

## **Rett Syndrome after Twenty-five Years: Where it started and where it is today**

### **Introduction**

Until 1984, only three US physicians had experience with Rett syndrome (RTT), a neurodevelopmental disorder that occurs predominantly in females. These physicians were Mary Coleman in Washington, DC, Vanja Holm in Seattle, and Alan Percy in Houston. Mary Coleman had attended a conference in Paris at which Jean Aicardi and colleagues presented the results of the soon-to-be published paper on RTT by Bengt Hagberg and other authors (including Aicardi) in the October 1983 *Annals of Neurology*. This paper was the first widely-circulated English language publication describing this unique disorder. Vanja Holm had visited Bengt Hagberg in Sweden and had discussed RTT with him. Both had diagnosed girls in the US with RTT prior to this publication. Alan Percy was involved in basic biochemistry research in 1983 when asked by Ina Desmond, the head of the Meyer Center for Developmental Pediatrics at the Texas Children's Hospital (TCH), to review the record of a young girl and a letter from her pediatrician that included a copy of the Hagberg paper. Dr. Percy visited this young girl in her home and, armed with the Hagberg paper, made his first RTT diagnosis. In January 1984, Dr. Percy brought this young girl into TCH to introduce RTT to his Baylor colleagues. As a result, Dr. Daniel Glaze, a fellow child neurologist, and Dr. Huda Zoghbi, a resident in the Child Neurology training program, were recruited to RTT research. Their enormous contributions to RTT over the ensuing years cannot be overstated. At that time, Dr. Zoghbi remembered another girl evaluated in the early 1980's with similar features. We knew nothing about RTT at that time. Thus, it was not surprising that a different diagnosis had been made based on a specific laboratory study. This girl also proved to have RTT. Very quickly thereafter, another four girls with RTT were seen in Houston and following an interview aired on CNN, many more diagnoses were made. This resulted in the establishment of the Rett Center at Baylor College of Medicine in Houston.

In 1984, Drs. Holm, Hugo Moser, and Percy attended an international meeting hosted by Andreas Rett in Vienna, the first time that US physicians discussed RTT with colleagues from across the world. Shortly thereafter, the International Rett Syndrome Association was formed by Kathy Hunter, Gail Smith, and Jane Brubaker and two meetings were convened in Baltimore to launch concerted research efforts in this country. With assistance from Congress generated by this grassroots support, the NIH created research programs to fund this research. In Houston, the RTT Center was fortunate to receive such funding from the lead Institute for RTT, the National Institute of Child Health and Human Development (NICHD), renamed last year as the Eunice Kennedy Shriver NICHD. By the time Dr. Percy left Houston for his current employment at the University

of Alabama at Birmingham (UAB), more than 100 girls and women with this disorder had been evaluated at Baylor together with Dr. Glaze and Rebecca Schultz, PNP. Dr. Percy had also been able to visit other RTT centers in the US, Canada, Europe, and Japan where he had evaluated several hundred more. Not long after his arrival at UAB, a comprehensive RTT Clinic was established there with the assistance of Jane Lane whose interest in RTT, due to a colleague with an affected daughter, preceded Percy's arrival. Ongoing research, supported in large part by NICHD and IRSA, resulted in very broad understanding of the clinical features and co-morbidities associated with RTT, identification of mutations in the gene, methyl-CpG-binding protein2 (*MECP2*) in the Zoghbi laboratory, and expansion of research activities in this country and abroad. Other centers in this country were located in Baltimore (Dr. Sakkubai Naidu), Portland, OR (Dr. Sudge Budden), and San Diego (Dr. Richard Haas); in Canada (Dr. Patrick MacLeod); in Sweden (Prof. Bengt Hagberg); in Germany (Prof. Folker Hanefeld); in the UK (Dr. Alison Kerr and Angus Clarke); in Spain (Dr. Merce Pineda); in Italy (Dr. Michele Zapella); in Japan (Dr. Yoshiko Nomura and Dr. Masaya Segawa); and in Australia (Dr. Helen Leonard and Dr. John Christodoulou). More recently, US clinics have been established in Boston, Los Angeles, New York, Oakland, and St. Paul.

IRSA remained an active supporter of these activities including the funding of a comprehensive *MECP2* mutation database in Sydney led by John Christodoulou (RettBase), a clinical data repository in Perth led by Helen Leonard (InterRett), and more recently, an international collaborative group led by Dr. Walter Kaufmann (RettSearch) and based at the Kennedy Krieger Institute in Baltimore. In 2007, the International Rett Syndrome Foundation (IRSF) was formed by the merger of IRSA and Research for Rett Foundation.

