

Review article

# Rett syndrome and the MECP2 gene

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First described by Andreas Rett in 1966,<sup>1</sup> Rett syndrome is a severe neurodevelopmental disorder which almost exclusively affects females. A genetic aetiology was suggested by MZ twin concordance and a case of vertical transmission of the disorder.<sup>2</sup> Although the prevalence is between 1 in 10 000 and 1 in 15 000 female births,<sup>3</sup> >95% of cases arise de novo so the disorder has been considered to be an X linked dominant which is lethal in hemizygous males. The few familial cases are almost invariably connected through the female line

### Clinical features

The uncertainty of diagnosis associated with the occasional presence of apparently affected males has always been complicated by the wide clinical spectrum presented by females, which ranges from the severely affected “classical” cases through a wide range of disability to a milder forme fruste. This variable severity has been partly ascribed to the degree to which skewing of X inactivation favours expression of the normal X chromosome. This, however, may not explain the specific variants such as the more mildly affected girls with retained speech or the more severe congenitally affected cases with early seizures.

Classically, Rett syndrome may be diagnosed when an affected girl has developed normally for about the first 6 to 18 months of life. At about this age her development begins to slow down and then regresses, with loss of skills already gained, such as speech and purposeful movement of the hands. Affected girls become subject to gross developmental delay, severe mental handicap, stereotyped hand movements such as hand wringing and flapping, an apraxic gait, and autistic behaviour.<sup>4</sup> There may also be acquired microcephaly, periods of hyperventilation, and difficulties with swallowing.<sup>5</sup> Finally, the regression period plateaus and

affected girls survive into adulthood without further deterioration. Table 1 summarises the criteria by which a clinical diagnosis of “classical” Rett syndrome may be made.

### Variants

Hagberg<sup>6</sup> described several variant forms of the disorder where affected girls do not manifest all of the typical features of the syndrome to the same degree. A milder, incomplete, and more protracted form of the syndrome occurring in ~10% of cases has been termed forme fruste.<sup>7</sup> This amelioration of symptoms is often seen in one of the probands from the few familial cases of the syndrome. One of the characteristic features of Rett syndrome is the loss during the regression phase of any speech which has been acquired by the affected girl. Occasionally, however, a few words can still be uttered. Although the words are not always used in context this represents a milder form of the syndrome, which has been termed the preserved speech variant (PSV). In contrast, a more severe, possibly congenital form of the syndrome has been described in which there are early seizures at <6 months.<sup>7</sup>

### Identification of the gene

The search for a gene for Rett syndrome was seriously hampered by a lack of familial cases as >95% of cases of the syndrome are sporadic, but by performing linkage analysis on the few available familial cases the region of interest was localised to Xq28 in 1998.<sup>8-10</sup> This localisation was followed by intense screening of the Xq28 region for likely candidate genes until in 1999 Amir *et al*<sup>11</sup> published the first report linking the syndrome to mutations in the MECP2 gene. Amir *et al*<sup>11</sup> reported mutations in the MECP2 gene in 5/21 cases of Rett syndrome.

Since publication of the original paper, there have been a series of confirmatory studies detecting mutations in the MECP2 gene in girls with Rett syndrome from several ethnic groups.<sup>12-20</sup> The numbers of mutations detected in these varies from 21% in the original paper to >90%. Obviously the detection rate in this type of study depends upon several factors including the accuracy of the original diagnosis, but the methodology used may also influence the outcome. Some groups have found mutations in >80% of girls by using preliminary detection methods such as SSCP, while others have gone directly to sequencing.

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Table 1 Criteria for the clinical diagnosis of Rett syndrome, according to Clarke<sup>20</sup>

Necessary criteria	Supportive criteria
Normal prenatal and perinatal development	Seizures
Loss of acquired skills such as communication and speech	Abnormal EEG
Normal head circumference at birth with acquired microcephaly	Apnoea
Marked developmental delay	Hyperventilation
Loss of hand skills	Breathing dysfunction with breath holding
Autistic features	Scoliosis
Gait apraxia	Growth retardation
Stereotypic hand movements	Cold feet
	Spasticity with muscle wasting

We ourselves found 50% of non-familial cases to have a band shift on a first screening using SSCP, but sequencing of the *MECP2* gene in the remainder detected mutations in a further six giving a total of 72% of cases. As denaturing high performance liquid chromatography (DHPLC) recently showed 100% concordance with sequencing analysis,<sup>21</sup> this may become the technique of choice for variation screening.

