



Rett Research Report

Issue 1

Newsletter from the Rett Syndrome Research Group

2 September 2000

From Dr Mike's Desk



Welcome from the Cancer Genetics Laboratory.

In this first Rett syndrome newsletter I hope to provide you with an update on recent research into Rett Syndrome from overseas, and here in New Zealand.

The Cancer Genetics Laboratory

Principal Investigators

| | |
|---------------------|--|
| Prof Tony Reeve | Professorial Research Fellow |
| Dr Mike Eccles | Senior Research Scientist |
| Dr Parry Guilford | Senior Research Scientist |
| Dr Ian Morison | Research Haematologist |
| Dr Michael Sullivan | Paediatric Oncologist/ Senior Lecturer in Paediatrics |
| Judy Norrish | Lab Manager |
| Sue Cleverly | Secretary/Data Manager |

The Cancer Genetics Laboratory was established over 20 years ago to research the genetic changes that occur in cancers of children and adults. The laboratory currently has 5 principal investigators and a full time staff of 26. Our research funding comes from grants from agencies such as the Health Research Council, the Cancer Society of New Zealand, the Lottery Grants Board, the Child Cancer Foundation, the Marsden Fund or from private donations

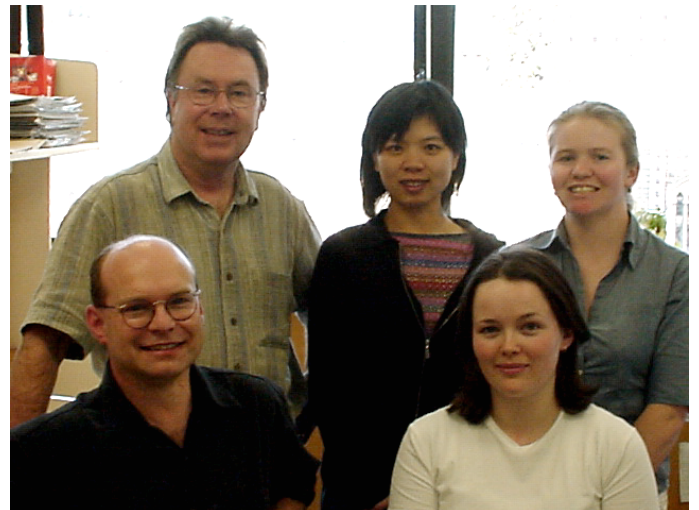
While our research over the last 7-10 years our laboratory has focused on the molecular genetics of specific cancers affecting children and adults, we have become increasingly interested in the genetics of a number of non-cancer conditions because of what these diseases can tell us about cancer.

We recently became interested in Rett syndrome when the gene was found - the Rett gene called *MeCP2* appears to be involved in the regulation of many other genes by a process called DNA methylation. It was coincidental then that our laboratory (my group in particular) has been very interested in the role of disturbed DNA methylation in cancer.

With the generous support of the Kristen Dean Endowment trust we have now established a group to research the genetics and molecular biology of Rett syndrome in New Zealand.

Our Rett Research Group

Dr Michael Sullivan MB ChB DCH FRACP PhD
Natalie Kerr MSc(Hons)
Libby Caygill (student)
Jenny Xu MB MSc(Hons)
Professor Tony Reeve PhD FRSNZ



Mike Tony Jenny Libby Natalie

What is Rett Syndrome

Rett syndrome is a severe and progressive neuro-developmental disorder that occurs almost exclusively in young girls (1, 2). Andreas Rett first described Rett syndrome in 1966 but recognition of the syndrome outside Europe did not occur until the early 1980s (3).

The incidence of Rett syndrome in New Zealand is unknown but a study in Australia estimates it may occur in about 1 in 10000 girls (about the same frequency as PKU) (4). We do not know the incidence of Rett syndrome Maori and Pacific Island girls. However Rett syndrome may be the second most common cause of severe developmental retardation in girls after Down Syndrome.



A young girl showing typical hand movements of Rett syndrome

Rett Syndrome: Clinical Features

Girls with Rett syndrome are normal at birth and have a period of normal growth and development during the first few months of life. The clinical features begin to develop between 6 months and 3 years of life. Over this period these girls begin to lose some of their previously acquired skills, there is a slow regression in language and communication skills and they develop stereotypical wringing hand movements (5). Other characteristic features include seizures, poor head growth, growth failure and abnormal breathing patterns. The clinical course for girls with *classical* Rett Syndrome is a progression through several stages of global neurologic decline. Ultimately girls affected with this condition become totally dependent and the majority will die in late adolescence or early adulthood although some girls have lived to over forty. The diagnosis of Rett syndrome is made according to a set of diagnostic criteria (5), but several clinical *variants* have been described and there is clinical overlap with other severe neuro-developmental conditions such as

Angelmans syndrome (4). The early period of normal growth and development presents an opportunity for early diagnosis and pre-symptomatic treatment if such a treatment becomes available.