In 2003, with funding from the NIH Office of Rare Disease (now ORDR) and the NIH National Center for Research Resources (NCRR), a consortium of investigators involved in Angelman, Rett, and Prader-Willi syndromes research was formed to study the natural history of these disorders and expand understanding of possible correlations between the respective mutations and the clinical features for each disorder. The RTT consortium consists of investigative teams from the Baylor College of Medicine (BCM), the Greenwood Genetic Center (GGC), and the University of Alabama at Birmingham (UAB). At the present time, nearly 750 individuals with RTT or with mutations in *MECP2* but without features of RTT have been enrolled. More than 85% of these have classic RTT, another 11% have atypical RTT, and 4% have *MECP2* mutation but do not have the features of RTT. Enrollment has been enhanced greatly by travel clinics, supported first by IRSA and now by IRSF, in Oakland, Chicago, New Brunswick (NJ), and Florida (mainly Miami). In addition, the UAB team has enrolled participants at the Children's Hospital Boston. Data gathered with each participant visit are then imported anonymously into a database managed by the

Data Technology Coordinating Center (DTCC) at the University of South Florida in Tampa. The assembled data have given us broader insights into the many features and associated problems experienced by girls and women with RTT and by the families or other caretakers. These include longevity or survival, growth, puberty, scoliosis, seizures, mutation frequency, males with *MECP2* mutations, and clinical severity. Surprisingly, a high number of mainly older individuals with RTT have not had mutation testing. These areas will be addressed briefly below.

### **Longevity or survival**

Few studies have been conducted on survival. Through the IRSA database as part of the Rare Disease study, we collected information on 1928 individuals with RTT from the US and Canada whose birthdates begin in the 1930's and extend to 2006. Using standard Kaplan-Meier survival curves, we found that overall longevity is significant in RTT with 50% living to about age 55 compared to age 77 for the average American female. Over time, these data may need to be revised upward as we learn more about day-to-day management such as nutrition and various therapies.

### **Growth**

We know that growth problems are pervasive in RTT including height, weight, and head circumference values that are significantly below those for the general female population. Data developed by a post-doctoral fellow, Daniel Tarquinio, DO, demonstrated that decline in head circumference may be seen as early as age 1 month, in weight as early as 14 months, and in length as early as age 21 months. One thing that is not seen in RTT is the typical pubertal growth spurt noted in most typically-developing females. An outcome of this study is the development of RTT-specific growth charts that we hope to have generally available later this year. Interestingly, the body mass index (BMI) curves for RTT do not deviate greatly from the typically developing female pattern indicating that despite poor overall growth, the deviation from normal is similar for both height and weight. We also know that hand and foot growth is less than in females in general, more so for feet than hands.

### **Puberty**

Puberty is a process that occurs normally in RTT. Data from the Rare Disease study indicate that the appearance of the so-called secondary sexual features including axillary and pubic hair and breast development as determined by Tanner staging may be accelerated in RTT. For example, the average age in the general population for appearance of Tanner stage 2 is about age 9 years, but in RTT it appears to be between 6-7 years. Nevertheless, the onset of menstruation is very similar: 12.9 yr for females in the general population and 13.1 yr for RTT. In a cooperative study conducted by investigators from Oregon, a specific mouse knockout interfering with a gene known to be regulated by *MeCP2* was shown to produce early signs of sexual maturity in females while

these females became fertile at the same age as their normal female littermates. These results suggest that MeCP2 regulates one or more genes that affect sexual development.

### **Scoliosis**

Data from 586 classic RTT participants, mean age = 10 years (0-57 yr), were analyzed. 292 (50%) had scoliosis; mean age = 15 yr with scoliosis and 6 yr without. By age 16, more than 80% had some degree of scoliosis. Using multiple regression analysis, greater clinical severity score, later acquisition, loss, or absent walking, and constipation were associated with scoliosis. Two *MECP2* mutations, R294X and R306C, had reduced risk for scoliosis. 12% of those with scoliosis required surgery. These findings corroborate previous reports on scoliosis frequency and extend understanding of co-morbidities, clinical severity, and relative risk reduction for specific mutations.

