression of Mecp2 protein and RNA in mice neuronal cultures in response to different stimulation conditions and in the presence of insulin-like growth factor1 (IGF1): a growth factor involved in brain development and plasticity. The stimulation protocols were selected according to their ability to induce different forms of synaptic plasticity: rapid depolarization, feed-forward plasticity (LTP, LTD) and feedback forms of plasticity (TTX, KCl). We find a significant reduction of Mecp2 protein nuclear expression in neurons in response to stimuli that induce a potentiation of neuronal response, suggesting that Mecp2 protein expression is modulated by neuronal activation. Application of IGF1 to the cultures induces an increase in the expression of Mecp2 transcript and nuclear Mecp2 protein in neurons. These results show that Mecp2 is responsive to neuronal stimulation and IGF1, and different stimuli have different effects on Mecp2 expression; this differential response may have downstream effects on functional mechanisms regulating brain development and plasticity.

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An electrochemiluminescence based assay for quantitative detection of recombinant Tat-MeCP2 fusion proteinP

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Background: Rett syndrome (RTT) is an X-chromosome-linked progressive neurodevelopmental disorder that affects girls almost exclusively, with an incidence of about 1 in 10,000. RTT is known to be caused in 95 % of the cases by sporadic de novo loss-of-function mutations in the MECP2 gene, encoding methyl CpG-binding protein 2 (MeCP2), a basic nuclear protein that is highly expressed in the brain, where it is specifically detected in neurons.

Objectives: Development of an electrochemiluminescence based immunoassay (ECLIA) for quantitative detection of recombinant transactivator of transcription (TAT)-MeCP2 fusion protein in murine brain, in order to be able to determine the amount of MeCP2 protein in normal neurons and to use these data as guidance for a supplementation therapy.

Methods: For our experiments we used a 96-well based ECLIA formatthat is able to measure in a highly quantitative, accurate and reproducible manner, with low intra- and inter-assay error througover a wide working range.

Results: The assay sensitivity for MeCP2 is 90 picogram per milliliter and it is able to detect endogenous MeCP2 and recombinant TAT-MeCP2 in murine brain. In addition, we confirmed the applicability of the assay for permeation studies of a recombinant TAT-MeCP2 fusion protein across a mouse blood-brain barrier in-vitro model.

Discussion: Here we report on the investigation to measure endogenous MeCP2 and recombinant TAT-MeCP2 protein levels through electrochemiluminescence immunoassay (ECLIA) in a 96-well plate format. This assay should provide a new quantitative tool to investigate appropriate dosage of MeCP2 in neurons of murine brain and to monitor the protein behaviour in neurons.

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Rett syndrome-genes, clinical or molecular diagnostics first?

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Background: With the clinical implementation of new molecular technologies we perform an increasing number of screening analyses (high resolution array CGH, NGS panels and exome sequencing). This enables earlier molecular diagnosis of patients with symptoms overlapping with the Rett syndrome (RTT) spectrum, especially when the phenotype is atypical and RTT is not suspected. The description on how to make the diagnosis in RTT and confirm it molecularly is important since these patients are rare and they are often seen first by paediatricians who do not necessarily have previous experience with the RTT phenotype.

The diagnostic rate of *MECP2* mutations in Denmark after 2008 is approximately 2.7 new patients per year [1]. We hypothesise that the diagnosis of *CDKL5* and *FOXG1* associated with RTT is harder to pinpoint and thereby the molecular diagnosis is established by screening analyses rather than with targeted single gene analysis approach.

Objective: To investigate method of analyses performed to make the molecular confirmation of Rett syndrome in Denmark.

Materials and Methods: We have registered 115 Danish patients with a *MECP2* mutation diagnosed at ages 15 mo. – 77 years. A *CDKL5* mutation has been identified in 10 patients diagnosed at ages 3 mo. – 51 years. A *FOXG1* mutation has been registered in two patients diagnosed 18 mo. – 4.5 years old. We looked into the method of analysis in all these diagnosed patients.