Rett Syndrome Genetics

Rett Syndrome: Genetics & Molecular Genetics

Two of the most curious features about Rett syndrome are its occurrence in girls and not boys, and the rarity of inherited cases. Nearly all the cases of Rett syndrome reported until very recently have been in girls. The absence of affected boys suggested that the disorder is X-linked (located on the female sex chromosome, the X chromosome) and lethal in male pregnancies. While it is clearly a genetic disease Rett syndrome is only rarely inherited in families.

Until late last year the cause of Rett Syndrome was completely unknown and no genetic or biologic marker had ever been found.

In October 1999 a group of researchers from Texas and San Francisco found the gene responsible for Rett syndrome. The gene, which is located at the tip of the X chromosome (Xq28), encodes for a protein called *MeCP2* (6) Figure 1. Curiously the *MeCP2* gene and the protein were already well known to those working in the area of gene control and regulation - including our group, but no one had suspected it would be involved in a disease, especially one like as Rett syndrome.

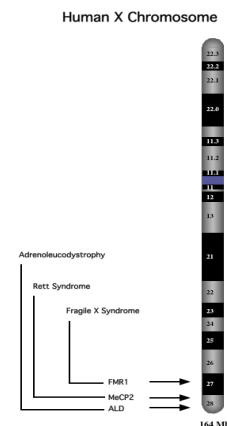


Figure 1 Location of the *MeCP2* gene on the human X chromosome

The *MeCP2* gene is one of a group of genes that are crucial for controlling the activity of many other genes by interacting directly with methylated DNA.

DNA methylation and *MeCP2*

In humans, and most other higher organisms, the very long DNA molecule is packaged into a complex

of DNA and protein called chromatin. Chromatin is a convenient way of condensing DNA so that it doesn't break and can be replicated easily. Chromatin is held together in larger structures - the chromosomes. In humans there are 22 pairs of chromosomes - called autosomes (1-22), and a pair of sex chromosomes called X and Y (Figure 2). All females have two X chromosomes while all males have only a single X chromosome and a Y chromosome. A gene is simply a string of the DNA arranged in such a way as to have a defined start point, a coded middle and a defined stop. The gene code contains a biologic message (made into messenger RNA) which is deciphered by the cell to make a protein.

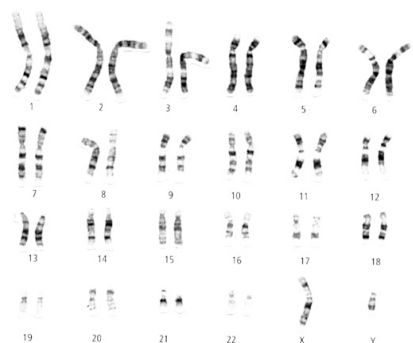
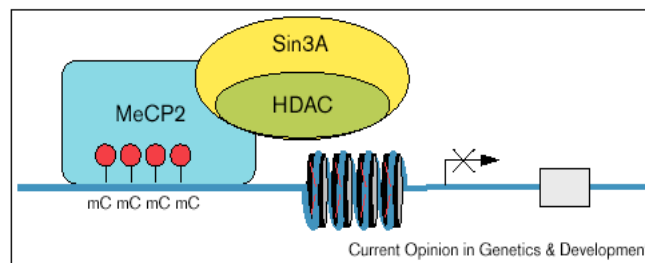


Figure 2 Normal human chromosomes

To help condense DNA into chromatin, the DNA molecule is methylated by an enzyme (DNA methyltransferase), which adds a molecule of carbon and hydrogen (CH₃) to the outside of the DNA double helix. Methylated DNA can bind specific binding proteins, which in turn bind other proteins to form chromatin. Another role of DNA methylation is to switch off the activity of genes by inhibiting to production of messenger RNA. The *MeCP2* protein, is one of these DNA methyl binding proteins and is able to form chromatin and switch off gene expression.

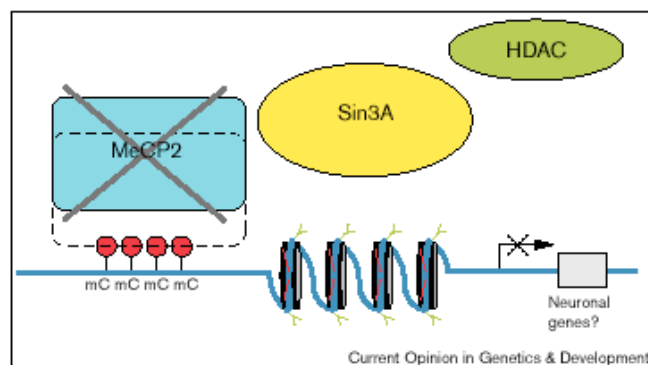
The *MeCP2* gene is located on the X chromosome in a region associated with several other developmental disorders such as the Fragile X syndrome.



