Letters to the Editor

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Mutation analysis of the methyl-CpG binding protein 2 gene (*MECP2*) in patients with Rett syndrome

EDITOR—Rett syndrome (RTT, MIM 312760) is a neurodevelopmental disorder characterised by normal early psychomotor development followed by a period of regression, the loss of acquired purposeful manual and speech skills, hand wringing, gait disturbance, and growth retardation.[1] As RTT occurs exclusively in females and almost all patients with RTT are sporadic, it has been proposed that RTT is caused by an X linked dominant mutation with lethality in hemizygous males.[1] Recently, DNA mutations in the methyl-CpG binding protein 2 gene (*MECP2*), mapped to Xq28, have been detected in some patients with RTT.[2 3] We carried out a mutation analysis in 40 Japanese patients with RTT to confirm that *MECP2* is the gene responsible for RTT and to detect common mutations in *MECP2*.

All patients screened in this study were sporadic cases, 38 patients with clinically typical phenotypes of RTT and two patients with preserved speech variant of RTT.[4] Genomic DNA was extracted from the peripheral blood of 40 patients with RTT, their parents, and 105 healthy Japanese women. Primer pairs for polymerase chain reaction (PCR) amplification, designed using the genomic sequence of *MECP2* (Gen Bank accession number, MeCP2 locus, AF030876, AJ132917), and the sizes of the products are shown in table 1. PCR amplification was performed in a final volume of 25 µl with PCR buffer, dNTPs, *Taq* polymerase, and each primer set. PCR conditions were: initial denaturing at 94°C for three minutes followed by 35 cycles of denaturing at 94°C, annealing at 56°C, and extension at 72°C for one minute each, and final extension for five minutes. To detect sequence variations, the products of PCR were analysed by electrophoresis on 6.5% polyacrylamide gels containing 0-100% linearly increasing denaturing agents at 60°C using a Bio-RAD D GENETM system and by direct DNA sequencing.

We found 15 different DNA mutations of *MECP2* in 36 (90%) of 40 patients with RTT (table 2). These mutations were not found in parents of patients or 105 healthy Japanese women, indicative of non-polymorphic variations. Three nonsense mutations, R168X, R270X, and R294X, were common in patients with RTT and have been identified in 12 (33.3%) of 36 patients with RTT (fig 1, table 2). In addition, four missense mutations, R133C, P152R, T58M, and R306C, were also detected in 16 cases with RTT (44.4%) (fig 2, table 2). Mutations with a nucleotide deletion resulting in a frameshift with a premature stop codon were detected in five patients with RTT. The clinical courses and symptoms in RTT patients with mutations of *MECP2* did not differ from those in four RTT patients who had no mutations detected in this study. Recently, Amir *et al*[2] and Wan *et al*[3] reported 10 mutations, five missense and five nonsense, in *MECP2* in patients with RTT. Among the five missense mutations, four mutations (R106W, R133C, F155S, and T158M) were in a highly conservative methyl binding domain (MBD) of *MECP2* and the R106W, R133C, and T158M mutations were also detected in patients in our study. In addition, three new missense mutations, L124F, S134C, and P152R, were detected in the MBD region of *MECP2* in Japanese patients with RTT. Methyl CpG binding protein 2 (MeCP2), an abundant chromosomal protein with a high affinity for methyl CpG pairs, is a transcriptional repressor[5] and is essential for embryonic development in mouse.[6] Since MBD, which consists of 85 amino acids, is essential for chromosomal localisation of the protein as determined by a deletion study of *MECP2*,[5] these amino acid changes may decrease its binding affinity for chromatin.[3]

As a result of an extensive mutation search of CpG hotspots based on the *MECP2* coding sequence, five R to X mutations, R168X, R255X, R270X, R294X, and R452X, were predicted by Wan *et al*.[3] All mutations resulted from a C to T substitution in an Arg codon (CGA). Two nonsense mutations, R168X and R255X, were identified in a previous report.[2 3] Five RTT patients in our study had the nonsense mutation R168X, resulting in a loss of the transcriptional repression domain (TRD) of *MECP2*. Furthermore, two similar nonsense mutations, R270X and R294X, were detected in three and four patients, respectively, with RTT (table 2). Twelve (33.3 %) of 36 patients with known DNA mutations of *MECP2* had one of these nonsense mutations in our series and had a truncated C-terminal half of MeCP2. The C-terminal half of the protein is needed for the efficient repression of transcription in vitro.[7] These data suggest that a C to T substitution in an Arg codon (CGA) is the most common mutation in *MECP2* in patients with RTT.

