

ELECTRONIC LETTER

Developmental delay and the methyl binding genes

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The report by Amir *et al*¹ that Rett syndrome (RS) is associated with mutations in the *MECP2* gene permitted laboratory diagnosis of this devastating yet common neurodevelopmental disorder. Hitherto the paucity of familial cases of the syndrome and the failure to identify the syndrome in males despite fairly wide clinical criteria had defined it as an X linked dominant disorder with male lethality.² Soon, however, reports from the few families in which RS is segregating showed that male family members who inherited the same mutation in the *MECP2* gene as their affected female relatives did sometimes survive to birth and beyond, but they did not, as the females did, develop Rett syndrome but were subject to an entirely different syndrome of severe male encephalopathy.³⁻⁴ In the meantime, attempts were being made to relate the severity of the clinical picture presented by girls with RS (ranging from "classical" to "forme fruste")² with the individual mutations. No correlations were observed either with the type of mutation or with its position within the *MECP2* gene save that N-terminal changes tended to be more severe than those located further downstream.⁵ The gene is divided into a methyl binding domain (MBD), a transcription repression domain (TRD), and a proline rich C-terminal domain. Mutations cause loss of function by interfering either with DNA binding or with the downstream association of MeCP2 with its transcriptional corepressors Sin3A and HDAC.⁶ To date, >200 different mutations have been detected in girls with RS, >95% are de novo, and they are found in all of the domains. Those in the MBD are predominantly missense while those in the TRD tend to be nonsense or truncating mutations. Downstream C-terminal mutations may be individual and are often of the insertion or deletion type. Despite the wide range of changes reported, it has been suggested that >60% are the result of R106W and T158M (in the MBD), R168X (between the MBD and the TRD), and R255X, R270X and R294X (in the TRD).⁷

Studies on other types of developmental delay, both familial and de novo, have indicated that mutations in the *MECP2* gene may be more common and related to more types of handicap than had previously been anticipated. Two studies of

children referred with a clinical diagnosis of Angelman syndrome (AS) but without an abnormality in 15q11q13 showed that 4/46 of the girls in one case⁸ and 5/40 in the other⁹ actually had Rett syndrome. Of these nine probands, only one was later found to have a clinical presentation inconsistent with the laboratory diagnosis. This may not be surprising given that in very small girls the two syndromes may present with overlapping clinical features and so be difficult to differentiate on clinical grounds alone.

Familial cases of developmental handicap indicate that males often show changes in the *MECP2* gene which would normally be considered to be SNPs and so unrelated to the clinical symptoms.¹⁰ Females in these families who are carriers of such changes are nearly always unaffected or very mildly so, most probably because of the effect of X inactivation.¹¹

Thus, females with mutations in the *MECP2* gene which affect the function of the MeCP2 protein may develop RS or other forms of severe developmental delay. Males with this type of mutation will, if they survive to birth, develop lethal encephalopathy. If the mutation in males is in mosaic form or is located within an XXY genotype, then classical Rett syndrome may result.^{5, 12, 13} In addition, a series of "milder" changes which would certainly not be expected to affect gene function markedly, if at all, in heterozygous females, may in the hemizygous male be associated with a milder disorder (table 1).

Although mutations in the *MECP2* gene account for >90% of Rett syndrome, in view of the range of clinical severity, there may be a significant minority of cases of "atypical" disorder or other types of developmental delay which are related to the other methyl binding proteins MBD1 and MBD2. Both MBD1 and MBD2, which is a component of the MeCP1 complex, are, like MeCP2, able to repress transcription by binding to methylated CpG.¹⁴

We therefore attempted to answer the following questions concerning the relationship between changes in the *MECP2* gene and developmental delay. How many children of either sex referred for molecular testing for AS but with no detectable molecular changes in 15q11q13 have mutations in