Results: *MECP2* analysis was performed with single gene analysis in all of the 115 patients; none of them was diagnosed by screening analysis. *CDKL5* mutations were detected using single gene analysis in five of 10 patients, four patients were diagnosed through a large NGS panel for epileptic encephalopathies and one patient was diagnosed by high resolution array CGH analysis (50 % diagnosed by screening analysis). One patient with *FOXG1* mutation was diagnosed by exome sequencing, and the other patient by NGS panel for encephalopathies.

In children born after 2012, where large scale screening analyses including clinical exome sequencing was gradually implemented in routine diagnosis in Denmark: *CDKL5* mutations were found in 3 patients (30 % of the total no of patients and 0.7 per year). *FOXG1* mutations was found in one patient (100 % of the total no of patients and 0.2 per year).

Discussion: The number of patients in this survey is relatively small, but the results indicate an increasing diagnostic hit rate of *CDKL5* and *FOXG1* mutations with new large scale screening methods. Since MECP2 is still mainly detected by single gene sequencing, this method is the most cost-efficient when the diagnosis Rett syndrome is clinically suspected. We assume that in the future the large scale screening methods will however increase the incidence of patients with *CDKL5* and *FOXG1* mutations compared to those with *MECP2* mutations.

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The use of accelerometry in two females with Rett syndrome

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Background: Recent papers on Rett syndrome (RTT) have emphasized the importance of improving and maintaining gross motor skills and physical activity (PA) levels. Activity levels can be assessed using accelerometry. The validity of the StepWatch Activity Monitor (SAM) as a measure of daily stepping activity has been established in females with RTT. A pilot study of the ActivPAL has shown that it is valid when measuring sedentary time in females with RTT. Preliminary results from a Danish population-based study using SAM and ActivPAL found that females with RTT aged 5–60 years have reduced levels of daily PA and increased levels of sedentary behavior. The mean daily step count was 6300 steps ($n=28$) and the mean hours spend sitting was 10h6min ($n=48$) corresponding to 83.3 % of their waking hours.

Objectives: This case report describes and interprets the accelerometer measurements derived from the SAM and ActivPAL in two females with RTT.

Methods: One of the participants was classified as a functional ambulator and the other as a therapeutic ambulator according to Hoffer Ambulation Scale (HAS). Both participants wore the ActivPAL for 7 consecutive days and the woman who could walk in the community also wore the SAM for 7 consecutive days. Assessments also included the Rett Syndrome Gross Motor Scale (RSGMS) and the Clinical Severity Scale (CSS).

Results: Case 1 was a 10 year-old-girl with a large deletion, a CSS score of 25/58, a RSGMS score of 13/45 and was classified as a therapeutic ambulator (HAS = III). She could walk short distances at home and in school with maximal support. ActivPAL data showed a sedentary time average of 10h31min corresponding to 92.6 % of her waking hours. Prolonged periods of sedentary time (>4hours) occurred during school hours and at home in the afternoon. Her parents have applied for a wheelchair with an integrated standing function to break prolonged sedentary time.

Case 2 was a 35 year-old-woman with an early truncating mutation, a CSS score of 20/58, a RSGMS score of 34/45 and was classified as a community ambulator (HAS = I). This woman was able to walk short distances independently or with minimal support. Her mean daily step count was 2635 and she was sedentary for 88.2 % of her waking hours. Her daily steps accumulated during morning and day center hours with much less activity occurring in the afternoon. The longest sedentary period was 2 hours and she often had short breaks of light activity (1–5min) every hour. Her family has applied for additional man-hours to increase standing and walking activities.

Discussion: The use of accelerometry provided valuable and useful information. The visual presentation of data guided families and the local habilitation team about when and where to focus on breaking sedentary periods and replacing it with light PA. However, accelerometers are expensive equipment and data collection and analysis are time consuming. Further studies of the effectiveness of a PA intervention with pre-and post measurements including simpler activity measures are warranted.

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The relationship of gastrostomy and spinal fusion among Rett syndrome patients

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Introduction: Clinicians are frequently asked difficult questions regarding the necessity and timing of possible interventions such as gastrostomy and spinal fusion that for many patients with Rett Syndrome (RTT) appear inevitable in their care continuum. Parents and clinicians alike are interested in knowing which Rett Syndrome characteristics increase the child's risk of having one or both procedures and at what age. With this knowledge, we can improve the counseling of our families.

