ABSTRACT BOOK
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PLENARY SESSIONS

Clinical Overview

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RTT (MIM 312750) is a unique neurodevelopmental disorder caused by a novel pathogenetic mechanism, i.e. disturbance of epigenetic gene regulation via DNA methylation. First recognized as a distinct clinical entity in 1966, RTT is a leading cause of cognitive, communication, and motor impairments in females. RTT occurs in all ethnic groups as a sporadic disorder (secondary to de novo mutations) in more than 99% and is recognized almost exclusively in females following apparently normal psychomotor development during the first six months of life. Diagnosis is based on specific clinical criteria. Consensus criteria for classic RTT include apparently normal early development, postnatal deceleration of head growth in most, loss of purposeful hand skills, stereotypic hand movements, psychomotor regression including communication dysfunction and early, short-term presence of autistic features, and gait dysfunction characterized by dyspraxia and jerky truncal ataxia. RTT remains a clinically rather than genetically defined condition. Presence of a mutation in the MECP2 gene confirms the clinical diagnosis. Children fulfilling clinical criteria for RTT may (95% or more) or may not have MECP2 mutations. MECP2 mutations may also be seen in children who do not have classical RTT. Revised clinical guidelines provide accurate and reliable diagnosis by primary care providers and clinical investigators. Mutations in MECP2, the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2), represent the genetic cause of RTT in most. MeCP2 mediates transcriptional silencing through its methyl-CpG-binding (MBD) and transcriptional repression domain (TRD). Studies of individuals with RTT and relevant mouse models indicate that RTT is a complex, multisystem disorder involving a variety of phenotypes that result from dysfunction of multiple pathways. Phenotypes related to MECP2 mutations in females vary from normal or mild learning disability to classic RTT, depending on mutation type and pattern of X chromosome inactivation (XCI) as well as unknown factors; phenotypes in males vary from fatal infantile encephalopathy to familial X-linked mental retardation. Rarely, classic RTT occurs in males with somatic mosaicism or with Klinefelter syndrome. Because girls with RTT commonly survive into adulthood, this disorder produces a significant societal burden, underscoring the need for effective therapies.

What is MeCP2 really doing in the Brain?

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Since the discovery of MECP2 mutations as the cause of Rett syndrome in 1999, our understanding of the function of MeCP2 has evolved considerably. While

MeCP2 appears to have multiple functions, this presentation will focus on trying to define the precise role for MeCP2 that is lacking in Rett syndrome brain. Recent experiments and conclusions will be summarized that are beginning to address some of the following questions: Where does MeCP2 localize in neuronal nuclei and what types of genes does it regulate? What is the essential role for MeCP2 in neuronal maturation? How does MeCP2 deficiency affect neuronal function? How does the complex mixture of cells in a mosaic female Rett syndrome brain affect the pathogenesis of MECP2 mutations? How can we apply what has been learned over the past decade of MeCP2 research towards developing better treatments for Rett syndrome? The presentation is designed to be a general overview of Rett syndrome pathogenesis and future directions for the field.

Other Phenotypes

Walter Kaufmann

Autistic features constitute an important component of the Rett syndrome (RTT) phenotype, particularly at the early stages of the disorder. This led to the inclusion of RTT as a subtype of Pervasive Developmental Disorder in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSMIV). The continuous inclusion of RTT among the autism spectrum disorders (ASD) is a highly controversial issue in the RTT community. There is consensus in the RTT literature on the transient nature of the autistic features, which are typically no longer present by early adolescence. Despite these data, few studies have formally investigated the clinical overlap between RTT and autism/ASD of unknown cause. Using data from the Australian Rett Syndrome Database (ARSD) and the International Rett Syndrome Phenotype Database (InterRett), we recently reported that girls who were diagnosed as having ASD before their RTT diagnosis were relatively more mildly affected and continued to have milder motor involvement, and further, were more likely to have p.R306C or p.T158M MECP2 mutations. These data, in conjunction with other studies such as those on autistic features in the Preserved Speech Variant, begin to delineate the similarities and differences between RTT and ASD. RettSearch has represented the RTT community in the deliberations of the DSM-V committee, formally requesting the exclusion of RTT among the autistic disorders in the new set of guidelines. At the center of the RTT-ASD issue is the ambiguity of whether ASD represents a multiple-etiology behavioral syndrome, which could even be temporary, or a biological entity with distinctive attributes other than the so-called autistic behavior. These and other issues regarding autistic features in RTT will be discussed in the presentation.

The male phenotype

Hilde Van Esch

Initially, RTT syndrome was considered an X-dominant condition lethal in males. However, this view was changed by the first description of a male patient with a MECP2 mutation. Subsequently, several additional male patients have been identified. The phenotypes in these males cover a large spectrum of neurodevelopmental disorders, ranging from classical RTT syndrome in male patients to lethal neonatal encephalopathy. Also milder cases have been reported, resulting in moderate, nonspecific to profound mental retardation or psychiatric disorders in males. Deficits in language and motor skills, autistic features, and epilepsy are also common in this latter group.

More recently, we have shown that not only a loss of function of MECP2 but also a gain in MECP2 dosage results in a severe neurodevelopmental phenotype in males. Patients with a duplication of Xq28 that include MECP2 suffer from severe mental retardation with facial and axial hypotonia, progressive spasticity, seizures, recurrent respiratory infections, and often premature death.

International collaborations: setting the scene

Walter Kaufmann

RettSearch is an international, multi-center collaborative network of clinicallyoriented researchers. Its mission is to promote the development of new therapeutic approaches for Rett syndrome (RTT) by helping to promote the coordination of the collection of information and pursuing research in areas of relevance to clinical trials in RTT. The idea of creating such a consortium originated from discussions during the 2nd International Scientific Research Workshop, at the Swedish Rett Center in Froson, Sweden, in June 2003. In addition to continuous electronic communication between members, RettSearch has held two in-person meetings (San Francisco, May 2006; Chicago, June 2008) in which key issues regarding the planning and implementation of multi-centers initiatives, mainly treatment trials, have been discussed. The outcomes of these meetings and other activities of the consortium, as well as future RettSearch plans, will be discussed during the presentation.

InterRett

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InterRett is an international database that contains clinical and genetic information on individuals with Rett syndrome. It is funded by the International Rett Syndrome Research Foundation and managed by the Australian Rett syndrome study team. The InterRett study was initiated to provide a valuable data resource for the research community. Since Rett syndrome is a rare condition affecting only 1/8500 females there are statistical limitations to the research which can be carried out using data from one country. By collecting data contributed by individual families and clinicians and by combining datasets from around the world we are creating sufficiently large sample sizes to allow meaningful statistical analyses. Since the establishment of the database in January 2003 we have gathered data on over 1600 individuals from over 30 countries.

Information on individuals with Rett syndrome can be collected from clinical records and research surveys. It may be in different formats, some written, some electronic and may be recorded in different ways. This can make it hard to combine information for research. There are also many issues that need to be addressed including ensuring the most efficient use of data and resources, consumer involvement, patient privacy and intellectual property rights of researchers. Furthermore, having collected information from families we have a strong obligation to analyse these data and publish our results. This requires further funding which is difficult to obtain. The InterRett team is keen to maximise technology and work collaboratively with others to find the best solutions to these challenges. In addition to the InterRett project we have used an online facility to work with families and clinicians to develop guidelines for the management of scoliosis. We are now in the process of using a similar approach to develop a standardised scale for describing Rett syndrome symptoms. During the World

Congress we will showcase our methods and put forward suggestion for future collaborative endeavours.

The European Network on Rett Syndrome

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E-RARE is an European initiative to promote research on rare diseases. It is a network of 9 partners from 8 countries (Belgium, France, Germany, Israel, Italy, Spain, Netherlands, Turkey) wanting to promote trans-national network research on rare diseases. This program is funded by the European Commission under its 6th framework program since 2006.
The first call was launched in spring 2007. In this context, 17 laboratories decided to join their forces to propose an european network on Rett Syndrome. We called our network EuroRETT. After a long internal and external evaluation process, our application was retained for funding among 13 other networks (among more than 100 applications reviewed).
250 girls affected by the classical form of Rett syndrome are born each year in the member states of the European Union (5 new cases each week). Among these cases, approximately 90% will have a mutation in the MECP2 gene and a small proportion will be carrier of a mutation in CDKL5 or FOXG1 (mostly in early onset seizures variant and other atypical Rett syndrome patients).
Rett Syndrome is a model disease for several reasons. The causative genes possibly play a role in chromatin remodelling mechanisms which are of major importance to understand genome regulation, expression and dynamics. Rett syndrome is also a severe phenotype for which there is currently no efficient treatment but that could be reversible. Also, it is a model of dysfunction of mature neurons. These reasons, combined to the strong commitments of parent associations to support research, have generated a huge interest for the condition and an important basic and clinical research effort. This interest is obvious at the european level but the different groups working on these aspects were not organized to exchange and collaborate efficiently. They are now organized, through this multidisciplinary, trans-national research network called EuroRETT that will be funded for a 3 year period.

The Italian Rett Syndrome Biobank and Database.

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During the last 9 years, the Medical Genetics Unit of Siena, thanks the strict collaboration with the Child Neuropsychiatric Unit of Siena, has evaluated more than 200 Rett patients. For all of them, based on the phenotype, the analysis of the MECP2 gene or the CDKL5 gene has been performed. Patients and their families received genetic counseling by one of us (AR or FM). For all patients and their parents the genomic DNA and the lymphoblastoid cell line have been collected and stored. The bank actually contains biological samples of 197 mutated Rett patients: 128 classic patients (32 with a MECP2 missense mutation, 66 with an early truncating mutation, 13 with a late truncating mutation and 17 with a MECP2 gene deletion), 24 patients with the preserved speech variant and either a missence or a late truncating mutation in the MECP2 gene, 4 patients

with the “forme fruste” variant, and 32 patients aged less than 4 years for whom a definitive clinical classification could not be achieved yet. Nine patients with the early onset seizures variant of Rett and a proven CDKL5 mutation are also present in the bank. In addition, the bank contains several negative Rett patients for whom a combination of DHPLC and MPLA analysis failed to demonstrate any mutation in either MECP2 or CDKL5. This cohort of patients is of great value in order to identify new genetic causes of Rett syndrome. The uniqueness of this bank is that all patients have been evaluated by the same group of clinicians and the molecular analysis has been performed by the same laboratory, allowing a uniform clinical and molecular data collection. These characteristic are particularly useful in order to perform genotype-phenotype correlations. The rapid enlargement of the samples collection required the establishment of an online database for data management and sharing of resources with the scientific community. The Rett database is actually available at www.biobank.unisi.it.

Abnormalities of Locomotion in Rett syndrome; Its Pathophysiological Importance

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Rett syndrome (RS) is a unique developmental neuropsychiatric disorder. The characteristic features of RS suggest the specific neuronal systems are involved from early infancy.

Among various symptoms of RS, the difficulty of crawling and walking is the early and key features to understand what are happening very first in the brain of RS. Crawling and walking are controlled by the specific neuronal system in the brainstem and spinal cord. In the spinal cord there is a system called locomotion center, and for the activation of this center tonic innervations from brainstem, that is serotonergic neuron for postural augmentation, is necessary. Dysfunction of this neuron in RS is suggested by decrease of postural tone in early infancy. Whereas in early childhood muscle tone start to increase initially from the legs. This increase of the muscle tone is due to the decrease of the activity of dopamine.

In the early stage of the brain development, serotonergic system in the brainstem project not only to the spinal cord but to the pedunculo-pontinenucleus (PPN). The PPN activates dopaminergic (DA) system of the substantia nigra and the ventrotegmental area with its ascending projection. The decrease of the function of the PPN leads to decrease of the activity of the DA neuron which consequently leads to failure of the development of frontal cortex. These sequential processes provide rigid hypertonus, loss of purposeful hand use, stereotyped hand movement and regression in childhood.

Intervention to improve the locomotion (crawling and walking) is thought to be the earliest effort which may improve the course of RS.

WORKSHOPS

Breathing and autonomic dysfunction in RS

Clinical features of breathing and autonomic abnormalities in Rett syndrome

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More than 125 persons with Rett syndrome (RTT) underwent autonomic and clinical assessments in the Swedish Rett Center. It was a 10-year period of learning about brainstem function in this very complex disorder, and we also investigated possibilities of clinical management and medical intervention to improve quality of life. Rett syndrome demonstrates clearly the role of the brainstem in cardiovascular medicine and how this affects the quality of life.
We performed cortico-bulbar neurophysiology using the NeuroScope system. This is a non-invasive assessment of brainstem functions in which cardiorespiratory indices and breathing patterns are quantified and carefully categorised. Electroencephalogram is recorded concurrently to monitor the accompanying cortical activity.
We identified three cardiorespiratory phenotypes: Forceful, Feeble and Apneustic breathers. Each cardiorespiratory phenotype requires unique and special treatments and each phenotype has specific clinical risks. Some of the phenotypes can partly respond to pharmacological intervention while others do not. Unusual behaviours can appear in persons with RTT due to the abnormal experiences of sudden and large changes in arterial blood pressure and heart rate. Cardiovascular disturbances are caused by abnormal spontaneus brainstem activity (ASBA), which mimics epileptic fits. Feeble breathers can go into sustained central apnoea if sedated with Opioids or given Diazepam. These drugs are contraindicated in Feeble breathers. Apneustic or Feeble breathers have their central chemoreceptors re-set to operate at higher than normal pCO2 and can fail to restart spontaneous breathing post-operatively following prolonged artificial ventilation. Forceful breathers often go into hypocapnic attacks with tetany resembling epileptic seizures. They require carbon dioxide treatment and not anti-epileptic drugs.
Brainstem competence in RTT varies from neonatal to adult levels. Body homeostasis can be severely and variably deranged in the three phenotypes from chronic respiratory acidosis in Feeble and Apneustic breathers to chronic alkalosis in Forceful breathers. Medical intervention must therefore be planned on individual basis.
We conclude that clinical presentation of RTT is heterogeneous and early establishment of the cardiorespiratory phenotypes is the basis of objective medical intervention. Individualised medical care can improve the quality of life in persons with Rett syndrome.

Substrates of respiratory and autonomic dysfunction in mouse models of Rett Syndrome

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Analysis of Mecp2 null mice is beginning to reveal defects in brainstem and peripheral autonomic circuitry that may underlie respiratory and autonomic dysregulation in Rett Syndrome. Although the spectrum of these defects is diverse, some common themes are beginning to emerge, such as reductions in Brain-Derived Neurotrophic Factor (BDNF), norepinephrine and/or gammaamino butyric acid (GABA) in specific brainstem sensory, motor and pre-motor cell groups as well as increases in neuronal excitability in some of these same regions. Moreover, new evidence indicates that brainstem defects influence not only local circuits but may also contribute to forebrain dysfunction as well. In addition, defects in peripheral catecholamine homeostasis, relevant to autonomic control, have recently been identified. This presentation will synthesize recent findings and discuss translational studies aimed at identifying potential new therapeutic approaches to Rett Syndrome.
Supported by NINDS, IRSF and Cortex Pharmaceuticals, Inc.

Pharmacological treatment of autonomic dysfunctions in Rett syndrome, from *mice* to men.

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Rett Syndrome (RS) leads to severe cognitive and autonomic dysfunctions. To date, in spite of the increasing number of scientists dedicated to this field, the exact function of the Mecp2 protein is still unclear. Moreover, there is currently no pharmacological treatment available for Rett girls to alleviate their physiological deficits. Previously, using a Mecp2-deficient mouse, we and others have shown that these animals exhibit breathing disturbances that can be linked to noradrenergic deficits at the brainstem level. Using immunohistofluorescent labelling, we demonstrated a significant decrease in the number of noradrenergic neurones (tyrosine hydroxylase immunopositive) in two neuronal nuclei of the Mecp2-deficient mouse brainstem involved in the cardiorespiratory control. In a second study, we have shown that a treatment of Mecp2-deficient mice with desipramine, a noradrenergic reuptake inhibitor, stabilized the breathing pattern and imcreased their lifespan. These results suggest that a pharmacological stimulation of the noradrenergic system could be a promising approach for the treatment of respiratory dysfunction, that causes a significant proportion of death in RS patients. A proof-of-concept Ph.IIa clinical trial is undertaken with girls suffering from Rett syndrome in France. This clinical trial has been funded by the French Ministry for Health. Besides this first clinical intervention originating from the work performed on mouse models of RS, our work is now devoted to better understand the link between Mecp2 and the catecholaminergic deficits and to develop new treatments for the respiratory (and possibly motor) symptoms of Rett Syndrome. In the next 36 months, we will work with a pharmaceutical company to test new candidate drugs in the mouse used as a pre-clinical model and will undertake in vivo and in vitro evaluations to identify new molecules of putative benefit for the patients.
Supported by INSERM, AFSR, the E-RARE “Eurorett” network and ANR.

The clinical management of scoliosis in Rett syndrome

Introduction to the clinical management of scoliosis in Rett syndrome

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The majority of girls with Rett syndrome develop scoliosis and occurrence prior to adolescence is not unusual. Early predictive factors for the development of scoliosis include low muscle tone and never learning to walk, and the p.R294X mutation appears to be protective against scoliosis. Little is known about the natural history of curve progression and the factors influencing this such as age, genotype and mobility level. Conservative management includes regular monitoring from before the development of scoliosis, optimising weight bearing and physical activity opportunities, and supported seating. Spinal surgery is appropriate for the management of more severe curves and is associated with positive physical and functional outcomes. A holistic and multi-disciplinary approach can best support the family and patient prior to surgery, during the immediate surgical period and when providing rehabilitation opportunities. We will present an overview of the management of scoliosis over the life span from the perspectives of the family, orthopaedics, physiotherapy and occupational therapy.

The surgical treatment of Rett scoliosis: The Delphi's decision

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The participating group was able to arrive at a consensus regarding a number of aspects of the surgical treatment of Rett scoliosis. When asked when surgery was indicated, the surgeons polled stated that the surgical decision was made using Cobb measurement and age (skeletal maturity) as the primary determinants. The surgery decision paradigm should also include care taker discussion and general health and the likelihood that the patients' quality of life will be improved.
There was consensus that Rett scoliosis surgery (like other neurogenic scoliosis procedures) should be performed in a centre where specialized anaesthesia and intensive care are available. There is agreement that respiratory, cardiac, neurological, gastrointestinal and nutritional status must be evaluated preoperatively. Technical aspects of the actual surgery have been evolving even as the consensus group has been meeting. Posterior surgery has replaced the anterior procedures. Some of these evolving techniques include the use of thoracic screws and all screw constructs, various posterior vertebral osteotomies, use of BMP and iliac screws, thoracoscopic anterior surgery and new approaches to "growing systems" for young patients. The group identified intraoperative and postoperative management considerations that although not specific for Rett should be considered in the multispecialty plan for scoliosis surgical care. Surgical objectives in Rett are the same as they are in other neuromuscular deformity patient including a balanced spine in the coronal and sagittal plane, a comfortable patient who is easily positioned and demonstrates endurance in sitting. We need instruments that more accurately evaluate outcomes and those outcomes should include burden of care in addition to health related quality of life.

Physiotherapy strategies for scoliosis

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This presentation will describe two case studies that illustrate a physical therapy approach. The core of the management regime in both cases was an intensive, daily, asymmetrical activation of trunk muscles through equilibrium reactions. In the first case study X-rays will be presented (evaluated by four experienced orthopedic surgeons blinded to the intervention process) suggesting that the intervention was successful in reversing the progress of the scoliosis for the above-mentioned child. Discontinuation of treatment led to severe and rapid deterioration of the spinal curve necessitating the use of a corset. The second case is a young child with Rett syndrome who was treated intensively from the onset of spinal asymmetries detected by an orthopaedic surgeon. After a few months of intervention the spinal curvature was controlled and the treatment was discontinued. Two years later the child shows no spinal asymmetries. The suggested intervention might present an option for therapists working with individuals with Rett syndrome to control the progression of scoliosis using a daily intensive intervention program. Due to the fact that those are case studies, generalization of the results is limited and further research is needed to establish the efficacy of this intervention regime. For individuals presenting with severe scoliosis (Cobb angle above 500), daily interventions of positioning and stretch can also be used to maintain and increase flexibility of the spine.

Functional assessment and management during the peri-operative period

Eva-Lena Larrson
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When the postoperative results of surgery are evaluated - it has often a focus on the angle of scoliosis. However, the interests of parents are different including the girls' continued ability to walk, which sitting positions will be allowed, and whether surgery will affect the girls' daily activities postoperatively. In a recently performed study of a long-term follow-up of functioning after spinal surgery in patients with Rett syndrome, the results showed that the majority of patients improved on objective measures. Despite experiencing stiffness in the spine, the girls became more active and healthy after spinal surgery.
Recommendations regarding pre- and postoperative therapy relate to:

- Provision of pre-surgical information by mail to the family before the surgery
- Request for pre-operative report from the therapy services
- Evaluation of pre-operative status: parents view of the motive for surgery, angle of scoliosis, angle of pelvic obliquity, weight distribution on a seating surface, sitting balance, number of and what kind of seating supports in a wheelchair, and time used for resting during the day.
- Post-operative mobilisation: start to sit at the edge of the bed the day after surgery, after that to stand and take a few steps (if able to stand and walk), and as soon as possible start to sit in own wheelchair.
- Provision of post-operative information and instructions for parents

after surgery whilst on the ward.
• Send post-operative report to occupational and physiotherapist in the therapy services.
• Post-operative evaluation with the same measurements as preoperatively, and two open-ended questions of “What do you think has improved since the surgery?” and “What do you think is worse since the surgery?”

A parent’s perspective on the clinical management of scoliosis in Rett syndrome

Sue Hall
Parent, Birmingham, UK

My daughter Rachel had a posterior fusion in July 2007. The main points I see as important for the management of scoliosis include:
Good communication links - between the Community Paediatrician, Physiotherapist and Orthopaedic Surgeon.
Early diagnosis and assessment - especially as a parent when you are relying on the experience of the community team to know when to refer to the Orthopaedic team for assessment. We were referred too late and I feel this had a huge impact as Rachel had to be operated on at such an early age, the youngest in the UK and USA to date when she had her operation (July 2007). This made the decision to operate a very difficult one, for the surgeon and us as parents. Rachel found it difficult to tolerate a brace and the curve worsened rapidly. Pre-operatively, her curve was 93 degrees.
The timing of corrective surgery - If the spine has to be stunted with a fixed rod, the internal organs have to have enough space to grow into, therefore, a difficult decision to time the operation. The surgeon said if Rachel had waited a further 6 months, she wouldn’t have survived intensive care. The timing therefore is of paramount importance. Rachel spent 3 weeks in intensive care and was reintubated 3 times. She was unable to support herself breathing, because of low muscle tone, the inability to cough to clear secretions and shallow breathing, all factors of Rett Syndrome. The 4th attempt to extubate was successful
Preparation for rehabilitation after surgery - with good planning and strong links between the family, hospital and community, it makes the rehabilitation process from hospital to home effective.
Support at home - it was important to plan for the level of nursing and personal care needed whilst at home, and the impact this would have on family members, siblings and the need for extra carers to help, day and night.
Transition back to school life - School needed a lot of support to accept Rachel back, initially on a staggered return. Communication between the medical team and physiotherapists (risk assessment and manual handling) and families to make sure there was a transition period back in to school and normal everyday life.
Finally.... a successful operation has now led to a healthy and happy life. The surgery has transformed Rachel’s quality of life every single day.

Getting Older Living Well

Session 1 : The session will present a personal perspective from Yvonne Milne on the struggle to maintain good quality of life for her daughter Clare.
Session 2 : Ingegerd Witt Engerström will discuss medical interventions to address problems such as autonomic dysfunction, seizures, contractures.
Session 3 : Sheena Reilly will provide an overview of the range of therapy interventions which are of value with a particular focus on the management of feeding problems.
Each speaker will focus in particular on the cost-benefit to the women of the various treatments on offer. Specific questions to be addressed incldued :
When does clinical intervention improve quality of life, and when do the possible side effects, risks and complications outweigh the benefits? In some cases good symptom management becomes more important than major medical intervention.

We will then invite audience participation to discuss examples of good practice, variations in practice in different countries, quality of symptom care around the world, and some of the difficult decisions that parents and carers may have had to face or be in the process of considering.

Molecular Pathogenesis of Rett Syndrome

MeCP2 and DNA methylation Targets in Rett Syndrome and Cancer Models

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Mutations in the human MeCP2 locus are known to be causal for the X-linked neurodevelopmental disorder Rett Syndrome (RTT). The biochemical properties of MeCP2 (and those of other MBD family members) regarding its ability to bind methylated DNA and to associate histone deacetylases led to a model proposing that loss of MeCP2 function would lead to local transcriptional activation of methylated genes. Interestingly, initial characterization of the potential impact of MeCP2 deficiency on the transcriptome has revealed a relatively small number of genetic targets, although convincing evidence of MeCP2 as a specific corepressor has been shown. More recent data has indicated a role for MeCP2 as an architectural component of the chromatin. Also, recently it has been demonstrated the association of MeCP2 with transcriptionally active genes. The issue of the exact roles of MeCP2 and its relation with the biology of DNA methylation is a question of inherent interest. Our laboratory has focused in the characterization of MeCP2 and other MBD proteins in various contexts including different cancer and RTT models. Here, the relevance of DNA-methylation dependent regulation mediated by MeCP2 will be discussed.

MeCP2-dependent Transcriptional Repression is required for Behavioral Modalities Mediated by the Basolateral Amygdala

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Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder that results from loss of function mutations in the methyl-CpG binding protein 2 (MECP2) gene. Using viral-mediated basolateral amygdala (BLA) specific deletion of Mecp2 in mice, we show that intact Mecp2 function is required for normal anxiety behavior as well as some types of learning and memory. To examine whether these behavioral deficits are the result of impaired transcriptional repression, since Mecp2 is believed to act as a transcriptional repressor in complex with histone deacetylases (HDACs), we infused a HDAC inhibitor into the BLA of wild-type mice. We found that HDAC inhibition produces similar behavioral deficits to those observed following the deletion of Mecp2 in the BLA. These results demonstrate a key role for Mecp2 as a transcriptional repressor in the BLA in mediating behavioral features of RTT.

Neuron-specific expression of Setdb1 histone methyltransferase in a mouse model of Rett syndrome

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Mutations of the gene encoding methyl-CpG-binding protein 2 (Mecp2) result in Rett syndrome (RTT). Chromatin remolding mechanisms thought to be regulated by Mecp2 involve histone methyltransferases (HMTs) and repressive chromatinassociated histone lysine methylation, including the H3K9me mark. Notably, the HMT, SET domain, bifurcated 1 (Setdb1), is specific for the H3K9 mark and in addition, contains its own methyl-CpG-binding domain (MBD). Setdb1 is involved in transcriptional repression via interacting with KAP-1, ERG, HDAC1/2, mSIN3, MBD1, and HP1 proteins. We observed that the expression pattern of Setdb1 is complementary to that of Mecp2 during mice brain development, as reflected by robust expression in prenatal brain followed by a rapid decline during the first few weeks postnatally. Therefore, we hypothesized that over-expression of Setdb1 in postmitotic neurons will functionally compensate for Mecp2 deficiency in a RTT mice model. To test this hypothesis, we generated mice expressing a myc-tagged Setdb1 transgene under control of the CaMKII promoter that drives neuronal expression in the cerebral cortex, striatum, hippocampus and other areas of fore- and midbrain. The transgene was introduced in mice with a Nestin-Cre mediated, CNS wide conditional deletion of Mecp2. In terms of body weight, locomotion, motor coordination, and life span, no significant improvement of the Rett-like phenotype was observed. This may be due to the fact that not all neuronal populations express the transgene. To overcome this limitation, experiments are underway to examine whether or not Setdb1 expressed through the tau locus (which results in pan-neuronal expression in CNS) could affect the phenotype of Mecp2-deficient mice.
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Gastrointestinal (feeding, swallowing and digestive ability)

Oral Dysphagia, the SOMA and Rett syndrome

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Girls and women with Rett syndrome demonstrate substantial change in their co-morbidities as they get older. This is particularly the case for gastro-intestinal co-morbidities. Inadequate nutrition is common with 20 percent of individuals requiring supplemental feeding. Feeding and nutritional issues can be a major concern to families and lead to additional co-morbidities. Dysphagia is frequent with oral phase dysphagia often being the most apparent. In an observational, population based video study using the Schedule for Oral Motor Assessment (SOMA) to assess oro-motor dysphagia, 102 of 212 families provided video for analysis. The participants had a mean (SD) age of 12.9 (7.1) years. Higher scores on the SOMA indicative of worse dysphagia were associated with increasing age and with decreasing BMI for age z score. Worsening dysphagia was additionally associated with poorer general mobility, presence of epilepsy and surgically managed scoliosis. Thus worsening dysphagia is a warning sign for poor nutrition. This workshop will explore the influence of oral, pharyngeal and oesophageal stage dysmotility in the aetiology of under nutrition in girls with Rett syndrome, and will discuss the potential influence of aspiration, constipation, and bloating from air swallowing on dysphagia and inadequate nutrition.

Prevalence of feeding impairment associated with Hypergag in 221 girls with Rett syndrome, a national prospective survey within the French National Association AFSR.

Catherine Senez
Toulouse, France

Feeding impairment is often a complication of the clinical course of RS.Some factors are well-known, but few data exist exploring the oro-pharyngeal dysfunction as the hyper gag response to a food stimulus. We determined the prevalence of hyper gag and defined associated factors through a prospective survey of members of the French association, AFSR.
We defined hyper gag by the presence of a Gag reflex triggered during meals, or poor appetite. Variables were quoted using a Likert scale. A total of 221 questionnaires were analysed using descriptive and comparative methods. Six questionnaires (3%) had missing data on the principal variables defining hypergag. The total population had a mean age of 15.67 ± 9.43 years with 33.6% over 18 years of age, 23% [12-18], 24.4% [7-12] and 18.9% under 7 years. Hypergag was reported by 53.8% of the parents. The prevalence of Gastroesophageal Reflux Disorder (GERD) was 21.7% (under anti-reflux treatment) and 27.15% (without treatment and having GERD symptoms).
There was a significant association ($p<0.05$) between hyper-gag and a higher prevalence of 'grimaces in the tasteful changes' (83.1% vs 54.2%), 'grimaces with cold food' (44.1% vs 23.4%), 'preferences for tepid food' (82.1% vs 62.4%), 'gag reflex during the brushing of the teeth' (45.3% vs 21.76%), 'feeding difficulties' (14.5% vs 4.2%), 'smooth food or in segments' (77.4% vs 64.0%) and 'preferences for sweet or salty food'(56.8% vs 43,2%). No age effect was noted.
Our results confirmed the high prevalence of feeding impairment and specifically

hyper-gag in more than 122 patients. Identification of associated factors with hyper gag will allow a simple tool like a questionnaire to be used to detect and to provide appropriate educational management taking into account the patients[1] preferences.

Feeding problems and consequences for Rett syndrome

Sue Fyfe
Curtin University of Technology, Perth, Australia

Feeding difficulties in Rett syndrome are complex and linked to difficulties in swallowing. Using questionnaire data from a population-based cohort of subjects between 2-29 years (n=201), we described the feeding experiences and examined the factors affecting growth. The mean weight, height and BMI zscores in subjects with Rett syndrome were below that of their age group and decreased steadily with age. Twenty percent of subjects had enteral nutrition support (ENS) using nasogastric tube or gastrostomy, which was more common in older age groups. Those with early truncating mutations (p.R255X, pT158M) had significantly higher prevalence of ENS than other mutation groups with no subjects with the pR294X and pR306C mutation using ENS. Lower mean BMI z-scores were associated with low mobility and increased frequency of breathholding and hyperventilation. Routine monitoring of growth should continue to determine the severity of nutritional problems in Rett Syndrome and active nutritional management is recommended.
In a separate study data were collected about feeding problems and how they affected daily life from six months of archived postings to an Internet list-serve called Rettnet and from responses to two questions posted directly to Rettnet. 109 parents or carers contributed postings to Rettnet. The average age was 11.4 years (SD=8.7 years) with an age range of 2 to 42 years. Common difficulties reported included inability to swallow and chew, aspiration and reflux, often causing dehydration and malnutrition. Many parents felt they were doing everything they could to help their child but that they were failing, as many children suffered from reflux, aspiration and air swallowing and were unable to take their medication effectively. Poor weight gain was extremely common in these children and a great concern to parents and carers. Over half the children (51.7%) had a gastrostomy inserted. The decision and difficulties surrounding the decision to have a gastrostomy inserted for the child was the most common issue raised and the decision and surgery was traumatic for the child and parent. Parents felt that the gastrostomy emphasized the child's difference and dependence. However, they felt that it had generally positive effects and provided relief that nutrition, hydration and medication could be managed safely.

A Parent's perspective of feeding problems in Rett syndrome

Kathy Hunter

Feeding problems are frequent concerns in Rett syndrome, as individuals seldom develop mature patterns of chewing and oral motor function. Swallowing may be difficult, especially with liquids. Abnormal tongue movements, low or high tongue tone, increasing shoulder girdle tightness, and neurological problems may contribute to feeding difficulties. Gastroesophageal reflux is also a common problem. Swallowing requires coordinated muscle contractions and it is necessary to close the mouth to swallow, which can be difficult. Thin liquids and large pieces of food are more difficult to swallow.
Deceased energy/nutrient intake leads to poor linear growth. Loss of hand use and

self-feeding skills increases caretaker time and feeding time. Increased salivation leads to an increased risk for dehydration and increases need for fluids. Seizures and medications may interfere with feeding and nutrient interaction and may alter vitamin and mineral needs. Scoliosis may cause a distorted gastrointestinal anatomy. Breathing disturbances may add to feeding difficulties and poor nutritional status.

An oral motor therapist should do an assessment to look at patterns of the lips, tongue, jaw, and cheeks for eating, drinking, facial expression, and speech, and to see if any of these patterns interfere with lip closure, mouth opening, and forming a seal around a feeding utensil. Thickening foods and drinks can allow more time for swallowing. Thickening increases their texture and encourages a wider range of foods. They also help reduce reflux because the weight and thickness of the food make it easier to keep down. Positioning is important to enhance swallowing during mealtimes and to avoid the risk of aspiration.

Any of the following problems should be evaluated promptly:

•ability to keep the lips closed on food
•defective chewing, defective swallowing
•choking; involuntary or obstructing movements
•vomiting or regurgitation; excessive secretions
•poor appetite; and dependence in feeding

If there are serious swallowing problems, a gastrostomy button often is recommended to avoid problems of aspiration or pneumonia and to decrease the long and difficult process of oral food intake. The idea of using alternative feeding methods is often upsetting for parents. It is hard to consider letting go of traditional feeding methods for a way that seems “unnatural.” Some parents feel that they have failed or look at it as yet another skill that she will have to lose. These are all normal reactions.

Cognition and Communication

Augmentative and Alternative Communication Intervention with Girls with Rett Syndrome

Judy Wine
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Clinical experience testifies that girls with Rett Syndrome (RS) have a rich inner world. They very often, albeit inconsistently, give clues to understanding – laughing appropriately, moving in response to a question, following what is going on with their eyes, etc. They have strong emotional responses to situations and will choose to participate depending on their perceptions and relationships with the persons involved. Their fundamental physical disabilities together with their very delayed reaction times interfere with their ability to indicate what they understand.

Repeated contact with RS girls has also shown that they have a need and desire to communicate. Their frequently used natural communication strategies include vocalizations, laughter, crying, eye gaze, facial expression, and body movement. However, these informal communication strategies are not enough to enable them to express everything they might want to say – to tell what happened at school, to ask a question, to say what is hurting, to express emotion, etc. It is thus necessary to identify additional communication strategies which will enable

a full range of communicative possibilities, depending on the needs and abilities of each specific girl. The field of augmentative and alternative communication (AAC) provides the strategies for communicative expression which are so essential for RS girls, to enable social participation, interpersonal interaction, language and cognitive development, development of feelings of self-worth and confidence, increased motivation, reduced frustration, and a multitude of other possibilities. These aided strategies include communication charts made up of objects, pictures, and/or the written word, single and multiple message voice output communication aids, and computers. The critical point in using these aided strategies is to determine the means of access most effective for each girl, whether it be pointing with her hand, forehead or nose, or eye gazing, always taking into account the slow response time typical of these girls.
In this presentation case examples with video clips will be used to demonstrate the communicative skills of girls with RS.

Assessing and improving communication skills-Some exciting advances

Nigel Livingston
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Our research focuses on the development and application of a number of devices and technologies that improve the ability of subjects with acute special needs to communicate.
Foremost amongst these is an eye-tracking system. This system employs an infrared camera and light emitting diodes (LEDs) to detect both the pupil and a glint (reflected infrared light off the corneal surface). The system accommodates significant head movement and, most importantly, does not require a calibration step whereby a subject is asked to look directly at specific markers or points on a computer screen. Further, no special training is required and the system can be set up within minutes either within a subject's home or workplace, or in our research laboratories.
Trials are being undertaken with a number of subjects who have Rett Syndrome. In each case it has been possible to detect when the subject focuses on or "selects" a particular image that is presented on screen. Further, subjects are able to follow these images as they are moved across or up and down the screen. There is a clear indication that subjects are able to indicate a "choice" by selecting appropriate images.
Our next step is to develop the system so that users are able to select icons, words/ letters, colours or images and thereby exercise control over their computer or a range of external devices or switches. In addition, we are designing trials that will be used to access the cognitive function and processes.

Measures and empowerment of cognitive abilities in Rett Syndrome

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Currently there are few structured proposals for cognitive intervention which can assist the sensitive and interested physician in helping to ensure the best possible quality of life for the patient with RS. Besides, some RS theoreticians

hypothesize that the behaviours that are neurologically driven are not open to modification. Despite these claims, the aim of this study is to show that RS girls can reach higher developmental levels in cognitive area than those widely known in literature. Well structured procedures, consistently repeated every day for a certain period of time, and mutually connected hierarchically so that each level constitutes the basis for the next one, can help RS patients get better. We report 12 cases of Rett syndrome envolved in a psycho-educational intervention based on cognitive empowerment through the improvement of attention process, temporal and spatial relationship, the ability to discriminate basic emotions and the reduction of the stereotypies. This activity was carried out into five phases with ABABA multiple Base-line design for clinical research. Results point out that RS girls show an improvement in selective attention, in the ability to recognize temporal and spatial relationship and a decrease in the amount of help needed during the interventions. Also the A.A.C. abilities (Augmentative, Alternative, Communication) improve. This work confirms that the cognitive abilities of girls with RS can be modified and the quality of their life can be enhanced.
The characteristics and implications of cognitive empowerment methods, their possibility to be measured and applied in school and in family settings are also discussed.

Outcome Measures for Clinical trials in Rett syndrome

Hand use in Rett syndrome as a measure of functional ability

Dr Jennepher Downs
Telethon Institute for Child Health Research, Perth, Australia

A new and sensitive measure of hand function for measuring change in clinical interventions in Rett syndrome.
Loss of hand skills is one of the core diagnostic criteria in Rett syndrome. Measures describing hand function in Rett syndrome lack detail or have used Likert scales in which the meaning of the cut-points is not clear. Video in naturalistic settings was used to describe hand function in females with clinically verified Rett syndrome identified through the Australian Rett Syndrome Database (n=121) using a standard protocol. Hand skills were observed when the subjects were offered a range of large and small objects, during feeding and everyday activities. Effects of age, genotype, mobility and frequency of hand stereotypies were investigated.Film of hand function was provided in 107/121 (88%) cases and mutations were identified in 78/103 (76%) of those tested. Eight levels of hand function were defined from 1 (poorest) to 8 (best). The median level of hand function was level 4 in those aged under 8 years, level 5 in those between 8 and less than 13 years and in those between 13 and less than 18 years but dropped to level 2 for those aged 18 years and over ($p=0.07$).
Hand function level was higher in association with better current mobility level ($p<0.001$), later onset of hand stereotypies ($p=0.04$), and with less frequent hand stereotypies ($p=0.01$). The median level of hand function varied by mutation and was level 6 for those with p.R133C (n=9) and p.R294X (n=9), level 5 for p.R306C (n=5), level 4 for C terminals (n=7), level 3 for p.T158M (n=7) and large deletions (n=5), level 2.5 for p.R255X (n=6) and level 2 for p.R270X (n=6) and p.R168X (n=10). The p.R168X mutation was associated with decreased hand function compared with the baseline CT delection group and increased mobility was associated with increased hand function.