### The MeCP2 protein

Methylation of the CpG dinucleotides in 5' CpG islands is an important factor in the transcriptional silencing of genes. Methyl-CpG binding protein 2 or MeCP2 is an abundant DNA binding protein which may act as a global repressor of transcription by binding specifically to methylated CpG dinucleotides in DNA. This can be achieved by one of two possible mechanisms. The first, simpler mechanism would be the direct interference by the bulky methyl group in the binding of transcription factors. The second, indirect mechanism involves the methylation of the promoter region preventing gene transcription via a secondary protein which binds to methylated CpG but not to unmethylated CpG, thereby preventing the formation of the transcription complex. Evidence favours the latter indirect mechanism, with the MeCP proteins providing the secondary step. MeCP2 was first isolated from rat brain by Lewis *et al*<sup>22</sup> in 1992. Working with mouse tissue, Nan *et al*<sup>23</sup> showed that the protein contained a methyl binding domain which bound specifically to methylated DNA but did not bind if the DNA was unmethylated. MeCP2 was also found to be concentrated in the pericentromeric heterochromatic regions so could well be involved in the formation of inert chromatin. It was then reported that, in *Xenopus*, methylation of DNA silenced transcription by altering the chromatin structure, condensing the nucleosome array.<sup>24</sup> After binding specifically to the methylated CpG in the target DNA, MeCP2 recruits Sin3A, a co-repressor, and histone deacetylase into a complex.<sup>24 25</sup> This complex then deacetylates the tails of histones H3 and H4 within the nucleosomes. Deacetylation of histones causes the chromatin to compact and become inaccessible to transcription (fig 1). Silencing can be prevented by inhibiting the histone deacetylase, showing that DNA methylation repression of transcription directly involves chromatin modification. The effect on the cell of MeCP2 may be the repression of transcriptional noise which arises because of a low level of illegitimate transcription rather than the targeted repression of specific genes.

The chromatin binding protein of 486 amino acids is divided into three domains. The methyl

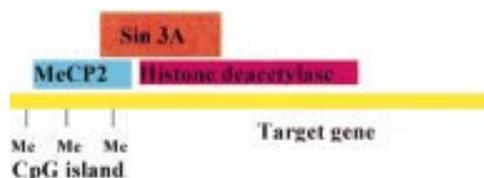


Figure 1 Transcriptional silencing by MeCP2.

binding domain (MBD)<sup>23</sup> which is 85 amino acids in length specifically binds to CpG dinucleotides which are methylated at position 5 of cytosine, that is, 5mC. The protein is found ubiquitously as it can bind anywhere in the genome where there is even a single 5mC, but it prefers to bind to 5mC rich CpG islands. Downstream from the MBD is the 104 amino acid long TRD or transcriptional repression domain. Once the protein is bound to the target gene, it is this domain which recruits both the co-repressor sin3A and the histone deacetylase.<sup>24 25</sup> The final C terminal segment of the gene product contains evolutionarily conserved poly-his and poly-pro regions. Although this region has as yet no known protein domain, it may also be involved in the interaction of MeCP2 with the nucleosome core.<sup>26</sup> The human and mouse 3'UTR have eight regions of closely conserved sequence similarity which has led to the suggestion that they may be involved in stabilising the transcript and in post-transcriptional regulation.<sup>27</sup>

### The *MECP2* gene

The *MECP2* gene (AF030876) is 112 756 bp long, but the cDNA (X99686) is only 1533 bp long. The gene, which has four exons (a further upstream exon has recently been described),<sup>28</sup> is transcribed from telomere to centromere with the coding region contained in exons 2-4. The 5'UTR is short with its 167 nucleotides situated in exon 1 (69 nucleotides) and part of exon 2 (98 nucleotides). The coding sequence for the methyl binding domain (MBD), a region comprising 85 amino acids, is split between exons 3 and 4 while the transcription repression domain (TRD), stretching from amino acid 207 to 310, lies entirely in exon 4.<sup>29</sup> There are two nuclear localisation signals (NLS), one lying between nucleotides 173 and 193 and the other in the TRD.<sup>30 31</sup> The very long conserved 3'UTR contains at least two polyadenylation signals that are differentially used.