Table 1 Mutations in the *MECP2* gene reported in males with developmental delay

Study	Familial or sporadic	Mutation
Imessaoudene <i>et al</i> ⁸	Familial non-fatal, non-progressive encephalopathy	G428S
Couvert <i>et al</i> ¹¹	Family MRX16	E137G
	Family T36	R167W
	Sporadic	A140V
	Sporadic	P399L
	Sporadic	R453Q
	Sporadic	K284E
Orrico <i>et al</i> ⁹	Familial severe XLMR	A140V
Klauck <i>et al</i> ²⁰	PPM-X family	A140V
Meloni <i>et al</i> ²²	Familial severe XLMR with spasticity	E406X
Hoffbuhr <i>et al</i> ⁵	Sporadic	1154(del 132)
Yntema <i>et al</i> ²³	Familial mild XLMR	In frame 1161-1400 (del 240)
Watson <i>et al</i> ⁶	Motor delay, seizures	Mosaic 241del2

Table 2 Primers from the MBD1 and MBD2 methyl binding domains used for SSCP analysis

Gene	Exon	Product size	Primer
<i>MBD1</i>	2	135 bp	5'-ctactgctgttccctgtgg 5'-cagtaacctctggtaatagg
<i>MBD1</i>	3	277 bp	5'-ggtagtgagggtgggctg 5'-gcacatccacccctacctag
<i>MBD2</i>	1	285 bp	5'-agcctgtccctgcccctg 5'-agcctgtccctgcccctg
<i>MBD2</i>	2	173 bp	5'-ttctctagtccaagtg 5'-aacctattttgattgagagg

the *MECP2* gene, with the implication that the girls should be tested for these mutations routinely? What types of molecular changes are associated with developmental delay in males? Can changes in MBD1 and MBD2 be detected in cases of developmental delay in which the *MECP2* gene shows no abnormality?

METHODS

A cohort of 66 children (38 female, 28 male) who had been referred to the Regional Genetics Laboratory with a request for molecular testing for Angelman syndrome (AS) were studied. No molecular abnormality had been detected in 15q11q13 in any of the cohort. DNA samples were amplified by PCR using the nine sets of primer pairs described by Imessaoudene *et al.*⁹ The products, which ranged in size from 182-296 bp, covered the coding region and the exon/intron boundaries of the *MECP2* gene. In several cases the primers published by Amir *et al.*¹ were also used. These produced larger products ranging in size from 340-413 bp, which made the SSCP analysis less efficient, but they covered more of the non-coding sequences within the gene. For single strand conformational polymorphism (SSCP) analysis, products were separated on an 8% polyacrylamide non-denaturing gel at 4°C and subsequently stained with silver nitrate by standard methods. When a shift was detected on the SSCP gel, genomic sequencing was carried out using both sets of primers individually using an ABI big dye terminator cycle sequencing kit.

DNA obtained from 100 normal males was also subjected to SSCP mutation analysis exactly as described above.

Samples in which no shift was detected were subsequently screened by SSCP analysis using primers (table 2) covering the methyl binding coding regions of the MBD1 and MBD2 genes.¹⁵

RESULTS

Three of the 38 girls (8%) with a possible diagnosis of AS had mutations in the *MECP2* gene. One girl had the T158M missense mutation and two others had R168X. Both of these mutations are strongly associated with Rett syndrome. Two of the girls were 2 years of age or less while surprisingly the third

was 11 years 9 months of age. Two of the girls have now developed a clinical phenotype consistent with Rett syndrome but the third has not. A fourth girl carries 21746C→T within intron III of the gene. As her father was subsequently found to have this change, it is unlikely that it contributes to her phenotype (table 3). Of the 28 boys studied, two had an apparent change in the coding region of the *MECP2* gene, S194S (C656→T) and A439T (G1389→A). No changes were observed in the coding region of the *MECP2* gene after SSCP analysis of 100 normal males.

S194 is the final amino acid in a region of high conservation between human (NM 004992), mouse (AF158181), and *Xenopus* (AF106951) MeCP2 protein. Beyond this point, although human and mouse remain identical for some distance the *Xenopus* protein diverges completely. Similarly the seven amino acids immediately preceding A439T are conserved in all three species, but again the *Xenopus* protein diverges totally beyond this point while human and mouse do not. The alanine moiety itself, however, is retained in the *Xenopus* but has become a methionine in the mouse.

No abnormalities were detected in either MBD1 or MBD2 after screening of 60 probands in whom no changes were previously observed in *MECP2*.