Developing statistically-based measurement of severity using existing questionnaire data

Ami Bebbington
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Severity scales for Rett syndrome (RTT) generally involve the use of composites of categorical items measuring the presentation and effect of RTT symptoms. The weighting of symptoms within severity scales has previously been based on clinical experience rather than statistical methods. Appropriate measurement of severity is required for clinical research, in clinical trials and phenotypegenotype relationship studies. Improvement in RTT symptoms is an objective of clinical trials, and therefore, the use of an appropriate outcome measure is critical. Psychometric techniques, such as principal component analysis (PCA) can be used to develop a statistically-based measurement of severity using existing questionnaire data. The results of such analysis would include: an overall severity model with item weightings determined by the variability of presentation in cases already collected; separate “severity components” whose variability is independent; and a “minimally determinative” set of characteristics of the RTT phenotype, which could be used to streamline the data collection process.

GENE THERAPY

Ectopic Reintroduction of MeCP2 into Forebrain Neurons of Heterozygous Mecp2-Deficient Female Mice Improves Their Rett-Like Phenotype.

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Rett Syndrome is a neurological disorder affecting primarily young girls that impairs both motor and cognitive functions. Mutations in the gene encoding the transcriptional repressor MECP2 account for most Rett Syndrome cases. Several different lines of Mecp2-deficient mice have been generated, and each develops a Rett-like condition. Interestingly, mice lacking MeCP2 in only forebrain neurons also develop a Rettlike condition. This led us to hypothesize that reintroducing functional MeCP2 into forebrain neurons of Mecp2-deficient mice would improve their behavior. To test this hypothesis, we generated transgenic mice that express an epitope-tagged MeCP2 transgene in forebrain neurons at a level of approximately 0.5X that of endogenous MeCP2. These transgenic mice developed delayed behavioral impairments by about 9 months of age, illustrating that only nominal increases in MeCP2 prevalence in specific neuronal populations is sufficient to promote negative consequences. We then inter-crossed these transgenic mice with heterozygous Mecp2-deficient mice, and followed the behavioral properties of female mice from the crosses in a genotypeblinded
manner for approximately one-year. The results revealed that the open field behavioral impairments of female Mecp2-deficient mice expressing the MeCP2 transgene in forebrain neurons had been restored wild-type mice. These data therefore indicate that at least some aspects of the Rett-like behavior of female Mecp2-deficient mice can be improved by reintroducing MeCP2 selectively into forebrain neurons.

Deficits in synapse maturation and their therapeutic reversal in a mouse model of Rett Syndrome

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Rett Syndrome (RTT) is caused by mutations in the gene coding for methyl CpG-binding protein 2 (MECP2). We examined the hypothesis that MeCP2 deficiency leads to a deficit in synaptic maturation in MeCP2 null mice. Consistent with the hypothesis, we found reductions in synaptic amplitudes measured by patch clamp recording from cortical neurons, reductions in the postsynaptic density protein PSD95 measured with immunohistochemistry, and reductions in the density of spines on cortical neurons. An in vivo assay of synapse maturation and developmental plasticity in visual cortex revealed that synapses had abnormally prolonged plasticity in MeCP2 null mice. Microarray analyses have shown that the Insulin-like Growth Factor 1 (IGF-1) signaling pathway has a key role in the stabilization and maturation of cortical synapses (Tropea et al., Nature Neuroscience 9: 660, 2006). MeCP2 null mice and RTT patients express aberrantly high levels of an IGF-1 binding protein (Itoh et al., J Neuropathol Exp Neurol 66: 117, 2007), which would in turn inhibit

IGF-1 signaling. Thus, we reasoned that upregulation of the IGF-1 pathway would reverse symptoms in MeCP2 null mice. Systemic treatment of young MeCP2 mutant mice with an active fragment of IGF-1 increased synaptic amplitudes, upregulated cortical PSD95 and stabilized cortical plasticity to wild-type levels. In addition, the treatment extended the life span of the mutant mice, restored regularity in heart rate, and improved locomotor function. Our results suggest that truncated or full-length IGF-1 are promising candidates for pharmacological treatment of Rett Syndrome, and potentially of other CNS disorders caused by delayed synaptic maturation.
Supported by the Simons Foundation and the Autism Consortium (MS), and the Rett Syndrome Research Foundation and the NIH (RJ).

Abnormal expression of MeCP2 undermines mouse development

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Variations in the number of copies of the genomic region containing MECP2 have been associated with neurological abnormalities. Patients with duplicated MeCP2 exhibit infantile hypotonia, severe mental retardation, progressive spasticity, language deficiencies and recurrent infections, while triplication of the MECP2 region results in a more severe phenotype. Further, transgenic mice that over-express MeCP2 (twofold) develop a progressive neurodevelopmental disorder.
Accumulating evidence suggests that most phenotypes associated to MeCP2 abnormalities are caused specifically by dysfunction of mature neurons (refs) arising from mis-expression of MeCP2 target genes in the brain. However, a role for MeCP2 outside of the CNS has not been ruled out and it is conceivably that certain anomalies may result from MeCP2 dysfunction in somatic cells. For example, the prolonged corrected QT interval exhibited by most Rett Syndrome patients could be related to MeCP2 dysfunction in the heart rather than in the CNS.
We demonstrate here that perturbations of the normal pattern of expression of MeCP2 in the mouse heart cause embryonic lethality with cardiac septum hypertrophy, indicating that proper interpretation of DNA methylation signals are important for normal heart development.

Sleep and Behaviour difficulties in Rett Syndrome

Introductory Remarks

Alison Kerr
University of Glasgow & Royal Hospital for Sick Children, Glasgow, Glasgow, Scotland

Types and frequencies of sleep and behaviour problems presenting in Rett disorder.

Emotional and Behavioural Aspects of Rett Syndrome: Clinical Update

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Interest in the genetic, physical and neurological aspects of Rett Syndrome predominates. In contrast and there have only been a few studies to define the associated behavioral and emotional features. Some of these compared individuals with Rett Syndrome to individuals with autism or with severe to profound mental retardation and aimed to distinguish behavioral phenotypes. Apparently there is a high prevalence of behavioral and emotional problems in Rett syndrome, such as episodes of anxiety, low mood and self-injury. Several studies have tried to establish an association between behavior patterns and the well-known common mutations. The principal data of the literature will be set out.

Emotion and Behavior in Rett Syndrome

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Rett syndrome is a neuro developmental and behavioral disorder. The MECP-2 mutations reported have a distinct effect on brain maturation resulting in cortical and autonomic disturbances.

Emotion and behaviors change and evolve with the growing child. From being a quiet, passive baby who sleeps more than other infants her age, sucks poorly and has a weak cry parents observe loss of language followed by decreased hand skills and onset of stereotypes.

Frequently intermittent crossing of the eyes, anxiety, irritability, agitation and screaming with hair pulling, biting or hitting occur with associated hyperactivity, rapid random pacing, and toe walking.

Sleep disturbances are accompanied by short periods of laughing or screaming and breathing problems become more obvious.

Older children demonstrate increasing intensity of gaze, loud moaning and screaming suggestive of distress. Adolescent girls may present with moodiness, sleeplessness, poor appetite, loss of weight, lack of interest and unexplained crying; suggestive of possible depression.

Research in mutant mice reveals 25% reduction in total brain volume and in specific regions such as the amygdala, hippocampus, striatum and hypothalamas which are responsible for emotion, behavior, attachment and anxiety and stress response. Norepinephrine, dopamine and more specifically serotonin play a major role in these regions and probably explain the emotional and behavioral observations.

Younger children have transient elevation of lactates, pyruvates and alanine with low levels of carnitine suggesting metabolic stress. These resolve but elevated CSF Glutamine persists.

Management presents a challenge to the clinician who must take into account not only the known neuro-physiological changes affecting emotion and behavior but

also determine whether there are underlying medical conditions aggravating these behaviors and treat them appropriately.

Sleep and behavioural problems

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Common sleep disorders of Rett syndrome (RS) are irregular times of going to sleep and rising, sleeping during daytime, and awakening from sleep, sometimes associated with screaming or laughter.
Behavioural abnormalities of RS are autistic features in infancy and early childhood, anxious screaming, anxiety or over-excitement in childhood, and in some patient depression in adulthood. As patients grow severe mental delay predominates.
What are the bases of these age related specific features of RS?
Sleep is a state in which specific neuronal activities, particularly monoaminergic neurons in the brainstem and midbrain take place. The sleep-wakefulness rhythm is a physiological phenomenon controlled by the biological clock and environmental stimulations, such as sun light, eating and other social cues.
Thus, evaluations of sleep components by polysomnography (recording of EEG, electro-oculogram, body movements by surface EMG, and other parameters during sleep) and sleep-wakefulness rhythm are useful to examine the activities of those neurons and neuronal systems.
Polysomnography of RS showed that the sleep components of REM sleep are present, suggesting that the brain in RS matures normally up to between gestational age 38 weeks and postnatal age 4 months.
Recordings of sleep-wakefulness rhythm of RS show increased sleep during daytime in infancy and childhood. This causes a lack of response to environmental stimulation resulting in “a good baby without much crying”.
Awakening during sleep often occurs at times indicating an associated with REM sleep.
Polysomnography also showed the components of REM sleep are abnormally observed during nonREM sleep, suggesting the hypofunction of the brainstem serotonergic and noradreneregic systems. Older children show the components of REM sleep regulated by midbrain dopaminergic (DA) system are abnormal with DA receptor supersensitivity. This DA receptor supersensitivity is the base of the sleep and bahavoural abnormalities observed in childhood.
Thus we speculate that the age related monoaminergic disturbances underlie the age related abnormalities of sleep and behaviour in RS. The early monoaminergic disturbances also lead to the characteristic motor dysfunction and higher cortical dysfunction in RS.

Moving Forward on International

Collaborations

Collaboration in Science and Support for Rett Syndrome

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There is a well-developed system of collaboration among the community of scientific and clinical researchers who work on Rett syndrome or who have found themselves working on it by accident. This involves the sharing both of clinical and scientific information and of biological samples and other physical resources. Annual meetings held in USA through and with the support of the RSRF/IRSF have greatly assisted with this process, which has substantially accelerated the pace of progress in this field. In addition, there are less frequent European meetings we hope will develop further collaborative links among European researchers. Collaborations that have benefited research in Cardiff have included the pooling of clinical data on patients with specific mutations (including InterRett via Helen Leonard), sharing of DNA samples from patients with particular clinical features or particular mutations (including Mark Bailey, John Christodoulou) and granting access to monoclonal antibodies by researchers in USA (Janine LaSalle).

Possibilities for collaboration among family support groups and Associations are more problematic as the needs of families in different countries tend to differ according to geography, systems of health or social care in place and the overall level of economic development. Social differences in relation to patterns of family and community support add further to differences in the roles that family support groups need to undertake. Despite this, we see great potential benefit from developing common approaches to problems at the supranational, 'continental' level – perhaps more than at the global level. In Europe, for example, socially as well as in terms of the science, there are opportunities for common working and resourcing that could be very productive.

We hope that this Congress will give us the opportunity to make progress on such efforts at the European as well as the more fully international level. We would especially like to explore how Associations and Researchers can work more closely together to ensure organizational and research development can go hand in hand, including joint planning meetings at realistic intervals. This in turn will help avoid possible duplication of effort – or missed opportunities – and provide a stronger platform for collaborative development.

Rett Syndrome: From Patient to Parents- Through Parents to Knowledge. Polish – Belgian Experiences from Medical Genetist's Point of View

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Since detection that Rett syndrome is caused by mutations in a single gene named MECP2 the clinical geneticist has been equipped by many diagnostic tests confirming the clinical diagnosis of Rett syndrome. It opened the way for counselling of families, similarly as for families with other genetic disorder. Genetic counselling is a complex process consisting of many biological, medical, psychological, educational, juridical,

bioethical and economical problems. Among them the relation between parents and specialists is crucial. The future of the child is determined not only by genetic factors but also by environmental interactions starting with the parents, through the close family, up to different social groups and levels of healthcare. Efficacy of medical and paramedical care depends on the national health organisation. The collaboration on an international and interpersonal level is significant in the process of knowledge gaining. This is outmost important in the search for effective management strategies and problem solving in specific genetic disorders. The aim of this presentation is to show our Polish - Belgian experiences of collaboration between the clinical geneticist and parents of Rett syndrome girls. Everything started in the physicians cabinet, through meetings in rehabilitation centers, in scientific conferences and in national and international parental support group activities. Meaningful are the social relations obtained through the media, the educational work with students and the contact with different medical and paramedical professional societies. This kind of collaboration is important for the recognition of the important role which parents play in the development of knowledge about genetic disorders and of public awareness.

Consensus Building from a Panel of Experts for a Rett Syndrome Severity Scale

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Background: Previous clinical research has used a variety of severity scales comprising additive composites of categorical items based on various Rett syndrome symptoms and characteristics. Severity scales should measure symptoms and characteristics that are considered clinically relevant; should be sensitive to longitudinal and interventional change; and should use appropriate measurement of signs and symptoms. Increased involvement in the development of a new outcome measure for Rett syndrome for the research community would result in increased ownership by the community, allowing for greater opportunities for collaboration, more uniform data collection and smoothing the multi-centre clinical trial process. Through the use of the Delphi technique and a panel of experts, we have developed clinical guidelines for scoliosis management in Rett syndrome. It is proposed that this technique, and the existing online questionnaire structures, can be used to develop consensus guidelines from Rett syndrome experts for a severity scale for clinical trials. This online-questionnaire based study will gather information form an international panel of Rett syndrome researchers on which symptoms and characteristics should be included in the severity scale, and how the severity of those symptoms should be measured.
Method: A two stage Delphi-technique will be used to develop consensus on the severity scale items and measurement. First, the international panel of researchers will be assembled. Then a draft version of the characteristics, based on previous severity scales used in research, will be piloted on a small group of researchers, before being sent out to the international panel. The views and responses of the international panel will be analysed for consensus and a second draft collated. The second draft will then be sent out the international panel. After re-evaluation, a final draft will be developed and distributed for final comments and endorsement. The clinical management of scoliosis in Rett syndrome guidelines project has shown this process to be effective.
Summary: The overall aim of the development of the consensus severity scale is improvement in data collection, international collaboration, and the development of an appropriate clinical severity scale for clinical research in Rett syndrome

Epilepsy in Rett syndrome: Diagnosis, clinical and genetic correlates and management

Epilesy in Rett syndrome in our Childrens Hospital

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We have done a retrospective study of 40 RTT patients with epilepsy, diagnosed in our hospital. Up to a 70% present epilepsy, especially in clinical stage III in our serie.
Median age at RTT diagnosis was 4.5 years. MeCP2 mutations identified were (R255X in 4, R270X in 2, T158M in 2, R306C in 3, R294X in 2, Y141X in 2, big deletions in 2, other mutations in 11) and a mutation of CDKL5 gene (cromosome 1) in one.
A 75 % of the patients presented epilepsy by 12 years old. Almost two thirds (19/30) of these children presented seizures before the diagnosis of RTT. The earliest age at onset was 53 days of life; 4/40 patients showed clinical seizures by 6 months; 10/40 by 2 years old, and 16/40 by 3 years. After 6 years old, new epilepsy diagnosis were less frequent, with the latest onset of our group at 12 years old.
Different types of seizures were observed at onset: generalized tonicclonic seizures in 11 patients, partial seizures in 8, tonic seizures in 4, clinical absences in 4, myoclonic epilepsy in 2, West syndrome in one.
EEG background was inespecific, with generalized slow activity, and bifrontal paroxisms, with great variability, even for the same patient. Also bilateral fronto-central paroxisms, or unilateral parietal, or multifocal paroxisms. In general, abnormalities observed during awake showed an important activation during sleep.
Medical treatment was iniciated based on clinical seizures and video-EEG registration: the first-choice antiepileptic drugs used more frequently on monotherapy were valproate and carbamazepine The association of Valproate, etosuximide, and levetirazetam were helpful for the most difficult cases. Clinical control was achieved in 73% of the patients. Two patients present a seizure “self-induction” mechanism, through tactil stimulus.
Molecular diagnosis may orientate about the probability of developing epilepsy, or age of onset. The choice of the antiepileptic drug should be based on the clinical presentation and electroencephalographic data. Video-EEG records are important for differential diagnosis of breathing disorders or motor paroxisms associated to RTT. Night polysomnography is helpful to define infraclinical seizures.
A good control of the seizures determines the gain of new habilities as the quality of life of these children.

Epilepsy in Rett Syndrome

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Epilepsy is frequently one of the most challenging of the comorbidities in people with Rett syndrome - a genetic neurodevelopmental disorder that affects mainly women. The clinical spectrum of the seizure disorder from different perspectives, difficulties in distinguishing epileptiform vs nonepileptiform episodic events and optimal management will be discussed. The characteristic EEG features, overlap at times with features in Angelman’s syndrome will be outlined. The influence of genotype (MECP2 / BDNF /CDKL5.) on the epilepsy phenotype will be explored.

Epilepsy in Rett syndrome- the experience of the Israeli

Rett Center

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Purpose: Rett syndrome, X-linked dominant neurodevelopmental disorder caused by MECP2 mutations, presents with autistic regression, loss of hand usage, hand stereotypies and microcephaly. Epilepsy is frequent, but course and treatment are controversial .Characterizing these features longitudinally in a large cohort contribute to better definitions and management. Methods: Charts, EEG's and v-EEG's retrospective review of 96 patients (18m-42y), followed at the Israeli Rett Clinic. Results: 73% of patients have epilepsy, divided into three groups: 5 - early epileptic variant (first year of life), 41 - onset at 2-5 years (regression stage), 20 - late onset (after 5 years). Early epileptic variant had severe seizure types in first year of life, followed by typical Rett picture. All were MECP2 negative, 1/4 had CDKL5 mutation. In the second group epileptiform EEG before age 2y was followed by intractable seizures of various types. Nine developed ESES, evolved in 2 to awake non convulsive status, and in one to Epilepsia partialis continua (EPC). Late onset group had milder epilepsy consisting mainly of PCS with or without generalization.. V-EEG was crucial in excluding non-epileptic behaviors and discriminating partial seizures from stereotypic behaviour in rhe EPC patient. Mutation presence, type, head circumference or speech preservation did not influence epilepsy course. Valproate, the most commonly used drug, was relatively effective, controlling seizures and ESES. Conclusions: Epilepsy appears earlier and is more severe then previously described. ESES is relatively common and has negative clinical impact. Controlling seizures and epileptiform activity may improve alertness and communication. Use of carbamazepine is guarded in young patients due to tendency to spike wave generalization. Valproate, despite putative detrimental role in Rett pathogenesis (HDAC inhibitor), was helpful and did not aggravate symptoms.

West syndrome and Angelman-like absence seizures: Atypical presentations of epilepsy in typical RTT syndrome

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Epilepsy is a current feature of RTT and represent one of the most challenging of the comorbidities associated with RTT. EEG features are well described, but electroclinical descriptions of epilepsy are scarce. We present here two patients with typical RTT that showed dramatically different epilepsy presentations.
The first patient was referred to us at 2 years of age for West syndrome. From the age of 6 months, parents reported delay in motor milestones. Before the onset of spasm, she was able to sit without support, to transfer objects and babbles. At age of 2 years, symmetric infantile spasm occurred with loss of eye contact ability to sit and hand use. EEG showed typical hyspsarhythmia, that disappeared with corticosteroids. Progressively, visual contact and axial tone improved, but she never acquired hand

use and babble. At 2.5 years, she demonstrated secondary microcephaly, axial hypotonia with spasticity. She had good eye fixation, no babbling, hand stereotypies, and hypotrophic extremities. Video EEG showed the combination of spasm, massive myoclonia, and tonic seizures, with multifocal spikes and no physiological activity.
The second patient was referred to us at 6 years of age for refractory atypical absence. From birth, the girl was described as hypotonic but she never experienced regression. She developed behavioural disturbances with happy demeanor, crying spells and sleep disturbances and stereotypic hand movements. Head growth slowly decelerate between 3 and 5 years. Epilepsy started at 4 years with repeated episodes of atypical absences. At 6 years, myoclonic, atonic and tonic-clonic seizures also occurred with periods of 10-15 seizures per day. Interictal EEG showed monomorphic theta activity during wakeness, that was highly suggestive of Angelman syndrome pattern. With valproate and lamotrigine, seizures frequency decrease, and at 12 years, she presented only few seizures per years.
According to the established RTT diagnostic criteria, both patients were classified as classic RTT, confirmed by MECP2 screening. We discuss the electroclinical pattern both patients and compared them to the typical RTT EEG pattern.

Therapy of Epilepsy in Rett Syndrome

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Epilepsy is very frequent in Rett syndrome patients and often difficult to treat. To find the optimal anticonvulsive treatment for patients with Rett Syndrome, we performed a retrospective study on the response to antiepileptic drugs (AEDs) in 110 patients with confirmed MECP2-gene mutations. Results: 58% of our patients had a history of epilepsy and 55% received AEDs. 54% of the females showed a reduction in seizure frequency of more than 50% after taking the first AED and 36% were seizure free for at least six months The second AED monotherapy lead overall to a seizure reduction of > 50% in only 38% of patients and a seizure free period of six months or longer in 22%. Sulthiame, carbamazepine and valproate were administered in an adequate frequency to allow statistical analysis regarding their efficacy. 71% of patients showed a seizure reduction of 50% or more when carbamazepine was used as the first AED, 56% were seizure free for more than six months. Treatment with valproate resulted in a seizure reduction of more than 50% in only 38% of patients and a seizure free period of longer than six months was seen in 6%. Sulthiame was found to have an efficiency in between carbamazepine and valproate. The rate of side effects was equivalent in all three anticonvulsants. In conclusion we found that the there is a large percentage of patients with Rett syndrome in whom the epilepsy is difficult to treat regardless of the AED used. Of the drugs that were administered frequently enough for statistical analysis carbamazepine was most effective. Sulthiame was slightly less effective and had the same rate of side effects. Valproate was least effective and should therefore not be used as the first AED.

ORAL COMMUNICATIONS

Mouse Models 1

Preserved inhibitory connectivity patterns in the Mecp2-stop mutant mouse hippocampus

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The molecular genetics of Rett syndrome (RTT) are well understood and models of the disorder in mice are providing potent resources for investigating the genotype – phenotype pathway. The underlying alterations to neuronal network behaviour that lead to the clinical picture in patients, however, are still poorly understood. Amongst the prominent clinical features of RTT are abnormal EEG patterns and a predisposition to epilepsy. Recent laboratory studies have shown alterations in the balance between excitation and inhibition within cortical and hippocampal circuits. The aim of the current study was to assess connectivity patterns within excitatory and inhibitory microcircuits in Mecp2 mutant mice.

Brains from wild-type and hemizygous male mice that do not express Mecp2 due to introduction of a neostop cassette (Mecp2-STOP mice, Guy et al., 2007) were fixed by intracardiac perfusion and coronal sections processed for immuno-cytochemistry. A range of neurochemical markers that enable discrimination between discrete populations of GABAergic interneurons, including somatostatin, cholecystokinin, parvalbumin, calbindin and calretinin, were examined in the hippocampus. In further experiments, major efferent excitatory pathways were labelled with neurobiotin delivered via patch clamp electrodes.

Subsequent analysis revealed no overt differences in the connectivity patterns of interneuronal subtypes between symptomatic Mecp2stop/y hemizygous mice and age-matched wild-type littermates. The patterns of distribution were similar in terms of the localization of soma, gross dendritic morphology and laminar termination of efferent axons. Both biocytin-labelled major excitatory pathways and interneuronal axons observed in the Mecp2 mutant brain conformed to the wild-type pattern in relation to laminar boundaries.

These findings suggest that the targeting of synaptic connections in the Mecp2-null hippocampus is not disrupted in an overt manner and suggest that the observed alterations in excitation / inhibition may reflect more subtle aberrations in synaptic physiology and structure.

Brain anatomy phenotypes in Mecp2-mutant mouse models of Rett Syndrome

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Rett syndrome (RTT) is caused by mutations in the X-linked gene MECP2. While patients with RTT show widespread changes in brain function, relatively few studies document changes in brain structure and none examine in detail

whether mutations causing more severe clinical phenotypes are linked to more marked changes in brain structure. To study the influence of MeCP2-deficiency on the morphology of brain areas and axonal bundles, we carried out an extensive morphometric study of two Mecp2-mutant mouse models (Mecp2B with complete loss of the Mecp2 gene generated in A. Bird's lab and Mecp2J with deletion of exon 3 of the Mecp2 gene generated in R. Jaenisch's lab). Compared to wild-type littermates, striking changes included reduced brain weight (~13% and ~9%) and the volumes of cortex (~11% and ~7%), hippocampus (both by ~8%), and cerebellum (~12% and 8%) in both mutant mice. At three weeks of age, most (24 of 47) morphological parameters were significantly altered in Mecp2B mice; fewer (18) were abnormal in Mecp2J mice. In Mecp2B mice, significantly lower values for cortical area were distributed along the rostrocaudal axis, and there was a reduced length of the olfactory bulb (~10%) and periaqueductal gray matter (~16%). In Mecp2J mice, while there was significant reduction in rostro-caudal length of cortex, this parameter was also abnormal in hippocampus (~10%), periaqueductal gray matter (~13%), fimbria (~18%) and anterior commissure (~10%). Our findings define patterns of Mecp2 mutationinduced changes in brain structure that are widespread and show that while some changes are present in both mutants others are not. These observations provide the underpinning for studies to further define microarchitectural and physiological consequences of MeCP2 deficiency.

Dendritic and axonal phenotypes in Mecp2-mutant mouse models of Rett Syndrome

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Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked gene MECP2. While young girls with RTT show widespread changes in brain function, relatively few studies document changes in dendritic, spine and axonal morphology. Previously, we provided evidence for changes in brain weight and volumes of brain areas and axonal bundles in two Mecp2-mutant mouse models of RTT (Belichenko et al., 2008). Here we continue to study the influence of MeCP2-deficiency on the morphology of dendrites and axons in two Mecp2-mutant mouse models (Mecp2B with complete loss of the Mecp2 gene generated in Adrian Bird's lab and Mecp2J with deletion of exon 3 of the Mecp2 gene generated in Rudolf Jaenisch's lab). Neurons in the fascia dentata (FD), CA1 area of hippocampus, and motor cortex were studied by confocal microscopy. Structure of dendrites and spines after Lucifer yellow microinjection and of axons after carbocyanine dye tracing were examined. At three weeks of age, most (33 of 41) morphological parameters were significantly altered in Mecp2B mice; fewer (23 of 39) were abnormal in Mecp2J mice. Striking changes were evident in the density and size of the dendritic spines and in the density and orientation of axons. In Mecp2B mice, the density of dendritic spines was decreased in FD (~11%), in hippocampus (14-22%), and motor cortex (~16%). A decrease in the size of spine heads (~9%), an increase in the length of spine necks (~12%), and increase dendritic swelling (~2.1 times) were found in Mecp2B FD. In addition, axons in motor cortex of Mecp2B mice were disorganized. In Mecp2J mice, significant reductions in spine density were evident in hippocampus (14-26% less). In studies detailing the changes in FD, CA1 and motor cortex, the elongation of spine neck affected the entire population,

resulting in the presence of spines whose neck was greatly increased. Electron microscopy showed abnormalities in dendrites, axons and mitochondria in both mouse mutants. Taken together, the findings document widespread abnormalities of dendrites and axons that recapitulate important features seen in RTT brains.

Region-specific synaptic abnormalities in a mouse model for Rett Syndrome

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Mouse models of Rett Syndrome, carrying deleted Mecp2, show functional alterations in basal synaptic transmission and plasticity as well as in the homeostatic balance between excitatory and inhibitory circuits in cortical regions. However whether these functional abnormalities are associated with structural changes of synaptic contacts in MeCP2-knockout (KO) animals is still unclear. In this study, we examined the morphological organization of excitatory synapses in the brain of MeCP2-KO mice at different postnatal ages (2, 4 and 8 weeks). Dendritic spines were visualised and analysed both in diolistic labelled brain sections and in a line of transgenic mice expressing green fluorescent protein (GFP) under Thy-1 promoter. In MeCP2 mutants, there was a striking decrease in the density of spines in layer V pyramidal neurons of the primary somatosensory (S1) cortex at 4 and 8 weeks. In contrast, no differences in spine number were detected in CA1 pyramidal neurons in the hippocampus. Interestingly, we found some degree of spine dysmorphogenesis, but not in spine density, in 2 weeks old animals. Moreover, electron microscopy revealed region-specific changes in the ultrastructure of axo-spinous contacts in 8 weeks old MeCP2-KO mice, consisting in a decreased size of spines and axon terminals and reduced PSD length in CA1 stratum radiatum and in S1 cortex. On the contrary we found an increase of the size of Purkinje cell spines and PSD in cerebellum. Finally, the analysis of the density and distribution of synaptic vesicles in presynaptic terminals revealed significant abnormalities in these mutants. Altogether, these results disclose striking neuron-specific changes of synapse organization in the brain of MeCP2-KO mice that may cause broader defects in the function of neural circuits underlying behavioural and motor abnormalities associated with the diseases, and indicate that architectural alterations of excitatory synaptic connectivity is an important pathological sign of Rett Syndrome.

Detection of Mecp2 gene targets using chip-on-chip

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MeCP2 is a transcription factor that when mutated causes Rett syndrome (RTT), a severe neurodevelopmental disorder which predominately affects females. A number of specific MeCP2 targets have been identified such as Bdnf and Dlx5. However, the molecular mechanisms by which MeCP2 deficiency leads to the pathophysiological defects associated with RTT remain largely unknown. This

study aims to identify genes in the mouse brain which are likely to be directly controlled by Mecp2.
Mecp2 immunoprecipitated chromatin (ChIP) from wild-type mouse brains was used to probe a mouse promoter array (chip) and has identified 2,816 genomic regions bound by Mecp2 out of over 28,000 promoter regions. Immunohistochemistry and qPCR using wild-type and symptomatic male Mecp2-null mouse (Mecp2tm1Tam) brains are used to verify if specific genes of interest are true Mecp2 targets and might therefore contribute to the Mecp2tm1Tam phenotype. Genes of interest include those involved in synapse function, cytoskeletal dynamics, dendrite architecture, axonal guidance, neuronal differentiation, mitochondrial respiratory chain function, cellular signalling, apoptosis and neural tissue-specific transcription factors. We have so far identified seven genes from the ChIP-on-chip array which show reduced expression in the Mecp2tm1Tam brain. Three of these genes are involved in regulating the activity of neuronal receptors at the synapse, two are part of the mitochondrial respiratory chain, one gene has a role in synaptic formation and maintenance, and the other gene is involved in neuronal conduction of action potentials and maintaining axon integrity.
The identification of those genes under the direct and positive transcriptional control of Mecp2 in the mouse brain shows that MeCP2 may function as a transcription activator and provides us with a unique opportunity to understand novel cellular functions regulated by MeCP2. Our study will provide insights as to how MeCP2 deficiency may lead to synaptic dysfunction, which may be relevant not only in RTT but also other disorders associated with mental retardation. This in turn will open up new investigative avenues for the discovery and development of targeted therapies for RTT and related neurodevelopmental disorders.

MECP2-protein substitution therapy in a mouse model of Rett Syndrome:

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Rett syndrome is an untreatable neurodevelopmental disease caused by loss of MeCP2 protein function. We produced novel recombinant MeCP2e1 and MeCP2e2 proteins fused at their N-termini to a protein transduction domain and assessed their therapeutic potential for Rett syndrome in Mecp2-/y mice. In vitro, both isoforms reverted the pathological hyperacetylation of H3 and H4K16, a hallmark of MeCP2 dysfunction. The fusion proteins were injected intraperitoneally, crossed the blood-brain-barrier and translocated to neuronal cell nuclei. Administering the TAT-MeCP2e1 and TAT-MeCP2e2 isoforms significantly extended the lifespan of Mecp2-/y mice from 55.5 to 92.6 or 84.8 days, respectively. The reduced volume of the hippocampal neurons in Mecp2-/y mice was rescued in TAT-MeCP2e1-treated animals. Wheel running measurements showed improved motor learning abilities of Mecp2-/y mice reconstituted with TAT-MeCP2e1 protein. These findings reveal that biologically active recombinant TAT-MeCP2 proteins can reach the brain after peripheral injection and can restore neuronal abnormalities in Mecp2-/y mice. Our study opens possibly therapeutical prospects for treatment of Rett syndrome.

Genotype-Phenotype

The phenotype of Rett Syndrome cases with deletions in the c-terminal region of MECP2

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Background: It is thought that deletions within the C-terminal region of the MECP2 gene may be associated with a milder Rett syndrome phenotype. This study will use the combined resources of the InterRett and Australian Rett Syndrome databases to describe the diagnostic criteria and severity of symptoms of cases with C-terminal deletions within MECP2.

Methods: Cases will be sourced from the Australian Rett Syndrome Database, a population-based longitudinal database of phenotype information collected from families and carers of people with Rett syndrome in Australia, and from InterRett, the International Rett syndrome phenotype database, with information submitted by families and clinicians. Deletion size and position in the C-terminal region has been collected in both databases. Eighty-seven (58 from InterRett and 29 from Australia) cases with frame-shift deletions (of 20 or more base pairs) within the C-terminal region of MECP2 will be included.

Outcomes: Cases will be classified according to the Rett syndrome variant delineation model, with comparison of criteria met for cases with different Cterminal deletions, and with the MECP2 mutation positive cohort as a whole.

Severity of overall phenotypic presentation will be measured for each case using the severity scales of Kerr, Percy and Pineda (as adapted by Colvin et. al. in 2003), and compared within the C-terminal deletion group, and with the MECP2 mutation positive cohort. The effect of deletion size and position on severity will be examined. Specific aspects of the Rett syndrome phenotype will be described, such as hand function, mobility and behaviour, in order to identify to what extent the phenotype of cases with C-terminal deletions differs from the phenotype of cases with other MECP2 mutations.

Rett Syndrome in Spain, genothype-phenotype correlations of the most frequent mutations

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Rett syndrome is an X-linked dominant neurological disorder, which affects mostly females. It is associated with mutations of the MECP2 gene. In Spain, 363 Rett patients have been diagnosed, all of them females except two males. The patients' phenotypes have been classified as classical (84,5%), preserved speech form (3,5%), late regression form (2,5%), early epilepsy form (3%), congenital form (6%) and frustre form (0,5%). Recurrent mutations have been identified in a 85,6% of the patients with an identified molecular error.

The genetic studies show that the most frequent mutations in Spain, in order of frequence, are R255X, T158M, R168X, R306C, R270X, R133C, R294X,

R106W.
Genothype-phenotype corelation is based on our severity scale checklist (score 1 to 21): mutations with higher scores are nonsense mutations (R 270X, R168X and R255X), with scores over 15 points in 38% of the cases. Nonsense mutations are associated to the most severe clinical forms.
Otherways, missense mutations have shown to be milder forms with scores below 14 points, even if they present with deceleration of head growth, observed on 89% patients with the mutation R306C and on 68% of patients with T158M mutation. Deaceleration of head growth is not a marker of severity. Detailed phenotypes shall be described and will show to be usefull, as the knowledge of the genetic studies (mild, severe, very severe mutations), for a prognostic orientation.
We think phenotype-genotype correlations can be a great help for the habilitation team to planify the goals in the intervention therapy and the additional cares of this patients with this severe neurodevelopmental disorder.

Molecular testing of MECP2 gene in Greek children with MR

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Rett syndrome (RS) is an X-linked dominant neurodevelopmental disorder affecting mostly girls. It is characterized by normal early development, followed by psychomotor regression and gradual onset of microcephaly. Most Rett cases (70-95%) are caused by mutations in MECP2 gene (Methyl-CpG-Binding Protein 2) which is also implicated in a variety of other mental retardation (MR) phenotypes, including X-linked Mental Retardation (XLMR), Fragile-X like Syndrome (FXS) and Angelman-like Syndrome (AS) phenotypes. Recently another gene, CDKL5, lying on Xp22, has also been correlated with the Rett phenotype and is being tested in several laboratories when RS analysis is requested. The aim of this survey was to evaluate the incidence and spectrum of MECP2 mutations in Greek children with RS phenotype or atypical Mental Retardation (MR) .
Screening of MECP2 exons 3 and 4 was performed by GAP-PCR, Denaturant Gradient Gel Electrophoresis (DGGE) and a novel protocol called Enzymatic Cleavage Mismatch Analysis (ECMA), followed by direct sequencing. A total of 247 patients were studied comprising: (A). 128 FXS like children (104 boys / 24 girls), and 55 AS like children (17 boys / 38 girls) for whom molecular testing did not confirm clinical diagnosis of FXS or AS respectively, (B). 25 classic and 28 atypical RS girls; and C. 11 boys referred with possible RS.
Molecular alterations, consisting of both common mutations (~ 61%), novel alterations (~10%) and polymorphisms (~10%), were detected in 80% of classic and 20% of atypical RS cases respectively. MECP2 mutations were also present in ~ 10% of the AS like and ~2.4% of the FXS-like cases, and one XLMR case as well. Finally, 4 out of the 11 boys were found to carry aberrations of MECP2, including the typical R106W mutation, a 46 bp deletion in exon 4 (c.1140del86) and an already known polymorphism T203M. MECP2 gene analysis provides an appropriate diagnostic tool for RS and contributes to the research into MR of unknown aetiology. .

Extending the phenotype associated with MECP2 mutations in females

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Rett syndrome has been traditionally defined in children with features concordant with the classical criteria for this condition. However following the identification of the link with the MECP2 gene it became clear that clinical variability was much greater than originally thought. To allow for this broader phenotype the criteria were amended in 2003. Till eight months ago all two hundred or more cases in the Australian Rett Syndrome Database (ARSD) with confirmed MECP2 mutations had characteristics consistent with clinical Rett syndrome. However in the past eight months three of the fifteen MECP2 positive cases ascertained by the ARSD were much less recognizable as Rett syndrome.

In these young women diagnosed at the ages of 13, 16 and 24 years the following heterozygous MECP2 mutations were identified: a frameshift (c.1157_1188del32: p.Leu386fs); an in-frame deletion (c.1143_1208del66 : p.Leu328_Pro403del22); and a complex rearrangement resulting in a frameshift (c.1152del56 followed by an insertion after base 1261 of bases 1123 to 1261 : p.Pro385fs). All subjects are intellectually disabled but only in one was there a history of regression. None have hand stereotypies. All three can walk with an unsteady gait and speech, although limited, has been preserved. The two older subjects have developed scoliosis and all three have seizures.

Age of diagnosis	13 years	16 years	24 years
Onset of seizures	3 ½ years	2 years	15 years
Regression	At 5-6 years affecting motor and language skills	No	No
Speech	Yes but significant difficulties	Yes- 50 words	Yes- responds appropriately to questions
Walking	15 ½ months	Delayed- not at 2yrs	Not delayed- 11 months
Dysmorphic features	Yes syndactyly and few unusual facial features	Yes broad base left thumb and stubby nail	Yes occipital prominence stellate pattern of the iris, simian crease left palm.
Head circumference	Normal	>90th centile	>90th centile
Mutation	p.Leu386fs	p.Leu382_Pro403del22	p.Pro385fs

These young women all clearly have a MECP2-related disorder but are unlikely to meet the clinical criteria for Rett syndrome. Some might debate whether or not to attach this diagnostic label. What we believe is more important is to establish how common MECP2-related disorder is amongst the general population of intellectual disability where a high proportion of children and adults currently have no identified cause.

A new family case of Rett Syndrome: two half sisters and one brother with identical MECP2 mutation, but with different clinical presentations.