Exon 4 of the *MECP2* gene also contains the large 3' untranslated region (UTR) which in the human gene is 8.5 kb long. This UTR contains several polyadenylated sites which would allow for the production of multiple transcripts of different sizes. It is not known whether different transcripts are produced at different stages of development or whether specific transcripts are produced in different cell types. Coy *et al*<sup>27</sup> first described the differential expression in human brain and other tissues of the alternative splicing patterns available to the 3'UTR. The 10.1 kb transcript which predominates in fetal brain contains the longest 3'UTR. This group also found that in the mouse *Mecp2* was expressed in early organogenesis so had a regulatory role in gene expression,<sup>27</sup> while Tate *et al*<sup>32</sup> found *Mecp2* to be essential for embryonic development in mice.

The finding of tissue specific differences in expression levels of the alternate transcripts may not correlate with the concept of the MeCP2 protein as a controlling factor of transcriptional noise rather than as a gene specific

Table 2 Mutations of the MECP2 gene in girls with Rett syndrome

Point mutations in the MBD		Point mutations in the TRD	
Total different	17	Total different	13
Missense	16	Missense	8
Nonsense	1	Nonsense	5
Total number	71	Total number	96
Missense	69	Missense	26
Nonsense	2	Nonsense	70
Loss of cytosine	65/71 (92%)	Loss of cytosine	93/96 (97%)
C>T	48/71 (68%)	C>T	86/96 (90%)

transcriptional suppressor. As it is as yet uncertain which genes are targeted by MeCP2, we cannot distinguish between non-specific noise repression, which is consistent with the ubiquitous expression of the gene, and control of a small number of important genes involved in development, which is consistent with alternate transcripts.

### Mutations in the MECP2 gene in sporadic cases of Rett syndrome

Rett syndrome is the first known disorder which is associated with defects in a protein involved in the methylation of DNA and abnormal chromatin packaging. To date, there have been more than a dozen published studies which report mutations in the MECP2 gene in patients with Rett syndrome. The following data summarise the results from these studies.<sup>11-20 33</sup>

#### POINT MUTATIONS (TABLE 2)

More than 70 different mutations have been identified and many are missense or protein truncating types which mainly involve C→T transitions. Even though a mutation in the MECP2 gene has still not been found in ~20-25% of girls with classical Rett syndrome, large deletions or intragenic inversions would not be detectable by the methodology used. Some limited FISH and Southern blotting studies aimed at detecting such large mutations have so far not done so.<sup>34</sup> The very large 3'UTR has also not been studied

Despite the fact that the great majority of mutations detected are de novo, there are eight relatively frequent mutations which account for ~60% of those found in girls with classical Rett syndrome (fig 2). All of these are C→T transitions. Small deletions, mainly in the C terminal region, account for a further 10% of mutations. This is presumably because of the presence of a series of CCACC repeats at the 3' end of exon 4.

In the region located between the MBD and the TRD, there have been 41 reported mutations. However 38 of these are the result

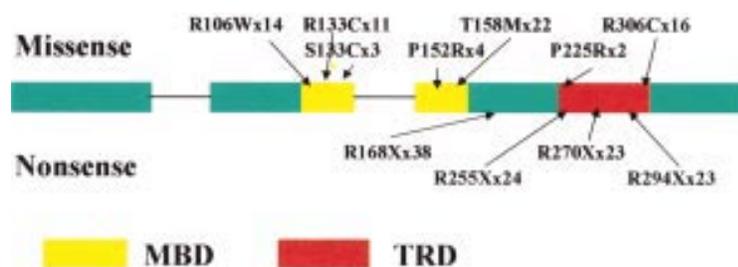


Figure 2 Hotspots for mutations in the MECP2 gene in girls with Rett syndrome.

of the single mutation R168X (502C>T), which is the most frequent mutation detected so far in Rett syndrome. Two missense and a further nonsense mutation complete the total of 41.