Methods: An IRB approved retrospective review was performed on the health records from 2013–2016 on 27 and 32 patients with classic RTT grouped based on gastrostomy status (no G tube = controls or G tube) respectively. Demographic, anthropometric, genetic, and clinical data were gathered. Statistical analysis was performed on the results.

Results: The mean age at gastrostomy placement was 12.7 years (range 5 months to 43.8 years). The most common reasons for the gastrostomy were: dysphagia, weight loss, inadequate fluid intake, aspiration, medication administration, and venting. There was no statistical significant difference in the type of MECP2 genetic mutation between the two groups. Features reported more frequently in the G tube group compared to the control group included: frequent vomiting (46 % vs. 7 %; $p=0.002$); reflux (90 % vs. 33 %; $p=0.0001$); and the inability to walk independently by age two (62 % vs. 22 %; $p=0.009$). In the G tube group, 36 % had all three features of bruxism, frequent vomiting, and reflux compared to only 7 % of the control group ($p=0.02$). Another feature, ambulation without any wheelchair assistance, was less in the G tube group compared to the control group, and approached significance (17 % vs 44 %; $p=0.067$). The G tube group was more likely to have spinal fusion compared to the control group (41 % vs. 8 %, $p=0.006$). Out of the 11 patients with both interventions, when the spinal fusion was performed first, the procedure occurred later in life compared to gastrostomy (19.2 years vs. 7.5 years; $p=0.004$).

Discussion: Even in a small cohort of RTT patients, we were able to show that certain characteristics such as frequent vomiting, reflux, and inability to walk independently by age two, appear more common in those who received a G tube compared to those who did not have a G tube. Also, patients in the G tube group more frequently had all three gastrointestinal characteristics of bruxism, frequent vomiting, and reflux than the control group. We also highlight the medical fragility in RTT patients who have a G tube procedure and go onto need the spinal fusion compared to the control group. Of those that need both procedures, we showed that if the gastrostomy is performed first, the age at the first procedure was much younger than the age if the first procedure was the spinal fusion. Knowing these characteristics can help clinicians counsel the families of patients with

RTT regarding prognosis and risk of having either or both a gastrostomy and spinal fusion.

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Peripheral innervation & vascularization in Rett syndrome

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Introduction: The long-standing clinical observations and diagnostic features of 'cold hands/feet' associated with Rett syndrome have not received sustained scientific scrutiny. There are well known autonomic regulatory issues associated with Rett, but no work has directly investigated whether there is a peripheral basis underlying the observations. To begin to investigate, we have performed epidermal punch biopsies with girls affected by Rett.

Methods: Three adolescent girls with clinically confirmed Rett syndrome (ages 12, 13, 15) participated. Single punch epidermal biopsies were obtained from the postero-medial calf to compare directly with normative samples from approximate age-matched, body-site matched control children without developmental disability. Epidermal nerve fibers were traced, from confocal images, using Neurolucida software (MicroBrightField, Colchester, VT.) according to established counting criteria and reported as number of epidermal nerve fibers per mm in a 50 um thick section.

Results: We observed merkel cells that were quite large; abnormal/atypical vascular innervation, elongated mast cells, densely innervated hair follicles, and abnormal/atypical looking epidermal nerve fiber (ENF) branching. To begin to quantify, we derived ENF density value estimates were 12.2 ENFs/mm, 39.3 and 53.2 for the three girls, which cf. 8 female controls (age 12–17), the average ENFs/mm was 27.3 (SD = 9.7; range = 15.3–41.1) in an adolescent sample without developmental disability.

Discussion: All observations were preliminary and were not uniform across all three girls. The apparent differences in the degree of peripheral innervation in general and the atypical patterns specific to arterioles warrants further investigation and are consistent with a recent report regarding microvascular abnormalities in Rett.

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European policy on rare diseases and the role of national Rett syndrome parent associations and expertise centres

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Background: In recent years there has been an increasing focus in Europe (and worldwide) on developing informal links between researchers, clinicians, therapists, individuals with Rett syndrome (RTT) and their families/caregivers to maximise approaches towards identification, treatment and long-term management of the disorder. At the same time, developments in European policy on rare diseases have opened up the way for more formalised collaborations and pathways to be established, both within and between countries.