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We report a □gRett□h family with two maternal half sisters and one brother, all harbouring the same MECP2 C-terminal truncating mutation (c. 1159C□¨ Tdel C). The mutation introduces a frameshift, leading to a premature termination of the MeCP2 protein. This type
of mutation is typical found in females with classic sporadic Rett syndrome (RTT), but the carrier females in this family do not show any RTT symptoms except mental retardation, while the boy has RTT features.
Since the discovery of the correlation between RTT and MECP2 mutations in 1999, there has been an intense mutation screening of RTT patients. This has broadened the understanding of the spectrum of clinical outcomes that can be associated with MECP2 mutations. Mutations causing classic RTT typically lead to premature truncation or amino acid changes in conserved areas/domains of MeCP2. The milder mutations lead to a phenotype distinct from classic RTT are often changes of conserved amino acids located outside MeCP2 functional domains or late truncating mutations that preserve a significant portion of the coding region.
The boy of this family is 13 years old, severely mental retarded and suffers from epilepsia. In his first years he presented with some symptoms of Angelman syndrome, but over the last few years he has developed RTT like symptoms, such as stereotypic hand movements
and respiratory irregularites. His two sisters are 8 and 17 years old. The older half sister is mentally retarded and is unable to take care of herself. The younger sister has a mild mental retardation. The mother to the three children, who is an obligate carrier, has a clinical
presentation somewhat in between her two daughters. The mother has not yet decided whether to be tested for the familial mutation. Therefore ethical consideration has to be taken
into account before performing further molecular investigations in this family.
This family present a link between classic RTT and asymptomatic carriers for a MECP2 mutation.

A male Rett patient and five females with preserved speech variant share the same MECP2 mutation

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About 80% of female patients with Rett syndrome (RTT) carry a mutation in the MeCP2 gene. Recent studies indicate that MeCP2 mutations are not necessarily lethal in males since the prenatal stage. The observed phenotype is variable and three main groups can be distinguished. A first group, characterised by severe neonatal encephalopathy, carries mutations usually found in females; a second group includes Rett-like patients and results from mosaic MeCP2 mutations or Klinefelter syndrome males. Within this group only mutations that affect Arg133, commonly referred in patients with a mild phenotype, have been reported in

females. The third clinically heterogeneous group comprises patients with mild to severe mental retardation, carrying mutations inherited from their unaffected mother and never reported in affected females. We refer on a maternally inherited C-terminal deletion, c.1163del44, p.Pro389X, in a boy with severe encephalopaty, precocious onset of epilepsy, hyperventilation and breath-holding, who never joined a purposeful hand-use and regressed his motor skills. X inactivation test disclosed a bordeline value in the mother, who shows a very mild intellectual impairment which was not scored by specific tests. RT-PCR on RNA from lymphoblastoid cells detected the presence of an abnormal transcript, translatable into protein, as confirmed by Western-blot. A mosaic condition was excluded by the lack of any normal RNA transcript and wild type protein. Interestingly we could detect the observed mutation, yet unreported in males, in 4 RTT girls with preserved speech (PVS) and a peculiar clinical evolution: three of them showed a balanced pattern of X inactivation, while one was partially skewed. Cognitive evaluation and clinical parameters were collected. Other PVS cases carrying this mutation and classified in the group of mild C-terminal mutations, that spare MBD, TRD and NLS domains, have been reported. A residual function of the protein might account for such a clinical subset fulfilling many criteria of RTT with mild evolution. Methylation array profiling is in progress on established cell lines to highlight which genes are up and downregulated in these patients

Phenotype and Function

Is there an early specific developmental trajectory of Rett disorder?

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Objective: Changes in the normal quality of general movements (GMs) are proven indicators of brain dysfunctions. Abnormal GMs such as cramped-synchronized GMs or an absence of fidgety GMs predict cerebral palsy already during the first weeks of life (Lancet 1997;349:1361-3; Clin Dev Med 2004;Vol.167). Also girls with Rett syndrome have abnormal GMs (Brain Dev 2005;27:8-13). In addition, they do have other early abnormal signs, such as abnormal finger movements, bursts of abnormal facial expression, bizarre smiling, tongue protrusion and asymmetric eye opening or closing (Pediatr Res 2005;57:1-5). The aim of this study was to compare the very early developmental trajectory of girls with Rett syndrome to infants with acquired brain lesion and normal infants. Method: We analyzed videos of 84 infants, born at term, and recorded several times during their first 6 weeks of life and again during 3-4 months of life. Their neurological outcome (median age: 12 years) was Rett syndrome (N=14), cerebral palsy (N=21), complex minor neurological dysfunction (N=19), or normal (N=30). Results: Abnormal general movements and postural stiffness during the first 6 weeks of life were associated with later neurological impairment, but not specifically with Rett disorder. Longlasting tongue protrusion ($p<0.05$) and asymmetric eye opening following a blink ($p<0.0001$) were mainly observed in infants with Rett disorder. At 3-4 months, all typically developing infants exhibit fidgety movements (LR neg=0.05) whereas the absence of fidgety movements predicts cerebral palsy (LR pos>51). None of the infants with Rett disorder had normal fidgety movements. They were either absent (N=4) or abnormal, i.e. jerky and too slow (N=10). Conclusion: Tongue protrusion and/or asymmetric eye opening during the first 6 weeks postterm age in combination with abnormal fidgety movements at 3-4 months are most likely early specific signs for Rett disorder.

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Gross motor skills of 99 females with Rett Syndrome

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Background: Gross motor impairment is a fundamental but variable component of the Rett syndrome phenotype.

Methods: This study used video supplemented by parent-report data from the Australian Rett Syndrome Database to describe gross motor skills in females with Rett syndrome (n=99,) and investigated effects of age, genotype, scoliosis and hand stereotypies on gross motor skills. Principal Components Analysis was performed to reduce the set of 15 sitting, standing, transfer and walking items into a smaller number of factors.

Results: Most subjects were able to sit, slightly less than half were able to walk

and a minority were able to transfer without assistance. Strong item loadings in the factor analysis enabled the calculation of general gross motor and complex gross motor skills scores. General gross motor skills declined with age and were poorer in those who had surgically treated scoliosis but not conservativelymanaged scoliosis. Complex gross motor skills did not decline with age and were better in those without scoliosis. Those with a p.R133C, p.R294X, or a p.R255X mutation appeared to have better gross motor skills overall than those with a p.R270X or large deletion mutation. Motor scores were not related to the frequency of hand stereotypies.
Summary: This information is useful for the clinician and family when planning support strategies and interventions. The two gross motor subscales can be used in clinical practise to measure responses to interventions.

General development in females with Rett Syndrome, focusing on abilities, deformities and management: the Swedish Rett center survey

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The aim of this study was to make a description of the early development in individuals with the diagnosis Rett syndrome using parents´ information. Information received from 125 cases of Rett syndrome in Sweden in 1997 provided us with families´ description of early development in gross motor function, fine motor function and communication/social interplay. Best abilities before regression were presented, 62% lost their best abilities, 22% kept them and 5% kept them with deterioration. Seventy-three per cent learnt to walk, 20% stopped walking and 2% retrained walking. Concerning feeding, 69% learnt to feed themselves, 57% lost this ability, 7% retrained the ability and 5% learnt to feed after regression. Sixty-four per cent were one year or younger when there was a deviation in development. Sixty answers reported the girl was late in developing functions while 35 reported sudden loss of reached abilities. Seventyfour per cent developed a scoliosis and 83% reported other deformities, of these, deformities in feet were the most common. Postural control was poor since all but 15 girls/women leant in different directions when sitting. Transitional movements were difficult to perform. In 80% of cases the families were those who suspected early that something was wrong in the child's development. Because of this it is essential that medical staff is aware of the different ways RS develops in order to give families early appropriate support and a plan for intervention. Since there is loss of function in this group but also kept abilities, retrained abilities and abilities achieved after regression, more research has to be focused on management and treatment to help persons with Rett syndrome keep and develop abilities according to their individual resources.
Keywords: Autonomic dysfunction, foot deformities, loss of function, motor development, physiotherapy, posture, regain function, scoliosis
This study was approved by the ethical committee at the University of Umeå (§15/97, dnr 97-1)
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Autism and Rett Syndrome - How different the differences are?

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The paper aims to present an overview of the differences and similarities between the two clinical entities in respect to genetic background, clinical presentation, interventions and management. Clinical diagnosis of Autistic Disorder and Rett Syndrome according to DSM-IV and ICD-10 has been compared and discussed. Literature review of the genetic factors and their relationship with the environmental factors for the both clinical categories has been presented. Continuity and discontinuity of the symptoms and its relevance to clinical interventions and management have been outlined. Different interventions and programs have been compared. Did we really succeed to build together a world where is a place for them?

Rett Syndrome and Rett disorder: an attempt to redefine phenotypes

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Background: The diagnosis of Rett syndrome (RTT) is based on a set of clinical criteria, irrespective of mutation status. The aims of this study were (1) to define the clinical differences existing between patients with Rett disorder (RD), when there is a known MECP2 mutation, and Rett syndrome (RTT), when aetiology is unknown, and (2) to characterize the phenotypes associated with the more common MECP2 mutations.

Patients and methods: We analyzed 88 patients fulfilling the clinical criteria for RTT. All were observed and videotaped by the same paediatric neurologist. Seven common mutations were considered separately, and associated clinical features analysed.

Results: Comparing RD and RTT, we found differences concerning psychomotor development prior to onset, acquisition of propositive manipulation and language, and evolving autistic traits. Based on age at observation, we found differences in eye pointing, microcephaly, growth, number of stereotypies, rigidity, ataxia and ataxic-rigid gait, and severity score. Patients with truncating differed from those with missense mutations regarding acquisition of propositive words and independent gait, before the beginning of the disease, and microcephaly, growth, foot length, dystonia and severity score, at the time of observation. Patients with the R168X mutation had a more severe phenotype, whereas those with R133C showed a less severe one. Patients with R294X had a hyperactive behaviour, and those with T158M seemed to be particularly ataxic and rigid.

Conclusion: A clear regressive period (with loss of prehension and language, deceleration of growth) and the presence of more than three different stereotypies, rigidity and ataxic-rigid gait seemed to be very helpful in differentiating RD

from RTT.

Diagnostic criteria for the Zappella variant of Rett Syndrome (the preserved speech variant)

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The preserved speech variant is the milder form of Rett syndrome: affected girls show the same stages of this condition and by the second half of the first decade are making slow progress in manual and verbal abilities. They walk without help, and may be able to make simple drawings and write a few words. Most of them can speak in sentences. Autistic behavior can often be observed. We previously described several cases in the pre-molecular era and subsequently reported a survey of 12 cases with MECP2 mutations. Seventeen new patients with the preserved speech variant and a proven MECP2 mutation have been clinically evaluated. Additional clinical data of our previously described cases are reported. These 29 preserved speech variant cases were compared with 129 classic Rett patients using a clinical severity score system including 22 different signs. There was both statistical and clinical evidence of the existence of this variant. On the basis of their abilities these girls can be distinguished as low-, intermediate- and high-functioning. Girls of the last two groups show a greater homogeneity: they speak in sentences, use their hands more easily, have normal somatic features, mild neurovegetative abnormalities, with autistic behavior in 76%, epilepsy in 30%, while girls of the first group are closer to classic Rett syndrome. The majority of patients carries either missense mutations (especially the p.R133C change) or late truncating mutations in the MECP2 gene. In the original article describing the first cases, the name of preserved speech variant was initially suggested by reviewers. Increasing experience has shown that language is not “preserved” but represents a later improvement as well as a subsequent advance in hand use, in contrast to classic RTT where these symptoms do not improve. In addition, this variant differs from the classic RTT not only as regards language abilities but also for other aspects including somatic features and cognitive abilities. Thus, the term preserved speech variant is in some way misleading and we propose to change it in favor of Zappella variant of RTT (Z-RTT). Renieri A. et al. Brain and Development. 2008. In press.

Management of Epilepsy and Cardiorespiratory Challenges in Rett Syndrome

EEG characteristics of Rett Syndrome

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Objective: Rett syndrome is a neurodevelopmental disorder that almost exclusively affects females. Most of the patients had electroencephalogram (EEG) abnormalities and clinical seizures. This study aimed at elucidating the EEG characteristics of Rett syndrome.
Methods: EEGs of 31 patients with Rett syndrome were retrospectively studied. All the cases were diagnosed by Department of Pediatrics, Peking University First Hospital from 1997 to 2007. Four hours video-EEG were performed on 27 cases, and 30 minutes sleep-EEG on 4 cases, using international 10-20 system, without sedation. EEGs were analyzed by two examiners.
Results: All the cases are female, aged from 16 months to 14 years (average age is 3.8 years). Two patients were in clinical stage I, 17 patients were in clinical stage II, 12 patients were in clinical stage III, and none in clinical stage IV. EEGs were abnormal in 29 cases in clinical stage II and stage III. Only two cases in clinical stage I had normal EEG. The abnormalities include (1)slowing of background activity; (2)loss of the non-rapid eye movement sleep characteristics; (3)frequent occurrence of focal spike or sharpwave discharges, as well as, generalized spikeand-wave discharges; (4)rhythmic central theta activity. Epileptic form discharge was observed in 26 cases (83.8%). Seizures identified events which represent the patient's typical 'seizures' were recorded in 12 cases during monitoring, 10 (83.3%) of them were not associated with epileptic form discharges in their EEG record.
Conclusion:The EEG pattern of Rett syndrome is characteristic and correlated to the clinical stage of the disease. Some episodic events of the patients may misdiagnosed as seizures. Therefore, the diagnosis of epilepsy should be well evaluated and the video-EEG monitor is recommended.

Epilepsy in Rett Syndrome: a study of association between phenotype and genotype

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Aim: Rett syndrome, a neurodevelopmental disorder mostly affecting females, is caused by mutations in the MECP2 gene. Seizures have been reported in more than 80% of subjects, as have breath holding episodes, which may resemble seizures.
The aim of the study is to determine the type and frequency of seizures in Rett syndrome and their association with the type of MECP2 gene mutation.

Method: Information was obtained from the UK Rett syndrome database, of subjects who been identified as having a mutation in the MECP2 gene. 137 subjects were selected from this as having the nine most common types of mutation. A questionnaire was designed to record detailed information about seizures and breath holding. Interviews were conducted by two investigators and seizure types identified by three experienced epileptologists.
Results and conclusions: 89 patients responded, giving a response rate of 65%. The ages of subjects ranged from 5 to 43 years and the average age was 17 years. 74% of subjects had been given a previous diagnosis of epilepsy according to their families.
The prevalence of epilepsy ranged from 100% (gene type R 270 Xc 808 C>T) to 50% (gene type R 294 Xc. 880 C>T).
Breath holding spells varied according to gene type from 100% in gene type R 106 Wc. 316 C>T to 50% in gene type R 133 Cc.397 C>T.
Generalised tonic clonic seizures ranged fron 0% to 77% according to gene type.
Secondarily generalised tonic clonoc seizures ranged from 0% to 100% according to gene type.
Data will be presented for all seizure types, breath holding and the association between mutation types and all seizure types

Epilepsy in Rett Syndrome

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Purpose: Rett syndrome (RS) is a neurodevelopmental disorder with epileptic seizures reported in 50- 90% of cases. The aim of this study was to characterize the clinical presentation of epilepsy in a series of Rett syndrome patients.
Method: This is a retrospective, single centre, observational, uncontrolled study of 76 consecutive patients diagnosed as RS in the Institute from 1988. until 2006. Twenty patients, not followed regularly, were not included in the study.
Results: A history of epilepsy was present in 44 (78,57%) of 56 patients. Seven patients (14,2%) died, one after status epilepticus. The median age of seizure onset was 6 years, range o,3-12 years. The most common seizure types were generalized tonic- clonic (28 -63%) and partial (24 - 54,5%). One third had only one type of seizure and others two or more. Three had status epilepticus. Sixteen patients (36,3%) used one antiepileptic drug and 28 (63,6%) used 2 or more. Most commonly used drugs were valproate (24), karbamazepine (21) and clobazam (12), and lately topiramate (10). The prevalence of epilepsy in older age group is influenced by difficulties in availability of local epileptologists and transport of RS girls with reduced mobility.
Conclusion: The degree of severity of epilepsy in our RS patient is significant. The long course of epilepsy reveals apparently active epilepsy in 75% of young adult patients.

The common BDNF polymorphism may be a modifier of disease severity in Rett Syndrome

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Rett syndrome (RTT) is caused by mutations in the transcriptional repressor methyl CpG-binding protein 2 (MECP2). BDNF is one of the genes affected by MeCP2, with BDNF levels being reduced in the brain of the Mecp2-null mouse model as a result of complex interactions . BDNF is a neurotrophic factor that plays a major role in neuronal survival, neurogenesis and neuronal plasticity, and has a common polymorphism (Val66Met [p.V66M]), which has been found to correlate with the severity and course of several neuropsychiatric disorders. Based on these observations, we have examined the association between disease severity scores and the BDNF polymorphism in 125 patients with RTT from the Australian Rett Syndrome Database (ARSD) and the Israeli cohorts, all carrying the commonest MECP2 mutations. We found that those who were homozygous for the wild-type (Val/Val) BDNF polymorphism were slightly less (but not significantly) severe than those who were heterozygous (Val/Met) (coefficient -2.08, p=0.09). When severity was examined for those with the p.R168X mutation, there was 6 point decrease in the severity score for those who were homozygous for the wild type (Val/Val) BDNF sequence. Age of onset of epilepsy was not significantly different for the entire cohort, but individuals with the p.R168X mutation and heterozygous (Val/Met) for the BDNF polymorphism were at an increased risk of epilepsy (Cox proportional hazard ratio 5.32 p=0.006, 95% CI 1.60 – 17.65), and had a decreased time to onset of seizures (2 yrs vs. 7 yrs) than cases homozygous for the wildtype BDNF allele.
Conclusion: In addition to mutation type and the degree of X-chromosome skewing, the common BDNF polymorphism appears to be an epigenetic modifier of RTT severity. This suggests that BDNF function may play a significant role in the pathogenesis of RTT.

Epilepsy in Rett Syndrome

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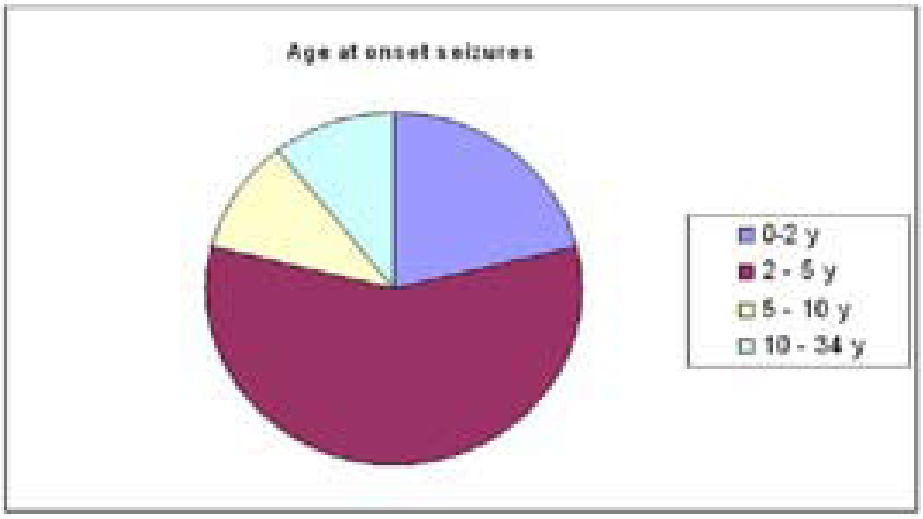
Epileptic seizures have a considerable impact on the quality of life in Rett Syndrome (RS).
Although epilepsy occurs in about 80% of patients with RS, there has been little research into the features of seizures and the use of antiepileptic drugs.
Literature data show that seizures tend to be worst in those subjects who are already generally severe for other parameters and that seizure rates are reduced

in subjects with p.R294X, p.R255X and C terminal deletions.
Regarding optimal treatment strategies for epilepsy in RS currently only limited data are available. Carbamazepine, sodium valproate, sulthiame and lamotrigine are reported as the most commonly used AED. Nevertheless, no clinical studies have compared the effectiveness of various AEDs in RS. So far the choice of an AED is based on the preference and individual experience of the physician in charge of the patient.
Aims:
- to define clinical history of epilepsy in RS, (the age-related profile, the relationship with genetic characteristics);
- to clarify the efficacy of different AEDs, thus to improve the treatment approach of subjects affected by RS.
Methods: It has been proposed a questionnaire for each child to obtain longitudinal data including clinical information on seizures, EEG findings and treatments. It was compiled by the physician in charge of the patient.
Results

Subjects	50 females
Age	18 ms-34 years (media 13 y)
Classic forms	39 (78%)
Variant forms	11 (22%) (4 Hanefeld, 3 PSV, 4 Fruste)
MeCP2 +	38 (76%)
CDKL5 +	3 (6%)
genetic investigations negative	9 (18%)
Epileptic patients	41 (82%)
Epileptic patients not including CDKL5 ones	38 (76%)



Most patients are in stadium 3 or 4. Medium age at onset seizures is 4.5 years.
The age range of onset seizures was 0.4-20 years (media: 4.7 y)
The presence of epilepsy does not appear to be correlated neither to clinical phenotype, nor to the kind of mutation or functional protein domain involved.
Neverthless in our study we observed a high frequency of epilepsy in patients with R294X mutation and a lower rate in Preserved Speech Variant patients.
First treatment was a monotherapy for all patients.
Valproate was the most administrated first drug and it resulted efficacious in 31% of cases.
Epilepsy was farmacoresistant in 53% of patients.
Afterwards, although the contribution of new AEDs, epilepsy is still an important problem for the most affected Rett patients.

Clinical update addressing the cardiorespiratory challenges in medicine posed by Rett Syndrome: the Frösö declaration

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Rett syndrome (RTT) is a genetic neurodevelopmental disorder with brainstem immaturity that affects 1 in 10,000 females. It demonstrates the importance of the brainstem in cardiorespiratory medicine. There is a lack of understanding of the cardiorespiratory turmoil in RTT in the wider medical community, which makes managing RTT patients such a challenge. Therefore, an international group of medical practitioners from various fields with a minimum of ten years experience treating RTT gathered in the Swedish National Rett Centre in Frösön to collate experience and provide a practical management strategy for all healthcare tiers. Establishing the cardiorespiratory phenotype in RTT requires detailed neurophysiology. The primary pathophysiology is defective control mechanism of carbon dioxide exhalation causing respiratory alkalosis or acidosis. Comprehensive management has a significant impact on the health and longevity of RTT persons. Good management requires the involvement of many different specialists engaged in an individualised approach. The Frösö Declaration promotes the need to understand the nutritional and cardiorespiratory requirements of these patients in order for them to receive appropriate and effective treatment. Parents are asking for such treatment throughout their interactions with health care providers from primary through secondary to tertiary centres. We believe that some aspects of treatment in RTT are now beyond the anecdotic stage.

Oxidative stress in Rett Syndrome

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Background: The molecular targets of MECP2 and the genotype-phenotype relationships in RETT syndrome (RS) remain to be elucidated. Several lines of evidence suggest a relation between hypoxia/oxidative stress and RS: (1) The presence of an oxidative imbalance in RS has been previously suggested by high MDA concentrations and low SOD enzyme activities (Sierra et al., Brain Dev 2001;23;S1:S236-9); (2) An unexpected MECP2 function of protecting the cerebellar granules from hypoxia /glutamate-excitotoxic damage (Russel et al, Neuroscience. 2007;150:563-74), and (3) a role for MECP-2 has been revealed in the epigenetic regulation of the E2 receptor alpha-promoter in the cerebral cortex following ischemia (Westberry et al., Neuroscience. 2008;152:982-9).
AIMS: We hypothesized that oxidative stress and subclinical hypoxia are key features in RS.
Methods: A total of 44 RS girls (age: M±SD, 14.7±7.8 yr) with MECP2 or CDKL5 gene mutations and age-matched controls were enrolled in the study. Erythrocyte Desferioxamine (DFO)-chelatable free iron (IE-NPBI), plasma DFO–chelatable free iron (p-NPBI), plasma free F2-isoprostanes (p-F2-IsoPs) and plasma protein carbonyls (p-PCs) were determined. Data were expressed as means ±SD, or medians (inter-quartile range). Differences between RS and controls were tested by Student's t- or Mann-Withney tests. Spearman rank correlation was used to explore univariate associations between variables.
Results: RS girls had higher IE-NPBI [1.7 (1.2-2.4) vs. 0.8 (0.65-0.90) nmol •mL -1, P=0.0005], p-NPBI [1.0 (0.8-1.2) vs. 0.2 (0.65-0.90) nmol •mL -1, P<0.0001], p-F2-IsoPs [56 (47.3-64.4) vs. 24.5 (19.65-31) pg •mL -1, P<0.0001] and p-PCs concentrations [0.08 (0.045-0.13) vs. 0.017 (0.011-0.064) nmol •mg -1, P=0.021] than controls. The inverse correlations IE-NPBI/ SpO2 (rs=-0.85 , p<0.0001), p-NPBI/ SpO2(rs= -0.39, p=0.0162), and p-F2-IsoPs/ SpO2 (rs=-0.36 , p=0.034) were observed.
CONCLUSIONS: Biochemical signs of a hypoxia-induced free radicals damage are present in RS, thus suggesting that systemic oxidative stress is likely to be involved in the pathogenic mechanisms of the condition.

Family Issues & Experiences

Communication as a basis and means for interaction, play, and learning for girls with Rett Syndrome

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Communication is the ability to transfer and receive messages that have a common meaning. In the communication process there are two active participants.
The basis for communication is connection and trust. In order for communication to exist, there must be a common base of understanding.
Communication begins in infancy, from the moment a child is born- Communication is a developmental process; we are born with the will to communicate as part of our survival instinct.
Girls with Rett Syndrome have an early experience of normal communication development until the stage of regression. Therefore, they have the will and motivation to communicate.
Due to their Apraxia, loss of speech and hand function, communication is difficult for them and they need the structure and adjustment of a communication system which will enable them to express their potential, considering both the communicative and cognitive aspects.
Research and clinical experience both agree that girls with Rett Syndrome comprehend language; their reaction may not be direct, but given sufficient time and an appropriate communicative environment, they will react to a situation.
Girls with Rett Syndrome have different learning abilities, a strong emotional interest in their surroundings and a will for social interaction- Because of that it's important for them to communicate. They try to initiate, communicate, and express feelings, but they cannot express their desires conventionally through the use of speech or gestures.
But, due to their functional difficulties, girls with Rett Syndrome are unable to verbally express themselves and thus bear witness to their intellectual level.
There is, therefore, a need to find the appropriate tools and strategies to enable them to communicate - to take an active part in an interaction, express opinions and standpoints, refer to another person, play, find enjoyment, and express their cognitive ability and comprehension.
This video presentation will demonstrate situations in which adjusted and accessible communicative environments enabled girls with Rett syndrome to take an active part in a communicative interaction – to play, talk, share, learn, and demonstrate their communicative and cognitive potential, as well as the enjoyment and motivation they feel when they communicate and are understood

Limitations or possibilities – a case study

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In the daily work with Rett patients, there is a great need for professional competence to evaluate the patients and in an interdisciplinary cooperation to suggest how to manage further development. This need gives the professionals the power to decide the patient's room of possibilities and developmental potentials, because the professionals are expected to be the ones, who know

the truth concerning the prognosis according to the diagnosis, which will often become the background for the possibilities of treatment offered to the patients. This issue creates a lack of balance in the relation between the professionals and the family. It is expected that the professionals' optic is a valid reason to define the patient's developmental possibilities.
Our case from the Danish Center for RTT is an 8 years old girl with a R255X mutation in MECP2.
She has been attending a special day care and has had daily contact with therapists and teachers. From early age it is described that she needs help in all Gross Motor functions. According to this she is daily placed in a stand with full support, with back shield and foot orthoses. According to her therapists she never had the strength to stand unsupported, never walked, no longer crawls, and now only sits with support.
During our examination we find, that she can stand alone in crawling position, and guided on her hands she has a crawling pattern. She can stand on her knees without a persons support with her hands on a couch. She can stand on her own for more than a minute supported by a couch with two pieces of tape on every knee.
We find that her main problem according to the Gross Motor functions is instability and dyscoordination of the muscles around pelvis.
In our case it seems that the professionals interpret RTT as a progressive disease and have taken a determined attitude towards the patient. This bias limits the possibilities of development and stigmatizes the patient as a person having RTT instead of being a patient, who needs professional help to find her developmental potentials.

Living with Rett Syndrome: family stories of coherence and resilience

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For families with children suffering from severe disabilities such as Rett syndrome, it is of crucial importance to reach a high level of adaptation to continuing challenges to be faced over a long period of time. This study explores the relationship between family sense of coherence and measures of family functioning, coping strategies, levels of functional impairment and demographic indicators. Participants were 51 families with girls (under 18 y.) with Rett-Syndrome. Measures contributing to the separation into groups of families with high, medium and low family coherence were identified. The highest discriminatory power had the utilisation and perception of social support and levels of family functioning. In a further step of analysis, resilience-related narratives of families were explored. Two types of resilience narratives were reconstructed, reflecting different resources, strategies and changes in family beliefs that help families in the process of adaptation. While the findings of this study are limited by the small sample and selection of families from a parent selfhelp organisation, they provide an inside perspective of resilience processes and implications for treatment and counselling.

The quality of life in the girls with Rett Syndrome

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Purpose: Rett Syndrome (RS) is a gentic disorder affecting mainly females.In the majority of cases,it caused by a mutation in MECP2, an X-linked gene. Its product, methyl-CpG-binding protein 2, plays an important role in the regulation of gene expression and chromatin structure.RS is a neurodevelopmental disorder of early postnatal brain growth in girls.Patients show a normal neonatal period with subsequent developmental regression and a loss of acquired skills,decerelation of head growth,and development of typical hand stereotypies. RS have an hard impact on ill children's life style, psych and physical development.In this study we evaluated the impact that the RS has on children and adolescents' quality life. Method:For this study,we have used the Impact of Childhood Illness Scale (Hoare and Russell,1995). It includes 30 questions that value 4 aspects of the child and family's style:illness and its treatment, impact on the child, impact on parents and impact on the family. For every question,it considers the frequency of the problem and the degree of the worry that it causes.We grave the questionnaire to 16 couples of parents' children with RS (age range 6-11 years). The majority of cases (12 girls) are caused by de novo mutations in an X-linked MECP2 gene. Results: All parents compiled the questionnaire. The obtained results showed that developing age subjects, who are affected by RS, can have emotional and behavioural difficulties that have an effect on subject and on his family. Thei parents live in a continuous stress state because chronic pain and anxiety cause depression, a sense of inadequancy and frustrazion. Conclusion: As in every chronic physicall illness, the sick child and his family are obliged to face a series of physical, behavioural and emotional changes. Further research will be undertaken to assess the usefulness of the questionnaire on a larger and more representative group of children with RS.
Key words: Quality of Life - Rett Syndrome - Child

Rett Syndrome – To teach me is to understand me

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This lecture discusses ways of learning for girls with Rett syndrome (RS). Learning is a basic and essential need for every human being. For girls with RS, learning and communication are sources of strength; however due to the complexity of the syndrome, they require methods of learning and teaching which are suited to their needs, in an environment that provides communication and communication aids. Girls with RS require an adjusted learning environment, together with a clear knowledge and belief in their abilities on the part of their teachers.
How to teach a child with RS:
"Look at children with the normal development and ask not why, but how"
Principles for teaching girls with RS:
1. A basic belief should be that they understand
2. Accessibility of the educational and communicational environment
3. Provide strategies for choosing and communication
4. Acknowledge the Apraxia element of the syndrome;.
5. Find motivational factors – the girls learn better in a relevant situation.

6. Understanding – provide options for learning - Teach, don't test
7. The girls need challenge, repetition and mediation..
8. They need peer learning for enjoyment and play –. Acknowledge the fact that they learn from exposure, but try to create active interactions.
9. Use creative and varied teaching aids
1) Relevant learning areas for girls with RS:
2) Development of communication aids for expression and choosing
3) Expansion of world knowledge and vocabulary
4) Exposure to written language in their daily environment and through book reading
5) Learning language characteristics – letter recognition, rhyming, similar words, etc.
6) Teaching of age-appropriate academic subjects
It is our duty to open the door for them, and not their duty to guess what we expect them to do.

The experiences of parents who have a child diagnosed with a genetic syndrome: Rett Syndrome

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Little has been published about the experiences of parents who have a child with Rett Syndrome. This research explored the experiences of 26 parents and 1 sibling of a child with Rett Syndrome recruited as part of the Cardiff Rett Syndrome Project. Semi-structured interviews generated in-depth data and thematic analysis revealed 9 major themes forming a common storey in the participants' experiences. This story began with the parents' initial concerns about their child and their feelings on receiving a diagnosis and ended with their beliefs about the future, including their uncertainty, need to take each day as it comes, and their hope that a cure for Rett Syndrome will be found. From these themes, five key areas of discussion emerged: Feelings of hope yet dread; experience of both certainty and uncertainty; feelings of luck despite being 'unlucky'; striving to lead a 'normal' life whilst life is 'different'; and beliefs about Rett Syndrome. The findings will be presented to give an insight into the experiences of parents of a child with Rett Syndrome.

Mouse Models 2

Expression of methyl CPG binding protein 2 (MECP2) during the postnatal development of the mouse brainstem

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MeCP2 is a member of the methylated DNA binding protein family that is able to modulate the transcription of target genes. Mutations in MECP2 lead to a wide range of neurological phenotypes and the better known of these diseases is Rett Syndrome. All patients having a mutation in MECP2 have mental retardation and most of them exhibit important defects of autonomic functions. The brainstem represents the key part of the brain involved in the autonomic regulation and an immaturity of the brainstem has been proposed in Rett Syndrome. To better understand the role played by MECP2 in the development and maturation of the brain, different studies have examined the spatiotemporal expression of Mecp2 mRNA and protein in the brain excepted in the brainstem. In the present study, we decided to study Mecp2 during the postnatal development of the mouse brainstem in order to establish correlations between changing in Mecp2 expression and affected functions in the Mecp2 deficient mice. We selected different areas known to regulate breathing, deglutition, heart rate, blood pressure and arousal such as, the ventrolateral medulla, the ventrolateral pons, the area postrema, the nucleus tractus solitarius, the trigeminal nucleus, the dorsal vagal nucleus, the hypoglossal nucleus and the locus coeruleus. We immunoquantified the Mecp2 staining level directly in the nucleus of each area selected using densitometric analysis. For Mecp2 mRNA, we microdissected the different areas and we quantified Mecp2 mRNA by real time PCR. Our results show that all neurons are stained and the level of staining is highly variable depending of the area studied. The developmental pattern is mainly characterized by a postnatal decrease of the Mecp2 mRNA and an increase of the Mecp2 staining level showing a clear discrepancy between the Mecp2 protein and the Mecp2 mRNA levels. We tried also to correlate changing in Mecp2 staining level and the postnatal appearance of autonomic dysfunctions however, we were not able to make such correlations. Finally, the spatial and developmental heterogeneity of the Mecp2 distribution is in favor of a more specialized function depending of each specific brainstem areas.

The mecp2-null mouse hippocampus is prone to hyperexcitability and displays an altered basal inhibitory rhythm

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Rett syndrome is an autism-spectrum disorder caused by loss of function mutations within the gene encoding methyl CpG-binding protein 2 (MeCP2). Although MeCP2 is expressed throughout the body, the primary deficits of Rett syndrome arise from alterations of nervous system function. Although subtle decreases in synaptic plasticity have been detected within cortical and hippocampal neurons of Mecp2-null mice, relatively little information exists on how the loss of MeCP2 affects neuronal network activity in the brain. Using the isolated

hippocampal circuit as a model network system, we tested whether the intrinsic network activities of symptomatic Mecp2-null mice would differ from wild-type. Extracellular and whole-cell patch recordings revealed that although spontaneous IPSP-based rhythmic activity is present in Mecp2-null slices, its frequency is significantly reduced from wild-type. This reduction was not associated with alterations in the gross electrophysiological properties of hippocampal neurons, but was associated with a diminished level of basal excitatory drive within this recurrent circuit. In addition to showing this network alteration, we also show that the Mecp2-null hippocampal network is paradoxically over-responsive to excitatory stimuli, and that it possesses a high propensity for generating sharp waves – an excitation-dominant and self-sustained population event that arises from subtle alterations in the basal excitatory / inhibitory balance in the CA3 circuitry. Taken together, these results indicate that the Mecp2-null hippocampal network has a diminished basal inhibitory rhythmic activity, but that the circuitry is inherently prone to becoming hyper-excitable.

Cortical and hippocampal EEG recordings from MeCP2-deficient mice reveal alterations in basal brain wave activity, and heightened sensitivity to agonistinduced epileptiform discharge

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Rett Syndrome is a neurodevelopmental disorder caused by mutations in the Xlinked gene encoding transcriptional repression factor MeCP2. Rett Syndrome is characterized by a host of neurological features including mental retardation, apnea, motor impairments, abnormal baseline EEG and intractable epilepsy. Mutant MeCP2-deficient mice exhibit at least some of the cardinal behavioral features of Rett syndrome. However, it remains to be determined whether or not these mice display baseline EEG abnormalities reminiscent of clinical Rett Syndrome. To address this question, cortical and hippocampal EEG activity was compared among MeCP2-/+, MeCP2-/Y, and age-matched control mice. MeCP2-/+ mice were further categorized into young / pre-symptomatic mice (under 9 months) and older / symptomatic mice (12-16 months). Recording electrodes were positioned in the hippocampal CA1 and contralateral somatosensory cortex, and brain wave activity was investigated while the animals were in movement/ exploratory and immobile-awake behavioral states. Power spectrum analysis of the EEG CA1 activity during movement/exploration reveals significant lower frequency of theta rhythm (by 0.5 – 2.5Hz) in each of the MeCP2-deficient mice. This effect was most pronounced in the MeCP2-/Y group, where the average theta frequency was reduced from 8.7 ± 0.8 Hz in WT to 6.2 ± 0.9 Hz. In addition, irregular activity in both somatosensory cortex and hippocampal CA1 were detected in the majority of the older symptomatic-aged MeCP2-deficient mice. These irregular activity occurred during the immobile-awake state, typically lasted several hundred seconds, and had frequencies of 2-5 Hz. Furthermore, each of these older MeCP2-/+ mice also displayed brief oscillations of 6-9Hz in the somatosensory cortex in the immobile-awake state, which were exacerbated by the GABAB receptor agonist Baclofen (3mg/kg). Thus, these immobile state-related cortical oscillations appear to be mediated at least in part by metabotropic GABA receptors, but further examinations will be required to determine if they are of an "absence-like" nature. Collectively, these findings indicate that a deficiency in MeCP2 function in mice leads to alterations in the

normal brain wave activity with similarities to what has been observed clinically in Rett syndrome patients.

Introduction of heterozygous Reln deletion results in extended lifespan and delayed onset of cognitive deficit in MeCP2-deficient male mice

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MeCP2 is a global transcriptional regulator, and complete MeCP2 deficiency changes the expression level of many genes. One of the genes affected by loss of MeCP2 function is Reln. Its protein product, reelin, plays important roles during brain development and in signaling pathways that regulate neurotransmission at synapses. In gene expression microarray and real-time qRT-PCR studies we showed that Reln transcript levels are increased in the cerebellum of Mecp2-mutant mice (Jordan et al, BMC Medical Genet 8:36, 2007). By chromatinimmunoprecipitation (ChIP), we found that MeCP2 binds to the Reln promoter in brain tissue. We, therefore, hypothesized that Reln overexpression in MeCP2-deficient mice may be responsible for part of the phenotype. To reduce the level of Reln transcripts in MeCP2-deficient mice, we carried out a genetic complementation experiment by breeding males heterozygous for a Reln deletion (rl+/-) to Mecp2+/- females. We then compared the four genotype groups in male offspring: (1) Mecp2+/y, rl+/+ (WT); (2) Mecp2-/y, rl+/+; (3) Mecp2+/y, rl+/- and (4) Mecp2-/y, rl+/-. Real-time qRT-PCR using RNA from brain tissue revealed that Reln expression was increased in group 2, decreased in group 3 and not different from WT in group 4. In both groups 2 and 4, some mice started to die around 6 weeks of age, but the overall survival curves are significantly different with more mice in group 4 surviving longer. Since the reelin signaling pathway is involved in modulating synaptic activity in memory and learning, we performed neurobehavioral tests. The level of anxiety, as measured by an openfield test, was correlated with the level of Reln transcript. In a Y-maze test at 4.5-7 wk of age, group 4 mice performed as well as groups 1 and 3, while group 2 mice performed poorly. At 10 wk, however, group 4 mice performed as poorly as group 2 mice. No phenotypic rescue was observed for brain weight, performance on rotarod and nesting behavior by reducing Reln transcript levels. We conclude that genetic reduction of Reln transcripts in Mecp2-mutant male mice results in a somewhat extended lifespan and delayed onset of cognitive decline.