Eleven of the 17 different mutations in the MBD have been reported only once, but there are three hotspots: R106W (316C>T) has occurred 14 times, R133C (397C>T) 11 times, and T158M (473C>T) 22 times.

The pattern changes in the TRD where nonsense mutations leading to truncated proteins tend to predominate. Further downstream, however, the mutation type reverts to missense. There are three hotspots for the truncating mutations: R255X (763C>T) has occurred 24 times, R270X (808C>T) 23 times, and R294X (880C>T) 23 times.

All three of these involve loss of a highly conserved arginine codon and all are C>T. The very high (but as yet unexplained) mutability of CpG dinucleotides in the MECP2 gene would target arginine codons for mutation although there is another conserved arginine at position 268 which has as yet no reported mutation. This indicates either that there is specificity in the selection of arginine codons for mutation or that mutations of R268 are lethal. The most frequent missense mutation in the TRD is R306C (916C>T), which has occurred 16 times, also involves loss of an arginine codon, and is also C>T. Loss of the same arginine codon R306H (917G>A) has been reported twice.

In summary, 33 different point mutations have been reported, 208 in total. Of the substitutions, 173 (83%) are C>T and 142 (68%) involve an arginine codon (table 3). This either results in incorporation of an incorrect amino acid in the protein chain or a truncation of the protein. In the normal protein, of 486 amino acids only 33 or 7% are arginine.

#### VARIANTS

Three variants have been reported, 656C>T, 1307C>T, and 1189G>A. The latter, which results in E397K, has been detected on three occasions. A single case of loss of the final stop codon leading to chain elongation has also been reported, 1461A>C X487C.<sup>17</sup>

#### INSERTION/DELETION MUTATIONS

Five insertion/deletion mutations have been found in the MBD and 12 in the TRD. Only one of these has been detected on more than a single occasion; 806delG>288X, leading to a stop codon, has been found in four patients.

The majority of insertion/deletion mutations are in the C terminal segment where >25 different types have been reported but so far only one has occurred more than once. Two

Table 3 The loss of specific arginine codons in the MECP2 gene

Region of the gene	Different mutations	Total mutations
MBD	4/17 (24%)	27/71 (38%)
MBD-TRD	3/4	40/41 (98%)
TRD	5/12 (42%)	85/96 (89%)
Total	12/33 (36%)	142/208 (68%)

Table 4 X inactivation in girls with mutations in the *MECP2* gene

Proband	Methodology			Mutation
	M27 $\beta$	Humara	FISH	
1	Random	Random		R106W
2	NI	NI	50:50	T158M
3	70:30	Skewed		R168X
4	75:25	NI		R168X
5	70:30		67:33	R198X
6	70:30	Skewed		Q208X
7		Skewed		R255X
8	60:40	Random		R255X
9	Skewed			R255X
10	NI	Random		R270X
11	60:40			R294X

cases of variant Rett syndrome with preserved speech had deletions in this region of the gene.<sup>15</sup>

In total there have been 33 different point mutations reported, 208 altogether, 42 different insertion/deletions, 46 in total, and four splice site mutations. Thus, 79 different mutations have been described in 279 subjects.

### Methodology

The majority of studies reported so far have used various gel separation techniques followed by sequencing of ensuing band shifts. Some, however, may have used direct sequencing only,<sup>12-14 16 20</sup> but the percentage detection is not markedly different from that of the studies with preliminary band shift screening of cases. Although the detection rate has reached >90% in some reports,<sup>13</sup> no group has as yet reported 100% and in some cases it is much lower. Difficulty in the differential diagnosis of the syndrome may account for some of the discrepancies, as also could the presence of mutations in hitherto untested regions of the gene such as the very long 3'UTR.

In order to check the methodology, we sequenced all of the cases of Rett syndrome in our study,<sup>19</sup> which had not shown a band shift using previous SSCP. A further six mutations were detected in addition to the 15 already reported, indicating that sequencing is the method of choice. There were still, however, several cases of "classical" Rett syndrome with as yet no molecular defect detected in the *MECP2* gene, although two had the E397K variant.