Objectives: To review the current situation in Europe with regard to RTT and to identify key players in the field.

Methods: During the 3rd European Rett Syndrome Conference in Maastricht in 2013 Rett Parent Associations from across Europe came together to share information regarding RTT in their own countries. Since then updates have been procured through meetings of organisations such as *Rett Syndrome Europe (RSE*, an umbrella organisation for the European Rett Parent Associations) and via email, phone and in-person contacts between country representatives and the Rett Expertise Centre Netherlands (Maastricht).

Results: As of 2016, 5–10 European countries have a national Rett Expertise Centre or specialised multidisciplinary Rett Clinics, which maybe within centres for rare diseases. A number of experts and researchers from these centres collaborate through the *European Scientific Rett Research Association* (ESRRA). Most countries have a Parent Association which offers support to families.

Discussion: EU policy on rare diseases promotes pan-European collaboration between stakeholders. National Rett Parent Associations and the Rett Expertise Centres that exist in many Member States have an important role to play in this, as do umbrella organisations such as RSE and ESRRA. European (and international) conferences also offer valuable opportunities for these groups to come together to engage in discussion and dissemination of latest research, treatment and management techniques. Rett50.1 is such an occasion.

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Development of international clinical guidelines for the management of communication in individuals with Rett syndrome

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Background: The number of specialist centres for the care and management of individuals with Rett syndrome (RTT) is growing internationally. There is huge variability in knowledge/expertise and clinical practice between and within countries. Guidelines are needed which provide information to families and which can be followed by clinicians.

Objective: To develop clinical guidelines for the assessment, intervention and long-term management of communication in individuals with RTT.

Method: In February 2016 an international consortium (see authors), led by the Rett Expertise Centre Netherlands, began this two-year project following the methodology used to develop already-published guidelines for the management of scoliosis, growth & nutrition, and bone health in RTT. It combines available evidence with expert consensus, paying particular attention to the needs of families and individuals with RTT. This approach includes a systematic review of published & grey literature, an inventory of clinical practices through the deployment of surveys, interviews and focus groups, the extraction of themes & statements upon which to draft guidelines, and the application of a Delphi process with an expert group to finalise the guidelines.

Results: The guidelines will be published in 2017.

Discussion: The guidelines will need to be flexible and responsive to variations between countries in culture and language, and economic and political situations which influence and shape societal attitudes towards individuals with rare diseases and which determine differing national healthcare and education policies. Fundamental to the effective implementation of the final guidelines is the involvement of individuals with RTT, their family members and professionals who work in the field of communication and RTT. This project draws on such knowledge and experience from as many countries as possible. Once published the guidelines also have the potential to be adapted and utilised for other Rett-related disorders.

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Mitochondrial dysfunction, impaired antioxidant enzymatic activity and compromised proteasome function

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Background: In the last few years, a strong relationship between oxidative stress (OS) and Rett syndrome (RTT), a rare and orphan progressive neurodevelopmental disorder affecting girls almost exclusively, has been well documented. However, to date the source of OS and the effects of the redox imbalance in this disorder remain to be explored.

Methods: Using freshly isolated skin fibroblasts from RTT patients and healthy subjects, we have demonstrated in RTT cells high levels of H2O2 and HNE protein adducts.

Results: These findings correlated with the constitutive activation of NADPH-oxidase (NOX) and that was prevented by a NOX inhibitor and iron chelator pre-treatment, showing its direct involvement. In addition, also the mitochondria are affected in RTT. In particular, we demonstrated a reduced mitochondrial bioenergetics coupled with an increased mitochondrial oxidant production. The molecular pathways of mitochondrial biogenesis and the fusion/fission dynamics are also impaired as demonstrated by altered levels of mithofusin1-2, Park and OPA-1. Further, we found that the activity of the key cellular defensive enzymes, namely glutathione peroxidase, superoxide dismutase and thioredoxin reductases, were also significantly lower in RTT. Finally, RTT cells shown compromised proteasome activity.

Conclusion: Taken all together, our findings suggest that the systemic redox imbalance in RTT can depend from both the pro-oxidant enzyme activation as well as by the mitochondrial dysfunction and the decreased activity of defensive enzymes. Due to these defective processes and also the proteasome activity impairment, the consequent accumulation of post-translational oxidatively modified proteins can lead to cell damage with systemic implications in RTT.