MECP2 directly regulates insulin-like growth factor binding protein 3 expression in brains

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Objective: Rett syndrome (RTT) is a major developmental degeneration characterized by mental retardation and specific autistic behavior. Mutations of the MeCP2 gene, encoding methyl-CpG binding protein 2, cause the disease. The pathomechanism of MeCP2 dysfunction leading to RTT phenotype is unknown. We discovered a novel MeCP2-downstream gene, which could be associated with the disease.
Methods and Results: As materials, we used mecp2-null mice and normal littermates for comparative expression study, and also human samples from Harvard Brain Tissue Resource Center. This study was permitted by the animal

ethic committee of our institute and was performed under informed consents. The promoter of IGFBP3 had a MeCP2 binding site, from the result of chromatin immunoprecipitation. Over-expression of IGFBP3 was observed in mecp2-null mice brains, using real-time PCR analysis. Although the number of IGFBP3-positive cells in the wild-type mouse brain might immunohistochemically decrease with age, mecp2-null mice showed wide distribution of IGFBP3-positive cells and fibers in the cerebral cortex. Western blot analysis showed over-expression of IGFBP3 was not only in mecp2-null mice, but also in human brain with RTT.
Discussion: Our study indicates that IGFBP3 is a MeCP2-downstream gene and MeCP2 can directly contribute to the transcriptional expression of IGFBP3 in brain. Moreover, pathological features of mecp2-null mice have some similarities of IGFBP3 transgenic mice, which show reduction of early postnatal brain growth. Over-expression of IGFBP3 due to lacking of MeCP2 may lead to delay brain maturation.

Prevention of cortical synaptic alterations and improvement of behavioural deficits of male and female mecp2 null mice by early environmental stimulation.

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Mutations of MeCP2 cause the majority of Rett syndrome (RTT) cases, an Xlinked disorder that affects 1:10000-15000 girls worldwide. MeCP2 mouse mutants recapitulate many features found in human syndrome. MeCP2 KO, for instance they are characterized by a short life span, breathing defects, motor impairments, deficits in synaptic plasticity, and alterations in neuronal morphology. Environmental enrichment (EE) is a housing condition that promotes cognitive, social and motor stimulation. EE has been found to be beneficial in animal models of brain disorders such as Parkinson's disease, amyotrophic lateral sclerosis, fragile X syndrome, Down syndrome and schizophrenia. In addition, EE is known to enhance BDNF production, raising the possibility that EE might be beneficial for MeCP2 KO phenotype. Indeed, recent data show that MeCP2 KO mice have reduced levels of BDNF in the brain and that crossing MeCP2 mutants with BDNF overexpressing mice prolongs their life span and causes a normalization of several morphofunctional features. To analyze the effects of EE on a murine model of RTT, we reared MeCP2 KO in large groups in cages containing toys and wheels from P10. In male mutants, we found that EE strongly ameliorated motor coordination as assessed with the rotarod test. Electron microscope analysis showed increased synaptic density in EE KO cerebellum and S1 cortex with respect to KO reared in standard conditions. EE was also able to compensate for the LTP deficits showed by MeCP2 KO in S1 slices and increased cortical BDNF levels. Finally, we observed a trend for a prolonged life span in EE KO. Female heterozygous mutants have been suggested to have higher than normal levels of anxiety. Using the open field test, we found that, at difference from standard MeCP2 KO female, EE mutants showed a behaviour similar to that of wt. These data demonstrate that MeCP2 KO phenotype can be attenuated by environmental stimulation.

Natural History

Movement disorders in Rett Syndrome: an analysis of 60 patients with detected MECP2 mutation and correlation with mutation type

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Rett syndrome (RS) is one of the best human models to study movement disorders: patients evolve from a hyperkinetic to a hypokinetic state, and a large series of abnormal movements may be observed along their lives: stereotypies, tremor, chorea, myoclonus, ataxia, dystonia and rigidity.
The aim of this work was to analyze movement disorders in RS patients with a detected MECP2 mutation, as well as their correlation with genotype, in a clinically and genetically well characterized sample of patients, and thus contribute to redefine the clinical profile of this disease.
We included in this study 60 patients with detected MECP2 mutations. These were categorized and grouped for analysis, according to: (1) type of change (missense or truncating, including nonsense and frameshift but also large deletions); and (2) location of the mutation.
Differences were found concerning the frequency of independent gait, dystonia, type of tremor, and global score severity when comparing the group of patients with missense and truncating mutations.
We also found differences in the presence, distribution, severity or type of movement disorders in the two groups of patients according to the median duration of the disease (less than 60 months; 60 months or more)
We conclude that movement disorders seem to reflect the severity and rate of progression of RD, patients with truncating mutations presenting a higher rate and more severe dystonia and rigid-akinetic syndrome, when comparing groups with similar time of disease evolution.

Evolution of stereotypies in adolescents and women with Rett Syndrome

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Stereotypies in Rett syndrome (RTT) are a diagnostic hallmark and are usually present in all stages of the disease. It is now accepted that hand stereotypies coincided with or sometimes preceded the loss of purposeful hand movements in early development of RTT girls. On the other hand, descriptions of movements disorders in adults with RTT are very scanty.
Since in our experience hand stereotypies remain present during the follow-up of older patients with RTT and, in a few cases, led us to molecular diagnosis in adult patients, we reviewed all adolescents and adults with RTT in order to describe their movement disorder.
Among 30 patients followed-up at present time at San Paolo Hospital in Milan, we selected those aged more than 14 years. We thus analysed 15 patients (median

age 18.8 years, range 14-31) affected by RTT, with or without MECP2 mutations. Information on early development was achieved by clinical charts and/or parents reports.
Classification of stereotypies was made using the one of Fernandez-Alvarez and Aicardi. All patients were video-recorded and informed consent was obtained by their parents for all video registrations.
Mean age at stereotypies onset was 17 months (range 8-24 months); stereotypies at onset tend to be maintained during evolution while new stereotyped movements can be detected in the follow-up.
All patients still present motor stereotypies involving separated or joined hands: most frequent hand movements were wringing, mouthing and twisting. Three patients showed leg involvement. Whole body stereotypies were detected in 2 patients (trunk rocking). In 6 pts stereotypies became simpler with a direct correlation with worsening of rigidity.
We confirm that in RTT pattern of manual stereotypies is maintained throughout different ages; in some patients, but not in all, stereotypies tend to become simpler with increasing age as patients become more rigid. Stereotypies as well as other movement disorders encountered in RTT have to be interpreted as a sign of dysfunction of nigrostriatal-dopaminergic pathway.
Moreover we underline that stereotyped movement persist in older patient and can be useful in order to suspect the RTT diagnosis in adult age in otherwise unclassified patients.

Rett Syndrome in adult women: data from the British Isles Rett Syndrome Survey (BIS)

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The few previous studies of adult women with Rett Syndrome (RTT) suggest that: the four main predictors of severity, reflected in survival, are: ability to walk, epilepsy, scoliosis and symptoms of brainstem over-excitability. There are some features which tend to improve, some which remain unchanged and others which deteriorate in adult RTT. We present a descriptive study of the clinical features that characterise the adult survivors of Rett syndrome, recruited to the British Isles Rett Survey (BIS).
Clinical data for 266 women of 20+ years with a clinical diagnosis of RTT (modified Hagberg criteria) were ascertained. Most were aged 20–29 years (158 patients), 79 were 30–39 years, 22 were 40–49 years and 7 were 50–59 years. Learning disability was present in 100% cases. 97.7% had hand stereotypy, 49.2% could walk. Those on antiepileptic medication diminished from 70% in their twenties to 29% in their fifties. A further 13% had previous seizures. Severe scoliosis was present in 42.9%, (75% of whom were in their 20's). 41.4% had less severe scoliosis and 11% did not have scoliosis. Breathing irregularity was found in 78% women and vacant spells in 72%. Features representing a milder phenotype included: 7% with preserved speech, 4% without regression and 7% with preserved head growth.
The paper reports the causes of death in 25/37 women who died.
MECP2 mutation analysis was carried out in 118 patients and a mutation identified in 89 (=75.4%). These mutations included 41 missense, 26 early truncating, 4 late truncating, 11 C-terminal deletions and 7 large deletions.

We found a predominance of patients with milder phenotypes in our population. Walking is even more common in older women than reported. Epilepsy tends to improve with age and may resolve. However breathing difficulties persist. The oldest patients tend to have less severe scoliosis. All common MECP2 mutations were found in the study group.
There is a particular need to increase awareness of RTT and its clinical variability amongst staff caring for adults with disabilities, so that they can identify and meet the needs of their adult patients with Rett Syndrome.

Survival rate of the originally described females with the Rett Syndrome.

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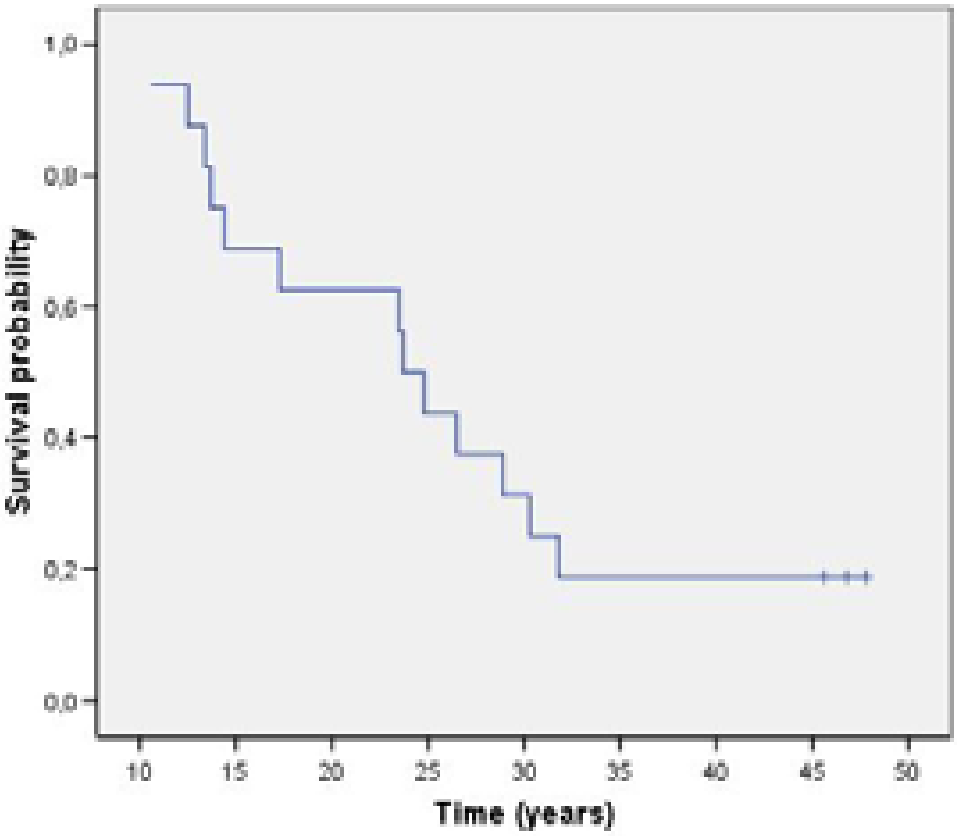
Rett syndrome (RTT) is a severe neurodevelopmental disorder typically affecting females. The condition was recognized and described in 1966 by Andreas Rett, but went relatively unnoticed for 17 years until the publication by Hagberg et al. Despite recent epidemiological data, little is known about the longevity of females with RTT. Reports from the Australian Rett syndrome Database (highest age of 28 years) give a survival rate of 98% at 10 years and 77.8% at 25 years. We investigated the survival age of the 22 females originally described by Andreas Rett in 1966 and were able to establish the age at death of 16/22 patients. The mean age of death was 20.0 years (range: 10-32 years). Survival after the age of 25 was 50% (illustrated in a Kaplan-Meier graphic). Three of these patients are still alive at ages of 48 years (information of functioning is given in two cases). Contrasting these results to those from the Australian study suggests better early survival rate with recent improvements in symptomatic management.



Aging in people with specific genetic syndromes: the Rett Syndrome

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The aging process of people with intellectual disabilities has been a topic of interest in recent years. Nevertheless little is known about the aging process of people with specific syndromes, like Rett syndrome. Good knowledge of the specific healthcare problems in adults with intellectual disabilities and anticipating on these problems are important issues in providing healthcare for these persons.

In association with the Dutch Rett syndrome parent association, 70 postal questionnaires were sent to the contact persons of the females aged at least 16 years with a clinical diagnosis of Rett syndrome. The questionnaire consisted of general questions, questions about living conditions, skills, physical and psychiatric morbidity. The response rate was 76% (n=53).

In general adults with Rett syndrome seemed to be reasonably healthy, whereas neurological, respiratory and behavioral morbidity appeared to be of great influence. High dependency needs are confirmed. In contrast with underweight, overweight is a relative unknown problem. Kyphosis increases with age, whereas autonomic dysfunction and non verbal communication reach a plateau or improve with age.

The adult RTT group has a more or less stable condition sometimes for many

years to come. In order to get a better understanding of the aging process and the individualized approach in RTT at every age longitudinal studies are of great importance.
Features of autonomic dysfunction deserve more medical attention, especially the interrelation between quality of sleep, respiration and behavior in RTT.

The relationship between morbidity and health service use over time in a Rett Syndrome population

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Background: The relationship between clinical variation of Rett syndrome and MECP2 mutation has not been studied longitudinally. This study aimed to investigate the trajectories over time of health status and health service use of people with Rett syndrome according to mutation type.
Methods: Data was obtained from four waves of questionnaires administered over a period of six years on 256 subjects from the population-based Australian Rett Syndrome Database. Health status (episodes of illness and medication load) and health service use (general practitioner and specialist visits and hospital stays) were summarized into composite scores with Principal Component Analysis. Linear and mixed regression models examined effects of mutation type and other variables on these scores over time.
Results: For some mutation types (p.R255X,p.R168X) health status was poorer at a younger age and improved over time, while for p.R133C it was better at a younger age and deteriorated with time. Health service use also varied by mutation type and age. Those with p.R133C had the highest health service use at the age of 25 years and the lowest at the youngest age. With other mutations, such as p.R255X, p.R270X, p.R294X, C terminal and p.R306C, there was a high level of health service use at a younger age, but this dropped off significantly by the age of 25 years to little or no use.
Conclusions: Health service use generally declined in parallel with deterioration in health status, although this pattern differed by mutation type, demonstrating important variability in the course of Rett syndrome.

Clinical and Therapeutic Management

Clinical intervention; regaining or retraining function and examples of motivating activities

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Studies for my Licentiate thesis show loss of motor abilities and many difficulties for someone with Rett syndrome. This research also shows the possibility to keep, regain/ retrain and develop new motor functions after the regression period for some. To distinguish between regain and retrain in Rett syndrome may be a problem sometimes because of dyspraxia which makes it difficult to use existing abilities. To regain or retrain a small ability is as important to the individual as a more complex ability may be to another person.
Individual analysis and individually planned intervention is important to help persons with Rett syndrome keep and develop abilities according to their individual resources. Motivation, having fun, is important in order to have positive result.
Clinical intervention will be shown through photos and videos on:
1. Retraining/regaining walking after many years for a grown up woman with Rett syndrome.
2. Regaining sitting without support. After surgery twice for scoliosis this girl could no longer sit without support. One year after the last surgery she was assessed at the Swedish Rett Center where she after intervention could sit unsupported again. And she has kept the ability.
3. Activities in the pool and on treadmill will be shown.

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Long-term follow-up of functioning after spinal surgery in patients with Rett Syndrome

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Summary: In a prospective study, twenty-three consecutive girls with Rett syndrome and neuromuscular scoliosis were evaluated for functioning at a longterm

follow-up. The patients had mostly improved, which was confirmed by their parents.
Introduction: Rett syndrome is associated with neuromuscular scoliosis and has a typically long C-shaped thoracolumbar kyphoscoliosis. Prospective long-term follow-up studies related to these patients' total situation are sparse. Most studies focus on the Cobb angle of the scoliosis, whereas parents are mainly concerned about the girls' continued functioning.
Methods: Twenty-three patients with Rett syndrome and neuromuscular scoliosis were evaluated preoperatively from 1993 to 2002. At follow-up, nineteen patients remained in the study. Three patients died (not due to surgery), and one patient could not participate because it was too far to travel. Mean follow-up time was 74 months (range 49-99 months). The assessments comprised the angle of scoliosis, sitting balance, seating supports in wheelchair, weight distribution, time used for rest, and care given. Follow-up questionnaires and two-open-ended questions about the positive and negative effects of surgery were sent to parents.
Results: Cobb angle, sitting balance, number of seating supports in wheelchair, weight distribution, and time used for rest had all improved after surgery. The parents assessed improvement in seating position, daily activities, time used for rest, and cosmetic appearance.
Conclusions: Most patients showed improvement in objective parameters. Despite experiencing stiffness in the spine, the girls became more active and healthy after spinal surgery. They were followed for more than four years, and the small number of discontinuations strengthens the results.
The evidence of positive surgical effects is of great importance to the family in the preoperative decision-making process.
Keywords
Rett syndrome, neuromuscular scoliosis, long-term follow-up, functioning

Surgery for scoliosis - and afterwards. Case reports

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Research reports on the risk of loss of motor abilities for someone with Rett syndrome, but there is also research reporting on the possibility to keep, regain/ retrain and develop new motor functions for some.
A questionnaire to all 178 families in Sweden with a girl or a woman diagnosed with Rett syndrome in 1997 provided us with 125 reports on families´ description of early development and development over the years. Seventy-four per cent developed a scoliosis of different severity and 24% reported kyphosis. Reported age when scoliosis started ranged from 0-20 years of age. Few were able to use braces, and surgery was performed on 19.
Clinical experience and studies report difficulty in using braces for someone with Rett syndrome and surgery is needed when scoliosis advances.
After surgery for scoliosis it is also important to be aware of dyspraxia, to remember earlier abilities, make individual analysis and individually planned intervention.
Case reports will be presented on 3 girls with Rett syndrome after surgery for scoliosis. The girls had different abilities before surgery – one not able to walk by herself, one walking with support and one walking by herself.
One girl using braces will be reported.

Clinical intervention will be shown through photos and videos.

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Improving functional skills and physical fitness in children with Rett Syndrome

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Background: Rett syndrome (RS) is the second most common multi-handicapped syndrome in females after Down syndrome. It is believed that the fourth stage of RS; late motor deterioration, is due to reduced activity levels rather than to an actual stage of this syndrome. Therefore, if functional and ambulatory abilities in this population are preserved and enhanced, the fourth stage might be avoided or delayed, thereby enhancing the individual with RS and her family's quality of life.

Aim: To investigate the feasibility and the results of a physical fitness program for persons with RS.

Methods: A daily treadmill, training program was implemented with four children with RS over a period of two months with three tests performed, two months apart with intervention taking place between tests 2-3.

Participants were four girls with RS (Mean age: years, (SD): 10, (±1.5)), attending an educational facility at a special education center in Israel. All participants had independent mobility and with typical characteristics of RS stage III. The training took place on a treadmill (by Trimline Company, capable of very low speeds <0.5km/h) by non-professional personnel. Pulse was monitored constantly during exercise by a polar pulse transmitter belt. The change in pulse measurements at rest and during training were considered as evaluators of aerobic physical fitness.

Functional measurements were based on a scale especially designed for the present study. The scale consisted of 27 items which measured different motor functioning of the participants.

Results: The intervention results showed that physical fitness of the children at the end of the training program had significantly improved ($P< 0.05$). Tests showed that general functional abilities had also significantly improved ($P< 0.05$). Moreover, specific items on the functional scale (knee walking, going up and down

stairs and speed of walking) also showed statistically significant improvement. High negative correlation (r=-0.76) was found between the reduction in pulse (suggestive of improved physical fitness) and functional improvements.
Conclusions: Physical fitness program executed on a daily basis is capable of improving functional ability of children with RS. Nonprofessional personnel can execute such a program under the supervision of a qualified physical therapist.

Postoperative care after vertebral arthrodesis of children with Rett Syndrome

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Between 2005 and 2008 we treated in our intensive care unit 10 children affected by Rett syndrome associated with severe scoliosis after receiving a vertebral arthrodesis (10 children with posterior approaches and one child with an additional anterior approach). The mean patient age was 14 +/- 2 years. Nine cases had deglutition problems of different severity, with one patient needing tracheotomy and gastrostomy prior to operation. Spiro metric testing revealed satisfying lung volumes, however, blood gases in two patients demonstrated elevated pCO2. Two patients needed walking aids prior to operation.
Mean time of postoperative assisted ventilation was 5 days. Respiratory care after extubation included manual respiratory physiotherapy, mechanical physiotherapy using Cough Assist and intrapulmonary percussion ventilation and a ventral position of the patient (luge).
Assistance for alimentation included tube feeding followed by careful restart of oral alimentation under guidance from a speech therapist.
The clinical evolution was generally uncomplicated. However, we observed one case of uncomplicated pneumonia, which resolved quickly following antibiotic treatment. No infection of the operation site was observed.
Remarkably, 10 years ago, the postoperative outcome of a similar patient cohort was complicated by a more than 30% rate of morbidity and mortality mainly following severe deglutition problems, which led to refuse arthrodesis operation for these patients.
We conclude that the improvement of the postoperative care widens the indication for arthrodesis operation towards patients with a previously unfavourable outcome.

The effectiveness of light-weight dynamic thermoplastic hand splints in reducing stereotypical hand-movements in 3 girls with Rett Syndrome

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Objective: To establish whether the daily use of light-weight, custom-made, thermoplastic dynamic splints can aid in decreasing stereotypical handmovements in girls with Rett syndrome.
To establish whether the daily use of thermoplastic dynamic splints can lead to increased functional hand movements during school activities.
Methods:
- Single subject A-B-A design
- Daily wear of splints for 2 hours each morning and 2 hours each afternoon

Results:
- Some increase in functional hand use
- Some decrease in stereotypical hand movements
- Some carry over when splints not worn

Conclusions: The use of light-weight dynamic thermoplastic hand splints does decrease the stereotypical hand movements exhibited by girls with Rett syndrome, when worn daily for 4 hours.

There is slight increase in functional hand use during school activities.

More research is needed in this area to add to the empirical data to support the use of dynamic hand splints in girls with Rett Syndrome.

CDKL5

The CDKL5 disorder - a description of 19 pathogenic mutations and associated phenotype

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We describe the clinical features of 19 patients (18 females and 1 male) identified in Cardiff with pathogenic CDKL5 mutations, 9 of which have not been reported. Twelve were referred by clinical geneticists and 7 by paediatric neurologists, on the basis of profound learning disability and early onset seizures. The age range of patients was 1 - 18 years (mode 7 years).

We identified 19 novel pathogenic mutations in the CDKL5 gene: four deletions/ insertions, four nonsense, four missense (all within the conserved catalytic domain) and seven which alter splicing.

Detailed clinical data were available for 15 patients. All had hand stereotypy, with limited hand skills in all but one. 86% had acquired microcephaly. Five made no developmental progress and only two learned to walk. One patient learned to talk in phrases, though this was not useful for communication. Some eye contact was present in three. Hyperventilation or Valsalva breathing was seen in three, but none of these had vacant spells. Mood lability was seen in five and did not correspond to those with breathing irregularity. Five had scoliosis, six had cold feet and three had gastro-oesophageal reflux. The male phenotype was indistinguishable from female.

All presented with epilepsy by 12 weeks of age (range 12 hours - 12 weeks). Initial epilepsy was varied with brief seizures gradually increasing in frequency and intensity. Five developed infantile spasms, none of which responded to treatment. All but three developed severe intractable epilepsy evolving, in the majority, in to myoclonic encephalopathy. Two had seizures which were reasonably well controlled on a single anti-epileptic drug and one had no seizures, aged four years.

Dysmorphic features were noted, including a long face with deep-set eyes, flattened mid-face, thick lower lip and thin upper lip, with gaps between the teeth.

Our data confirm that infantile onset epilepsy in a male or female with profound learning disability, should prompt the clinician to think about CDKL5 analysis, particularly where myoclonic seizures are present. The sources of referral for CDKL5 analysis suggests a need for increasing awareness amongst clinicians caring for adults with learning disability.

Mutation analysis of the X-linked cyclin-dependent kinase-like 5 gene (CDKL5) in patients with early-onset epileptic encephalopathy and Rett-like phenotype.

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Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5; OMIM 300203) gene have been detected in children with severe encephalopathy and intractable early-onset seizures often accompanied by clinical features reminiscent of the Rett syndrome phenotype.
We are conducting a collaborative study aimed at investigating the molecular bases of encephalopathy of the first year of life and defining the role of different disease genes in the etiopathogenesis of these clinical entities.
We report here the results of the molecular analysis of the CDKL5 gene, so far performed in 24 individuals, 10 males and 14 females, with a clinically heterogeneous phenotype ranging from early epileptic encephalopathy to atypical Rett syndrome with early occurring seizures. All the studied individuals had previously tested negative for mutations of the MECP2 gene. Mutation scanning of the CDKL5 gene was conducted by PCR amplification of the entire coding sequence, exons 1-21 and intron-exon boundaries, followed by dHPLC analysis (Transgenomics Wave Denaturing High-Performance Liquid Chromatography), and direct sequencing of altered fragments.
We have found 3 known polymorphic variants in two patients. Two of these variants (c.3003 C>T and c.3084 G>A), had been reported in Caucasian individuals as belonging to a rare conserved haplotype. We have detected 3, de novo, disease-causing mutations: two missense mutations in female subjects and a late truncating mutation in a male individual with a 47, XXY karyotype (individually reported in a separate abstract). None of the putative pathogenic mutations we have detected have been previously described in literature. The results of our study, even though preliminary, underline the relevant role played by CDKL5 in the etiopathogenesis of clinical conditions bridging epileptic encephalopathy and Rett syndrome features.

CDLK5 mutations in Rett patients with early onset epilepsy

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MeCP2 mutations have been idenfied in 50% of atypical cases of Rett syndrome (RTT). Atypical RTTs include a subset of more severe forms characterised by the onset of the disease shortly after birth, congenital hypotonia and infantile spasms. The finding of apparently balanced X/autosome translocations disrupting the CDKL5 gene in two unrelated females, clinically characterised by early-onset generalized seizures, hypsarrhytmia and mental retardation has been instrumental to identify a second gene responsible for the most severe forms of RTT.
CDKL5 is predicted to code for a serine/threonine kinase sharing homology with members of the MAP- and cyclin-dependent ,CD- kinase families and has been demonstrated to have a crucial role in MeCP2 phosphorylation in vitro; the two proteins seem to belong to the same pathway and directly or indirectly interact.
Up to now 32 unrelated patients have been reported to be mutated in CDKL5:

mutations span the whole gene mainly affecting the catalytic domain and the large COOH region. We refer on mutational screening of 115 patients, 18 males and 97 females, recruited on the basis of early onset seizures and sorted from three main cohorts: atypical Rett, negative after MeCP2 sequencing, Angelman syndrome, with unknown mutations, and autistic patients. Six patients carrying novel mutations were identified among the Rett cohort: a splicing mutation skipping exon 4, a two exon deletion, two stop and two missense mutations, all de novo and with balanced X inactivation. Interestingly one of the missense mutation shows mosaicism in blood. All girls had a precocious onset of epilepsy with daily mioclonic seizures and one of them showed phenotypic signs fitting Angelman criteria.
Lymphoblastoid cell lines were established from four of the mutation carriers to assess the consequences of the mutations. RT-PCR showed the presence of the aberrant transcript in all cases apart those with truncating mutations, in keeping with NMD (nonsense mediated decay) rules. The phenotype seems to be more severe in the presence of aberrant transcripts.

Early onset seizure variant of Rett Syndrome: toward the definition of the clinical diagnostic criteria

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We report here a detailed clinical investigation of 8 CDKL5-mutated girls in order to delineate the clinical diagnostic criteria for the Hanefel variant of Rett syndrome. Four were previously reported (Scala et al. J Med Genet 2005 and Mari et al. Hum Mol Genet 2005) and 4 are novel (p.R178W, p.Q347X, p.N71D, pV132G). All patients were evaluated by two different clinical geneticists (FM and AR). Patients range from 14 months to 9 years and have a similar clinical course and comparable degree of severity. All present epilepsy with an onset variable between 10 days and 3 months of life. The type of seizure at onset is different and during the course of the disease each patient experiences multiple seizures types. After the introduction of a new antiepileptic drug patients have a short free period but epilepsy progressively relapse despite the treatment. The patients do not have a classic regression period due to precocious timing of seizure onset. Parents refer that even if the perinatal period was apparently normal the girls were irritable, easy to cry, drowsy and poor sucking. All patients show typical persistent stereotypic hand movements affecting severely the ability to grasp thing. It is to note that the psychomotor development is severely impaired in all cases: the older girls can sit unaided but they can not stand up neither with support while the younger girl, 14 months old, has not yet acquired the ability to maintain the head. Interestingly, all the girls have an head circumference within the normal range both at birth and at the time of clinical examination indicating that head growth should be considered a minor criteria for the Hanefeld variant of Rett syndrome. Each patient were classified using a severity score that include the evaluation of 22 different clinical signs (for clinical score see Renieri et al Brain Dev in press). Results were compared with

128 classic MECP2-mutated patients and 25 PSV MECP2-mutated patients, all evaluated by the same clinical geneticists. Both the statistical and the descriptive approach were used to delineate clinical diagnostic criteria for the early onset seizure variant of Rett syndrome.

A novel CDKL5 MUTATION in a 47,XXY boy with the early-onset seizure variant of Rett Syndrome

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Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene have recently been found in patients with severe neurodevelopmental disorders, including infantile epileptic encephalophathy, severe X-linked infantile spasms (ISSX) and mental retardation, autism, and the early-onset seizure variant of Rett syndrome. As part of a clinical and molecular study we are conducting on encephalopaties of the first year of age associated with epilepsy, we have ascertained a child presenting with a severe early-onset epileptic encephalopathy, mild dysmorphic features, global developmental delay, and profound intellectual and motor impairment reminiscent of Rett syndrome. A standard karyotype analysis showed the presence of two copies of the X chromosome (47XXY by ICN 2005). A mutation scanning and quantitative analysis of the MECP2 gene coding sequence resulted negative, while the molecular analysis of the CDKL5 gene allowed the identification of a de novo heterozygous mutation at nucleotide 1675 of the coding sequence (c.1675C>T) resulting in the creation of a premature stop codon (p.Arg559Stop). The pattern of X chromosome inactivation was found to be balanced. This pathogenic CDKL5 mutation, never previously described, truncates the large COOH-terminal region of the gene, crucial for the proper subcellular localization of the CDKL5 protein.
We report the second case of intragenic CDKL5 mutations found in a male subject, the first one detected in an individual with 47,XXY karyotype. We would like to draw attention on the importance of considering the causal involvement of CDKL5 in males showing early onset seizures and Rett-like clinical features, as well as other phenotypes that have been related to mutations of this gene in females.

MECP2 regulates CDKL5 expression by binding to its methylated gene promoter

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We recently characterized the mode of action of cocaine and fluoxetine, that involves the induction of several epigenetic factors, including the methyl-CpGbinding proteins MeCP2 and MBD1, as well as the histone deacetylase HDAC2. Mutations within the MeCP2 gene is associated with Rett syndrome, while a Rettlike syndrome has been associated with mutations within the cyclin-dependent kinase-like 5 (CDKL5) gene, suggesting that the two genes are somehow related. Since CpG islands are present in the 5'-flanking region of the CDKL5 gene which was found to be repressed by cocaine, we examined whether CDKL5 was regulated by cocaine through a mechanism comprising DNA methylation. Using real-time RT-PCR, CDKL5 repression was found in rat striatum in response to acute cocaine treatment and was maintained after 10 daily injections. Bisulphite

genomic sequencing analysis revealed the presence of methylated CpG within the CDKL5 proximal promoter, in which an 73 % increase in CpG methylation level was observed following chronic cocaine treatment. The increase was associated with removal or addition of methyl residues on specific CpG sites, which was confirmed by methylation-specific PCR. Accordingly, using chromatin immunoprecipitation experiments performed with anti-MeCP2 antibody, MeCP2 was found to bind to the CDKL5 promoter and a 2.5 fold increase in binding was observed in response to cocaine. The data demonstrate that cocaine repressed CDKL5 expression by a direct interaction of MeCP2 with CDKL5 promoter. They also establish that treatment with a pharmacological agent is sufficient to modify the methylation pattern of a given gene in adult brain cells, which was acquired during development. The identification of CDKL5 as an in vivo MeCP2 target gene provides new insights into the mechanism by which mutations within the two genes lead to common neuropathological symptoms.

Therapeutic Management

The musement: music/motor function

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Musement is a music and movement program developed by music therapist Märith Bergström-Isacsson and physiotherapist Gunilla Larsson at the Swedish Rett Center. The songs and movements are intended for young children with or without disabilities integrated in group activity. Experience has shown that music is a good path to communication and understanding for children with Rett syndrome and other disabilities (1). Music in itself is movement involving sound fluctuations that reach our ears and our body that in turn generates and supports movements (2).

Working with body movements purposefully, beginning when children are small, may help prevent future problems. The movements are integrated to specially written songs, providing participants with an enjoyable experience. Each song and movement is accompanied by brief description of how to perform the movements, and the objectives they are intended to achieve.

Musement has been carefully formulated and each exercise contains objective both for music therapy and physiotherapy. Musement works with multiple senses, both from the inside out and the outside in. The material includes a book and an accompanying animated DVD film.

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The value of music in everyday life working with Rett Syndrome

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Music is important for persons with Rett Syndrome — as attested to by Andreas Rett himself — but how can music be used? This presentation will give an overview of the possibilities for how to use music in everyday life, and how it can be applied more specifically as a therapeutic intervention for persons with Rett Syndrome.

Throughout history, and in all parts of the world, wherever remains of human beings have been unearthed or discovered, alongside have been found ’remains’ of music. We don’t know exactly why but music seems to be a biological need, something people will always require in their lives. This paper will connect the general biological human need of music to the value of music for persons with Rett syndrome (1). Research to date provides arguments for music preference (2), and the importance of music in communication and learning situations (3) for persons with Rett Syndrome.

Rett Syndrome is a neurological disorder that is found all over the world and the syndrome affects basic human functions such as communication,, movement and intellectual functioning. Rett Syndrome is one of the few diagnoses where the original medical authority, Andreas Rett (as early as 1966), reported music as a necessity and as a need for this population.

The presenter will illustrate different occasions where musical interventions are

important for the results with the help of video excerpts from clinical work.
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Specialised education from a multi professional prospective

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Specialised education

The St Margaret's Developmental Curriculum provides specific teaching strategies. The curriculum covers the development and integration of all the senses of taste, smell, touch, vision, hearing and bodily experiences. By the stimulation and awakening of these senses, those with Rett syndrome are enabled to make sense of the outside world and learning can take place.

Each student has their own specialised Individual Education Plan and associated tasks are completed in class groups, with school activities or 1:1 sessions. Activities are based on a multiprofessional approach, timetabled to give structure and promote anticipation through repetition.

Each class has an on-site named physiotherapist, occupational therapist and speech & language therapist. Students have individual therapy programmes designed to maximise their physical potential, independence, communication and purposeful hand use.

Music therapy can help children with Rett syndrome to develop their social interaction skills and is provided by our on-site music therapist on a one-to-one basis or in small groups.

Aromatherapy is provided on an individual basis by our qualified on-site aromatherapist.

How we support pupils with Rett Syndrome

Hand skills

- educational, therapy & care programmes are developed to encourage purposeful hand function;
- intervention programmes are developed where necessary to support students who may self harm.

Balance, standing & walking skills

Students are supported to maintain these skills by taking part in the following:

- independent walking programmes;
- splinting programmes;
- individual physiotherapy programmes;
- horse-riding, cycling and hydrotherapy;
- independent sitting programmes.

A wide range of aids are used as appropriate to individual needs (e.g. handling belts, walking frames, Bambach chair, balance boards).

Unveiling hidden resources - communication and

learning in individuals with Rett Syndrome through music therapy

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Individuals with RS are often speechless; however this doesn't mean they have nothing to say. Unveiling the person's wishes and desires could place special challenges as she may not exhibit understanding or other communicative capabilities. Revealing hidden communicative and learning abilities in RS call for the use of different motivational and expressive mean that are meaningful to the individual. Music therapy can be one of the means in which communicative and learning development can take place as it is greatly loved by them.
This presentation would like to illuminate that despite severe developmental disability, most individuals with RS appear to have developed normally at first. We can then presume that they have had the early communicative, interactive and emotional experiences necessary for learning. When the progress of their condition is compared to Daniel's Stern's account of the development of 'he 'five senses of self' in infancy, it appears that many individuals with RS do acquire what Stern defines as an 'emergent self', the 'core self with others', the 'intersubjective self' and some may have begun to develop the 'verbal self'.
As a result of the drastic regression at stage II, a change in her interaction with others will occur, resulting in their responses and expressions toward her. This however does not mean that the she has lost the skills she had acquired till then. Music therapy can help to unveil the hidden resources and skills and more so, develop and enhance new ones.
This presentation will illustrate through many video excerpts communicative and learning abilities through musical interactions with individuals with RS. It will take into account music therapy research and years of clinical experience with this population; while integrating Daniel Stern's developmental psychology theory as well as Colwyn Trevarthen and Stephen Malloch's new psychology of movement in human communication which focuses on the intrinsic musical nature of human interaction.

Rebecca's story - positive change as a result of music therapy for a four year old child with Rett Syndrome

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At 18 months of age Rebecca's parents noticed changes in her behaviour,she became less interested in toys and games and lost the ability to play her toy drum. Rebecca's head growth slowed and she developed hand wringing. A clinical diagnosis of Rett Syndrome was confirmed following genetic testing. As Rebecca grew older,her parents sought music therapy. The evidence for music therapy indicates benefits in development and maintenance of glocal functioning. There is potential to stimulate cognitive development and give children a more fulfilling and sociable life (Elefant 2001,Hill 1997,Holck 2002,Wigram 2002). Rebecca's mother felt that the transformation was dramatic. It appeared that Rebecca's interest in music had been re-ignited. She would actively seek out her musical instruments in preference to other toys. She found the sessions

very stimulating and would continue to be vocal,chatty and very alert for some time after. Over a period of time,during sessions,her hand movements have become more purposeful. Rebecca now frequently holds a beater in her hand for sometimes as long as 8 seconds before discarding it. This skill has also been transferred to everyday living activities. She has tapped her hands in time to music,associated objects with songs,made choices through eye or finger pointing and has initiated turn taking activities. The relationship between Rebecca and her therapist has formed a central component of the treatment. Specific techiques have been used to develop eye contact with the use of Rebecca's name by varying pitch during improvised music making. Now,both in therapy sessions and at home music acts as a motivator to support the creative and meaningful movements Rebecca can make. Data recorded from video observation shows an increase in Rebecca's non-verbal communication,vocalisation,ability to hold an instrument or beater,take turns and initiative positive selection.

The Snoezelen approach for individuals with Rett Syndrome

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The multisensory treatment, also known as Snoezelen was established in the early seventies. The approach was initiated by Ad Verheul's, a Dutch art therapist. It emphasizes both the importance of the physical environment (an adapted sensory environment) and the attitude of the caretaker (an enabler).
The philosophy behind the multi-sensory treatment believes that when accepting the client as a person and adapting the environment to suit the client's needs, they become less stressed and more communicative. Today, the original understanding of the approach has been broadened. Few studies have been carried out trying to prove the efficacy of the multi-sensory philosophy as a therapeutic tool. So far results have been questionable.
In Israel the Snoezelen Approach has been used to intervene with individuals with Rett syndrome at different ages, with clients showing a variety of needs, for goals such as: improving hand function, improving balance and reducing falls, improving joint range of motion, and enhancing contact with family members.
The presentation will shortly address the principles for admitting patients for treatment, evaluation procedures, treatment plans, and will focus on measurable results.
The presentation will focus on three case studies:
1.A significant ($p < 0.05$) improvement in fall reduction
2.A significant enhancement of inter-personal contact and quality of familial bonding
3.A significant ($p < 0.05$) improvement in joint range of motion

Clinical Management

Retrospective analysis of patients attending Australia's first Rett Syndrome multidisciplinary management clinic

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Established in 2000, the Rett syndrome Multidisciplinary Management Clinic at the Children's Hospital at Westmead, Sydney, is Australia's first comprehensive clinic for the diagnosis and long-term management of females with Rett syndrome. Our team consists of a Clinical Genetist and Paediatrician, Genetic Counsellor, Dentist, Paediatric Dietician, Speech Therapist, Occupational Therapist, Physiotherapist, and Music Therapist. This unique clinic is regarded highly by both the professional community and the families it services. To date over 90 females and one male have been diagnosed and supported through the clinic aged between 18 months and 42 years.