### **Seizures**

Seizures are common in RTT. However, many clinical events that appear to be seizures may not be. After age 2, the EEG is typically abnormal in RTT, characterized by slow background rhythm and spike activity. Before beginning medication for seizures, it is important to confirm that this is the correct diagnosis. It is important to treat clinical seizures, but not the EEG just because it is abnormal. For events that are not seizures, the typical antiepileptic medications (AED) will be ineffective and represent an unnecessary exposure to these medications. From the natural history study, we found that approximately 60% reported having seizures at one time. This was true both for classic and atypical RTT. At the time of the natural history evaluation, the physician (Drs. Glaze, Neul, Percy, or Skinner) found that 48% had no seizures; 19 % no seizures with an AED. Of the 33% still having seizures, 14% occurred monthly, 11% weekly, and 8% daily. No significant difference in seizure occurrence by race/ethnicity was noted. A significant age relationship was seen: 15% < 2 years; 50-70% 5-20 years; 48% >30 years. Of the specific mutations, seizures occurred more frequently for T158M (74%) and R106W (78%), and less frequently for R255X and R306C (both 49%). 92% of those with seizures were receiving one or more AEDs: carbamazepine (37%), lamotrigine (33%), and levetiracetam (26%). 7% received no treatment, 6% also had a vagal nerve stimulator (VNS), and 6% were on the ketogenic diet. No significant difference in head circumference existed between those with and without seizures.

Individuals with seizures often had more difficulties with walking, hand use, and communication. In summary, seizures are common in RTT (about 50% overall), have an age related onset and occurrence, and vary by mutation. Seizures are uncommon before age 2 and rarely begin after age 20. Many may be seizure-free and on no AED. Reports from smaller RTT cohorts suggest greater seizure prevalence, namely, 81-94%. The ages of participants in these smaller cohorts may play a role in these differences, for example, if they contain mainly children from age 5-15 years. Overall, the age distribution of those with or without

seizures is the same. However, when examined by age group, we noted increasing percentage of seizure occurrence with advancing age. In the age group 15 to 20 years, 86% of the participants in our natural history study were reported to have seizures. This number is similar to that reported in the Australian Rett Syndrome Database. The period from three to fifteen years appears to be the critical window during which the onset and occurrence of seizures is most likely.

### **Gastrointestinal Problems**

Dr. Kay Motil and Dr. Glaze completed a survey of nearly 1000 families on the prevalence of gastrointestinal, nutritional, and neurological problems. Ninety-five percent of parents reported concerns that related to gastrointestinal dysmotility, including constipation (81%), oropharyngeal incoordination (63%), gastroesophageal reflux (38%), and gastroparesis or poor stomach emptying (14%). Sixty-two percent reported feeding problems, including poor chewing ability (55%) and swallowing dysfunction (43%), both of which may contribute to poor weight gain (39%). Parents reported the use of nutritional supplements in 47%; gastrostomy feedings were required in 28% and a ketogenic diet was administered to 7%. Thirty percent reported skeletal bone fractures. This is about 6 times higher than normal. Three percent reported biliary tract (gall bladder) disease. Other diagnoses such as cystic fibrosis or celiac disease were rarely reported. Dr. Motil also completed a study on the results of gastrostomy placement on growth in 92 girls and women with RTT showing that gastrostomy placement greatly improved growth and undernutrition, regardless of the age at which the procedure was performed. This study provides strong support for gastrostomies in individuals with RTT who are at increased risk for progressive growth failure and undernutrition. In summary, nutritional and gastrointestinal problems frequently complicate the clinical course of girls and women with RTT. Increased physician awareness of these problems should improve the quality of life.

### **Mutation Frequency**

The frequency of mutations in Natural History study participants is compared with those from the North American database acquired from a survey of IRSA members and from RettBase, the IRSF sponsored mutation database in Australia. These data are shown in the Table below. The agreement between these three different data sets is quite striking.

Comparison of <i>MECP2</i> Mutations from Participants in the Natural History Database, the North American Database, and RettBase				
Mutation	Natural History Study		North American	RettBase
	Number N = 533	%	% N = 918	% N = >2100
T158M	66	11.4	11.9	9.1
R168X	58	10.3	9.4	8.8
R255X	58	10.3	9.0	7.9
R306C	41	6.9	6.9	4.6
R294X	35	6.4	6.2	5.6
R270X	33	5.6	7.2	6.9
R133C	21	3.9	6.4	4.4
R106W	20	3.4	4.4	3.4
Large deletions	48	8.4	6.4	Not reported
C-terminal del	36	6.6	8.8	Not reported
Others	114	21.4	23.4	Not reported

The greater frequency of large deletions in the Natural History study is due to complete mutation testing in these participants versus the North American database. In order to be enrolled in the Natural History study, both sequencing and deletion/duplication testing of *MECP2* must have been performed.