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Discovery of new genes in Rett syndrome patients by WES

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Background: Rett syndrome (RTT) is a developmental disorder of early onset, genetic basis, dominant inheritance and X-linked. There are described three genes that cause RTT: *MECP2*, *CDKL5* and *FOXG1*. However, the etiology of 15 % of RTT patients still remains unknown.

Objectives: The aim of this project is to identify new candidate genes in a cohort of patients with RTT phenotype without genetic diagnosis by Whole Exome Sequencing (WES).

Method: The patient and healthy parents without genetic diagnosis and negative CGHarray Cytoarray Plus (180K) (Agilent Microarrays) were analyzed by WES with TruSeq Sample Preparation Kit (Illumina). The filtering criteria used were: search mutations with 1000 g MAF below 0.05 in genes with dominant inheritance, de novo, X-linked, autosomal subject to imprinting and/or with functional impact in the CNS. For the validated mutations in genes related with gabaergic pathways (SLC6A1 and GABBR2), we performed RT-qPCR (TaqMan Gene Expression) and Western Blotting assay of RNA and protein extraction from peripheral blood.

Results: Most of the validated mutations are genes expressed in the central nervous system: ion channels and GABA/gluta-

mate/acetylcholine pathways. The preliminary studies of the *SLC6A1* and *GABBR2* expression were not conclusive.

Discussion: We do not only identify 1 gene which causes RTT-like phenotype. Pathway of genes has to be address to understand overlapping phenotype, instead to one disease only. Although blood tissue has convenient extraction, we cannot detect RNA and protein of these genes. Our next studies are performing RT-qPCR and Western Blotting assays with RNA and protein extraction from fibroblasts.

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Genetic diagnosis of patients with overlapping clinic Rett-like by targeted panel of genes

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IntroductionBackground: It has been studied patients with clinical Rett (RTT) without genetic diagnosis using the Next Generation Sequencing (NGS). This type of diseases requires clinical diagnosis. The finding of a mutation confirms the diagnosis, but not necessarily established it.

Objectives: NGS using targeted panel of genes facilitates the simultaneous study of causative genes of RTT and others whose mutation produces a similar or overlapping clinic, such Pitt Hopkins and Ohtahara syndromes.

Methods: It has been designed a gene panel of 17 genes related to the clinical RTT-like presentation by HaloPlex Target technology. Enrichment System, for Illumina Sequencing.

Sanger sequencing was used in exons not well covered. If do not find any change, MLPA was done by causative RTT genes.

Results: We have detected mutations in genes that do not cause RTT pathology in 14 of 187 studied patients with clinical Rett-like. A total of 8 patients presented mutations in *STBX1* gene, related with Ohtahara syndrome and 6 patients were redirected as a Pitt-Hopkins, finding mutations in causative *TCF4* gene.

The database HGMD-professional, dbSNP, 1000 G and predictions pathology programs (Polyphen 2.0 and SIFT) were consulted.

NGS variants have been verified by Sanger sequencing and studied the origin of the mutation in the parents.

Discussion: The genetic study by NGS allows to study a larger number of genes associated with RTT simultaneously, redirecting genetic diagnosis to other syndromes. Significantly reduce response time and the cost of the study.

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MECP2 duplication: genetic and clinical study in spanish patients

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Background: The *MECP2* duplication syndrome (OMIM_300260) is a neurodevelopmental disorder X-linked characterized by severe to profound intellectual disability, early infantile hypotonia, autistic traits, seizures and recurrent respiratory infections. It usually affects boys, but also there are girls affected. Duplication could be de novo or inherited from asymptomatic carrier mother. It has been reported about 120 cases all over the world, without a known incidence.

Objectives: The aim of the study is to characterize a Spanish cohort with *MECP2* duplication syndrome to improve our knowledge of the disease and perform a genotype-phenotype correlation.