Currently, members of the clinic team are working towards a retrospective analysis of the patients who attended the Rett syndrome Multidisciplinary management clinic at the Children's Hospital at Westmead, Sydney between the time the clinic was established in February 2000, until December 2007. With approval from the CHW Ethics Committee we have reviewed all medical records of females who have attended the clinic since 2000. Demographic data; clinical phenotype and genotype have been entered into a purpose built database with the assistance of the members of the management team. From these analyses we will ascertain the frequency and nature of complications of Rett syndrome in this cohort. We anticipate that this will provide an insight into the services, aids and supports that may be required and help us to provide a better service for these families. This retrospective analysis will also enable us to move forward with our management clinic.

Since the clinic first started we have observed that the age of girls at diagnosis has dramatically decreased. Some girls are now are being referred to us from Paediatricians, Geneticists and GPs from as young as 18months of age. We anticipate that early diagnosis and intervention will improve the long term morbidity of the disorder. This will also provide a valuable opportunity to study the long-term benefit of early management and intervention.

Detection of alert factors of malnutrition Rett Syndrome: a national prospective survey within the French national association (AFSR)

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Malnutrition is frequently associated with Rett Syndrome (RS). A self administered questionnaire was filled by parents members of the french association. The study related to a population-based cohort composed of 144 girls ages 2 to 18 y.o and 73 adults ages 18 to 48 y.o. was aimed to define the prevalence of malnutrition and the associated factors linked to the nutritional status. We defined malnutrition with girls whose Z score Weight/Height was < - 2 and with adults whose BMI was < 18,5 kg/m².
The overall prevalence of malnutrition was 29.2% with a significant higher prevalence in adults 44.6%. versus 21.5% in children (Chi 2 test, P <0.01)
Maximum time of the meal was significantly higher in the malnutrition group: 50±30,39 minutes versus 41.41±15.97 (t test, p=0.015).
These data suggested that the malnutrition risk increase with age.
Four factors were significantly associated with malnutrition as “Bad or uneven appetite” 36% versus 22% (p < 0,05), “Self feeding unability” 98% versus 83% (p < 0,01), “Cought after eating and during the meal intercourse” 21% versus 9% (p < 0,05) and “Alternation of hard and liquid stools” 34% versus 18% (p < 0,05).
Surprisingly, in this study, in the malnutrition group, 79 % of the parents estimated that their children were well nourished.
Our results confirmed the high prevalence of malnutrition in RS and showed associated factors with malnutrition expressing parent’s fear and malpractice suggesting low food intake. Detection of alert factors invite us to promote an early nutritional assessment and to develop educational program towards families.

Factors affecting skeletal integrity in an Australia Rett Syndrome cohort

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Introduction: Individuals with Rett syndrome have been shown to have an increased risk of fracture. This study investigated whether individuals with Rett syndrome have decreased bone mass density (BMD) and bone mineral composition (BMC) and how genotype, mobility, pubertal stage, epilepsy diagnosis and medication influenced these skeletal parameters.
Materials and methods: Subjects between the ages of 4 and 30 were recruited from the 288 verified cases in the Australian Rett syndrome database (ARSD). The Lunar prodigy Dual energy x-ray absorptiometry was used in multiple centres throughout Australia to measure the BMC and BMD of the total body and BMD of the lumbar spine (LS), femoral neck (FN) of each lower limb, lean tissue mass and fat mass in each subject. Normative data were used to calculate Z-scores based on age, height or weight of the RS cases. Subject BMI was calculated and plotted against the BMD and BMC in the total body and regional locations. Mobility level, Tanner stage, epilepsy diagnosis, anticonvulsant medication and genotype data for each participant were obtained from the ARSD.

Results: Data were available on 97 subjects with an average age of 15.0 years (SD=7.16). Bone density was very low for all sites measured with age BMD Zscores ranging from Zage=-2.9 (LS) to Zage=-3.56 (LFN). Univariate analysis showed that the genotype R168X, low mobility, epilepsy diagnosis and post puberty Tanner stage were associated with low bone density ($p<0.05$ in all sites). However multiple linear regression identified the R168X mutation type as the strongest predictor of low BMD in all sites and total BMC. Lean tissue mass for height was low (Zheight= -1.092) showing reduced muscle mass in these subjects.
Conclusion: Genotype affected bone density and mineral composition more than any other factor with the phenotypically severe p.R168 mutation showing the strongest association.

Increased fracture rate and bone turn-over is associated with low bone mineral density in girls with Rett Syndrome

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In Rett syndrome (RS), recent data suggest that osteoporosis results from various factors including immobilization, antiepileptic drugs and the MECP2 mutation. To provide a better understanding of the bone metabolism in Rett syndrome, we propose, in a cohort of girls affected with MECP2 mutations, 1) to evaluate the incidence of fracture; 2) to characterize the bone metabolism of these girls; 3) to look for contributing factors of osteoporosis in French girls with RS.
Eighty-eight girls (17.6±1.1 yrs; min 5.2; max 46.7) with RS and MECP2 mutations were enrolled. The parents reported 26 episodes of fractures out of 54 patients which is 2.5 times higher than the general population (3 times higher in young girls less than 18). They received 1.3 ± 0.2 antiepileptic drugs; ~50% of the patients walked everyday; their IGF-I level is 299.9 ± 17.2 ng/ml; their mean urinary calcium/urinary creatinine ratio is 0.47 ± 0.06 mM/mM ($N<0.3$); 45% of the patients have 25-OH vitamin D level less than 10 ng/ml indicating vitamin D deficiency. The bone mineralization was assessed by two independent investigators on the hand x-ray and found moderately or severely altered in 18 out of 32. The mean cortical/diaphyseal ratio was decreased: 0.49±0.02 indicating that they had a decreased bone density. In this cohort, the following parameters were associated with the occurrence of fracture: age, urinary excretion of calcium, PTH level, number of anti-epileptic drugs and the cortico-diaphyseal ratio. However, the vitamin D level was not associated with the occurrence of fracture.
This study confirmed previous reports of increased incidence of bone demineralization and fractures in Rett girls. Our results suggest that an increased bone resorption (elevated urinary calcium excretion despite a low bone mineralization) is likely involved in the bone fragility of these children and sustain the use of antiresorptive molecules in the treatment of osteoporotic girls with RS. In addition, we identified vitamin D deficiency in about half of the patients; this contributive factor could be easily avoided.
The authors thank the patients and the french association for Rett syndrome

(AFSR) for their contribution to the study.

The Israeli Rett Syndrome center. Evaluation and transdisciplinary play-based assessment

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Rett syndrome (RS) is a neuro-developmental syndrome of genetic origin, affecting mainly females. Individuals diagnosed with RS exhibit a variety of functional difficulties which impair their quality of life. The complexity of impairments and the differences between each child make reduces the child from reaching her full functional potential, thereby necessitating the administration of a skilled, individually tailored treatment. Since the foundation of proper treatment is based on a structured, well administered, insightful assessment, the individual with RS with her complex array of difficulties should benefit from such a procedure. This notion has led to the establishment of the Israel Rett Syndrome Center. The center includes a medical branch located at the Safra Children's Medical Center at Tel Hashomer hospital and an educational/rehabilitation team, which performs assessments in special education facilities and residential settings throughout Israel. The assessment team works within an ecological frame using the concept of play-based assessment. This means that the team works as close as possible within the individual's naturalistic environment. The end result of a two hour evaluation process is a detailed report, suggesting holistic intervention possibilities with each child.
This presentation will present the working model used by the education/rehabilitation team at the Israeli Rett Syndrome Center (IRSC). The manner in which the IRSC team operates will be vividly presented by means of video vignettes.
The principles and working characteristics of the IRSC model will be suggested here as a potential model for establishing additional teams, presenting similar evaluation services for other individuals with RS.

Rett Syndrome experience in 62 Mexican patients

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Since 1995 when the Mexican Rett Syndrome Association (AMSRAC) was founded began the clinical registration supported by a pediatric neurologist at National Institute of Pediatrics (INP) in Mexico City. This institute is one of the two most important public hospitals for children with medical residence training and health research, that receives patients from all our country, in particular those with very low social and economic resources. Over the past thirteen years up to date the AMSRAC has attended as many neuroscience meetings as possible, all over the country for awareness of the RS criteria diagnosis.
Material and Methods
We included 62 Rett Syndrome (RS) patients from AMSRAC data that have been evaluated on clinical evidences (mayor and support diagnostic criteria work group) by pediatric neurologists. All cases but 3 have hospital (INP) code number: two patients were older than 18 years and the other had no resource to attend himself to Mexico City. During evaluation at INP, we excluded other diagnosis (brain CT scan or MRI, EEG, ophthalmologic evaluation and amino acid metabolic screening) and attempted to create an atypical features table based on clinical issues, supported by Hagberg`s concept MECP2-deviant phenotypes.

Last year, we began the molecular biology diagnosis to complete the most recently cases.
Results
Eighteen (18/62) female patients stopped calling on the hospital appointments, so we decided to include forty three cases (aged 2 to 22 years old) divided into two groups:
I) RS Typical or Classic: 21/43
II) RS Atypical or variant: 22/43 (two of them were males)
Four different MECP2 mutations (R168X, T158M, R270X and R306C) were screening in 25 patients:
I) RS Typical or classic: 11/25
II) RS Atypical or variant: 14/25 (included the two males)
These mutations were identified in only 12 female cases as follows:
T158M: 4/25 (three were typical) R270X: 3/25 (all typical)
R306C: 3/25 (two were typical) R168X: 2/25 (all typical)
Discussion
Despite of AMSRAC efforts, we presumed many families with RS patients have to emigrate because of lack economic resources. More than a half of our cases were RS atypical on clinical grounds. The R168X, T158M, R270X and R306C mutations were found in most of RS typical or classic cases. T158M was the most common of MECP2 mutation in this small series. The same missense mutation was also predominant in Chinese patients published H. Pan, Y.P. Wang in 2002. The Government has to improved health resources to avoid patients emigration, meanwhile we suggest routine mutations screening in MECP2 during the first clinical evaluation in order to detect the heterogeneity of our country to study phenotype-genotype correlations.

Plasma leptin concentrations and oxidative stress in Rett Syndrome

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Background: In Rett syndrome (RS), elevated leptin concentrations, unrelated to weight balance, have been reported (Blardi P. et al, J Pediatr. 2007;150:37-9; Acampa M. et al., Neurosci Lett. 2008;432:69-72). Although a link between hyperleptinemia and sympathetic overactivity has been reported (Acampa M. et al., Neurosci Lett. 2008;432:69-72), the clinical significance and underlying mechanisms for hyperleptinemia in RS remain unclear. Several studies indicate a leptin / oxidative stress relationship, with both anti-oxidant, and pro-oxidant activities having been reported. In particular, exogenous leptin has been reported to increase lipid peroxidation and to inhibit the antioxidant system in the mouse brain (Kutlu S, et al., Tohoku J Exp Med. 2005;206:233-6),while greater susceptibility to ultraviolet-induced oxidative stress in genetically obese leptindeficient mice has been described (Katiyar S.K.& Meeran S.M. Free Radic Biol Med. 2007;42:299-310).
AIMS: We tested the hypothesis that high leptin concentrations in RS are related

to an unrecognized oxidative stress.
Methods: A total of 42 RS girls (age: M¡ÓSD, 14.7¡Ó7.8 yr) with MECP2 or CDKL5 gene mutations, and 24 age-matched healthy controls with body mass index (BMI) between the 5th and the 85th percentiles were enrolled in the study. Plasma leptin was determined by an ELISA kit (Quantikine - Human Leptin Immunoassay, R&D Systems, Lille, France), and its concentrations were expressed as ng/mL. Erythrocyte Desferioxamine (DFO)-chelatable free iron (IENPBI), plasma DFO¡Vchelatable free iron (p-NPBI), plasma free F2-isoprostanes (p-F2-IsoPs) and plasma protein carbonyls (p-PCs) were also determined. Data were expressed as means „b SD or medians (inter-quartile range). In order to explore possible univariate associations between variables, Spearman rank correlation was used.
Results: Mean plasma leptin concentrations in RS patients were significantly increased as compared to controls (25.2 „b 20.1 ng/mL vs. 12.6 „b 5.0 ng/mL, p=0.0037). Positive leptin/ p-F2-IsoPs (rs=0.541 , p=0.0128) and leptin/IE-NPBI (rs=0.526 , p=0.034) correlations were observed. Conversely, leptin /p-PCs and leptin/ p-NPBI relationships were not statistically significant (rs=0.359 , p=0.082 and rs=-0.128, p=0.2886, respectively).
Conclusions: Increased plasma leptin concentrations in RS are associated with a previously unrecognized condition of systemic oxidative stress.

Molecular Genetics

MECP2 activates the expression of nuclear proteins associated with chromatin remodeling in vitro

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Rett syndrome is a severe X-linked neurodevelopmental disorder mainly affecting girls and is the second most common cause of mental retardation in girls. The methyl CpG-binding protein 2 (MeCP2), a ubiquitous transcriptional repressor interacting with the chromatin remodeling machinery, is considered the major causative factor of Rett syndrome and related phenotypes, including autism. Our present study focuses on interactions between MeCP2 and chromatin proteins leading to changes in chromatin architecture and gene expression. We have developed in-vitro systems overexpressing the normal human MeCP2 and MeCP2 containing Rett causative mutations, as well as a MeCP2 knock-down system using specific siRNA. Studying these systems with a comprehensive antibody array including nuclear proteins implicated in gene regulation, we demonstrate that MeCP2 over-expression and knock-down is synchronized with the expression of a specific set of nuclear proteins including the hBRM/hSNF2a component of SWI/SNF, HMGB1 high mobility group protein, G9a histone methyltransferase and PRMT1 protein arginine methyltransferase, as well as the known HDAC2 histone deacetylase. In addition by means of coimmunoprecipitation,
we demonstrate a direct interaction between MeCP2 and hBRM/hSNF2a component of ATPase-dependent SWI/SNF complex involved in global chromatin remodeling mechanism. Moreover, MeCP2 over-expression is associated with a delayed maturation of synaptic connections among cultured hippocampal neurons. Our findings suggest that MeCP2 acts through parallel mechanism of chromatin remodeling involving HDACs and SWI/SNF complex, thereby inducing local as well as large scale changes in chromatin architecture and compaction. More recently, we have investigated in these experimental systems the effect of valproic acid (VPA), a known anticonvulsant and HDAC inhibitor commonly used for the treatment of epileptic symptoms in Rett patients. Our preliminary data show that VPA partially restores siRNA-induced MeCP2 deficiency in vitro, an observation we would like to support by analyzing peripheral blood samples of VPA-treated and untreated patients with Rett syndrome.

Characterization of HIPK2 that, by associating with MECP2 might function as a modifier gene in Rett Syndrome

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Mutations in the methyl CpG-binding protein 2 (MECP2) gene on Xq28 are

responsible for most cases of classic RTT whereas less than half of the patients with variant RTT forms carry mutations in MECP2. It therefore seems that other genes are involved in causing RTT; moreover the fact that there are patients with milder phenotypes in spite of severe mutations argues that modifier genes might restrict the clinical outcome by regulating MeCP2 functions. To search for MeCP2 interacting proteins involved in RTT we performed a yeast two-hybrid screening and identified HIPK2 (homeodomain interacting protein kinase 2) belonging to a family of Ser/Thr kinases originally identified as corepressors for homeodomain transcription factors. HIPK2 has a role in regulating cell growth and genotoxic stress-induced apoptosis. Furthermore, its involvement in the nervous system is indicated by the neuronal defects of null mice partially overlapping those observed in Mecp2 ko mice.
Since important MeCP2 neuronal functions are regulated by its phosphorylation we wanted analyze the functional role of its interaction with HIPK2. We have confirmed that the two proteins associate and phosphorylation assays have shown that MeCP2 is phosphorylated by HIPK2 in vitro. Importantly, we have also shown that a specific serine within MeCP2 is a target of HIPK2. Functional assays have shown that ectopic MeCP2 causes an increase in cell death and an additive effect of the two proteins in inducing apoptosis in cultured cells was observed. Importantly, the role of MeCP2 in inducing apoptosis together with HIPK2 is lost when the serine is mutated or a kinase dead derivative of HIPK2 is used.
Presently we are analyzing whether MeCP2 is a target of the kinase in vivo and the role of the interaction for the nervous system. In favor of the hypothesis that the two proteins work in a common molecular pathway we have shown by immunohistochemistry experiments that the expression pattern of the two proteins in the brain of adult mice is highly similar. We therefore believe that these studies are relevant for understanding whether HIPK2 acts as a modifier gene influencing disease severity in RTT patients with mutations in MeCP2.

MECP2 contributes to microtubule stability through the tubulin deacetylation

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Methyl CpG binding protein 2 (MeCP2) is known to mediate gene expression by several mechanisms within the nucleus. MeCP2 also has a recently recognised cytoplasmic distribution, which is of unknown functional significance. Here, we report that MeCP2 maintains microtubule stability via the tubulin de-acetylation pathway. We show that MeCP2 associates with microtubules in the cytoplasm as well as on the mitotic spindle and within the midbody remnant. We have found that microtubule inhibitors alter the
cytoplasmic distribution of MeCP2 in wild type cells. In a MECP2 mutant cell line, we have detected impaired microtubule stability and dynamics together

with reduced amounts of acetylated tubulin. SiRNA knockdown of MeCP2 in wild type cells is associated with reduced amounts of acetylated tubulin, whereas MeCP2 overexpression causes a rise in the level of acetylated tubulin. We have since identified an interaction between MeCP2 and the microtubule deacetylase histone deacetylase 6. Our data reveal that MeCP2 maintains microtubule stability by inhibiting the tubulin deacetylase activity of HDAC6.

Epigenetic drugs modulate MeCP2 dynamics in living cells

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MECP2 is an X-linked gene coding for a protein functioning as a transcriptional repressor. The protein, MeCP2 (Methyl CpG–binding protein), is an abundant component of pericentric heterochromatin and its mutations or duplications are present in around 80% of patients with a neurological disorder known as Rett Syndrome, one of the most common causes of mental retardation in females (1/10000 live female births). No effective pharmacological treatment is as yet available for this disorder. MeCP2 is characterised by two important domains: the Methyl CpG−Binding Domain (MBD), permitting the binding to methylated CpGs and the Transcriptional Repression Domain (TRD) interacting with the Sin3A−histone deacetylase complex. There is little understanding of the MeCP2 dynamic interaction with chromatin. We recently demonstrated that MeCP2 binds strongly and reversibly to chromatin and that mutations in regions located downstream of the MBD modulate this affinity.

Heterogeneity in MeCP-2 binding capabilities might be therefore the underlying cause of the variable severity of the Rett symptomatology. In this prospective, an epigenetic pharmacological approach aimed at modulating the MeCP2 binding capacities to chromatin could prove useful to treat this disease and ameliorate its several symptoms

NIH3T3 cells were treated with two epigenetic drugs targeted respectively to DNA methylation (5aza−2'−deoxycytidine) and histone activations (Thricostatin) and then transiently transfected with GFP-MeCP2 or with for GFP-pathological mutants of MeCP2. Cells were then analyzed by strip FRAP (Fluorescence Recovery After Photobleaching). The time necessary for the fluorescence to reach half of the total recovery after bleaching (t ½) is an indication of the protein affinity to chromatin. Our data indicate that treatment with 5aza−2'−deoxycytidine, but not with thricostatin result in a significant decrease in t ½ in GFP-MeCP2 transfected cells. Interestingly, thricostatin treatment alters the immobile fraction in the nucleoplasm. Similar results were obtained with one of the pathological mutant (R106W)

In conclusion we demonstrated that epigenetic drugs may affect MeCp2 affinity to chromatin. Further, we established a method to test the potential of different compounds in rescuing the binding capabilities of MeCP2 mutants.

Disruption of the cellular regulation of CDKL5 might be relevant for Rett Syndrome

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Mutations in the human X-linked cyclin dependent kinase like 5 (CDKL5) gene have recently been identified in some Rett patients with the Hanefeld variant and in girls with mental retardation associated with early seizures. We have previously shown that CDKL5 works in a pathway common with that of MeCP2, the main cause of classic Rett Syndrome (RTT). In fact, the two proteins associate and the kinase mediates the phosphorylation of MeCP2 in vitro. This suggests that mutations in CDKL5 cause RTT in part because important MeCP2 functions are impaired. Furthermore, CDKL5 might play a secondary role in RTT by acting as a modifier gene thereby influencing disease severity in patients with mutations in MECP2.
Even though our results do suggest a common molecular pathway belonging to CDKL5 and MeCP2, we still have to reveal in which brain areas and when the two factors are communicating. Furthermore, we have to understand the functional relevance of the identified interaction.
Our immunohistochemistry and western blot experiments show that in adult brain the two proteins have overlapping expression patterns. However, whereas MeCP2 levels appear rather uniform through late embryogenesis and postnatal stages as well as in the different brain areas, CDKL5 levels appear to be dynamically modulated. In particular, the CDKL5 protein is virtually absent in the mouse embryo brain and is strongly induced in early post-natal stages. Furthermore, whereas MeCP2 is confined to the nuclear compartment, CDKL5 is found in both the nuclear and cytoplasmic compartments indicating a possible role in transmitting signals between these two compartments. Interestingly, the subcellular distribution of CDKL5 varies in the different areas of the adult mouse brain as well as during development. Experiments in cell cultures have allowed us to show that CDKL5 shuttles between the nucleus and the cytoplasm and that an active nuclear export mechanism depending on the C-terminal tail of the protein is responsible for the cytoplasmic localization. The relevance of this regulation seems to be demonstrated by the fact that all the late RTT truncating mutations identified so far lead to an abnormal accumulation of the kinase into the nucleus.

Proteomic analysis of CDKL5-deficient human fibroblasts

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Mutations in the human X-linked cyclin-dependent kinase-like 5 (CDKL5) gene have been shown to cause infantile spasms as well as Rett syndromelike phenotype. To date, fewer than 30 different mutations have been reported. CDKL5 encodes for a putative kinase, which is able to phosphorylate itself and to mediate MeCP2 phosphorylation. In the present study we thought to overcome some of the limitations profiling on complex tissues, such as brain, by studying

clonal cultures of non-transformed fibroblasts from CDKL5 mutation patients. Because CDKL5 undergoes XCI, this last feature allows the separation of cells that express the wild type CDKL5 from the active X from those that express the mutant CDKL5 from the active X. To identify consequences of a CDKL5 deficit, we compared by a 2-D gel based proteomic technique the protein expression profile in matched pairs of clonally derived mutant or wild-type CDKL5-expressing fibroblasts cultures from three unrelated girls with severe epileptic encephalopathy. Using MALDI MS-MS, 15 differentially expressed proteins were identified, which were found either up-regulated (n=6) or down regulated (n=9) compared to the wild-type cells. Differentially expressed proteins, that include cytoskeleton and oxidative stress proteins, can be related to mechanisms underlying epilepsia. We observed a significant decrease in the ribonuclease inhibitor concentration, which plays an important role in cell protection from per-oxidative injuries, and an increased expression of Prx VI due to putative oxidative signals. Moreover, we noted a significant decrease in transketolase which catalyses two of the three steps of the nonoxidative branch of the pentose pathway, thus serving a critical role in providing a reversible link between glycolysis and the pentose phosphate pathway, essential when the cell must modify requirements for NADPH reducing equivalents. Finally, oxidative stress can affect cellular cytoskeletal organization, maybe through proteolysis and/or abnormal changes in microtubules and intermediate filaments. Some of these proteins are currently being investigated by quantitative RT-PCR and western blot. On the basis of our results we could propose that a CDKL5 deficit induces oxidative stress susceptibility and cytoskeletal reorganization that could be involved in the brain damage characteristic of CDKL5 mutation patients.

Mouse models 3

Age- and region-specific disturbances of monoaminergic systems in the brain of mecp2-null mice.

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Rett syndrome (RTT) is a pervasive neurodevelopmental disorder that affects mainly females, caused by mutations in the methyl CpG-binding protein 2 gene (MECP2). A multitude of brain neural systems are affected in RTT resulting in an autonomic dysfunction (breathing and sleep), a characteristic loss of locomotor abilities and a movement disorder which includes dystonia and stereotypies, as well as profound cognitive impairments. This wide involvement may suggest that a dysfunction of the modulatory monoaminergic brain systems play a role in RTT pathophysiology. In fact, a deregulation of neurotransmitters such as norepinephrine, dopamine and serotonin have repeatedly, although not always consistently, been shown to be altered in the brain and cerebrospinal fluid of RTT patients. Furthermore, the Mecp2-null mice, a model of RTT, showed

reduced levels of these neurotransmitters and its metabolites both in total brain extracts and in the medulla oblongata as compared to wild-type mice. In order to clarify the contribution of monoamines to the different clinical components of the RTT phenotype, we performed a neurochemical study of different brain regions of the Mecp2-nulltm1.1Bird mouse potentially playing a role in RTT-like pathophysiology, at two different timepoints: before and after the establishment of overt symptoms. We found that the serotonergic and noradrenergic systems are affected in this model, with a reduction in the levels of the neurotransmitters and their metabolites, as well as a dysregulation of their degradation, already at three weeks of age. Additionally, we observed that the prefrontal and motor cortices were the primarily affected regions, whereas the hippocampus and cerebellum may play a role in later stages of the disorder.

Contribution of FXYD1, a MECP2 target gene, to the neuropathology of Rett Syndrome

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Rett syndrome (RTT) is a disorder of brain development that predominantly affects girls that, in most cases, is caused by a mutation in a gene called MECP2. MECP2 encodes a protein that normally “silences” other genes. We found that the brains of RTT patients, and that of mice lacking MeCP2, express higher levels of FXYD1, a gene encoding a protein that regulates cell excitability and is directly repressed by MeCP2. Brain neurons overexpressing FXYD1 show morphological abnormalities (i.e. reduced dendritic arborization) similar to those of RTT patients, further suggesting that an excess of FXYD1 contributes to the neuropathology of RTT. To test the hypothesis that at least some of the neuropathological manifestations of RTT seen in animals lacking MeCP2 would be ameliorated by genetically preventing the FXYD1 response to MeCP2 deficiency, we are breeding Mecp2 null mice or mice expressing a truncated MeCP2 (Mecp2{308/y}) with Fxyd1-null (-/-) mice and characterizing the offspring for behavioral or morphological abnormalities.
Our results show that disrupting Fxyd1 expression (+/- or -/-) does not improve the survival rate of Mecp2 null mice. Similarly to RTT patients, Mecp2 null mice exhibit a decrease in dendritic arborisation of the cerebral cortical neurons. Initial results comparing neuronal dendritic arborisation in the cortex of Mecp2 null -FXYD1{-/+} mice show an improvement in dendritic arborisation deficit compared to Mecp2 null mice.
A first cohort of animals derived from the breeding of Mecp2{308/y} to FXYD1{-/-} males has been tested for motor coordination, balance, fear conditioning, anxiety, and learning and memory behaviors. Initial results show that Mecp2{308/y}-Fxyd1{-/+}, and more clearly Mecp2{308/y}-Fxyd1{-/+}, present a selective improvement in learning and memory deficits observed in Mecp2{308/y} mice, without rescuing the other behavioral defects observed in Mecp2{308/y} mice.
These preliminary results suggest that deregulation of Fxyd1 expression contributes to the neuropathology of two major abnormalities detected in animals models of RTT, the loss of dendritic arborisation in Mecp2 null mice and the deficits in learning and memory affecting Mecp2{308/y} animals.

Evidence of central cholinergic hypofunction and rescuing of the behavioural phenotype by postnatal choline supplementation in the truncated mecp2-308 mouse model

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A perinatal supplementation with choline, an essential vitamin of the B-complex and acetylcholine precursor in neurons, has been recently proposed as a potential dietary treatment for Rett syndrome (RTT). Evidences from MeCP2Jae null mice suggest a choline-dependent improvement of motor coordination and locomotor activity phenotype. We investigated the efficacy of supplementation with choline (25 mM) from birth to weaning (pnd 25) in the truncated MeCP2-308 mouse model. When adult male offspring were tested for rotarod performance, a tendency for choline effects appeared: in the absence of changes in other groups, postnatal dietary choline increased motor coordination only in the mutant mice. Further, the finding of a significant genotype-by-choline interaction indicated a basal profile of reduced locomotor activity and of increased emotionality (indicated by low time spent in the intimidating central area of the arena) in mutant mice, compared to wild type (wt) controls. Remarkably, choline treatment compensated both these behavioural alterations, restoring wt-like levels. No effects of choline were highlighted in wt animals. To probe the functional status of central cholinergic system, mice were challenged with the specific cholinergic muscarinic antagonist, scopolamine (2 mg/kg). As expected, a hyperactivity profile was induced in wt subjects. This was not the case for mutant mice, thus revealing an underlying reduced cholinergic tone. Present findings suggest that dietary choline supplementation from early after birth improves behavioural symptoms in the mutant offspring, specifically for motor and emotional domains. This cholinergic alteration, so far never reported in RTT mouse models, appears in good agreement with previous observation in RTT human brains and paves the way to further therapeutic approaches.

Tyrosine hydroxylase deficits in the chemoafferent and the sympathoadrenal pathway of the mecp2 deficient mice

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Rett Syndrome (RS) is a severe neurological disorder, which could account for up to 10% of severe mental retardation of genetic origin in women. Mutations in the methyl-CpG binding protein 2 (MECP2) gene have been identified in more than 90% of sporadic RS cases.). It has been proposed that exaggerated and inadequate autonomic responses, specifically cardio-respiratory arrhythmia, could be responsible for the sudden death of 26% of RS patients. Using Mecp2 deficient mice, we have previously shown that their breathing disturbances are probably due to catecholaminergic dysfunctions at the brainstem level. Using immunohistofluorescent labeling, we also showed a significant decrease in the number of tyrosine hydroxylase (TH, rate-limiting enzyme in catecholamine synthesis) immunopositive neurons in the brainstem of the

adult Mecp2 deficient mice, likely due to the progressive decrease of the TH protein synthesis (Viemari et al, 2005 J Neurosci; Roux et al, 2007 Eur J Neurosci). In the present study we used TH immunoquantification associated to densitometric measurements, in several peripheral catecholaminergic tissues, the chemoafferent pathway (carotid body and petrosal ganglion) and the sympathoadrenergic system (superior cervical ganglion and adrenal medulla) of adult Mecp2 deficient mice. Our results show that the TH staining level is significantly decreased in the sympathoadrenergic system as well as in the chemoafferent pathway of Mecp2 deficient mice. The chemoafferent pathway responds to the decrease in the level of arterial oxygen tension by increasing the ventilation rate in order to counter the deleterious effects of hypoxemia. In order to evaluate in vivo, the chemoafferent pathway, we recorded the breathing of Mecp2 deficient mice in normoxia followed by a hypoxic challenge. Our results clearly show that the hypoxic ventilatory response is highly increased in Mecp2 deficient mice, correlated with the catecholaminergic deficits. Finally our study indicates that the catecholaminergic metabolim is also affected in the peripheral nervous system in absence of Mecp2. Such deficits can provide new insight to better understand the pathophysiology of Rett Syndrome.

Comparative gene expression analysis in the adrenal medulla of the mecp2-deficient mouse

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Methyl CpG binding protein 2 (MeCP2) is a member of the methylated DNA binding protein family. MeCP2 is predicted to modulate the transcription of target genes through direct promoter binding or via its involvement in chromatin remodeling mechanisms. We have previously shown catecholaminergic dysfunction in the brainstem of a mouse model of Rett Syndrome. Mecp2-deficient mice exhibit variable respiratory rhythm and frequent apneas due to reduced norephrinephrine content and a decrease in tyrosine hydroxylase (Th, the limiting enzyme of catecholamine synthesis) positive neurons of the medulla oblongata. The adrenal medulla is composed of chomaffin cells that are all expressing tyrosine hydroxylase. A previous study showed a defect in catecholamine secretion in adrenal medulla of Mecp2-deficient mice (Wang et al., 2006). Here, we decided to explore the peripheral catecholaminergic system and we performed gene expression analysis using expression microarrays and mRNA from Mecp2-deficient or wild type adrenal medulla. Mecp2-deficiency in the medulla is associated with a massive modification of the gene expression profile. We found approximately 1200 down-regulated genes with an expression ratio about 0,5 to 0,01 ($p<0,01$) and up to 600 up-regulated genes with at least a 2 fold increase in expression ratio ($p<0,01$). These results show that 1- the role of MeCP2 is probably not to be a simple transcriptional repressor and this protein could also be an activator of gene transcription as demonstrated in a recent comparative gene expression study performed in the hypothalamus of the Mecp2-deficient mouse (Chahrour et al., 2008) 2- MeCP2 deficiency, in addition to affecting the central nervous system, could also lead to a dysfunction of the peripheral nervous system, this being consistent with its ubiquitous expression 3- the adrenal medulla is implicated in various physiological pathway and our results suggest a possible implication of the adrenal medulla in the pathophysiology of

the Rett Syndrome.

Behavioural alterations can be unvealed in mecp2-308 mice already soon after birth

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In a mouse model of Rett syndrome (RTT) which expresses a truncated form of MeCP2 (MeCP2-308), we performed a longitudinal evaluation across the lifespan, starting from soon after birth till adulthood. During the neonatal phase, mice were followed for the expression of spontaneous general movements and emotional communicative behaviour – namely, ultrasonic vocalizations on postnatal days (pnds) 3, 6 and 9. The results obtained evidenced, as early as pnd 3, subtle anomalies in spontaneous general movements by Mecp2-308 male mice during the so-called pre-symptomatic phase. Specifically, mutant mice were found to exhibit more intense curling and more side responses than wild type (wt) littermates, an indication of impaired motor coordination in the righting reflex. More evident effects of genotype were highlighted on pnd 9, with Mecp2-308 male mice showing more pivoting and head rising behaviours than wt controls. In addition, a general picture of slight impaired communicative capacity emerged in mutant mice. In particular, on pnd 6, the age at which wt subjects show a peak in vocalizations, a significant decrease in ultrasonic vocalization rate characterized MeCP2-308 pups. During the early symptomatic phase at pnd 60, Mecp2-308 male mice displayed increased anxiety-like behaviours in the light-dark test, compared to wt controls: Mice in fact preferred to spend consistently more time in the dark compartment of the two-side apparatus, and in this compartment they also exhibited a selective reduction in locomotor activity. By contrast, performances in the cognitive passive-avoidance task and in rotarod test were apparently not affected by genotype. Present results suggest that an increased attention devoted to the characterization of the pre-symptomatic phase may be especially informative in mouse models of human neurodevelopmental disorders with neurological and emotional/communicative symptoms emerging during infancy. This analysis can provide precocious biomarkers of RTT and an early window of opportunities on which potential therapies could be tested.

POSTER COMMUNICATIONS

Genetics

GE01
Analysis of MECP2 gene mutations, pattern of xchromosome inactivation and BDNF P.VAL66MET polymorphism in female individuals with Rett Syndrome.

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Rett Syndrome (RTT) is an X-linked dominant neurodevelopmental disorder, with onset during early childhood that primarily affects females. Classic RTT is characterized by apparently normal development for the first 6-18 months of life followed by regression - with loss of acquired motor and speech skills - and the development of stereotypic hand movements, autistic features episodic apnea and/or hyperpnea, gait ataxia and apraxia, acquired microcephaly and seizures. A few clinical RTT variants have been described, including the “preserved speech variant”, the “forme fruste”, the “late regression variant, the “congenital variant”, and the “early onset seizures variant”, initially described by Hanefeld in 1985. The phenotype of affected individuals may nonetheless, even in “classic RTT”, be very variable. Mutations in the MECP2 (Methyl CpG binding protein 2) gene are found in up to 90% of classical RTT forms: the phenotype variability observed in these individuals must therefore derive from differences in mutation type and position, pattern of X inactivation or other modifying factors, as recently suggested for the BDNF gene Val66Met polymorphism.
We have analyzed these variables in a cohort of 19 female individuals referred to the Department of Paediatrics, University of Padua, with a diagnosis of pervasive developmental disorder, possibly associated with epilepsy, or with a more specific diagnosis of Rett syndrome. These individuals, who were subjected to complete molecular analysis of the MECP2 gene, resulted to carry 12 different pathogenetic mutations including 4 missense, 2 nonsense, 5 frameshift two of which never previously reported in literature, and 1 large deletion. The study of the BDNF polymorphism showed that Val66 represented 75% of all the alleles; Val66 homozygosity was found in 50% of the tested subjects, while only one individual was homozygote for the Met66 polymorphism. The study of the X chromosome inactivation pattern evidenced a balanced X-inactivation in only 30% of the patients.
The comprehensive evaluation of these data in relationship with the clinical features of the patients, and in particular with the occurrence of seizures, will be presented.

GE02
Spectrum of MECP2 mutations in classical Rett Syndrome patients of Indian origin

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Introduction: Rett syndrome (RS) is an X-linked neurodevelopmental disorder that primarily affects girls, and is one of the most common causes of mental retardation in females. Mutations in X-linked methyl-CpG-binding protein 2 (MECP2) gene, located on chromosome Xq28, have been found to cause RS. Mutations are found in 70% - 80% of patients with classical RS and in less than 50% of patients with atypical RS, although there are no reports from India. This is the first study from India on mutation spectrum of RS.
Objective: The present study aimed to investigate frequency and type of mutations of MECP2 gene in Indian patients with classical RS.
Methods: Selection of patients was done based on the diagnostic criteria of Rett syndrome (Hagberg et al. 2002). A systematic analysis of exon 2-4 of MECP2 in 34 sporadic patients with classical RS was performed by polymerase chain reaction (PCR), single strand conformation polymorphism (SSCP) and Restriction fragment length polymorphism (RFLP), followed by sequencing.
Results: 14 different mutations including three novel variants (c.50_51insA (p.D17fs), p.Q262X & p.G428G) were identified in 26 (76.47%) of 34 patients. Most of the mutations were nonsense mutations. p.R168X was found in 6 (18%) patients; p.T158M was found in 4(12%), p.R255X was found in 3 (8%) patients followed by p.R270X, c.806delG, p.R306C in 2 (6%) patients. Other mutations including c.50_51insA, p.Q262X, c.1157_1200del44, p.D156E, p.P152R, p.G428G, p.I125I, c.378-74C>T were found with a frequency of 3% each.
CONCLUSION: Altogether, we have identified a heterogeneous spectrum of mutations, including novel MECP2 mutations, in a high proportion (76.47%) for the first time in classical RS patients of Indian Origin. The p.R168X is the most common mutation in Indian RS patients followed by p.T158M, p.R255X, p.R270X, c.806delG, p.R306C.
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GE03
Genotype-Phenotype Correlation in Rett Syndrome
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Background: Rett syndrome (RTT) is a severe neurodevelopmental X-linked disorder characterized by variegated clinical spectrum (Classical and Variant forms). Also genetic features are heterogeneous: two genes (Mecp2, CDKL5) have been already identified as involved, but 20% of patients results negative at genetic investigation; the same mutation may show heterogeneous phenotypic expressions. Due to the still unclear genotype-phenotype correlation, individuation of clinical indications for genetic analysis becomes a priority in RTT. Moreover variegated clinical spectrum of RTT implies risk of misdiagnosing atypical forms.
Our study has the aim of examining genotype-phenotype correlation and contributing to a better setting of the clinical indications for genetic investigation in RTT.
Method: Rett patients were identified from those referred to our Institute in the last 6 years.