Normal function of *MeCP2*. *MeCP2* (light blue) binds methylated cytosine residues (mC, red circles) in CpG islands and recruits Sin3A (yellow) and histone deacetylase (HDACs, green). Deacetylation of the histone tails compacts the chromatin and silences transcription. Deacetylated histone tails are represented as red lines linking the nucleosomes to the DNA strand (in dark blue) over the nucleosomes (black rectangles).

***MeCP2* mutations in Rett syndrome**

In Rett syndrome about 75% of affected girls have mutations in the *MeCP2* gene (6-9). These mutations lead to the production of either a non-functional *MeCP2* protein or no *MeCP2* protein at all. It is not yet clear how mutations in the *MeCP2* gene cause the clinical features of Rett syndrome but is likely that it involves the disruption of gene regulation.



Putative influence of RTT-causing mutations on the function of *MeCP2*. (The same colour scheme and symbols are used as Figure 1) Mutated *MeCP2* protein may not properly bind or recruit Sin3A and HDACs (histone deacetylases), leading to partial loss of transcriptional repression. The hatched outline indicates possible absent or altered binding of *MeCP2* to methylated cytosine residues (mC); the large 'X' indicates decreased or absent function of *MeCP2*. The small arrow with the hatched cross indicates a possibly reactivated promoter of downstream target genes. The question mark denotes uncertainty about the identity of the *MeCP2* target genes affected in Rett syndrome.

Some rare families with affected girls have had boys who were born with very severe fatal neonatal encephalopathy (7). Mutation analysis has shown these boys have mutations in the *MeCP2* gene.

Rett - a genetic riddle explained?

The genetic riddle of Rett syndrome is now less of a mystery. The reason that Rett syndrome is not inherited is that girls with this disorder do not themselves have children and boys with mutations die before or soon after birth. All the cases of Rett we see are due to new mutations in the *MeCP2* gene which probably develop during the formation of the gametes - either the oocyte or sperm.

Why do girls get Rett syndrome and boys develop an encephalopathy? Boys have only one X chromosome and so have only the one copy of the *MeCP2* gene, while girls have two X chromosomes and hence two copies of *MeCP2*. An *MeCP2* mutation in a boy will cause a complete loss of the gene in all cells in the body with a lethal result. An *MeCP2* mutation in one of the two genes of a girl will lead to a partial loss of gene activity - which is obviously most severely manifest in the brain.

Rett Research Projects

DNA Diagnosis of Rett Syndrome

With the generous support of the Kristen Deane Endowment Trust we have set up testing for Rett syndrome by direct mutation analysis of the *MeCP2* gene. Eleven girls have been tested so far, with definite mutations found in 5 cases, no mutations in 3 cases and 3 cases yet to be completed. The test is done on a standard blood sample and can be taken by a GP, community laboratory or hospital and sent to our Dunedin Laboratory.

Current Research Plan

The last few months have very busy for us as we plan our research programme.

1. A National Clinical Study of Rett

We have submitted a proposal to the New Zealand Paediatric Surveillance Unit to do a national study of Rett syndrome over the next two years. In this study we plan to determine the frequency of Rett syndrome in New Zealand and identify the clinical and demographic features of the girls. We will also look for a wider range of *possible* cases who may not meet the *classical* diagnostic criteria but who may have *MeCP2* mutations.

2. Molecular studies of Rett syndrome

We were delighted to receive an invitation from the Rett Syndrome Research Foundation of USA to submit

an application for research into the molecular biology of *MeCP2* and Rett syndrome. We plan to apply the recently developed DNA microarray technology of the Otago Genomics Facility (Director, Professor Tony Reeve, Principal Investigator Dr Mike Sullivan) to look at the genes affected by *MeCP2* mutations. We also have also submitted a research proposal to the Lottery Grants Board for a similar project.

We are currently preparing a national ethics committee application after which we will invite families to participate in our research.

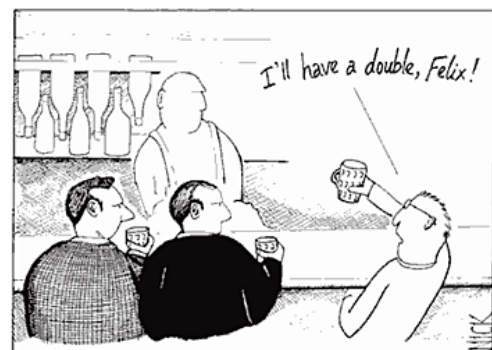
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In the next issue of the Rett Report

- Conference report and Update from the literature
- New genetic technology

Cartoon



Cambridge, 1953. Shortly before discovering the structure of DNA, Watson and Crick, depressed by their lack of progress, visit the local pub.

Cartoon by Nick Kim, used with permission