### X inactivation

There have been several studies of X inactivation in Rett syndrome girls and although there is the occasional report of skewed inactivation, the general finding has been that skewing is neither particularly frequent nor particularly marked in this syndrome.<sup>10 35</sup> Amir *et al.*,<sup>36</sup> in their study of 34 sporadic patients, found 31 (91%) of them to have random X inactivation and suggested that if skewing occurred it was associated with a milder phenotype. This implies that it is only necessary for a partial loss of activity of the *MECP2* gene (which is subject to X inactivation) to result in "classical" Rett syndrome. We have detected random X inactivation in girls with mutations in either the MBD or the TRD who have been diagnosed with the "classical" disorder (table 4).

However, despite random inactivation of the X chromosome being the most common finding in both mothers and normal sisters of sporadic cases of the syndrome, the mothers of two cases were found to carry the same mutation as their affected daughters. Both had marked skewing of X inactivation, which favoured expression of the normal X chromosome so preventing the disease.<sup>17 36</sup> In those families where two affected sibs and their normal mothers have all been found to carry a mutation in the *MECP2* gene, the unaffected mother has almost always been found to have marked skewing with the X chromosome carrying the *MECP2* mutation being predominantly inactive.<sup>10 12</sup> One family with three female carriers of a late truncating mutation presented with widely differing phenotypes; the mother was phenotypically normal, one of her daughters had classical Rett syndrome, and the other had a preserved speech variant. In each case, the phenotype could be ascribed to the degree of inactivation of the mutated X chromosome.<sup>37</sup>

Mitigation of what should apparently be a severe phenotype has also been found in cases of early truncating mutations. Since the majority of mutations in the MBD are missense, and the majority of truncating mutations are distal to the MBD, interest in the few truncating mutations which have been detected in the MBD allowed the demonstration of phenotype mitigation for Y141X and 411delG, both of which disrupt the DNA binding surface of the MBD.<sup>38</sup> The most proximal nonsense mutation yet, Q19X, reported in a patient with suspected Rett syndrome and autism, was also subject to mitigation via moderately skewed X inactivation.<sup>39</sup>

### Familial cases

More than 30 families with more than a single affected female have been studied so far. Surprisingly, in only five of these was the same mutation detected in more than a single proband.<sup>11-13</sup> In another four of the families, one of the affected girls was found to carry a mutation while her affected relative did not.<sup>13 19</sup> In many of the families (21/30), no mutation of the *MECP2* gene was detected at all. An exception to this is the family from Brazil with three affected girls. Wan *et al.*<sup>12</sup> studied two of the three affected girls and their mother and found all three to have the R168X mutation. The unaffected mother has almost total skewing of X inactivation.<sup>10</sup> Mutation in both members of familial cases has only been found in 29%<sup>36</sup> to 45%<sup>38</sup> of families, which is in contrast to the average of >90% in sporadic cases. This may imply that familial cases are even rarer than was originally believed as some younger family members may have been misdiagnosed on the "lightning does not strike twice" principle. In familial cases where an *MECP2* mutation has been described, asymptomatic or mildly affected family members have skewed X inactivation, whereas affected girls tend to have a random pattern. Germline

Table 5 Males with mutations in the MECP2 gene

Authors	Mutation detected	Inheritance type	Diagnosis	Comment
Orrico <i>et al</i> <sup>12</sup>	A140V	Familial	Severe non-specific MR	
Villard <i>et al</i> <sup>11</sup>	T158M	Familial	Severe neonatal encephalopathy	Sister has Rett syndrome
Watson <i>et al</i> <sup>13</sup>	241del2	Singleton	Severe MR progressive spasticity	
Meloni <i>et al</i> <sup>10</sup>	Q406X	Familial	AS	Somatic mosaic
Wan <i>et al</i> <sup>12</sup>	V288X	Familial	Congenital encephalopathy	Sister has Rett syndrome
Imessaoudene <i>et al</i> <sup>44</sup>	G428S	Familial	Non-progressive encephalopathy	

or somatic mosaicism have also been described<sup>12</sup> and both maternal and paternal mutations occur in sporadic cases.<sup>36</sup>

### Ethnic groups

The same mutations of the *MECP2* gene have been detected in diverse racial groups as would be expected if the majority are de novo. The groups studied have included Europeans (Russian,<sup>19</sup> Italian,<sup>15</sup> Swedish,<sup>14</sup> German,<sup>16</sup> French,<sup>17</sup> and British<sup>13 19</sup>), along with Chinese,<sup>20</sup> Japanese,<sup>18 33</sup> Brazilian,<sup>12</sup> and Pakistani<sup>19</sup> patients.