For each patient we investigated clinical presentation (Classical or Variant form), seizures, clinical evolution, stage, presence and type of genetic mutation, functional domain involved. A severity global score (SGS) was identified considering these parameters: purposeful hands use, deambulation, language, epilepsy. In order to obtain comparable data, we evaluated the clinical status at the end of the third stage.
Results: AND CONCLUSIONS: We identified 25 patients affected basing on European Society of Peadiatric Neurology Diagnostic Criteria. Patients age is included from 3 years and and 34 years (media 12,5 years). Patients expressing classic phenotype are 19 (76%); 9 of them (47.4%)don't present postnatal head growth deceleration and/or microcephaly.
A genetic mutation (Mecp2 or CDKL5) is found in 79% of patients; one patient presents a genetic polymorphism and one patient with Preserved Speech Variant (PSV) presents a triple delection never described before. More severe SGS is associated with mutations involving MBD or NLS domains, while PSV forms are associated with deletions involving WDR domain. According with other authors, R168X, R255X, R270X and R294X mutations aren't found in PSV and are connected, in our study, with Classic phenotype.
From clinical point of view we stress the low frequency of the postnatal head growth deceleration and/or microcephaly in our Classic RTT patients. According to Hagberg, probably collocation of this parameter into the necessary diagnostic criteria for Classic form would be revaluated.

GE04

Early infantile onset –"congenital"- Rett Syndrome variants, Swedish experience through four decades and mutation analyses

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The early infantile onset -congenital- variant of Rett syndrome presents with deviations of behaviour from very early infancy. Here, we report on a clinicalgenetic study in a collected series of 14 Swedish girls with the early infantile onset Rett syndrome phenotype. The clinical diagnosis was based on symptom onset before the age of six months and the probands fulfilled three or more Rett variant criteria and five or more supportive criteria. Genotype-phenotype correlation studies in the CDKL5-gene have recently shown clinical associations to early infantile onset Rett variants. Mutation analyses for both the MECP2-gene and the CDKL5-gene were therefore performed. Interestingly, we found a large deletion covering 2 exons in MECP2, which underlines the importance of MECP2 mutation screening even for the "atypical" early infantile onset variants of Rett Syndrome. All early infantile onset Rett syndrome patients in this study lacked previously well-known hotspot mutations in the MECP2-gene.

GE05

Male Rett Syndrome patient with mosaic MECP2 mutation.

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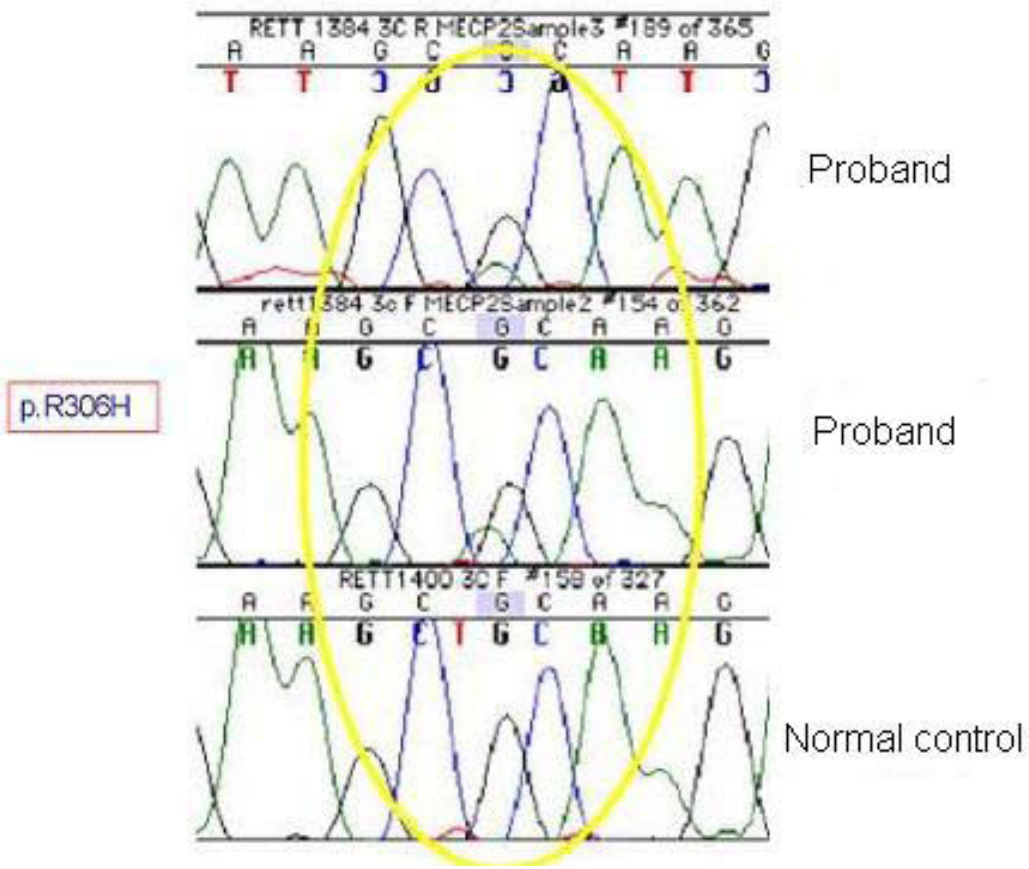
Rett syndrome (RTT [MIM 312750]) is a neurodevelopment disorder caused by mutations in MECP2. It has been mainly observed in females and the few reported males have an XXY karyotype or MECP2 mutations in mosaic state. Here, we report the clinical description of a male with Rett syndrome. The mother had a normal pregnancy and delivery. Auxological birth parameters were all in the normal range. The development was normal until 7 months, when showed frequent episodes of vomiting, hyperventilation, and started to loose interest in surroundings. He acquired the ability to walk unaided at the age of two years and he never developed language. Generalized convulsions appeared at 8 years that are still barely controlled by therapy. Brain MRI, karyotype, molecular analysis for FRAXA and FRAXE, methylation test for Angelman syndrome and UBE3A gene sequencing resulted negative. We firstly evaluated the patient at the age of 15 years old. He showed somatic hypoevolutism, microcephaly (OFC of 50 cm, <<3rd percentile), midline stereotypic activities, bruxism, recurrent episodes of apnea and hyperventilation, scoliosis and cold extremities. The patient presented typical features of Rett syndrome including a normal perinatal and early postnatal period, a phase of regression started at 7 months, postnatal microcephaly and typical stereotypic hand movements. DHPLC analysis and sequencing of MECP2 gene demonstrated the presence of the hot spot p.R306H mutation in mosaic state (~50%). In accordance with the somatic origin of the mutation, parents' DNA analysis resulted normal. This case underlines the importance to perform an accurate molecular analysis of the MECP2 gene in male cases with a strong suspicion of Rett syndrome given the possibility to find the mutation at different levels of mosaicism.



GE06

Rett Syndrome: our experience in molecular diagnosis in the north of Spain. Report of the point mutations and rearrangements in the MECP2 gene found in our patients.

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Rett Syndrome (RTT) is a neurodevelopmental disorder characterized by MR, regression of development, hand stereotypes and progressive microcephaly (1). Mutations in the methyl-CpG-binding protein 2 (MECP2) gene, located in Xq28, were reported to account for the majority of typical cases of RTT (2).

Five years ago we undertook in our laboratory a complete study of MECP2 in patients with typical and atypical RTT syndrome. The aim of this work is to present our results and compare them with other previously reported series in order to better understand the different clinical profiles of Rett Syndrome.

We studied a total of 62 females. MECP2 has been tested for both mutations and deletions in 46 of them, and for deletions only in 16 because they were previously studied for mutations in another laboratory.

To study point mutations, exons of MECP2 were amplified by PCR with primers and conditions as previously described (3), following by a heteroduplex analysis by Conformation Sensitive Gel Electrophoresis (CSGE). When an aberrant migration pattern was detected, PCR amplification product was directly

sequenced. MECP2 rearrangements were studied by Multiplex ligation-dependent probe amplification (MLPA) technique (Salsa P015C from MRC-Holland).
We found in total 12 pathogenic mutations (12/62=19.35%): 5 partial deletions, 1 partial duplication, 3 missense and 2 nonsense mutations and one novel silent change (R270R) that produces an aberrant splicing.
We also found the frequent polymorphism c.378-17delT in two cases (2/62=3.22%).
All of these findings will be discussed at the Congress with the complete description of the genotype-phenotype correlation of our patients.
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GE07

The phenotype of Rett Syndrome cases with large deletions of MECP2

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Background: Large deletions of MECP2 are a recognised cause of both classical and atypical Rett syndrome, however information on associated their functional severity and other morbidities has been limited to a few small studies (Hardwick et al 2007, Archer et al 2006, Scala et al 2007). This study will use the combined resources of the InterRett and Australian Rett Syndrome databases to examine the phenotype of Rett syndrome and large deletions of MECP2 in detail, including comparison with cases without large deletions of MECP2.
Methods: Cases will be sourced from the Australian Rett Syndrome Database, a population-based longitudinal database of phenotype information collected from families and carers of people with Rett syndrome in Australia, and from InterRett, the International Rett syndrome phenotype database, with information submitted by families and clinicians. Where possible, information about deletion size and location has been included. 44 cases with large deletions of exons 3 and/or 4 of MECP2 will be included in analysis.
Outcomes: Cases will be classified according to the Rett syndrome variant delineation model, with comparison of criteria met for cases with large deletions of varying sizes and positions, and with the MECP2 mutation positive cohort as a whole. Severity of overall phenotypic presentation will be measured for each case using three previously described severity scales (Kerr, Percy and Pineda) and compared within the large deletion group, and with the MECP2 mutation positive cohort. The effect of deletion size and position on severity will be

examined, including, where possible, comparison of cases whose large deletions extend into the IRAK1 gene. The presence of any other associated congenital abnormalities will also be evaluated. The large deletion related phenotype of this disorder will be described in detail, and its differences, if any, to the phenotype seen in cases with other MECP2 mutations will be examined.

Clinical Investigation

CI01
Neurophysiological, neuropsychological and behavioural functions within a trial of LCPUFA supplementation in girls with Rett Syndrome

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Phospholipids fatty acid are major structural components of neuronal cell membranes, which modulate membrane fluidity and hence function. Evidence from clinical and biochemical sources have indicated changes in the metabolism of fatty acid in several psychiatric disorders. Very long chain fatty acid and carnitine levels are decreased in Rett syndrome.
The aim of this work is to evaluate at three and six months, changes in LCPUFA (long chain poly-unsaturated fatty acid) status and functional neurophysiological, neuropsychological and behavioural functions within a trial of LCPUFA supplementation in girls with Rett syndrome.
21 girls with classical RS (mean age 13,25, range 6-20 years) have been treated. The patients were randomized to LCPUFA (250 mg below 15 Kg, 500 mg between 15 and 26 Kg, or 750 mg if > 26 Kg) or placebo (maize oil) for 6 months.
Patients underwent EEG video-polygraphic recordings during wakefulness using a computerized EEG System. Scalp electrodes positioned according to the international 10/20 system plus EMG electrodes for deltoid muscles and/or distal muscles, electrocardiogram and breathing effort. Behavioural observations were coded by a child-experimenter procedure designed to assess psychological function such as attention and recognition abilities.
With reference to neurophysiological parameters results show no significant effect, with reference to neuropsychological and behavioural functions, reduction of distraibility seems to be higher in LCPUFA group than in placebo group. In this abstract we report only preliminary results, since the trial is not completed. Data will be fully analized and will be reported completely at the conference.

CI02
Pulse oximetry-derived variables and phenotype severity in Rett Syndrome

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Background: Rett Syndrome (RS) is characterized by severe respiratory-control disorders, potentially leading to hypoxia and/or hyperoxia (Gaultier C. & Gallego J., J Appl Physiol 2008;104: 1522¡V1530). However, a systematic pulse oximetry evaluation in RS patients is lacking to date.
AIMS: We tested the hypothesis that an unrecognized subclinical hypoxia is present in RS and is related to phenotype severity.
Methods: A total of n=44 girls with RS (age: M¡ÓSD, 13.9¡Ó8.2 yr) with MECP2 or CDKL5 gene mutations and age-matched controls were enrolled in

the study, together with 40 age-matched healthy girls as controls. Continuous and noninvasive measurements of oxyhemoglobin (SpO2), perfusion index (PI), pulse rate (PR), carboxyhemoglobin (SpCO), Methemoglobin (SpMet) and Pleth Variability Index (PVI) were carried out by a MASIMO Radical mod. 7 pulse oximeter (Masimo Co, Irvine, CA, USA) with adequate fingerprobes. Randomly measured 1- hour data were obtained in triplicate and average data were used for statistical analysis. Phenotype severity in RS patients was measured by the Kerr, Pineda scale, and Percy scales (Colvin L., et al., Arch Dis Child 2003;88:38¡V43). Data were expressed as means „b SD, or medians (interquartile range).Differences between RS and controls were tested by Student¡¦s tor Mann-Withney tests. Linear regression analysis or Spearman rank correlation were used to explore univariate associations between variables.
Results: RS patients showed significantly higher SpO2 (93.5[89.3-96.5] % vs. 98[98.0-99.0] %, p<0.0001]), PR(98¡Ó19 bpm vs. 71¡Ó7 bpm, p<0.0001]), SpCO(6[3-9] % vs. 0[0-3] %, p<0.0001]), SpMet (1.0[0.3-1.8] % vs. 0[0-0.3] %, p<0.0001]), and PVI (32.0¡Ó11.9% vs. 18.3¡Ó4.6%, p<0.0001), together with lower PI(1.02[0.65-1.57] % vs. 3.0[2.9-4.0] %, p<0.0001]) values, as compared to controls. The following significant correlations were observed: Kerr/ SpMet (r=-0.6725 , p=0.0012), Kerr/SpO2 (r=-0.7927, p<0.0001), Kerr/PVI (r=0.8013, p<0.0001), Pineda /SpO2(r=-0.7134 , p=0.0004), Pineda/PVI (r=-0.7134, p=0.0004),Pineda/SpMet (r=0.5963, p=0.0055), Percy/SpO2 (r=-0.6104, p=0.0043),Percy/SpMet (r=0.4840, p=0.0306), and Percy/PVI (r=0.5599, p=0.0102).
Conclusions: Significant pulse oximetry changes are present in RS and are to be interpreted as a condition of subclinical hypoxia, associated with tachycardia, skin vasocontriction, and low cardiac preload. Pulse oximetry changes are related to phenotype severity, thus suggesting a role for hypoxia in the RS pathogenic mechanisms.

CI03

5 methyltetrahydrofolate levels and folate receptor autoantibodies in Dutch Rett patients

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Introduction: Low level of 5-methyltetrahydrofolate (5MTHF) in the spinal fluid of Rett syndrome patients have been reported. Recently, a Spanish group found decreased 5MTHF in 50% of Rett patients with severe epilepsy. The identification of blocking folate receptor (FR) autoantibodies in the serum of Rett patients from North-West Europe provides an explanation for low CSF folate whereby the antibody prevents binding of folate to the FR expressed on the choroid plexus and inhibits transport into CSF. We measured CSF 5MTHF levels and serum autoantibodies against FR in our Dutch (North-Europe) Rett population to evaluate the efficacy of folinic acid treatment in these Rett patients later on.
Material and Methods

We studied 17 Dutch Rett patients (age range 2-30; mean 8 years), genetically confirmed in 12. In addition to routine hematologic and biochemical analyses, we determined CSF 5MTHF and plasma free amino acids including homocysteine and compared these with aged matched controls. Folate blocking FR autoantibodies were measured in 12.
Results: CSF concentration of 5MTHF was normal in 16 out of 17 patients, (range 46-148 nmol/l), and low in one genetically proven adult patient (34 nmol/l, normal range 43-102), with good seizure control. All patients had normal peripheral folate metabolism.
Six young Dutch Rett patients (out of nine), with normal levels of 5MTHF in spinal fluid, demonstrated low
(0.2-0.5 picomoles/ml serum, negative<0.2), medium (0.5-1.0) or high titers (> 1.0) of FR autoantibodies in serum. Four of them experienced seizures and the Rett patients with the most severe seizures had the highest levels of FR autoantibodies (1.97 and 2.78 picomoles/ml serum respectively). Results were negative in 3 (More samples are still pending)
Discussion: One genetically proven (adult) Dutch Rett patient out of 17 had a decreased and two patients had low normal levels of 5MTHF. No relationship was found between severe seizures and low CSF 5MTHF in our population. Folate blocking auto antibodies were found in six young Rett patients with normal levels of 5MTHF. High titers were associated with severe seizures. Determination of FR autoantibodies in North West European Rett patients with severe seizures is recommended, despite normal spinal fluid levels of 5MTHF.

CI04

Evaluation of CSF neurotransmitters and folate in 25 patients with Rett disorder and effects of treatment

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Background: Rett disorder (RD) is a progressive neurodevelopmental entity caused by mutations in the MECP2 gene. It has been postulated that there are alterations in the levels of certain neurotransmitters and folate in the pathogenesis of this disease. Here we re-evaluated this hypothesis.
Patients and Methods: We evaluated CSF folate, biogenic amines and pterines in 25 RD patients. Treatment with oral folinic acid was started in those cases with low folate. Patients were clinically evaluated and videotaped up to 6 months after therapy.
Results: CSF folate was below the reference values in 32% of the patients. Six months after treatment no clinical improvement was observed.
Three of the four patients with the R294X mutation had increased levels of a dopamine metabolite associated to a particular phenotype. Three patients had low levels of a serotonin metabolite. Two of them were treated with fluoxetine and one showed clinical improvement. No association was observed between CSF folate and these metabolites, after adjusting for the patients age and neopterin levels.
Conclusion: Our results support that folinic acid supplementation has no

significant effects on the course of the disease. We report discrete and novel neurotransmitter abnormalities that may contribute to the pathogenesis of RD highlighting the need for further studies on CSF neurotransmitters in clinically and genetically well characterized patients.

CI05
Impaired lung oxygen exchanges in Rett Syndrome

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Background: Rett Syndrome (RS) is characterized by severe respiratory-control disorders, including irregular breathing pattern, episodes of hyperventilation, and life-threatening apneas. However, no information exists on the respiratory gas exchanges in RS.

AIMS: To determine lung gas (O2, CO2 and N2) exchanges respiratory rates and total ventilation in patients with RS.

Methods: A total of 16 RS girls (age: M¡ÓSD, 12.6¡Ó5.9 yr) with MECP2 or CDKL5 gene mutations were enrolled in the study. Respiratory rate, total ventilation and lung gas exchanges for O2, CO2 and N2 [(A-a) PO2, (A-a) PCO2, and (A-a) PN2] were investigated using a portable respiratory gas analyzer (Hanky Hapy, Ambra Sistemi, Pianezza, Turin, Italy). Gas exchanges were analyzed over a 60-sec time period, were carried out in duplicate, and average values were used for statistical analysis and compared to theoretical data predicted for a healthy, age-matched, control population. Continuous and noninvasive measurements of oxyhemoglobin (SpO2), was carried out by pulsoximetry. Arterial blood gas analyses were measured by an ABL520 Radiometer. High-resolution CT (HRCT) scans were obtained in case of impaired gas exchanges. Data were expressed as means „b SD or medians (inter-quartile range). In order to explore possible univariate associations between variables, linear regression analysis was used.

Results: Mean respiratory rates (29.2 „b 5.4 breaths/min, i.e., 1.68 „b 0.34 folds higher than normal) and mean tidal volumes (9.28 „b 4.24 ml/kg/min) were higher than predicted. Mean (A-a) PO2 was larger than predicted with 20.41 „b 11.88 mmHg (n.v.< 5.0 mmHg). Conversely, (A-a) PCO2 (4.40 „b 4.17 mmHg) and (A-a)PN2 (8.18[2.51-10.87] mmHg) values were within the normal range. Likewise Qs/Qt values were higher than expected (10.9[5.83-12.92] mmHg). Approximately 70% of the RS patients showed impaired lung O2 exchanges [(A-a) PO22: 24.5ƒn„b 6.6 vs. 3.9 „b 1.16 mmHg, P=0.0006), against normal diffusion of CO2 and N2. (A-a)PO2 was inversely related to SpO2 (r=-0.96, P<0.0001) and PaO2 (r=-0.61, P=0.047). Multiple, bilateral ground-glass opacities (GGOs) were observed at the HR-CT in about 60% of the RS cases with impaired lung O2 exchanges.

Conclusions: RS is significantly associated with lung O2 diffusion impairment, ventilation/perfusion mismatch, and non-specific lung abnormalities.

CI06
APOE as a potential modulation factor in Rett Syndrome

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Background: The phenotypic spectrum of Rett syndrome is widely variable. A number of studies on genotype-phenotype correlation yielded inconsistent results. The pattern of X chromosome inactivation (XCI) is considered to be an important factor determining the severity of RTT phenotype but it has some limitations in explaining all the phenotypic manifestations of RTT. We propose a hypothesis that there might be other genetic factors modulating the Rett phenotype. Apolipoprotein E is the major apolipoprotein in brain and the ε4 allele is strongly associated with the incidence, progression, and pathology of Alzheimer's disease as well as other pathological conditions. We performed molecular genetic analysis of APOE polymorphism in a large case-control study to evaluate our hypothesis.
Methods: We analyzed the frequencies of APOE alleles in a group of 75 unrelated female RTT patients with confirmed MECP2 mutation and 100 unrelated healthy female controls by PCR/RFLP method. In a selected group of 11 patients with the same T158M mutation in the MECP2 gene, we additionally performed the analysis of XCI pattern. Results were statistically evaluated by the test of binomial distribution.
Results: We found that the frequency of ε4 allele is significantly higher in RTT patients than in controls. Additionally, the manifestation of RTT among patients with the same MECP2 mutation T158M was severer in ε4 carriers than in those with ε3/ε3 genotype.
Conclusion: To our knowledge this is the first such study in RTT patients and further confirmation in experimental and epidemiological studies is necessary. ApoE is hypothesized to regulate many biological functions, resulting in significant changes in the onset and severity of numerous clinical conditions. Our results show that ε4 allele might be considered a candidate modulation factor of phenotype of RTT and other neurodevelopmental disorders. The study was supported by grants GA UK 257927 9270, IGA NR9215, and MSM0021620849.

CI07
Quality of movement during sleep in Rett Syndrome
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Sleep disorders are commonly observed in patients with RTT. One of the earliest sign is the delayed development of sleep-wake-rythm (R-W-R) and the lack of age-related decrease in total and day-time sleep, as expected in normal childreen. Studies of sleep structure showed that muscular tone is abnormal: often

lost during non REM stages (NREMs), but not always in REM sleep. So far, there is no report of RTT patients with REM sleep behaviour disorders (RBD), a parasomnia characterised by dream-enacting behaviours related to dreams and loss of normal REMs muscle atonia. We showed an improvement of movements and speech during RBD in patients with Parkinson's disease. Several parents or carreers of RTT patients reported that they observed that during sleep, patients could have smoother or faster movements than awaked. Therefore, we asked whether RTT patients have RBD and, when present, we paid a special attention on the quality of movements and speech in REM sleep.

We developed a screening self-administered questionnaire aim at identifying and characterizing (smoothness, strengh) movements during sleep in RTT patients and at comparing them with their own movements during wakefulness. The questionnaire was sent to the parents of the RTT patients from the French Association of Rett Syndrome (AFSR). 34 parents responded at the questionnaire. RTT patients mean age was 16.2 years (range from 5 to 42 years). 10 /34 patients were reported to have an improvement in them movements wich were smoother.

The early involvement of dopaminergic system was suspected in RTT (upward regulation of DA receptors as a consequence of the hypopfunction of 5HT and of NA neurons). Can we postulated that in RTT movements during RBD may by transmitted to lower motor neurons because of interrupting the pontomedullary pathways which mediate the REM sleep atonia? Videopolysomnographic recordings will complete this preliminary study.

Further investigations of REM sleep movement alterations in RTT patients may provide new insight into the pathophysiology of the disease.

Clinical Evaluation

CE01
Comparison of psychological profiles of young children with autistic disorder and Rett Syndrome: value of detailed specific psychological evaluation

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Rett syndrome and autistic disorder are included in the category known as pervasive developmental disorders (DSM IV-R). Rett syndrome is a severe and pervasive disorder of the development of the central nervous system. The clinical description comprises communication disorders and social withdrawal, absence of language development, severe psychomotor retardation, manual stereotypies, and apraxia affecting gait and voluntary use of the hands. Autistic disorder is characterized by qualititative impairment of social interaction, and verbal, nonverbal and emotional communication, with limited behaviours, interests and activities.

Clinical practice demonstrates that it is necessary to consider differential diagnosis with these two psychopathological entities in that signs of withdrawal and disorders of communication and interaction are common to both syndromes.

The aim of this study was to define several clinical forms in children with Rett syndrome or autistic disorder matched for age and overall development, receiving specialized treatment and education.

Ten young girls were therefore selected, five with Rett syndrome and five with autistic disorder evaluated by specific scales. All ten children (aged from 3 years to 10 years 3 months) had mental retardation, evaluated using the revised Brunet-Lezine scale (1998). Cognitive and socio-emotional developmental levels were evaluated by the Cognitive and socio-emotional battery, BECS (ECPA, 2007).

Analysis revealed differences between the developmental profiles of the two groups of children, and particular features of their psychological profiles. These differences warrant further study in order to obtain greater understanding of the neurodevelopmental disorders involved.

This study emphasized the need for clinicians working with children with pervasive developmental disorders to have evaluation tools that are sufficiently sensitive and discriminating, and applicable to the development of individual treatment plans.

CE02
Prevalence of GERD symptoms and associated factors in 216 girls with Rett Syndrome, a national prospective survey within the French national association AFSR.

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GERD is prevalent in Rett Syndrome (RS), often ignored and represents a real pre-occupation in daily life for parents. Expression of GERD is polymorph. Throughout our long experience working with parents having child with RS, we defined a questionnaire detecting GERD symptoms.

We attended to determine the prevalence of GERD and to define associated factors throughout a prospective survey using a questionnaire tested for better literacy with users before the study were filled by parents, members of the French association AFSR.
We defined 3 sub groups of GERD: group A, confirmed GERD (RS under PPI and/or anti-acid treatment), group B, suspicion of GERD by the presence of the 2 symptoms, cough triggered by a meal and sleep problems) and group c without GERD symptoms. Variables were quoted using a Likert scale.
A total of 216 questionnaires were analysed using descriptive and comparative methods.
The total population had a mean age of 15.63 ± 9.53 years without significant differences between the 3 sub-groups. Group A and group B represented respectively 21.7% and 27.7%.
Comparative analysis using Chi 2 test showed clearly significant association ($p<0.05$) between the presence of GERD and a higher prevalence of regurgitations and vomits (A 35.6%, B 15.5%, C 2.7%), otitis (A 18.2%, B 11.1%, C 0%) and teeth grinding (A 52.2%, B 49%, C 30.9%) . Surprisingly congestion of the throat was more prevalent in the sub-group without GERD symptoms (A 55.9%, B 49.0 %, C 80.5%).
Without endoscopy confirmation, our results confirmed the high prevalence of GERD. Identification of associated factors will allow to use a simple tool like as a questionnaire for an appropriate management of GERD to confirm the diagnosis and to start a treatment test.

CE03
Correlations between neurophysiological and psychological function in girls with Rett Syndrome

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Girls affected by RTT syndrome show different neurodevelopmental phenotype and the reasons for these differences are not well understood. The aim of the present study is to delineate if and how neurological and neurophysiological impairment can reflect behavioural and neuropsychological functions, in order to identify prognostic factors that could be important for clinical management of girls with RTT syndrome.
21 girls with RTT syndrome aged between 6 -18 at clinical stage 3-4 were entered into a database which contains demographic, antropometric, developmental, clinical, behavioural and genetic information. Patients underwent EEG video-polygraphic recordings during wakefulness using a computerized EEG System (Micromed System Plus, Micromed s.r.l., Mogliano Veneto, TV, Italy). Scalp electrodes positioned according to the international 10/20 system plus EMG electrodes for deltoid muscles and/or distal muscles, electrocardiogram and breathing effort. Behavioural observations were coded by a child-experimenter procedure designed to assess psychological function such as attention and recognition abilities.
Results show negative correlations between neurophysiological impairment and attention abilities. To our knowledge this is the first study that demonstrates a strict correlation between seizure frequency and neuropsychological impairment, in particular, with reference to selective attention and visual scanning in patients with RTT syndrome.

Clinical management in RTT syndrome should consider as prognostic factors epilepsy severity and EEG pattern suggestive of epileptic encephalopathy.

CE04

Prevalence of feeding impairment associated with hyper gag in 221 girls with Rett Syndrome, a national prospective survey within the French national association AFSR.

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Feeding impairment is often a complication of the clinical course of RS. Some factors are well-known, but few data exist exploring the oro-pharyngeal dysfunction as the hyper gag response to a food stimulus.

We attended to determine the prevalence of hyper gag and to define associated factors throughout a prospective survey using a questionnaire tested for better literacy with users before the study were filled by parents, members of the French association AFSR.

We defined hyper gag by the presence of Gag reflex triggered during meals or poor appetite (group A and group B as without hyper-gag). Variables were quoted using a Likert scale.

A total of 221 questionnaires were analysed using descriptive and comparative methods. Six questionnaires (3%) were with missing data on the principal variables defining hyper-gag.

The total population had a mean age of 15.67 ± 9.43 years with 33.6% > 18, 23% [12-18], 24.4% [7-12], 18.9% [0-7] years.

Hyper-gag was reported by 53.8% of the parents. Prevalence of GERD was 21.7% (under anti-reflux treatment) and 27.15% (without treatment and having GERD symptoms).

Comparative analysis using Chi 2 test showed clearly significant association ($p<0.05$) between hyper-gag and a higher prevalence of “grimaces in the tasteful changes” (83.1% vs 54.2%), “grimaces with cold food” (44.1% vs 23.4%), “preferences for tepid food” (82.1% vs 62.4%), “gag reflex during the brushing of the teeth” (45.3% vs 217.6%), “feeding difficulties” (14.5% vs 4.2%), “smooth food or in segments” (77.4% vs 64.0%), “preferences for sweet or salty food”(56.8% vs 43,2%). No age effect was noted.

Our results confirmed the high prevalence of feeding impairment and specifically hypergag in more than ½ patients. Identification of associated factors with hyper gag will allow to use a simple tool like a questionnaire to detection and to provide appropriate educational management taking into account the patients’ preferences.

CE05

Rett Syndrome: clinical study in 7 patients

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Introduction: Rett syndrome (RS) is a progressive neurodevelopmental disorder characterized by regression of motor and mental abilities in females after a period of normal development. It is principally caused by mutations in the gene encoding CPG binding protein 2 (MECP2) in Xq28.

Observations: We describe seven girls with Rett syndrome aged 3 to 13 years. All had an apparently normal development during the first six months of life. The

middle age at onset of disorder was nine months. The loss of purposeful hand skills with reduced communication were initial symptoms. These were followed by psychomotor regression, autistic features, stereotypic hand movements (hand washing or hand wringing) and irregular breathing with hyperventilation. Ambulation was possible in two patients but with ataxia and apraxia. Stereotypies other than manual, were also observed.
Hyperkinetic syndrome was predominant and the striking feature in two girls. Seizures were observed at the first stage of the disease in one girl and were intractable. EEG showed epileptiform abnormalities in all patients with or without seizures. Bruxism was observed in almost all patients and scoliosis in two girls.
Magnetic resonance showed cortical and sub-cortical atrophy in three patients Different mutations in MECP2 gene were identified.
Conclusion: Unusual features like major hyperkinetic syndrome or epilepsy at the first stage confirm the clinical variability in RS. All patients fulfilled the Rett syndrome criteria and mutations in MECP2 gene were also observed in girls with atypical onset of the disease.

CE06

Rett Syndrome and long-term disorder profile

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In a cohort of 103 females clinically diagnosed with Rett syndrome (RTT), 91 had a detectable MECP2 mutation. Emphasis on details of natural history facilitated grouping of individuals with the same MECP2 mutation and the development of so-called disorder profiles. Some examples of disorder profiles of different recurrent MECP2 mutations are discussed. RTT females with the frequently recurrent R133C and R306C missense mutations and those with intragenic deletions in the C-terminus of MECP2 deserve more attention in larger studies as their development is different and milder in the long term.

CE07

Use of parental consultation to develop a short form of phenotype questionnaire in Rett Syndrome

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Background: Questionnaires about symptoms, signs and functional ability in Rett syndrome typically contain a large number of items with variable relevance to the families' experiences. The Australian Rett syndrome study, a populationbased database of Rett syndrome cases in Australia born since 1976, has previously collected data with extensive follow-up questionnaires, developed in consultation with a consumer reference group. A brief questionnaire containing items relevant to parent views could improve the rate and quality of responses by reducing the burden on participating families, especially as the individual with Rett syndrome grows older. Symptoms and signs of Rett syndrome can be viewed

from many perspectives, such as severity of presentation; functional impact, and burden on daily life, often measured on a Likert scale. Item impact methods have previously been used to shorten questionnaires by retaining items occurring with the greatest frequency and those with the greatest perceived impact on daily life (Jokovic, 2006).
Objectives: This study aims to use item impact methods to develop a brief questionnaire for parents and carers participating in the Australian Rett Syndrome Database. A further aim is to create a measure of change for intervention studies that is meaningful to parents and clinicians.
Methods: In the first stage of this study, the consumer reference group will be consulted to collect their views on the objectives of the study, and a pilot questionnaire containing both severity and impact questions will be developed based on their perceptions of important items. This questionnaire will then be piloted on a larger group of parents and carers of people with Rett syndrome. The responses to this questionnaire will be analysed and a first draft version of the shortened questionnaire will be re-tested on the pilot participants, whose views will inform the final draft of the brief questionnaire. The brief questionnaire will be compared to the severity scale developed from principal components analysis on existing Rett syndrome phenotype data for items in common and psychometric properties, with the overall aim of developing a valid, brief and useful questionnaire that reflects parents' and carers' views of severity of Rett syndrome

CE08
Psychopathological assessment in girls with Rett Syndrome

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Purpose: Rett syndrome (RS) is a neurological disorder, affecting mainly females, caused by MECP2 mutations usually resulting in severe physical disability. The central nervous system is the primary organ system involved in RS. Neuropathologic findings indicate a failure of neuronal maturation with too small neurons and too dentritic arbors and no evidence of a neurodegenerative process. The combination of clinical and neuropathologic characteristics presents the profile of neurodevelopmental disorder. The present study investigated the neuropsychological preformance, emotional and relations status in girls with RS.
Method: The three girls with RS according to DSM-IV criteria were studied. All girls have had clinical, biochemical, EEG, neuroimaging and mutation studies. The following psychodiagnostic tests were used: Wechsler intelligence scale for children-revised, memory and digit span tests, Thematic Apperception test.
Results: Mutation of the MECP2 is present in all cases. The subjects had a total below average. attention deficit was also reported; cognitive deficit mainly affects verbal abilities, therefore memory and language functions. The general psychological problems of developmental RS patients are not very different from those of all children: they display, marked problems regrding body schema, family adaptation, peer relationship. An advanced stage of the disease can give rise to considerable psychopathological problems both in children and their family.Conclusions: However, it is difficult to understand the etiopathogenesis of neuropsychological disorders in children with RS since the girls examined so for have not been very rapresentative; the tests used can be affected by children's motor difficulties, the assessment is often carried out in particularly stressful

periods for the patient, and the regulatory mechanisms underlying the cognitive processes are often neglected; a global approach, considering biological, psychological and social factors might well cast some light on the causative mechanisms of such disorders.

Clinical Aspects

CA01
Seizures in Rett Syndrome: clinical and electrophysiologic aspects

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Purpose: Rett syndrome (RS), one of the leading causes of mental retardation and developmental regression in girls. The majority of cases of sporadic RS are caused by mutations in the gene enconding methyl-CpG-binding protein 2 (MECP2). Clinical characteristics consisti of microcephaly, stereotyped hand washing and seizures. Seizures occur in about 80% of subjects, seizure onset in RS is associated with early developmental factors and with genotype. cases without a delectable MECP2 mutation had a higher risk of seizure onset up to 4 years of age but a lower risk after 4 years (Jian L. et al., 2006; 2007). To evaluate seizure frequency, clinical and electrophysiologic aspects in RS and its relationship with others factors. This study aims to assess the clinical and therapeutic aspects of seizures in RS. Method: Ten girls (age range 6-11 years) with RS according to DSM-IV criteria were studied. All girls have had clinical (history of seizures), biochemical, video/polygraphic/EEG monitoring sessions, neuroimaging and mutation studies. Seizures were classified according to international League agastain epilepsy (ILAE, 1989).
Results: Mutation of the MECP2 is present in nine girls. Seizures were reported in all cases; the median age onset was 38 moths. The clinic semiology is: tonic and clonic generalized (4 cases),parzial complex seizures (3 cases), absence (1 cases) and myoclonic seizures (1 cases). The frequency is variable: continuous seizures in 3 cases, sporadic seizures in 3 cases, subnitrant seizures in 1 cases. All cases recevied antiepileptic drugs (AEDs): valproate (4cases), carbamazepine(2 cases), lamotrigine and sodium valproate (2 cases), sodium valproate and carbamazepine (1 cases). In 3 girls epileptic activity in the EEG was not associated with clinical seizures and many events presumed to be seizures have no EEG correlate during video-EEG motoring.
Conclusion: Seizure frequency in RS syndrome is age-dependent and associated with early developmental factors and with genotype.

CA02
Very late onset and prolonged regression in Rett Syndrome

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We present a 21 year old girl with Rett syndrome with an atypical presentation and conversational speech. As a child she was labelled with atypical autism with profound learning disability. However, at 16 years old, Rett was first suspected based upon her marked learning disability, severe scoliosis requiring surgery and intermittent hand stereotypy. This was confirmed by the finding of a p.1162-1172del11bp mutation in the MECP2 gene, with random X-chromosome inactivation.

Her early course was of slow but steady progress of a shy and anxious child. She was dyspraxic and had motor tics. She had intermittent hand stereotypy and a reluctance to use her hands for fine motor tasks. She could write and draw simple pictures. She had subtle breathing irregularity.
She began to regress age 16.5 years, which worsened following two episodes of acute illness for which she was hospitalised for 2 weeks, and is ongoing at 21 years. She started to stutter whilst talking. Stereotypy worsened and interfered with tasks such as self-feeding. She lost awareness of bodily functions with daytime soiling and nocturnal enuresis. Breathing irregularity became more apparent, during which aggressive outbursts were more likely. She became more unsteady on her feet and could only walk 20 metres. She became increasingly anxious and was forgetful and confused at times. She lost interest in activities that she previously enjoyed.
During the acute illness she had a neutrophilia and markedly raised CRP. CT head scan was normal. Her EEG was normal with no sub-clinical epileptic activity. Psychometric evaluation is ongoing.
Whilst regression may be part of her Rett syndrome, its prolonged nature is unusual and therefore alternative aetiologies are also being considered.