### Non-Rett and Males

*MECP2* mutations may be seen in females with classic or atypical RTT, in females who do not have the features of RTT, and in males. In females who have a mutation but do not have the features of RTT, most are associated with higher

functioning. In some cases, this is due to unbalanced X-inactivation in which a higher percentage of cells are expressing a normal copy of *MECP2* and fewer cells are expressing an abnormal gene. This group includes females who may have normal function, learning disability, mild cognitive problems with preserved speech, and significant behavioral problems including aggression and psychosis. Males with *MECP2* mutations may have 1) a severe infantile encephalopathy leading to early death, 2) cognitive impairment and motor difficulties, or 3) typical RTT. Those with typical RTT may also have Klinefelter syndrome or somatic mosaicism. Klinefelter syndrome involves an extra X chromosome (47XXY) whereas somatic mosaicism involves two cell populations, one with a normal X chromosome and one with a *MECP2* mutation. In both instances, these males are similar to females in having some cells with normal *MECP2* and some cells with abnormal *MECP2*. Both of these appear to be quite rare, whereas non-RTT females and males with the infantile encephalopathy may be much more common than we realize as many may not be tested because they do not fit the typical RTT profile.

The most common association of abnormalities in *MECP2* in males involves a duplication of the entire gene usually along with other genes in the same region on the X chromosome. These males tend to have mild to moderate cognitive problems, absent speech, and a shuffling gait. Unlike typical RTT, these males tend to be of normal to large stature. They also may have frequent upper respiratory infections and recurrent sinusitis due to duplication of one or more of the other X chromosome genes contained within this duplication. In general, the duplication occurs on the X-chromosome, but a small number in which the duplication has been attached to a non-X chromosome has been described.

### **Clinical severity stability**

Two measures of overall clinical severity are completed at each visit on all participants in the Natural History study. Analysis of these results in those seen on more than one visit indicates that they are very consistent or stable from one visit to the next after the pre-school years. For this reason, the protocol was recently changed so that participants will now be seen twice yearly through age five and then annually once they turn six.

In addition, a preliminary analysis comparing clinical severity scores with specific mutations reinforced the previous reports of Neul et al. and Bebbington et al. showing that specific mutations as a group have lesser severity. These are R133C, R294X, R306C, and c-terminal deletions.

### **Percent of North American database survey (survival study) not tested**

One of the outcomes of the North American database survey was the number who had not had testing for a *MECP2* mutation and the number of those who were tested but the parents did not know the results. Of 1928 participants, 763 (40%) were not tested. Some of this group had expired prior to the availability

of testing, but many parents were satisfied with the clinical diagnosis and did not pursue testing when it did become available. After all, RTT is a clinical diagnosis. *MECP2* testing is simply confirmatory of the clinical diagnosis. Interestingly, those born before 1990 were much less likely to be tested than those born later. At the present time, complete testing is very important for several reasons. First, participation in the initial clinical treatment trials will most likely require testing and identification of a mutation. Second, identification of a mutation is essential if we are to correlate specific mutations with specific clinical features. Third, while recurrence in one's family is rare, identifying a specific mutation may be required in the event that other siblings have neurodevelopmental difficulties however mild. Fourth, this information could be critical for guidance of other family members who may wish to seek genetic counseling.

Of the 1165 that were tested, results were not known by the parents for 106 (9%). We strongly believe that all parents or other caretakers should receive and retain a copy of the mutation results. This is an important document, one for which payment was made in one way or another. Parents should insist on receiving a copy if one is not offered.

### **Conclusion**

The information contained herein could not have been provided without the gracious participation of those individuals and their families who have enrolled in our Natural History study. We look forward to completing our planned enrollment of at least 1000 participants. We appreciate the opportunity to learn from you and hope this information provides you with a firmer understanding of this unique disorder and adequately explains the broad variability of conditions associated with mutations in *MECP2*. We also acknowledge the generous support of IRSA and IRSF and the critical funding of the National Institutes of Health (NICHD, ORDR, and NCRR)

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