Methods: The cohort consists in 13 patients of both sexes diagnosed in different Spanish hospitals. The duplications were detected by MLPA and/or CGH-array. The clinical characterization was carried out using a checklist designed for the project. The molecular characterization is divided into several steps: 1) Checking the duplication by qPCR-doses, study XCI and FISH; 2) If FISH shows tandem duplication, we narrowed the breakpoints through qPCR, PCR-long and Sanger sequencing; 3) Analyze the expression of the two MeCP2 isoforms by RT-qPCR.

Results: In this collaborative study has been characterized a heterogeneous cohort, with both sexes, with different phenotypes (Rett-like and duplication patients) and inherited or de novo duplication located in different regions (tandem, other region of ChrX or ChrY). However, this information is not enough to create a clear genotype-phenotype correlation.

Discussion: We suppose *MECP2* duplication syndrome is an underdiagnosed disease that needs further characterization studies in order to give a better genetic and clinical diagnosis.

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Age-dependency of antiepileptic drugs efficacy in 104 girls with Rett syndrome

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Background: Approximately 30–80 % of girls with Rett Syndrome suffer from epilepsy, which represents one of the most severe problems clinicians have to deal with, especially when patients are 7–12 years old. Drug resistance is reported in approximately 30 % of patients.

Objectives: to investigate efficacy and tolerability of antiepileptic drugs (AEDs) in patients with Rett.

Methods: In this study we evaluated the characteristics of 104 patients having a diagnosis of Rett syndrome, followed at our unit. Girls were divided into 5 groups according to age: 1–5, 5–10, 10–15, 15–20, >20 years; efficacy and tolerability in different age groups were compared. We searched for correlations with MeCP2 mutations.

Results: 104 girls were included, 89 had a mutation in MeCP2. Epilepsy was present in 83 patients (79.8 %). Mean age at onset was 4.1 years. 19.6 % of patients presented with daily seizures, 21.6 % weekly, 31.3 % monthly, 27.4 % sporadic. Drug resistance was present in 12 pts (14.5 %). Valproic acid (VPA) resulted as the most prescribed single therapy in young patients (<15 years), whereas carbamazepine (CBZ) was preferred by clinicians in older patients. The most frequently adopted association was VPA with lamotrigine (LTG) in the patients under 10 years and older than 15 years. The group aged 10–15 years resulted the most difficult to treat, with a mean of 3 different antiepileptic drugs (AEDs) used. In this group clinicians adopted associations of AEDs, mostly including VPA. In general, efficacy of treatment resulted in 65 pts (45 %).The most frequent adverse events reported are agitation, sedation and appetite loss.

Adverse events do not result to be specific for age groups and are not different from what expected in the general epilepsy population.

Discussion: VPA is reported as the most effective AED in younger girls (<10 yrs), whereas CBZ has the major efficacy in patients aged 15 years or older. Between 10 and 15 yrs is often necessary an association of AEDs, mostly including VPA. No specific profile for adverse event can be found in Rett population and in different age groups. No correlations between genotype and efficacy or tolerability of AEDs could be found.

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Communicative functions in the preserved speech variant of Rett syndrome: a case study

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Background: The atypical mild form of Rett Syndrome (RTT), the preserved speech variant (PSV), is characterized by relatively good speech-language development and communicative interactions. A longitudinal case study of an 11-year old child was performed in order to evaluate her linguistic abilities.

Objective: We assessed various aspects of speech-language and communicative functions of an individual with the preserved speech variant of Rett syndrome (RTT) to describe her developmental profile over a period of 11 years.

Methods: The following data resources and methods to assess speech-language and communicative functions during pre-, peri- and post-regressional development were incorporated: retrospective video analyses, medical history data, parental checklists and diaries, standardized tests on vocabulary and grammar, spontaneous speech samples and picture stories to elicit narrative competences.

Results: Despite achieving speech-language milestones, atypical behaviours were present at all times. We observed a unique developmental speech-language trajectory (including the RTT typical regression) affecting all linguistic and socio-communicative sub-domains in the receptive as well as the expressive modality. The child's linguistic abilities superficially appeared higher than they really are. Only very fundamental pragmalinguistic competences could be found, disturbed by a short attention span which usually limits topics to one sentence, even with a cooperative partner.

Conclusion: Future research should take into consideration a considerable discordance between formal and functional language use by interpreting communicative acts on a more cautionary note.

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