CA03

A descriptive study of Rett disorder throughout life: the British Isles survey for Rett Syndrome

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The British Isles Rett Syndrome Survey (BIS) has been an ongoing project since 1982, established by Dr Alison Kerr in Glasgow and now based in Cardiff. Its aim is to monitor the health of people with RTT in the British Isles and to enhance our understanding of the natural history of the disorder.
The survey consists of questionnaires completed by families and reports from physicians, as well as clinical data from Dr Kerr's numerous RTT Clinics (1982–2005). Information is recorded on each case longitudinally, from the perinatal period through to death, and includes the results of genetic, physiological and post mortem investigations when available.
BIS has registered 1291 cases over 25 years: 1253 females and 36 males.
462 (35.8%) have the clinical diagnosis of Classic Rett (including 67 deceased); 202 (15.6%) of incomplete CR (including 24 deceased); 250 (19.4%) of atypical Rett (AR) (including 25 deceased). 72 (5.6%) do not have the diagnosis of RTT (including 6 deceased) but their clinical features resemble RTT. In 305 (23.6%), patients the status is unknown due to incomplete data.
MECP2 analysis revealed mutations in 405/565 patients tested. Of these, mutations were identified in 209/248 (84.3%) with CR, 53/59 (89.8%%) with incCR, 77/154 (50%) with AR.and in 66/82 (80.5%) patients with uncertain clinical diagnosis. No mutations were found in those 22 patients who did not clinically have RTT.
BIS, with detailed clinical data gathered for >25 years, encourages international collaboration between researchers. Its particular strength lies in its combination of longitudinal clinical data and mutation results; we hope this will be of value in conducting clinical trials of potentially therapeutic interventions.
Because the Survey was developed before the recognition of the role of the MECP2 gene in RTT, the clinical and mutation data are not complete. We are seeking to arrange mutation testing where this has not happened and we make every effort to contact

families or carers to obtain additional clinical information

CA04
Motor function over time in Rett syndrome - loss, difficulties and possibilities

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Aim: Acquire more knowledge and understand more about the motor development in Rett syndrome, to report on successful intervention, develop and suggest appropriate interventions and to contribute to an early diagnosis.
Design: Study I three case reports, Study II a longitudinal case study, Study III a questionnaire answered by 125 families with girls and women with Rett syndrome in Sweden.
Result: It was possible to keep range of motion in ankle joints over time in a girl with Rett syndrome, re-train walking and getting up from the floor after several years for a grown up woman as well as to learn a new motor function for a third person with this syndrome. Knowledge of earlier abilities, motivation in each person and joint planning for the intervention was important as well as an understanding of these persons' dependence on other people's initiative. Parents' reports increased the knowledge about early development in Rett syndrome, what deviation first observed, and development over the years. In our study 60/116 parents reported their daughter was late in developing functions or not developing functions at all and 32/116 reported a sudden loss of functions.
Postural instability in sitting was reported for most and the most common contractures were reported to be in backs and feet. Seventy three per cent started to walk; some stopped walking, some deteriorated, and some retrained walking after a period of not walking. Eighty per cent of those who learnt to walk were still able to walk at the time of the questionnaire – in 27 per cent of these, deterioration was reported.
Conclusion: Good knowledge about the development of this disorder and the wide variation in expression is mandatory in order to be of support for the person with Rett syndrome, the family and carers. This includes individual analysis in order to attend to losses or deterioration as well as to realize the possibility to keep, retrain and develop abilities for some persons with Rett syndrome.
Keywords: Child development deviation, foot deformities, loss of function, physical therapy, posture, recovery of function, Rett syndrome, scoliosis.

CA05
Motor disabilities in the Rett Syndrome

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Purpose: Rett syndrome (RS) is a neurological disorder affecting mainly females. About 90% of cases involve a mutation in the methyl-CpG-binding protein 2 gene (MECP2). Patients with RS are characterized by array of neurological and orthopedic difficulties. Patients show a normal neonatal period with subsequent developmental delceleration of head growth, and development of typical hand stereotypies. Patients show a specific motor problems, hypotonia, loss of transitional movements, ataxia, motor apraxia, spasticity, kyphoscoliosis and foot deformites. Rehabilitation interventions to reserve physical imparirments include exercise of various types and different physical modalities.The present

study investigated the motor disabilities in children with RS. Method: Nine girls with RS (age range 6-11) according to DSM-IV criteria were studied. Mutation of the MECP2 is present in all cases. All the subjects controlled in the study underwent a physical and functional examination (Vignos functional scale, 1963), and neurodiagnostic examination. Results: According to Vignos functional classification (a 1-10 scale wich manly evaluates lower limb function and where higher scores correspond to more severe impairment), 4 cases were at disease stage between 7 and 8, 5 cases were between 4 and 6. Motor abnormalities were stereotyped movements and gait disturbance, seen in all girls. Clinical evaluation has relevealed the former as postural hypotonia with failure in locomotion (9 cases), ataxia (7 cases), motor apraxia (4 cases), loss of transitional movements (4 cases), spasticity (6 cases), Kyphoscoliosis (5 cases), loss of ambulation (6 cases), loss of hand function (4 cases), foot deformites (8 cases), spatial disorientation (4 cases). Rehabilitation interventions to reverse physical impairments include exercises of various types and different physical modalities. Supplementary intervention can support physical impairment by introducing adaptive techniques, environmental modifications, and assistive tecnologies. Conclusion: The core motor symptoms of RS consist of two aspects; firstly the unique developmental abnormalities of the discrepancy of crawiling and walking and secondly the pathognomic symptoms which include the abnormal muscle tone,psture, locomotion and stereotyped movement. The treatment of girls with RS suggest that physical therapy is useful in the management of these children to maintain or increase motor skills and control deformities.

CA06
Clinical and cognitive heterogeneity in little patients with Rett Syndrome

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Our predecessor Andreas Rett shared with us his astonishment in 1966, when in front of very young girls presenting well codified developmental anomalies that are recognisable in different progressive stages, and that he gathered into a syndrome.
Following this clinical description, with help from DSMIV, discovery of MECP2 gene in 1999 by Huda Zoghbi and its heterogeneity reminds us of cerebral plasticity: acquisitions in several enjoy us and ask some questions, epilepsy in others requests the better therapeutic and protecting choice, manual dyspraxia demands creation of rehabilitation and tools… excellent memory in others allows some questions about “cut and paste” work and hope of progress .
New techniques in functional imagery suggest this question of Andreas Rett in his first astonishment formulation:” in their eyes, they tell us their better comprehension’…which brain?, which function and what about decreasing of cranial perimeter?
We will discuss about several cases.

CA07
Atypical communicative development in Rett Syndrome: the preserved speech variant

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Background and Objective: Comprehensive studies on early communicative skills and their developmental trajectories of girls with unusual phenotypes of Rett syndrome are rare. We present a unique case report delineating the course of various speech-language abilities of a rare variant of Rett, the Preserved Speech Variant.
Subject and Methods: One girl with a MECP2 mutation (del(378-43)-964*ins965GA) meeting clinical criteria for Preserved Speech Variant, was longitudinally observed from 6 months of age to 10 years. As diagnosis was approved by mutation testing at the girl's age of 4 years, we retrospectively analysed videos and clinical history up to this age. Furthermore, we prospectively applied the following methods up to 10 years: the Austrian Rett Survey; behavioural observation in her natural surroundings (video data); Austrian Communicative Development Inventories (ACDI); spontaneous speech samples; active vocabulary test (AWST); language development test (SETK 3-5); test for the reception of grammar (TROG-D).
Results: Episodical events of atypical and stereotyped prelinguistic and linguistic behaviour increased over time and became predominant at two years of age. A peculiar quality of cooing and babbling, echolalia, impaired phonological and morphosyntactic abilities, and out of context speech were among the deviant patterns observed.
Conclusions: The comprehensive analyses suggest a qualitative deviation already during the pre-regression period that impacts on the entire developing neuro-cognitive system. The unique possibility to "look back" gives new insights into the genetic interference with normal brain development and proves that this variant too, manifests itself within the first months of life.
Supported by: FWF (P19581-B02); Lanyar Foundation (P325).

CA08
Rett Syndrome and spasticity
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The presentation is dealing with the phenomen of spasticity in girls who have acquired the Rett-Syndrome. Spasticity is the result of a Brain associated movement disorder. Spasticity is initiated by outer and inner stimuli. From a therapeutical viewpoint can it be considered as a sensibility disorder. Permanent spastic muscelcontractions lead to luxation of the hips and deformations of foot and spine, which should be under permanent neuroorthopaedic dupervision to maintain a quality of live at a high level. The possibilities of the different therapeutical options (physio- and ergotherapy / occupational therapie, means of aid and surgery) are discussed. At the end spasticity should minimised. The given range of motion of the entire body should be maintained or improved in order to prevent permanent damage.
The presentation shows physiological basics of spasticity, the following progressive damages and the different therapeutical options. Time: 60 Minutes

Management

MA01
Examining the effect of the listening programme on girls with Rett Syndrome

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Introduction/Objectives: Girls with Rett syndrome experience communication difficulties compounded by reduced auditory processing. Sensory Integration (SI) theory1 suggests that this underlies attention to sound, limited concentration or anticipation, anxiety associated with change of events, difficulty engaging in interactions or comprehending language.
'The Listening Programme'2 (TLP) was used with 5 girls with Rett syndrome. TLP claims to address these issues by increasing users' auditory processing abilities. Using audio CDs with digitally enhanced high/low frequencies, TLP claims to stimulate fuller auditory nerve activity and neural processing. It has been used successfully particularly with individuals with autism3.
Participants, Materials/Methods: A cross-over trial including 5 students with Rett syndrome (multiple single case study) was conducted at a residential school for PMLD and complex health needs. Listening took place for 15 minutes daily (X5 per week) for 20 weeks; participants were randomly allocated to two groups to examine effect of listening to regular classical music, versus TLP itself. Group A had 4 weeks listening to classical music, followed by the 16-week TLP; Group B did 16 weeks TLP, followed by 4 weeks regular listening.
Behavioural observations were recorded during Listening, plus the two hour period following Listening while engaged in class activities. Video recordings were made prior to entering the study and at 4,16 and 20 weeks. Observers (and chief investigator) were blind to which category of Listening the students were following. Observations were made of mood (calm versus anxious) and attention (task and person engagement versus distractibility). Detailed assessments were made using school curriculum4.
Results: Early results indicate that mood was positively affected in all students, during both ordinary music and TLP. In two students, this change was greater with TLP. The clearest trend was an increase in attention and person engagement, most marked with TLP, in all students. In three students this was not sustained with ordinary music alone.
Conclusions: There are strong indications that TLP had a positive effect on attention, particularly in person engagement, above the effects of music alone. Mood appears to have been positively affected by TLP but also by music alone. (full data available Sept 08).

MA02
The New Danish center for Rett Syndrome

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The Kennedy Center in Denmark is a national research center for genetics, visual impairment and mental retardation. For the years 2007-2010 KC has received 0,24 mill. € per year to establish a National Center for Rett syndrome (RTT). 96 patients aged 3-60 years are known in Denmark
We are now a professional team consisting of three medical doctors (one is a

PhD student with a research project on osteoporosis in RTT, two are part time consultants), a molecular geneticist, a physiotherapist, a part time social worker, a part time nurse and secretary assistant.
We base our work and activities on:
- Evaluating each patient's physical and social needs
- Giving specialized knowledge to the families and local professional contacts
- Diagnosing and giving special support to families with a newly diagnosed child
- Generating new knowledge of RTT through research.

Our first activity was to send out questionnaires about expectations to the center to parents and caregivers. 66 questionnaires were returned, showing genuine interest, hope and expectations. The replies indicate a serious need for careful evaluation of motor ability and regression, for an outgoing function giving instructions to professionals, coordinating treatments, and for centralizing the expert knowledge on RTT.
From December 2007 until June 2008 30 patients have been evaluated. 26 of these fulfil the criteria to be included in the PhD project. After each visit a report on the patient is made focusing on a careful physical evaluation and recommendations on physiotherapy, medical treatment, diet and the social situation in the family. The report is sent to the family and all professionals involved.
We have experienced that there is a substantial need for a center for a rare condition as RTT. In general it is unrealistic to expect that the local professionals have expert knowledge or years experience in RTT, which might also limit their expectations of the patients' possibilities of improvement in motor and social function.
Already now we see that our center creates a different perspective on possibilities of management and treatment for the patients in their network of families, caregivers and professional contacts.

MA03

Personnal development framework plans implemented to 4 grils with Rett Syndrome in a centre for child with a disability.

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The quality of presence is played in the non-verbal. It was built in the field of education, which developed the cognitive skills (recognition of shapes, colors, objects, the concept of space, scale ...) are the phases of construction of the vocabulary. What will fuel the basis of the communication by images (icons). This translates to different learning level of personal autonomy, social and domestic. All these new skills developed will be able to materialize in the various activities: cooking, music, walks, storytelling, class, drama, crafts, computers ...
It is in these areas that every youth in a group, can show what they can do and what he likes to do. This work is built in partnership with parents necessary for a comprehensive approach to the young. A schedule individual and a timer gives them a benchmark space-time structure their learning.
The main objective of « Quelque Chose Plus » is to establish educational approaches depending on age and illnesses of children accommodated within the institution in meeting the obligations of the Law of the right of children to have education, care.
We implemented personal development framework plans (PDFP) to 4 Rett

Syndrome (RS) girls in our centre. Taking into account specificities of RS the PDFP focused on the development of communication (receptive and expressive), autonomy (personal and social) and cognition skills. Beside the learning education program, a care program (physiotherapy, psychomotricity speech therapy) was added.
Benefits of such program allow shared assessment criteria to define the progress in learning skills.

MA04
Support and Integration
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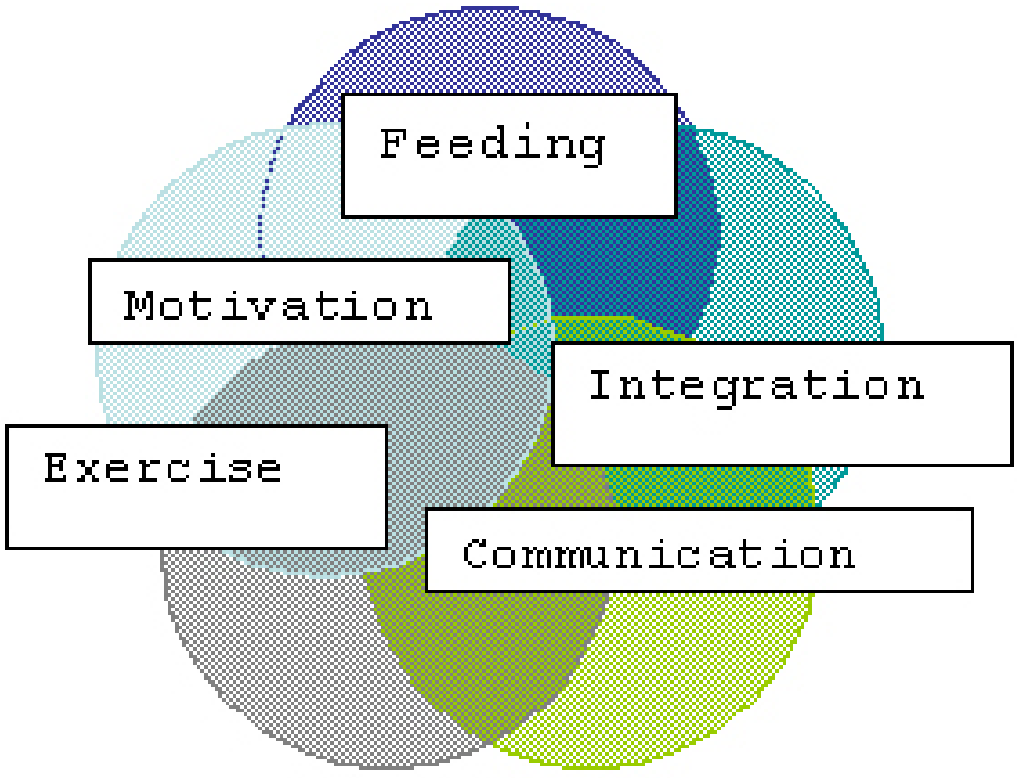
Sandra was born on the 8th of October 1982.When she was 18 months she was diagnosed with Autism, which actually turn out to be recognised as the RETT Syndrome when she was 7 years old.
She could only speak two words (“auto” and “micki”, the name of her cat) and started walking when she was 15 months. Since she was 3 years old we tried different therapies.
Now she lives in her own apartment within my house. Looking back over 23 years passed breading a girl with a RETT syndrome and trying diverse therapies, it is clear that my background as a nurse helped me a lot facing this situation.
Implementing a therapeutic model taking into account several dimensions, my experience could be summarized into 3 day to day management targets:
1. Give hope to all the people who are confronted with the RETT Syndrome in their daily life.
2. Explain the different therapies, which could help the women diagnosed with the RETT Syndrome to have a better life despite the handicap.
3. Give individualised information.



MA05
Rett association of Serbia - experiences and perspectives

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Rett Association of Serbia was founded in 1995 by a group of parents and professionals. Today it consists of over 100 members.
During the last 12 years the Association has carried out a number of activities, including detecting and mutual connecting of Rett families; organizing of educative conferences for the parents and professionals; purchase of wheelchairs; organizing of intensive therapy treatments in spas; organizing of a day-care center in Belgrade; support for the families in the form of special weekend care; collecting, translating and distribution of the literature; designing and maintenance of a web-site; informing the members on the current research and treatments; cooperation with ministries and governmental agencies; cooperation with similar associations in the country and abroad; etc.
Thanks to these activities, Rett syndrome is currently recognized in professional circles, but the aims are still not achieved. Many Rett children live under inconvenient conditions and without necessary therapies. The families are often without the funds due to the economic crisis and high rate of unemployment. Except two day-care centers (Belgrade and Nis), schools, support services, personal assistants are without the reach of Rett children in Serbia. Even their health protection is insufficient and inappropriate.
The Association is confronted with many problems. Due to small number of affected children, there are no permanent sources of financial support. Membership in the Association is free. The Association has no employees and engages only volunteers. Activities are organized and conducted only by few members, who are exposed to “burn-out” syndrome due to the high pressure. The situation imposes the problem of further Association functioning. New determination of priorities and redefinition of the strategy are required.

MA06
Conclusions from an intensive individual home training program

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I would like to present a unic home made individual therapy program with our daughter.
Therapy is made on daily base in one to one situation.....

MA07
Project to monitor the ossimetrici levels during music therapy treatment for a case of Rett Syndrome

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The final objective of our research is to evaluate whether Elisa, aged 12, affected by Rett Syndrome, is able to voluntarily control her own respiratory abnormalities.

Whilst considering which music therapy methodology would be most suitable to achieve this objective, I evaluated the possibility of developing a research project that could help me prepare the successive methodological work assessing Elisa's functional prerequisites related to respiration and thus the possible investigation of her communicative potential related to her vocal canal instruments including the flute.

The first aim of the research involves monitoring Elisa's breathing using a saturimetro.

The second aim involves recording Elisa's breathing activity, during a specific music therapy session which provides for the use of the flute.

This activity will consist of an elaboration in real time of a partitura which will reproduce Elisa's breathing pattern using the flute in accordance with the following scheme.

The final aim is to observe the presence of potential significant variations in Elisa's breathing along with a new type of musictherapy activity this time designed specifically to modify and control of the respiratory function. In this final phase, in fact, the task of the music therapist will be to use the flute a san instrument that imitates the breathing of a normal subject.

This research project is of Single Subject type composed of a monitoring phase in the domestic context a monitoring phase in the musictherapy context and a treatment phase .For each phase of the research 8 independent evaluations of subject E's ossimetrici levels will be made using a special instrument (saturimetro). These evaluations will then be compared with each other using the "C" test statistical technique (Von Neumann, 1941) for the analysis of the temporal series. This type of analysis will allow us to draw conclusions regarding the pattern of each individual phase (simple analysis) and the comparison made between the various phases (aggregated analyses).

Once the monitoring has been completed, we will collect the data we have obtained and continue the work in the hope that it will enable Elisa to use her own vocal canal in an increasingly spontaneous and deliberate way.

SATELLITE WORKSHOPS

Cardiorespiratory dysfunction and management in Rett Syndrome

Clinical determination of cardiorespiratory phenotypes in Rett Syndrome

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Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder that affects 1 in 10,000 females. Cardinal features that evolve due to immaturity of the brain include cortical features like severe mental retardation and epilepsy. Extra-pyramidal features like incoordination of actions, dystonia, orthopaedic deformations and secondary muscle wasting. Brainstem features dominate the RTT disorder and include monoaminergic dysfunction with dyspraxia, sleep disturbance, frequent daytime sleeping, night awakening and agitation. The abnormal breathing rhythms in the awake-state may be due to incompetence of the neuronal network of inhibitions in the brainstem secondary to immaturity. The resulting abnormal breathing rhythms and accompanying autonomic dysfunctions like a unique sympatho-vagal imbalance in which the sympathetic tone is normal but the parasympathetic tone is very low and remains at the neonatal level throughout life can explain the sudden deaths accounting for at least 25% of all deaths in RTT. The abnormal breathing in RTT together with the dysautonomia forms three distinct cardiorespiratory phenotypes that are important and vital for clinical management of the RTT disorder.
The cardiorespiratory phenotypes are determined during a one-hour corticobulbar neurophysiology in which cardiovascular vital signs like cardiac vagal tone (CVT), cardiac sensitivity to baroreflex (CSB), heart rate (HR), and mean arterial blood pressure (MAP) are recorded simultaneously in real-time. Respiratory vital signs like breathing rate and pattern, transcutaneous partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2) are also measured continuously. A total of 13 abnormal, awake breathing rhythms found in the RTT population are categorised into Feeble Breathers, Forceful Breathers and Apneustic Breathers and these constitute three unique cardiorespiratory phenotypes with different levels of autonomic tone and blood gases. Electroencephalogram and continuous video record time-locked with the physiological measurements are kept for behavioural analysis. The category of the dominant abnormal breathing pattern of the person with RTT determines the cardiorespiratory phenotype of that person. Valsalva's manoeuvres are common complications of breathing dysrhythmias in RTT and affect all the three cardiorespiratory phenotypes. Growth and development, nutritional needs and clinical risks associated with each of the three clinical phenotypes will be discussed.

Cardiorespiratory characterisation in Rett Syndrome: the Italian experience

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Introduction: 60 girls with Rett syndrome (RTT) were the subjects of the present study. Cardiorespiratory phenotypes were established and the International Scoring System (ISS) was used to assess the clinical presentations in each subject. The autonomic nervous system was examined using the NeuroScope (Medifit Instruments Ltd, London, UK), which allowed us to identify the specific cardiorespiratory phenotypes and genetic mutation was also investigated in each subject.
Results: 53 girls had MECP2 mutations (88.3%) and only 1 had a CDKL5 mutation. The remaining 6 girls (10%) were mutation negative according to our methods of detection. Body mass index and head circumference were significantly reduced compared to the normal population and the ISS score was higher in the classic form compared to formes frustes and to the preserved speech variant. Epilepsy was equally represented in all the clinical forms of RTT but drug-resistant cases were usually observed within the classic form. Forceful and Feeble cardiorespiratory phenotypes are extensively represented, 27/60 and 30/60 respectively, but only 3 cases (5%) were in the Apneustic cardiorespiratory phenotype. There was no statistically significant correlation between the different clinical variants of RTT and the cardiorespiratory phenotype and no correlation between subject's age and the cardiorespiratory phenotypes either.
Conclusions: We found cardiorespiratory abnormalities in all our subjects with RTT and since the specific phenotypes were not related to the subject's age, it implies that the phenotypes are stable and do not change with age. Our data also shows low ISS scores in the relatively benign clinical forms of Rett syndrome, especially in the preserved speech variant. We suggest that the NeuroScope system is useful in diagnosing Rett syndrome even in MECP2 negative cases.

Significance of cardiorespiratory phenotypes in clinical management of Rett Syndrome

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The NeuroScope system can be used for objective and quantitative monitoring of brainstem functions in Rett syndrome (RTT). The cardiovascular vital signs that can be measured are: cardiac vagal tone (CVT), cardiac sensitivity to baroreflex (CSB), heart rate (HR), and mean arterial blood pressure (MAP), all can be recorded simultaneously in real-time. The respiratory vital signs that can be measured are: breathing rate and pattern, transcutaneous mixed capillary partial pressures of oxygen (pO2) and carbon dioxide (pCO2). We have assessed more than 120 patients with classical Rett syndrome in the Swedish National Rett Centre and found the following: Three cardiorespiratory phenotypes, which we named Forceful, Feeble and Apneustic breathers. These were present in similar proportions in the Rett population but early developments measured by occipitofrontal head circumference (OFHC) and body mass index (BMI) were different among the phenotypes. This has implications in nutritional management. Overall growth indicated by height was similar among the phenotypes. Baseline levels of CVT and CSB were different among the cardiorespiratory phenotypes

indicating inter-phenotypic differences in parasympathetic activities. However, parasympathetic activity in the whole population was similar to that previously reported in Rett patients. Baseline levels of MAP and HR were similar among the phenotypes, which suggests no inter-phenotypic difference in sympathetic activity and is consistent with previous reports of little effects of Rett syndrome on baseline sympathetic tone. The reported adverse effects of opiate analgesics and idiosyncratic responses to diazepams were limited to Feeble Breathers while hypocapnoeic attacks were unique and specific to the Forceful Breather only. We conclude that Rett syndrome is made up of heterogeneous and distinct cardiorespiratory phenotypes that are important for clinical management of the disorder. We recommend early identification of these phenotypes to facilitate objective and appropriately targeted clinical management of the RTT disorder.

Cardiorespiratory influences on the EEG in Rett Syndrome

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The electroencephalogram (EEG) in Rett syndrome (RTT) generally shows a slow background of theta activity at 4 – 6 Hz. At times Rolandic spikes, focal, multi-focal or generalised epileptiform discharges can be seen. Relatively normal EEG characteristics including alpha waves can be seen in the more able persons with RTT.

Almost 50 % of persons with RTT are reported to have epilepsy. While some have true epileptic seizures, other seizure-like events are not associated with epileptiform features in the EEG. These are most likely due to abnormal activity in the brainstem and can be difficult to distinguish from epileptic seizures clinically.

We have studied the cardiorespiratory abnormalities in RTT that cause nonepileptic events to establish if they are associated with observable changes in the EEG.

During the involuntary Valsalva's manoeuvre in RTT, a common complication of breathing dysrhythmia during which intrathoracic pressure increases above 20 mmHg due to forceful expiration against a closed glottis followed by sudden expulsion of air when the glottis finally opens, reproducible attenuation of EEG activity followed by short bursts of rhythmical slow wave activity often in all leads accrues during or immediately following the Valsalva's manoeuvre. Increased partial pressure of CO2 (pCO2) caused by feeble or apneustic type of breathing can lead to widespread slowing of EEG activity, often affecting all leads. Decreased pCO2 caused by forceful breathing can induce attenuation of the background rhythm and if associated with hypoxia caused by central apnoea can obliterate EEG activity. The very common Abnormal Spontaneous Brainstem Activations (ASBA) seen in RTT very rarely spread to the cortex, therefore no changes are often seen in the EEG. Out of more than 200 cases we have monitored, only three had any cortical activity associated with ASBAs. These results demonstrate the clear need for concurrent cortico-bulbar neurophysiology in order to fully understand and diagnose EEG changes and seizure-like events in RTT.

Abnormal spontaneous brainstem activities (ASBA) in Rett Syndrome

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Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder that affects mostly females. Brainstem features caused by immaturity dominate the RTT disorder throughout life. Assessment of brainstem function may be important for understanding the clinical presentations in RTT. The cardiovascular vital signs measured are cardiac vagal tone (CVT), cardiac sensitivity to baroreflex (CSB), heart rate (HR), and mean arterial blood pressure (MAP), recorded simultaneously in real-time. Respiratory vital signs measured are breathing rate and pattern; transcutaneous partial pressures of oxygen (pO2) and carbon dioxide (pCO2), also recorded continuously. Electroencephalogram and continuous video record time-locked with the physiological measurements are kept for monitoring cortical activity and behavioural analysis.

Brainstem activations seen are: “Brainstem Storms” - spontaneous and simultaneous large increases in all cardiovascular indices coinciding with troughs in pO2 where the differences with pCO2 becomes very small, equivalent to tissue asphyxia. These may be chemoreceptor-driven brainstem activations. Other unexplained brainstem paroxysms includes repeated and generalised activations of the whole medulla oblongata affecting both rostral and caudal parts for more than one minute. We call these “Brainstem Epilepsy”. The affected patients are ill with subdued consciousness. Agitation and or screaming accompany the attacks. Mild single or multiple short attacks affecting rostral, caudal or the whole medulla is called sporadic ASBA. Brainstem deactivations are: “Brainstem Shutdown” - simultaneous sympathetic and parasympathetic withdrawal and HR is clamped to sino-atrial pacemaker rate while MAP drops towards 40 mmHg (5.3 kPa). It is equivalent to cardiovascular denervation and can last up to 3 minutes. The patient is semi-conscious, cannot keep upright and the skin is pale. This can be mistaken for hypotonic attack in epilepsy. Mixed deactivations and activations are: “Rage Attacks” (sham rage) - the rostral medulla oblongata is activated leading to increased sympathetic tone while the caudal medulla is deactivated leading to decreased parasympathetic tone. These are associated with dilated pupils, sweating and changes in moods often leading to screaming. We conclude that monitoring brainstem activity is necessary for understanding the complex clinical presentations in RTT and can be used for objective and rational clinical management.

Treatment of apneustic breathing in girls with Rett Syndrome- a pilot study

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Breathing dysrhythmia is a feature of brainstem immaturity in Rett syndrome

(RTT) and presents as three cardiorespiratory phenotypes of which apneustic breathing is one. We investigated whether neuromodulatory effect of the 5-HT1A receptors agonist (Buspirone) can correct apneustic breathing. Southern General Hospital NHS Trust Glasgow and Central Middlesex Hospital London Local Ethics Committees approved this study. Parents or carers received written explanation of our procedure and they provided written consents.
We used the NeuroScope system (MediFit Instruments LTD, London, UK) to assess brainstem function. Breathing movement was monitored using resistance plethysmograph, electrocardiogram, continuous non-invasive beat-to-beat arterial blood pressure and transcutaneous mixed capillary blood gases; pCO2 and pO2 were all recorded synchronously. Electroencephalogram and video were also recorded synchronously and time-locked to the physiological data. Initial screening test for “Apneustic Breathers” was carried out. An arbitrary cut-off point of 15% or more of apneustic breathing out of 30 min. screening period was the acceptable level of apneustic breathing and these patients were admitted into the study. Of the 20 girls with RTT screened, only 8 girls aged 3-26 years (mean age 8.9) were admitted into the study. One-hour brainstem assessment was conducted to establish baseline brainstem autonomic function before administration of the drug. Further brainstem autonomic assessments were at 48 hours, 2 weeks and lastly one month during which continuous Buspirone treatment was maintained. Buspirone treatment was started gradually at 5mg a day and increased by 5 mg every 48 hours to a total daily dose of 20 mg, which was maintained for the remaining one month.
We observed significant reduction in prolonged breath holding and protracted inspirations, but significant increase in the shorter regular breath holdings performed within normal respiration rates. These results suggest that Buspirone can be used to treat the prolonged forms of apneustic breathing in RTT and offers a novel therapeutic use in this disorder.
We wish to thank Dr Alison M Kerr, OBE, MD, retired Senior Lecturer in Paediatrics and Learning Disability, University of Glasgow, UK for advice and encouragement.

Detection of hypocapnic attacks and resuscitation in Rett Syndrome

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Persons with Rett Syndrome (RTT) presenting as Forceful Breathers tend to have fixed low levels of pCO2 (chronic respiratory alkalosis). Episodes of increased hyperventilation or Valsalva like breathing may cause the pCO2 to drop so low that hypocapnic attacks with tetany develop. This can often be mistaken for an epileptic seizure.
Hypocapnia can be detected by measuring mixed capillary blood gases transcutaneously concurrently with brainstem function using the NeuroScope system (Medifit Instruments Ltd, London, Uk). Persons with a pCO2 level of less than 20 mmHg (2.7 kPa) have high risk of developing hypocapnic attacks. Simultaneous EEG recording is required to monitor cortical effects of the attack,

thus the whole arrangement constitutes cortico-bulbar neurophysiology.
The short-term management of infrequent hypocapnic attacks is similar to panic attacks or hysteria and involves re-breathing into a 5 l bag, preferably attached to a tightly fitting facial mask. This will gradually increase the level of CO2 inhaled by the patient and thus the blood pCO2 level. For more severe and long-term cases, a constant increase in inspired CO2 is necessary. The safest source of this is Carbogen, a 5% CO2 in oxygen mixture administered continuously through nasal prongs. Treatment by reducing the tidal volume with Bi-level Positive Airway Pressure (BiPAP system) should in theory be feasible but person with RTT may not easily tolerate the delivery mask. Treatment with CO2 should be monitored closely; preferably by measuring blood gases and the blood level of carbon dioxide will determine the end-points of the treatment.
The long-term aim is to move the operational pCO2 level towards normal between 39 – 44 mmHg (5.2 – 5.9 kPa). Since the operational levels of pCO2 are very variable in RTT due to resetting of the central respiratory chemoreceptors, it is evident that regular cortico-bulbar neurophysiology must be part of clinical management in order to achieve a stable breathing.

SUDEP: Sudden Unexpected Death in EpilePsy: lessons from Rett Syndrome

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Sudden Death in Epilepsy (SUDEP) is the sudden unexpected non-traumatic and non-drowning death in patients with epilepsy. It is a leading cause of death in epilepsy, responsible for at least half of fatalities. There are only few studies in children, but the suggested risk factors include convulsive seizures, prone body position, seizures frequencies more than 50 a month, full scale Intelligence Quotient of less than 70 and multiple anti-epileptic drugs, at least more than two.
Cardiorespiratory mechanisms may be involved in SUDEP. Respiratory changes with central and obstructive apnoea, excess bronchial and oral secretions, pulmonary oedema and hypoxia have all been observed in seizures. Cardiac abnormalities are also well documented with reduced HRV in epilepsy; seizure-associated rhythm and repolarisation abnormalities and at post-mortem myocardial fibrosis is seen.
There is no unified explanation for SUDEP but lessons from Sudden Infants Death Syndrome suggest that a combination of respiratory and cardiac repolarisation abnormalities may be important in the causation. In Rett syndrome (RTT), there is a greater risk of death compared to the normal population, particularly after the age of 20 years with survival rates at 35 years of 70% compared to 98.4%. The majority of deaths appear to be sudden and more recently 22% of deaths reported to the International Rett Syndrome Association were sudden deaths of unknown cause.
Our studies of cardiorespiratory function in RTT have shown multiple breathing dysrhythmias both at rest and during seizures caused by brainstem immaturity. We have also shown a unique sympatho-vagal imbalance in which cardiac vagal tone is very low in association with normal sympathetic activity at rest in RTT. This suggests that the complex integrative inhibition of brainstem neurones required for vagal and respiratory functions are more affected than pacemakerdependent sympathetic functions and this could carry a greater risk of sudden

death. The complex cardiorespiratory abnormalities in RTT may provide a good opportunity to study and unlock the causes of SUDEP.

The use of cardiorespiratory indices in the management of patients with Rett Syndrome

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The autonomic nervous system is deeply involved in Rett syndrome (RTT) with dysfunctional central regulation. Breathing dysrhythmia, insufficient control of blood gases, unstable blood pressure and heart rate deranges bodily homeostasis. Other common disorders are: Oropharyngeal dysfunction, aspiration, gastrooesophageal reflux, oesophagitis, constipation, urine retention, cold discoloured feet, sleep problems, mood swings and agitation, all related to autonomic dysfunctions.

We used the Neuroscope system to assess autonomic function and facilitate rational management of RTT. Breathing abnormalities were categorised by rhythm to establish cardiorespiratory phenotypes. Baseline autonomic activity was measured during normal breathing, normal blood gases and no epileptiform activity in the cortex.

Variation level of brainstem competence, from neonatal to adult level with unstable respiratory oscillator and lack of sympathetic restraint were found. Unusual behaviours due to abnormal spontaneous activations of the braintem (ASBA) were common. Rolling of eyeballs and unsteadiness due to ASBA could easily be mistaken for clinical signs of epileptic fits. Three cardiorespiratory phenotypes were identified: Forceful, Feeble and Apneustic Breathers. Feeble or apneustic breathers may not restart spontaneous breathing post-operatively due to artificial hyperventilation. Feeble breathers are sensitive to sedation (Opioids and Diazepam) and these drugs can cause apnoea. Valsalva's manouver is a common complication in RTT. Nutritional needs can be doubled the normal value for forceful breathers. Assisted breathing (Bi-pap) can be used to prevent respiratory acidosis. We also found Theophyllin useful as a respiratory stimulant and Buspirone for apneustic breathers. Re-breathing in a bag or using carbogen can treat Hypocapnic attacks in Forceful Breathers.

For weak parasympathetic control, joyful physical activity every day and relaxing music were encouraged to build up parasympathetic tone.

We had good clinical outcomes from these objective measures in combination with further follow-up investigation.

AFSR (French Rett Syndrome Association)

Psychomotor management

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The purpose of the psychomotor management of RETT Syndrome is to improve the quality of life for disabled children. This requires a close relationship between parent / professional.
We will resume in the workshop principle bases of child development and suggest techniques and exercises in correspondence with each step. In a second section, we discuss a more detailed and precise, the basement of the psychomotor, ie the sensorymotor
approach.
It is important to highlight a few reminders about child development. We base then on the psychomotor development of children. It is from there that will build support in psychomotor management approach. The changing tone is linked to neurological maturation. It responds in particular to two laws of development:
- cerebrospinal tail law: motor control and positional grows from the top down of the body. (keeping the head is present before sitting…)
- proximo-distal law: control segments of changing members of the vertebral axis to the periphery.
(The shoulder is first used, then the arm, forearm and hand.)
Finally, these two laws are governed by the law of differentiation: motor overall refined and differentiates activities increasingly localized, fine and appropriate.
We must respect this timeline when we offer activities for girls with a RETT Syndrome. This implies a choice of exercises that both maintain stages acquired, and help move the following stages in postural control, dialogue tonic.
The sensory-motor approach as described by André Bullinger perhaps regarded as the bedrock of psychomotricity. His contribution lies in aspects of sensory and motor function in their archaic warning, orientation and formatting of the body making it possible conduits handling and consumption, this in perspective instrumental. Sensorymotor
skills are always present in human behaviour. «Their place and their importance vary depending on the tasks, in which the subject is committed» (A. Bullinger).
The interactions between the organism and its environment that will help build a set of performances that cover the body, objects encountered and the space that contains them. The sensory flow (gravity, tactile, olfactory-taste, sound, visual) are a privileged materials that fuel the mental activity of the individual.
The state and tonic postural will vary depending on the flow collected. The interactions between states tonics, postural and emotional dimensions raised will allow to create a balance sensory-tonic, said platform sensory-tonic (H. Walloon), from which instrumental actions are possible. This activity tonic first representation of sensory-motor can take place only if there is a balance sensory-tonic. A tone too high will freeze the body or cause discharge tonics, unlike a collapse tonic does not allow mobilization tonic. The human appeal through dialogue tonic (J. de Ajurriaguerra) with the body of the other will help a space merger that creates, to give meaning to its landfills tonic. «Gradually, dialogue with others will help build the capacity to regulate and contain these emotional states» (A. Bullinger).
During development, the objects of knowledge that the baby reaches evolve. The description of child development in terms of successive spaces allows us to identify

in different stages of development and disorders that can result in disruption or bad acquisitions. We recall that the term «space» is on the sensory-motor coordination that do exist. It is a common language that allows a dialogue between different sensory modalities and stabilizes representations of the body. It allows you to locate his body and objects in space.
«Thus the sensory-motor approach explores more particularly the role of the activity of the topic, the importance of covariations between modalities for sensory-motor control instrumental. «(A. Bullinger).
The sensory-motor approach is a way to assess but also to take charge of baby and child carrying deficits, disruptive sensory or motor.