### Affected males (table 5)

It has in the past been part of the dogma associated with Rett syndrome that because it is a condition which only affects females it is lethal in males. The presence of an apparently affected male within a family has tended to preclude a diagnosis of Rett syndrome in an affected girl. Wan *et al*,<sup>12</sup> however, reported a family in which an affected girl had a brother who died from a neonatal encephalopathy. Both sibs carried a V288X (806delG) mutation. Their mother, who had mild learning disabilities, had favourable skewing of X inactivation but her sister, like her daughter, had classical Rett syndrome. Other males with similar phenotypes have also been found to carry *MECP2* mutations.<sup>40</sup> Villard *et al*<sup>11</sup> reported a family in which a girl with Rett syndrome had two brothers who were affected with severe neonatal encephalopathy. All three affected subjects and their mother carried the T158M mutation. The mother was protected by marked skewing of X inactivation. Orrico *et al*,<sup>12</sup> however, reported a family in which a female with mild mental handicap and four males with severe non-specific mental retardation all had an A140V mutation in the *MECP2* gene. Although such an error in the single X chromosome may usually be lethal in utero, if a male does survive beyond birth then he presents a different clinical picture from that described in girls with Rett syndrome, even within the same family. As so many of the cases of Rett syndrome are sporadic (>95%), this

would suggest that there may be a cohort of males as yet undiagnosed who have a disorder attributable to mutations in the *MECP2* gene.

### Other phenotypes (table 6)

Since the discovery that affected males (and some females) do not present with the same clinical picture, even allowing for Rett variants, attention has focused on other similar conditions. These have included cases of apparent Angelman syndrome where no abnormality could be detected in chromosome 15 and families with severe, and even families with mild, mental handicap. Two studies of Angelman syndrome<sup>43 44</sup> have resulted in the detection of 5/47 mutations in the *MECP2* gene in one study<sup>43</sup> and 6/78 in the other.<sup>44</sup> Of the six detected by Imessaoudene *et al*,<sup>44</sup> only four were females compatible with a diagnosis of Rett syndrome. The other two, one male with a late missense mutation and one female with the common truncating mutation R270X, both had encephalopathy. In the other study, four females and one male had *MECP2* mutations. The early truncating mutation found in the male patient was mitigated by the presence of mosaicism.<sup>43</sup>

### Do mutations in the MBD and the TRD both produce "classical" Rett syndrome?

Cheadle *et al*<sup>13</sup> found an increase in severity with the nonsense mutations and with deletions when compared to the missense mutations, but other studies have not yet confirmed this finding. Amir *et al*<sup>36</sup> suggested that different *MECP2* mutations produce a similar phenotype and that X inactivation is important in the production of the classical syndrome. In any condition where there is at least a partial protein product from the mutated allele, the severity of disease may depend almost entirely on the degree of X inactivation rather than on the mutation itself,<sup>19</sup> although marked skewing has not been a consistent feature in Rett syndrome. The phenotype may also be influenced by other modifier loci, as yet undescribed.<sup>26</sup>

Mutations in the MBD of which the overwhelming majority are missense would be expected to eliminate or at least reduce binding of the MeCP2 protein to the target gene. The truncating mutations which occur in the region between the MBD and the TRD may lack a nuclear localisation signal and those in the TRD itself would be expected to fail to form complexes with Sin3A and histone deacetylase. As there appears to be no relationship between clinical severity and mutation type, both resulting in "classical" disorder, this would suggest that efficient binding of the MeCP2