DISCUSSION

A series of 66 children referred for laboratory testing for Angelman syndrome but without a detectable abnormality in 15q11q13 were tested for mutations in the coding region of the *MECP2* gene by SSCP analysis followed by sequencing of any abnormalities observed. Four girls were found to have a shift after SSCP analysis, three of whom were subsequently found to have a mutation commonly associated with Rett syndrome. Analysis of the *MECP2* gene for Rett syndrome was requested subsequent to a negative finding for AS in one of the girls with R168X and the one with T158M. The other girl with R168X and the fourth girl, who carried a polymorphism in intron III, were clinically not considered to have classical Rett syndrome. Watson *et al.*,⁸ Imessaoudene *et al.*,⁹ and this study have all found that approximately 8% of the girls referred for

Table 3 Mutations in the *MECP2* gene

Sex	Clinical description	Exon	Mutation
F	Sat 10 mth, stood 17 mth, mild developmental delay until 9 m, happy baby, bouts of laughter, can finger feed, drinks from a cup, no loss of behavioural skills, hand wrings	4	T158M
F	No history until chest infection at 8 mth, cannot walk w/o help, not microcephalic, did not lose skills, some hand use, hand wringing	4	R168X
F	Normal development until 20 mth then regression	4	R168X
F	Severe learning difficulties, microcephaly, severe epilepsy	IVSIII	21746C→T
M	Mild developmental delay, motor development normal, slow growth <3rd centile	4	S194S
M	Developmental delay, happy baby, variable tone, squint, HC <3rd centile	4	A439T

laboratory diagnosis of AS in fact have a molecular abnormality associated with Rett syndrome, illustrating the difficulty of differential diagnosis. In both Watson *et al*⁸ and in this study, mutations in the *MECP2* gene have been detected in girls who phenotypically do not appear to have Rett syndrome, making the decision to request further testing in such girls even more difficult.¹⁶

Two changes were detected in 28 male subjects. The study by Watson *et al*⁸ found a single change in 22 males. This boy had a mutation associated with Rett syndrome but, like other males with a severe mutation, was believed to carry it in mosaic form. Imessaoudene *et al*⁹ described a male in their cohort who had non-fatal, non-progressive encephalopathy and G428S. Like this latter mutation, the S194S¹⁷ and A439T changes detected in males in this study would normally be considered to be polymorphisms with no clinical implications. Interestingly, however, S194S (first described by Amir *et al*¹) and A439T are exactly the two changes reported by Cheadle *et al*¹⁸ in their study of girls with Rett syndrome. Cheadle *et al*¹⁸ dismiss these changes as not related to the disease as they also found 4/120 chromosomes studied to carry the S194S change. These presumably were all detected in heterozygous females, in some cases in affected girls, yet a control study of 100 normal males did not detect any polymorphisms in the *MECP2* gene. Amir *et al*¹ did find S194S in an unaffected father of two sisters with Rett syndrome but reported no changes in 96 non-Rett chromosomes. Several studies have now associated A140V with mental handicap in males while leaving carrier females either unaffected or only mildly handicapped.^{11 19 20} A functional study by Kudo *et al*²¹ using a transient expression system indicated that while the Rett syndrome mutation R106W significantly reduced expression of the *MECP2* gene, the milder mutations A140V and E137G, both of which have been detected in males with non-specific mental handicap, had milder impairment of function as they still retained some transcriptional repression activity. In hemizygous males, other subtle changes in protein structure or rate of protein production may also cause mild impairment of MeCP2 function. While they do not result in Rett syndrome itself, they may still cause the protein to function less efficiently. Since this is a protein which acts on the downstream expression of a wide range of genes, it is possible that there may be different neurodevelopmental disorders whose severity may depend upon the differential functioning of the MeCP2 protein.

MeCP2, MBD1, and MBD2 all belong to the same family of nucleoproteins which bind specifically to methylated DNA and repress transcription via a complex involving histone deacetylase (HDAC).¹⁵ Although the genes targeted by any of the three MBD domains have not yet been elucidated, mutations in either MBD1 or MBD2 might be expected to exert a similar effect on gene silencing as has been found for MeCP2. No mutations in either MBD1 or MBD2 were detected in a cohort of 60 children with developmental delay, indicating either that mutations in these genes are lethal or that the specificity of the target genes for these two methyl binding proteins is distinct from those bound by MeCP2. If this is so, then any mutations which occur in MBD1 or MBD2 may result in a completely different phenotype.

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