Psychomotricité

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Le but de la psychomotricité dans la prise en charge du syndrome de RETT est d'améliorer la qualité de vie des enfants polyhandicapés. Cela passe par une étroite relation parent/professionnel.
Nous reprendront dans l'atelier les principe de bases du développement de l'enfant et proposerons des techniques et exercices en correspondance avec chaque étape. Dans une seconde partie, nous aborderons de façon plus fine et précise, les soubassements de la psychomotricité, c'est-à-dire la sensorimotricité.
Il est important de souligner quelques rappels concernant le développement de l'enfant. Nous nous basons alors sur le développement psychomoteur de l'enfant. C'est de là que se construira la prise en charge en psychomotricité. L'évolution du tonus est lié à la maturation neurologique. Il répond en particulier à deux lois de développement :
- loi céphalo-caudale : le contrôle moteur et posturale se développe du haut vers le bas du corps. (le maintien de la tête est présent avant la position assise…)
- loi proximo-distale : le contrôle des segments de membres évolue de l'axe vertébrale vers la périphérie. (l'épaule est d'abord utilisée, puis le bras, l'avant bras et enfin la main.)
Enfin, ces deux lois sont régies par la loi de différentiation : la motricité globale s'affine et se différentie en activités de plus en plus localisées, fines et adaptées.
Il convient de respecter cette chronologie lorsque nous proposons des activités aux filles avec un Syndrome de RETT. Cela implique un choix d'exercices qui à la fois maintiennent les stades acquis, et contribuent à passer au stades suivants dans le contrôle postural, le dialogue tonique
L'approche sensori-motrice telle que la décrit André Bullinger peut-être considérée comme le soubassement de la psychomotricité. Son apport se situe au niveau des aspects
sensoriels et moteur dans leur fonction archaïque d'alerte, d'orientation et de mise en forme du corps rendant possible les conduites de manipulation et de consommation, ceci dans une perspective instrumentale. Les conduites sensori-motrices sont toujours présentes dans le comportement humain. « Leur place et leur importance varient suivant les tâches, dans lesquelles le sujet est engagé » (A. Bullinger).
Les interactions entre l'organisme et son milieu vont permettre que se construisent un ensemble de représentations qui portent sur l'organisme, les objets rencontrés et l'espace qui les contient. Les flux sensoriels (gravitaires, tactiles, olfactifs-gustatifs, sonores, visuels) sont un des matériaux privilégiés qui alimentent l'activité psychique de l'individu.
L'état tonique et postural va se moduler suivant les flux perçus. Les interactions entre les états toniques, posturaux et les dimensions émotionnelles suscitées vont

permettre que se créent un équilibre sensori-tonique, dit plate-forme sensori-tonique (H. Wallon), à partir duquel des actions instrumentales sont possibles. Cette activité tonique de première représentation sensori-motrice ne peut se dérouler que s'il y a un équilibre sensori-tonique. Un tonus trop élevé va figer l'organisme ou provoquer des décharge toniques, à l'inverse un effondrement tonique ne permet pas de mobilisation tonique. Le recours humain au travers du dialogue tonique (J. de Ajurriaguerra) avec le corps de l'autre va aider, par l'espace de fusion qu'il crée, à donner un sens à ses décharges toniques. « Progressivement, le dialogue avec autrui va permettre de construire les moyens de réguler et de contenir ces états émotionnels » (A. Bullinger). Au cours du développement, les objets de connaissances auxquels le bébé accède se transforment. La description du développement de l'enfant en termes d'espaces successifs nous permet de nous repérer dans les différentes étapes de ce développement et des troubles qui peuvent en découler lors de perturbation ou de mauvaises acquisitions. Nous rappelons que le terme « espace » est relatif aux coordinations sensori-motrices qui le font exister. C'est un langage commun qui permet un dialogue entre les différentes modalités sensorielles et stabilise des représentations de l'organisme. Il permet de situer son corps et les objets dans l'espace.
« Ainsi la sensorimotricité explore plus particulièrement le rôle de l'activité du sujet, l'importance des covariations entre les modalités sensori-motrices pour la maîtrise instrumentale. » (A. Bullinger).
L'approche sensori-motrice est un moyen privilégié pour évaluer mais aussi de prise en charge du bébé et de l'enfant porteur de déficits, de désorganisations sensorielles ou motrices.

Conductive Pedagogy
Annick Champolion-Puel,
AFPC
Each caregiver involved in the management of Rett syndrome wants to develop different functional areas defined in an attempt to assess the capabilities of these children: communication, motor skills and tone, perception, attention, intention and initiatives. All these fields are interrelated and dependent on each other, paramedical and educational activities must also be, and indeed, each professional can act in each area.
The professionals, educators, physiotherapists, psychomotor, occupational therapists, speech therapists, caregivers together in a multidisciplinary team, are dreaming to work with children and their parents, in a trans-disciplinarity, knowledge sharing and action without losing their professional specificity.
The conductive education, imagined in Hungary in 1940 by Professor Petö to enable disabled children with cerebral palsy to access to school, offers perhaps the possibility of putting into practice the concept of trans-disciplinarity. The experience of the team conductive leading groups of children is carried out in 2 settlements in the Ile de France region. Three girls with Rett syndrome have been able to participate in these groups.
AFPC (French association of Conductive Pedagogy) intends to submit his research, observations and reflections on this approach. After a theoretical presentation, we will comment on a film showing the application of the principles of Conductive Education with a group of disabled children.
Finally we will discuss developments in images of 3 young girls with their parents.

Pédagogie Conductive
Annick Champolion-Puel,
AFPC

Chaque personne intervenant dans la prise en charge d'enfants présentant un syndrome de Rett souhaite développer les différents secteurs fonctionnels définis pour tenter d'évaluer les capacités de ces enfants particuliers : communication, motricité et tonus, perception, attention, intention et prise d'initiatives.
Tous ces secteurs sont liés et dépendants les uns des autres, les actions éducatives et paramédicales se doivent de l'être aussi ; et, en effet, chaque professionnel peut agir dans chaque domaine.
Les professionnels, éducateurs, kinésithérapeutes, psychomotriciens, ergothérapeutes, orthophonistes, AMP réunis en équipe pluridisciplinaire, rêvent de travailler, avec des enfants et leurs parents, en transdisciplinarité et de pouvoir partager leur connaissance et action sans perdre leur identité professionnelle.
La pédagogie conductive, imaginée en Hongrie en 1940 par le Pr Petö pour permettre aux enfants infirmes moteurs cérébraux d'accéder à l'école, offre, peut être, la possibilité de mettre en pratique le concept de transdisciplinarité pour les enfants polyhandicapés.
L'expérience de l'équipe conductive animant des groupes d'enfants polyhandicapés est menée depuis 10 ans dans 2 établissements en Ile de France. Trois jeunes filles présentant un syndrome de Rett ont pu participer à ces groupes.
L'AFPC se propose de présenter ses recherches, observations et réflexions concernant cette approche.
Après une présentation théorique, nous commenterons un film montrant l'application des principes de la Pédagogie Conductive avec un groupe d'enfants polyhandicapés.
Enfin nous évoquerons en images l'évolution des 3 jeunes filles avec leurs parents.

Day to day management, implementing basal stimulation (described by Andreas Fröhlich)

Dany Gerlach,
trainer, basal stimulation method, CPME , French Rett Syndrome Association

People with Rett syndrome suffer, even if the diagnosis is the same for all, an evolution, even a regression very different from each other. When they reach the stage of polyhandicap, they become dependent, sometimes totally, a third person in all acts of everyday life. At this stage, care becomes more difficult and complex.
They need people who understand without verbal language, who accompany them and treat them with confidence, enabling them travel, movement and change. Above all, it is important to structure and organise the daily so that they feel well-being, motivation and joy of living and they find meaning to their lives.
The acts of daily life are, for these people, space time the longest in one day, they live at home, in a day or in a home. It is essential to take advantage of all these situations natural daily to enable them to make structural and rewarding experiences, both with their own body as the social and physical environment. The daily becomes - alongside activities more "therapeutic", medical and paramedical, - "a central living space", as A. Fröhlich, an opportunity to promote their development.
These ideas are exactly the objectives of the basal stimulation, an approach developed 30 years ago by Andreas Fröhlich, a professor of Special Education in Germany, which sparked international concern for many years.
After a short introduction on the concept and basic principles of this approach, leaders propose advice and practical ideas useful in daily support.
The experience of professionals will be complemented and enriched by a mother experience with a daughter suffering from Rett syndrome.

Approche au quotidien, des personnes ayant un polyhandicap, en référence à l'approche de la

stimulation basale® d'Andreas Fröhlich

Dany Gerlach,
Formatrice, méthode de stimulation basale, CPME , AFSR

Les personnes atteintes d'un syndrome de Rett subissent - même si le diagnostic est le même pour toutes – une évolution, voire une régression très différentes les unes des autres. Lorsqu'elles atteignent le stade du polyhandicap…., elles deviennent dépendantes, parfois totalement,d'une tierce personne dans tous les actes de la vie quotidienne. A ce stade, les aider devient plus difficile et plus complexe.
Elles ont besoin de personnes qui les comprennent sans langage verbal, qui les accompagnent et les soignent avec confiance, qui leur permettent déplacements, mouvements et changements. Surtout, il est important de structurer et d'organiser le quotidien de manière à ce qu' elles éprouvent du bien-être, de la motivation et de la joie de vivre et qu'elles trouvent du sens à leur vie.
Les actes de la vie quotidienne constituent, pour ces personnes, l'espace temporel le plus long dans une journée, qu'elles vivent à la maison, dans une structure journalière ou dans un foyer. Il est essentiel de profiter de toutes ces situations naturelles du quotidien pour leur permettre de faire des expériences valorisantes et structurantes, aussi bien avec leur propre corps qu'avec l'environnement social et matériel. Le quotidien devient – à côté des activités plus «thérapeutiques»,médicales et paramédicales, – «un espace de vie central», comme le dit A. Fröhlich, une occasion de favoriser leur développement.
Ces idées correspondent exactement aux objectifs de la stimulation basale, une approche développée il y a 30 ans par Andreas Fröhlich, professeur en pédagogie curative en Allemagne, et qui suscite un intérêt international depuis de nombreuses années.
Après une petite introduction sur le concept et les principes de base de cette approche, les animateurs proposeront des conseils et des idées pratiques utiles dans l'accompagnement au quotidien.
L'expérience des professionnels sera complétée et enrichie par le témoignage et l'expérience d'une mère ayant une fille atteinte d'un syndrome de Rett.

"Today I have a dream…" : Mr Luther King could have made such speech regarding CIT

Christian Schoen

(Without definitive solutions, the moderator will present its thoughts and proposals regarding CIT and social changes)
CIT are an inexhaustible information' resource of increasing quality, a global network to communicate and exchange, a medium occupying a growing space in the society, the family and each person life (time spent per day) and a social system without social barriers; because of that, no one should be left aside CIT development and opportunities.
CIT developed extremely rapidly and intensively, worldwide and in all domains but specifically in healthcare ("e-health"), and are becoming unavoidable although they are not always designed for all and everyone (see "Web for all" European program). With CIT, world and people are changing because of: simplicity, globality, accessibility, cost …
Recent developments may socially help specific groups such as rare diseases' patients and relatives, to have a greater role in the society and to break (few) social barriers.
CIT are transforming the way people are communicating. From a "businessto-consumer" model = from "experts" to learning ones (pyramidal model), we

are entering, because of Google, Wikipedia, UTube …, in the CommuniActing world = communicating within communities and being actors and not (only) spectators.
Communitying is a growing social fact, creating and animating communities. Within such community, each and every person is acting and interacting, networking and personalizing its environment, demanding quality … (Web 2.0 concept). Plus, if individual may be excluded from society because they act as individual, communities should and will “force” society to have a different perception.
Discussion

Eye motricity

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The anatomical and sensory vision are essential prerequisites of the lives of visual function. Recent advances in neuroscience allow us a much more acute in the analysis of messages received by this extraordinary body and their way of conduction.
In RETT Syndrome, ophthalmology and orthoptie occupy a place quite justified in a multidisciplinary care. The importance of a systematic vision management will be an integral part of any care program. Various profiles can exist in this syndrome: the absence particular eye disorder, the need for a port of corrective lenses, the presence of a deviation eye-motor or delays maturation of visual function. The fine structure of care rehabilitation will optimize the best visual capabilities of the patient with RETT Syndome.
The vision, an important tool in the non-verbal communication is the basis of any constructive exchange between the daughter RETT, his family and professionals and is a driving force in the care overall.
“The beauty is something in the eyes that expresses intelligence, and intelligence is something in the eyes that expresses the beauty” Bernard Werber.

Oculomotricité

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Orthoptie, Association Française du Syndrome de Rett

L’intégrité anatomique et sensorielle de la vision constitue des préalables impérieux des conditions d’existence de la fonction visuelle. Les progrès récents des neurosciences nous permettent une compréhension beaucoup plus aiguë dans l’analyse des messages perçus par cet extraordinaire organe ainsi que leur mode de conduction.
Dans le syndrome de RETT, l’ophtalmologie et l’orthoptie occupent une place tout à fait justifiée dans une prise en charge pluridisciplinaire. L’importance d’un contrôle ophtalmologique systématique doit faire partie intégrante de tout bilan. Divers profils peuvent exister dans ce syndrome : l’absence de trouble particulier, la nécessité d’un port de correction optique, la présence d’une déviation oculo-motrice ou encore des retards de maturation de la fonction visuelle. La structuration fine d’une prise en charge rééducative permettra d’optimiser au mieux les capacités visuelles de la patiente avec un Syndrome de RETT.
La vision, outil important dans les communications non verbales est la base de tout échange constructif entre la fille avec un Syndrome de RETT, son entourage familial et les professionnels et constitue un élément stimulant dans la prise en charge globale.

« La beauté c'est quelque chose dans le regard qui exprime l'intelligence, et l'intelligence c'est quelque chose dans le regard qui exprime la beauté » Bernard Werber.

Food and nutrition, evaluation and management in Rett Syndrome

Catherine Senez,
speech therapist et Irène Benigni, nutritionist, French Rett Syndrome Association

The nutritional status and accompanying meals for people with Rett syndrome are central concerns of families and professionals.
To meet the needs of children and adults with Rett Syndrome, an evaluation of their nutritional status, their swallowing capabilities, and difficulties encountered in everyday life is needed.
This assessment focuses on:
- anthropometric criteria, sometimes supplemented by biological criteria,
- evaluation of chewing and swallowing and capacity to feed and hydrate,
- oral dental status,
- dietary intake and energy expenditure,
- appetite and meal duration,
- digestive disorders (gastroesophageal reflux and constipation).

In the light of the study that we conducted in 2008 in partnership with the French Association of Rett Syndrome, which includes 221 children and adults, we will highlight the difficulties most frequently encountered and possible solutions. These solutions involve dietary and educative measures, adjust physical and medical care. Discussion will be illustrated by clinical cases. They are part of an individualized treatment strategy that takes into account the person as a whole and on all its accompanying partners, families and professionals.

Alimentation, nutrition et Syndrome de Rett : évaluation et prise en charge

Catherine Senez,
orthophoniste et Irène Benigni, diététicienne, Association Française du Syndrome de Rett

L'état nutritionnel et l'accompagnement des repas des personnes avec syndrome de Rett sont au centre des préoccupations des familles et des professionnels .
Afin de répondre au mieux aux besoins des enfants et adultes Rett, une évaluation de leur état nutritionnel, de leurs capacités oro-motrices, et des difficultés rencontrées au quotidien est indispensable.
Cette évaluation porte sur :
- des critères anthropométriques, complétés parfois par des critères biologiques,
- une observation de la mastication et de la déglutition et des capacités à se nourrir et à s'hydrater
- l'état bucco dentaire
- Les apports alimentaires
- les dépenses énergétiques
- l'appétit et la durée des repas
- les troubles digestifs (reflux gastro-oesophagien et constipation).

A la lumière de l'étude que nous avons réalisée en 2008 en partenariat avec

l'Association Française du Syndrome de Rett et qui concerne 221 enfants et adultes Rett, nous ferons le point sur les difficultés les plus fréquemment rencontrées et les pistes de solutions possibles.
Ces solutions font appel à des mesures diététiques, à des mesures rééducatives, à des adaptations matérielles ainsi qu'à une prise en charge médicale et seront illustrées par la présentation de cas cliniques.
Elles s'inscrivent dans une stratégie thérapeutique individualisée qui prend en compte la personne dans sa globalité et qui concerne tous les partenaires de son accompagnement, familles et professionnels.

BIOGRAPHIES

ADACHI Megumi
Dr. Megumi Adachi obtained M.S. in Biochemistry at the University of Rhode Island and Ph.D. in Biochemistry at the Oregon Health and Science University. She is currently a postdoctoral fellow in the laboratory of Dr. Lisa Monteggia at UT Southwestern Medical Center. Her work is aimed at understanding the role of MeCP2 in animal behavior and synaptic function linking pathophysiology of Rett syndrome.

AKBARIAN Schahram
Dr. Schahram Akbarian is the Associate Director of the Brudnick Neuropsychiatric Research Institute at the University of Massachusetts Medical School. He received his graduate training at the Freie Universitaet Berlin in Germany, and did postdoctoral research in the laboratories of Dr. Edward G. Jones and Dr. Rudolf Jaenisch.He is a board certified psychiatrist trained at the Massachusetts General Hospital in Boston.

AL-RAWAS Sami S F
Consultant in Clinical Neurophysiology and Clinical Autonomic Investigation in the Department of Clinical Physiology/Clinical Neurophysiology.

BAHI-BUISSON Nadia
Dr Bahi- Buisson is an associate professor in Pediatric Neurology unit of Necker Enfants Malades Hospital, Paris France that is involved in clinical follow up of Rett patients since 2003 and build a multidisciplinary consultation in Necker with high interest on epileptology and movement disorder in Rett syndrome. In parallel, she participates in molecular projects on Rett, including phenotype genotype correlation and search for new molecular bases of Rett, but also in developing therapeutic approaches. She is the president of medical scientific committee of the French association of Rett, and strongly involved in EURORETT

BAIKIE Gordon
Gordon is a paediatrician in the Department of Developmental Medicine at the Royal Children's Hospital, Melbourne, Australia. His research interests include aspiration, dysphagia and Rett syndrome. He is the paediatrician in the multidisciplinary Rett syndrome clinic.

BALLESTAR Esteban

Esteban Ballestar leads the Chromatin and Disease Group of the Cancer Epigenetics and Biology Programme (PEBC) at the Catalan Institute of Oncology. In the past years, he has been working in association with Manel Esteller at the CNIO after a postdoctoral period in Alan Wolffe's lab at NIH.

BEBBINGTON Ami
Ami is a biostatistician with Rett syndrome study. She is new career researcher who has had a signficant role in the Australian Rett syndrome study. She loves mathemtics.

BENIGNI Irène
Dietician specialized in severely multi handicapped people.
She has worked for nearly twelve years with severely multi handicapped and cerebral palsied people. She is the president of the paramedical Council of the French Rett Syndrome Association and she is in charge of an adult continuing education programme in a training institution called CESAP (Paris).
In 2005, she has followed a degree course about malnutrition and tube feeding at the academy of medicine in Lille. She is contributing to research studies dealing with a nutritional status assessment for severely multi handicapped adults. She has contributed at the collective book called: "Le Syndrome de Rett, une maladie génétique" published by the French Rett Syndrome Association.

BEN-ZEEV Bruria Ghidoni
Dr Bruria Ghidoni Ben-Zeev, Pediatric Neurologist, Head of Pediatric Neurology unit and the Israeli Rett Ctr in Safra pediatric Hospital, Sheba Med. Ctr. Ramat-Gan, Israel. Assistant Professor in Sackler School of Medicine, Tel Aviv University. Main fields of interest both in clinal work and in research are epilepsy and neurogenetic disorders.

BERGSTROM-ISAACSSON MARITH
MT, MA studied pedagogy and music instruction in Härnösand and music therapy at the Royal University of Music in Stockholm, Sweden. She finished her MA degree in June 2005 at the Centre for Research in Music Education (MPC) in Stockholm. Now she is a music therapist at the Swedish Rett Centre since 1996, as a part of an interdisciplinary team. At the Rett Centre she is responsible for the music part in specialized healthcare, research and information/education.

BUDDEN Sarojini
Medical Director Rett Syndrome Clinic, Oregon Health & Sciences University & Legacy Emanuel Children's Hospital, Pediatric Development Program, Portland, Oregon

CASS Hilary
Hilary Cass is Consultant in Paediatric Disability at Great Ormond Street Hospital for Children and Head of School of Paediatrics for London. She runs a national Rett syndrome service in the UK, is medical adviser to the RSAUK and has a particular interest in service provision and personal choice in the management of complex disability.

CELESTIN Elisabeth
Elisabeth is the mother of Ilona, a 9 years old Rett Syndrome girl, and has two

other children. She is the president of the French Rett Syndrome Association.
She is born in 1970, studied marketing in Paris. She worked in a pharmatical company; afterward she stopped working to grow her children.
She now has a mid-time job to guide disabled children in the classroom.

CHAMPOLION-PUEL Annick
MD, specialised in rehabilitation, working with people with multiple
since 1992 in various establishments. These schools are places of life,
of discovery, learning and care; pedagogical approach seems to me
to meet the needs of these people to enhance and develop their skills.
We then created the French Association for Conductive Education in 1998 to develop this approach.
Médecin de rééducation fonctionnelle, travaillant auprès de personnes polyhandicapées depuis 1992 dans différents établissements. Ces établissements sont des lieux de vie, de découverte, d'apprentissage et de soin; l'approche pédagogique me semble répondre aux besoins de ces personnes pour mettre en valeur et développer leurs compétences. Nous avons alors créé l'Association Française de Pédagogie Conductive en 1998 pour développer cette approche.

DELAMONT Robert S
Consultant Neurologist & Neurophysiologist with a long standing interest in Epilepsy and Episodic Disorders with particular reference to Sudden Death in Epilepsy.

DOWNS Jenny
A physiotherapist by background, Jenny works with the Australian Rett Syndrome Study and is interested in the areas of mobility, hand function, fracture and scoliosis. Recently, she co-ordinated the development of clinical guidelines for the management of scoliosis in Rett syndrome.

EUBANKS James
Dr. Eubanks is a Senior Scientist at the Toronto Western Research Institute and an Associate Professor at the University of Toronto. He received his BSc degree from the University of California, Davis in 1985, and his PhD from the University of California, San Diego in 1991. He is currently a member of the International Rett Syndrome Foundation Scientific Advisory committee, and a Scientific Advisory Board Member of the Ontario Rett Syndrome Association.

EVRARD Philippe
Born in 1942.
Trained in Louvain (University of Louvain Medical School – Hôpital Universitaire Saint Raphaël), in Paris (Hôpital Saint Vincent de Paul and Hôpital de la Salpêtrière) and in Boston (Massachusetts General Hospital – Harvard Medical School).
Main current appointments:
Professor, University of Paris 7 Denis-Diderot Medical School
Chief of the Service of Paediatric Neurology and Metabolic Diseases, Hôpital Robert-Debré (AP-HP)
Founder of the INSERM Research Laboratory of Developmental Neuroscience, Hôpital Robert-Debré, Paris
Honorary Professor, University of Louvain, Brussels, Belgium

Member of the Belgian Royal Academy of Medicine
Founder, board member and former Secretary general, EPNS (European Paediatric Neurology Society)
Board member of the ICNA (International Child Neurology Association)
Former President of the European Society of Pediatric Neurology
Main research contributions:
A new ontogenic and phylogenic neocortical unit
Mechanisms, disturbances and diseases of neuronal migration
New concepts and tools for “neuroprotection”
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FYFE Sue
Sue Fyfe is the Head of the School of Public Health at Curtin University of Technology in Perth, Western Australia. She is an anatomist, speech pathologist and epidemiologist with current research interests in disability, models of data collection in rare diseases and outcomes of teenage pregnancy. Her background working in the developing world during the 1980’s sparked her interest in public health but also in the problems associated with children and families living with disability.

GAUDY Martine
Martine is the mother of a 13 years old girl with Rett Syndrom and is Vice-President of the French Rett Syndrome Association.
She represents the AFSR in the Alliance Maladies Rares (Rare Deseases Alliance) and in the Rett Syndrome Europe.
Born in 1952 near Limoges, she studied in the Institut d’Etudes Politiques of Bordeaux and prepared the National School of Administration (ENA).She started a career overseas for diplomatic services, then worked in several ministries in France, mainly Education and Research . Human Resources Manager for 10 years in a national research institute, she has now retired to look after her daughter Agathe and struggle in the associative field, where she has numerous engagements.

GUERIN Pascaline
Child psychiatrist, Chief of Child & Adolescent Psychiatry, Chartres Hospital. Instrumental in developing a unit for diagnosis and the treatment of Pervasive Developmental disorders. Interested in the phenotypes of Rett Syndrome. Engaged in studies of autistic disorders (genetics, therapy, pharmacology).

HALL Sue
Sue is mother to Rachel, aged 9 years, with Rett Syndrome and Alex, aged 3 years, and is married to David. Rachel developed scoliosis and the family have experienced both conservative and surgical management strategies. Sue is passionate in all that she does, and is devoted to optimising Rachel’s quality of life. In addition, she has have worked as a Medical Secretary in the Paediatric Oncology Department at Birmingham Children’s Hospital for 15 years.

HANSEN Stig
Consultant Clinical Scientist at the Institute of Neurological Sciences, South

Glasgow University Hospitals, Glasgow, Scotland working with particular interest in quantitative sensory testing, autonomic function testing and Rett Syndrome.

HORTON Antony
Dr. Horton gained his Doctoral degree at St. Andrews University in Scotland U.K., where he was trained in the areas of neuroanatomy, developmental neurobiology and neuronal cell survival. Following this, he conducted 4 years of post-doctoral research on neurodegenerative diseases at the Rockefeller University in New York. Dr. Horton has published on aspects of neurodegeneration and neuronal cell survival in a number of research papers and journal articles. Prior to joining IRSF, Dr. Horton gained valuable experience in research management spending 5 years at the Juvenile Diabetes Research Foundation where as a Program Director, he led a small team that helped set the translational research agenda on Diabetic Complications. In addition, Dr. Horton spent two years at the Alzheimer's Drug Discovery Foundation where he worked in a Venture Philanthropy setting on the development of drugs for Alzheimer's disease and related dementias.

HUNTER Kathy
Kathy Hunter founded the International Rett Syndrome Association in 1984 following the diagnosis of her then ten year-old daughter. She has been a leader in the field of advocacy and support for the last twenty-five years. Kathy has been the curator of the first international database for patients with Rett syndrome, and has maintained a strong liaison with researchers. She authored two editions of The Rett Syndrome Handbook, comprehensive texts on every aspect of information, care and management of the syndrome.

HUPPKE Peter
Peter Huppke born in 1967 in Heilbronn, Germany, medical study in Goettingen, Germany, 1989-1995, specialization in pediatrics 1995-2000, specialization in pediatric neurology 2000-2004, consultant for pediatrics and pediatric neurology since 2004, Prof. for pediatrics in Goettingen since 2008.

JULU Peter O.O.
Dr Peter Julu is a Consultant Physician at Breakspear Hospital UK, and a Consultant Researcher at the Institute of Communication in Aalborg University, Denmark. He is the Neurophysiologist in charge at the Swedish National Rett Centre at Frösön Sweden, an Honorary Consultant Autonomic Neurophysiologist at the Royal London Hospital, and an Honorary Senior Clinical Lecturer at both the Wingate Institute of Neurogastroenterology and the Royal London Hospital UK.

KATZ David M.
Professor of Neurosciences, Chairman of the SAB of IRSF. His work on Rett Syndrome is focused on understanding the role of MeCP2 in regulation of BDNF expression and secretion and on the development of BDNF-based therapies for respiratory dysfunction.

KAUFMANN Walter E.
He is the Director of the Center for Genetics Disorders of Cognition & Behavior at the Kennedy Krieger Institute and a Professor of Pathology, Neurology, Pediatrics,

Psychiatry, and Radiology at the Johns Hopkins University School of Medicine. Dr. Kaufmann has been an active researcher in the Rett field since 1994, where he has contributed in the areas of neuroanatomy, neurochemistry, molecular biology, and neuroimaging. More recently, he has coordinated RettSearch, the consortium of clinically-oriented Rett researchers and has collaborated in several clinical projects focused in better defining the Rett phenotype.

KEBBAL Nadia
Orthoptist specialized in the visual handicap and working in the service of ophthalmology of the Civil Hospitals of Colmar for 17 years (department of Doctor Wipplinger. The sphere of activity is wide including functional explorations of the vision (electrophysiology, retinal angiography, OCT, field of vision), in the surgery field, in the squint and the other aspects of care and oculodriving disorders / Orthoptiste spécialisée dans le handicap visuel et travaillant dans le service d'ophtalmologie des Hôpitaux Civils de Colmar depuis 17 ans (du Docteur Wipplinger). Mon champ d'activité est large puisqu'il s'étend des explorations fonctionnelles de la vision (electrophysiologie, angiographie rétinienne, OCT, champ visuel), à l'assistanat opératoire, aux strabismes et autres prises en charge de troubles oculo-moteurs.

KERR Alison
Edinburgh Medical School & University. Senior Lecturer Paediatrics &. hon Consultant Learning Disability, Glasgow University & Royal Hospital for Sick Children. (retired). 1982-2005 engaged in clinical, epidemiological and neurophysiological research for Rett, developed & conducted the British Isles Rett Survey.

KOSTKA Philippe
Psychomotricien Diplômé d'Etat, cadre de santé, expert reconnu en psychomotricité.

LARRSON Eva-Lena
Eva-Lena is an occupational therapist and since 1992, has worked in a spinesurgery unit in Sweden with patients with neuromuscular and idiopathic scoliosis.
In 2002 she made her medical dissertation "Pre- and postoperative evaluation of function and activity in patients with paralytic scoliosis". Eva-Lena has recently collated data to describe outcomes after spinal surgery for the patient with Rett syndrome.

LEONARD Helen
Qualified in medicine and public health, obtaining high distinctions in all three epidemiology and two of the three biostatistics units in the public health course, she has focussed her epidemiological research in the area of childhood disability over the past ten years and has now become a leading authority in the epidemiology of intellectual disability and specific associated syndromes. She has taken the major role in establishing a population-based intellectual disability database (known as IDEA) in Western Australia (WA).
Since commencing her active research career in 1997 she has co-authored 75 publications in peer-reviewed journals (and in over two thirds was first or senior author), as well as thirteen book chapters and five reports. Her track record was instrumental in helping her, along with a comprehensive interdisciplinary team

of co-investigators (including national and international collaborators), to be awarded a grant from the National Institutes of Health to study the determinants of outcome and burden in Rett syndrome. She has now made a significant contribution to understanding the relationship between genotype and clinical variability as well as using tools to assess functioning, quality of life, economic burden and impact on the family.
Since 2002 Dr Leonard has managed the InterRett, the first international collaborative initiative in Rett syndrome, which is funded by the International Rett Syndrome Foundation. This database now includes more than 1500 cases and its data are making a major contribution to the literature. She presented at the Rett Syndrome Clinical Trials workshop, held in San Francisco in 2006, the aim of which was to set up an infrastructure for clinical trials in Rett syndrome and also attended a follow-on workshop in Chicago in 2008.
Whilst maintaining an impressive level of productivity in research she has placed a high priority on developing close connections with families as well as fostering links with other experts in Rett syndrome to facilitate international collaborations. With her clinical background, she is passionate about the translation of research findings into better clinical practice. She is the coordinator of the scientific program for the World Rett Syndrome Congress in Paris in October 2008.
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LOTAN Meir
Meir is a physiotherapist working at the Israeli National Rett Syndrome evaluation team. He has a special interest in physiotherapy for persons with intellectual disability, with an emphasis on individuals with Rett syndrome. Meir has published books and articles on the clinical aspects of Rett syndrome and was awarded in 2000 by the IRSA (Int Rett Syndrome Association) for his contribution to individuals with Rett syndrome.

MARDYCS Pierre
Pierre Mardycs, physiotherapist, specialised in training of stress management. Teacher involved in University Course (DU gestion des situations non programmées) of crisis mamangement (Paris Bichat). Large experience of workshops in stress management for physicians.

MILNE Yvonne
Yvonne Milne (BSc, MBE) founded the UK Rett Association UK, and is Honorary President, as well as being President of Rett Sydrome Europe, and non-executive director of an NHS Mental Health and Learning Disability Partnership Trust in the UK. In 1997 she was awarded an MBE for services to health care.

NAGARAJAN Lakshmi
Lakshmi nagarajan is the HOD, Neurology at Princess margaret hospital for Children in Perth, WA . Lakshmi directs the WA Child and Adolescent Epilepsy Program. LN's research interests include Epilepsy in RTT syndrome, Neonatal seizures, EEGs in childhood, VNS and management options for childhood epilepsy.

NAIDU Sakkubai
Dr. SakkuBai Naidu is a research scientist at the Kennedy Krieger Institute. She is also a Professor in the Departments of Neurology and Pediatrics at the

Johns Hopkins University School of Medicine. She is a trained pediatrician and neurologist with special interest in developmental and neurogenetic disorders affecting children and adults. Combining careful clinical analysis with technological advances in neuroimaging, genetics and neuroscience, Dr. Naidu is able to accurately characterize neurogenetic disorders. Her activities are at the interface between clinical neurology and basic sciences.

NGUYEN Gerard

Gérard Nguyen is qualified in Medicine from Paris University, Lariboisière Saint Louis Medical School. He is Consultant Physician in Internal Medicine and endocrinology in University Hospital Avicenne, Assistance Publique-Hôpitaux de Paris. He has published on aspects of hypertension, prevention of cardiovascular and metabolic diseases with a specific interest on the non compliance and quality of life assessment. He was working also in clinical research and also as a medical director within top ten pharma in France and as co-founder of a CRO (contract research organisation). He is today the Executive Manager of PCO (professional congress organiser). He has implemented his experience in research, methodology, medical education and in scientific events into the management of specific events for the involvement of Patient Associations. He was the vice president of the last EACD congress 2005 in Monaco with Philippe Evrard. He is the European coordinator of a ehealth project for the European Commission, also a FP7 expert for project in pediatry involving Parent Associations and a member via Rett Syndrome Europe of the patient and consumer working party at EMEA.

NOMURA Yoshiko

She is a child neurologist working in the Segawa Neurological Clinic for Children, Tokyo Japan since 1975.
Her association with Rett syndrome began in 1982 when I visited Prof. Rett in Vienna. Through the research and medicine on Rett syndrome, she has learned very much about brain and human behaviour and been privileged to enjoy the wonderful association of and friendship with colleagues world-wide.

PERCY Alan

Alan Percy is the Bew White emeritus Professor of Pediatrics, Neurology, Neurobiology, and Genetics and Associate Director of the Civitan International Research Center at the University of Alabama at Birmingham (UAB). Dr. Percy has been involved in the study of Rett syndrome (RTT) since making his first diagnosis at Baylor College of Medicine in 1983, establishing a RTT center at Baylor and recruiting Huda Zoghbi to RTT studies that identified mutations in MECP2. He is currently Director of the RTT Center at UAB and PI of the RTT consortium for the NIH-funded Rare Disease Clinical Research Center.

PINEDA Mercedes

Dr Mercé Pineda is currently Associate Professor of Paediatrics at the University of Barcelona, Spain and works within the Neuropaediatric Department at the University Hospital, Sant Joan de Deu and the Centre for Biomedical Research on Rare Disease (CIBERER) in Barcelona.
Having graduated in medicine at the University of Barcelona she went on to specialize in the area of paediatrics, specialising in neuropaediatrics. Her particular research and clinical interests are in rare neurological conditions, more specifically those related to Rett syndrome and mitochondrial disorders; and has widely published in the fields of

metabolic and genetic research into these conditions

PINI Giorgio
Dr Giorgio Pini is a doctor of medicine and specialist in Child and Adolescent Neuropsychiatry and in Clinical Psychlogy. He is the Director of the Department of Child Neuropsychiatry at Versilia Hospital, Lido di Camiore and of the Rett Centre of North West Tuscany, Italia.

REILLY Sheena
Sheena Reilly is Professor of Paediatric Speech Pathology at the Royal Children's Hospital in Melbourne, Australia. She has published widely on Rett syndrome and has a particualr interest in dysphagia.

ROQUE Christiane
Christiane is the mother of a 23 years old Rett Syndrome girl with high disabilities called in France "polyhandicap" and is the president of honour of the French Rett Syndrome Association.
Engaged early in associative movements, she is so-founder of the Association "Enfant du Monde", Alpes Maritimes Committee in 1995.
Born in 1956 in Tarroudant, Marocco, she is mastered of Art Degrees and worked for a while in the French National Education before leaving it in order to attend to her daughter Charlène and her day to day care.
She now enjoys a good rhythm between her association struggles and a mid-time job as translater in law office.

ROUX Jean-Christophe
Research scientist at INSERM, he is investigating breathing dysfunction in RS using mouse models. He is involved in the development of pharmacological approaches for the treatment of autonomic dysfunction in RS.

ROYE David
Dr. Roye is the Director of Pediatric Orthopaedics at The New York Children's Hospital and the St. Giles Foundation Professor of Pediatric Orthopaedics at Columbia University College of Physicians and Surgeons. Dr. Roye has had special interest in the treatment of scoliosis and correction of spine deformity in children and has published widely in this area. Dr. Roye also has a long-standing interest in providing medical services and teaching in developing countries, participating annually in overseas medical delivery since 1987 in Kenya, Romania, and China.

SCATTIN Luciana
Luciana Scattin, née le 14 mars 1970 à Venise Italie
2002 Spécialisation en Médecine Physique et rééducation
Depuis 2005 employée à :Associazione "La Nostra Famiglia", I.R.C.C.S E. Medea, Conegliano (Treviso) Italie.
2004-2007 médecin responsable "IMP -Les Amis de Laurence" Paris, France
Octobre 2003: «Nuova proposta nel trattamento conservativo della scoliosi secondaria» ; XI° congresso nazionale SITOP (Italie)
Mars 2004: Prix Queneau 2004 (remis par le GES): "Scolioses "idiopathiques" évolutives de l'adulte: aspects qualitatif e quantitatif. Pronostique individuel. Etude longitudinal chez 51 sujets"

Avril 2004 : présidents comité scientifique congres “Scuola francese e italiana a confronto: la scoliosi dell’adulto e la scoliosi neuromuscolare” Abano Terme (Italie)
Juin 2005: “Trattamento conservativo delle scoliosi secondarie con corsetto BSO” XXVIII congresso nazionale GIS Pesaro (Italie)
Juin 2006: “Scoliosi idiopatiche evolutive dell’adulto. Aspetti qualitativi e quantitativi (Natural history of adult scoliosis)” XXIX congresso nazionale GIS Padova (Italie)
Juin 2007: “Natural history of adult scoliosis”, Spine
Depuis 2006 membre du CMS de l’association française syndrome de Rett

SCHOEN Christian
Dr Christian Schoen is a French general practitioner who is:
Personably and professionally concerned by rare diseases,
Managing I.T.I (Information & technology Intelligence),
Working in CIT domain related to health (“e-health”) : “creation and management of expertise and knowledge”,
Developing a European project regarding social networking and e-health.

SENEZ Catherine
Catherine Senez is a swallowing therapist, she specialised in cerebral palsy with the Bobath School. She has worked in a developmental care unit in the Timone children’s hospital in Marseille. She is now in charge of an adult continuing education programme and a member of the European commission “EuforPoly”. As a member of the paramedical Council of the Association Française du Syndrome de Rett, she contributed at the collective book: “Le syndrome de Rett, une maladie génétique” published by the AFSR.She is the author of a book published in 2002 by SOLAL: “Rééducation des Troubles de l’Alimentation et de la Déglutition” (Rehabilitation of Eating and Swallowing Disorders).

SMEETS Eric E J
Dr Eric E J Smeets MD, PhD is a pediatrician and child neurologist at the Department of Clinical Genetics of the Academic Hospital Maastricht, Maastricht University, in the Netherlands. He is a Consultant Pediatrician at the Center for Human Genetics, University Hospital Gasthuisberg, Leuven, Belgium. He has a long standing experience with developmental disorders and genetic syndromes in children and adults with intellectual disability. He is the author of several articles on Rett syndrome and MECP2 related disorders.

SUR Mriganka
Mriganka Sur is the Newton Professor of Neuroscience and Head of the Department of Brain and Cognitive Sciences at the Massachusetts Institute of Technology (MIT). Professor Sur studies the development, organization and plasticity of the cerebral cortex of the brain. Recently, his group has demonstrated novel mechanisms underlying developmental brain disorders, and proposed innovative therapeutic approaches to treating such disorders.

VILLARD Laurent
Research scientist at INSERM, he is the coordinator of an european network on Rett Syndrome (EuroRett). His laboratory is studying Rett syndrome and human brain malformations.

WITT-ENGERSTRÖM Ingegerd, MD
Physician and pediatrician with special education in child neurology and habilitation (rehabilitation), Medical Director of the Swedish Rett Center, she has contributed to many areas of RTT research, including the delineation of clinical features. She founded a center of Excellence for Rett syndrome, performing pioneer work on non-pharmacological interventions in RTT, such as music therapy. She brought research on the central autonomic control in Rett syndrome to Sweden with Dr P. Julu; The Frösö Declaration on intervention was published in June 2008.

YOUNG Juan
Juan Young earned his PhD from the Universidad de Buenos Aires, Argentina and trained as a postdoctoral fellow at Baylor College of Medicine, with Dr. Huda Zoghbi (2001-2004). He is presently an Assistant Professor at the Centro de Estudios Cientificos, Valdivia, Chile.