Table 6 Other phenotypes with mutations in the MECP2 gene

Author	Mutation	Diagnosis	Type
Orrico <i>et al</i> <sup>12</sup>	A140V	Mild MR	Familial
	T203M	Autistic Rett variant	Sporadic
Watson <i>et al</i> <sup>13</sup>	1230del44	AS	
	P101R	AS	
	1330del52	AS	
	Y141X	AS???	
Imessaoudene <i>et al</i> <sup>44</sup>	R106W	AS	
	R255X	AS	
	R255X	AS	
	803delG	AS	
	R270X	Severe early onset encephalopathy	

protein is necessary, but once achieved is insufficient by itself to silence the target gene as a change in chromatin conformation is also essential.

Ballestar *et al*<sup>45</sup> found R106W and R133C, which are located on the  $\beta$  sheets of the MBD, cause a 100-fold reduction in the affinity of the MeCP2 protein for methylated DNA. Although T158M had only a two-fold reduction, this threonine is very highly conserved and is not found in any other MBD in other methyl CpG binding proteins.

Yusufzai *et al*,<sup>46</sup> in an elegant study, reported that four missense mutations in the MBD (R1096W, R133C, F155S, T158M) abolished selectivity for methylated DNA. The mutated MeCP2 protein would still bind but had lost its ability to differentiate between methylated and non-methylated CpG dinucleotides. By contrast, a more downstream missense mutation, R306C located in the TRD, did not abolish this selectivity. The L138X truncating mutation in the MBD did not bind to DNA at all and failed completely to repress transcription. The nonsense mutations in the TRD failed to repress transcription of a reporter gene but the R306C and E397K missense mutations were found in this study to cause repression in a manner similar to the wild type. The authors suggested that the mechanism for failure to repress transcription lay in a decrease in the stability of the truncated protein. The MeCP2 proteins with C terminal deletions were also found to be less stable than the wild type, but again not R306C or E397K. These findings caused the authors to suggest that E397K (which had been detected on several occasions in Rett syndrome) was a polymorphism rather than a mutation.

In the mouse, embryonic stem cells with early truncating mutations are still viable and will even differentiate in culture, but if they are inserted into chimaeric embryos those with a high percentage of mutant cells die showing that MeCP2 is essential for embryogenesis.<sup>32</sup>

It has been suggested that the prevalence of the syndrome and the predominance of affected females indicates an increased mutation rate in the paternal rather than in the maternal *MECP2* gene.<sup>47</sup> When cell lines from three affected girls which had retained only the mutated X chromosome were studied, two were found to be of paternal origin and the third of maternal origin.<sup>32</sup>

Rett syndrome thus appears to be the result of abnormal remodelling of chromatin with a subsequent effect on one or several genes which are not themselves structurally abnormal. Other examples of this phenomenon include imprinting defects, X inactivation, and the recent finding of a defect in phosphorylation of histone H3 in Coffin-Lowry syndrome.<sup>48</sup> It remains to be seen whether there is one, or if there are several, such affected genes involved in the aetiology of Rett syndrome and then to determine the function which they perform in the brain.

## Future research

Questions which remain unanswered include, do the girls with Rett syndrome who apparently do not have a mutation in the *MECP2* gene have a mutation that lies in the 5' promoter region, the very large 3'UTR, or in another gene entirely? If there is a second gene could it be a target gene bound by MeCP2? It has also been suggested that mutations in other members of the MBD family may have a similar pathogenesis since MBD2 and MBD3 also form complexes with deacetylases.<sup>49</sup>

Once the transcriptional consequences of mutations in the *MECP2* gene have been determined then we will know the number and the type of critical genes which are under the control of MeCP2. Then answers to questions such as why are there so many different phenotypes associated with the mutations, how can this type of loss of transcriptional control cause a neurodevelopmental disorder syndrome such as Rett syndrome, and how can such an apparently identical range of mutations be involved in the other disparate phenotypes may become clear. Indeed, there may be further conditions associated with mutations in the *MECP2* gene. The true picture in males is not yet clarified; does a male variant of Rett syndrome exist or is this phenotype lethal and if so why this particular one and not others? How does the loss of transcriptional control disrupt development rather than prevent it from birth or before?

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