Seven mutations, R133C, P152R, T158M, S306C, R168X, R270X, and R294X, in exon 3 of *MCEP2* were detected in 28 cases (77.8%) in our study. Six mutations resulted from a C to T transition and only one C to G

Table 1 PCR primer sets for amplifing exons of MECP2

Exon		*Primer sets*	*PCR product (bp)*
Exon 1	F	5' ACAGAGGCCAAACCAGGAC 3'	416
	R	5' TAGAGGTGACAAGGCTTGTG 3'	
Exon 2	F	5' CTCCATGAGGGATCCTTGTC 3'	463
	R	5' AGACTGGCATGTTCTCTGTG 3'	
Exon 3-1	F	5' ACATTGCTATGGAGAGTTCTC 3'	427
	R	5' GTTTGATCACCATGACCTGG 3'	
Exon 3-2	F	5' GAAGCTCCTTGTCAAGATGCC 3'	477
	R	5' TTTGGGGACTCTGATGGTG 3'	
Exon 3-3	F	5' ACTGAAGACCTGTAAGAGCC 3'	632
	R	5' AATGCTCCAACTACTCCCAC 3'	

F: forward primer, R: reverse primer.
DNA sequence cited from Gen Bank accession number AJ132917.

Table 2 Mutations of MECP2 identified in patients with RTT

Mutation type	*DNA change*	*Predicted protein change*	*No of patients*
Missense	C391T	R106W	1
	G447C	L124F	1
	C472T	R133C	3
	C476G	S134C	1
	C530G	P152R	3
	C548T	T158M	5
	C991T	R306C	5
Nonsense	C577T	R168X	5
	C883T	R270X	3
	C956T	R294X	4
Frameshift and stop	450(C)	1 bp deletion	1
	770(G)	1 bp deletion	1
	771(C)	1 bp deletion	1
	829-832(GGCA)	4 bp deletion	1
	882(C)	1 bp deletion	1

DNA sequence cited from Gen Bank accession number AJ132917.

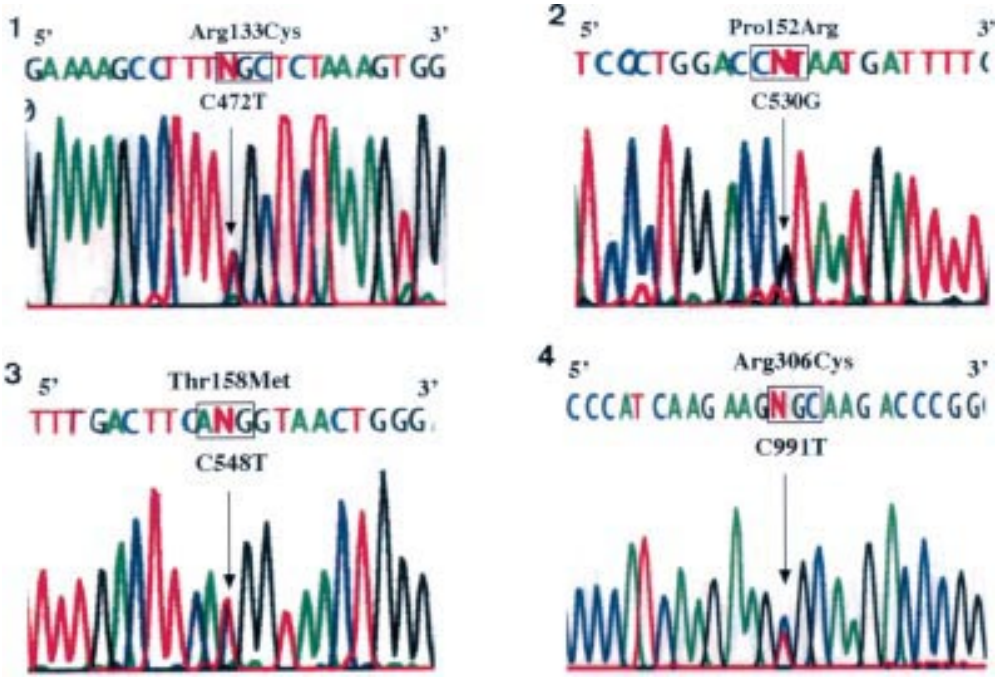

Figure 1 Nucleotide sequence of four common missense mutations of MECP2 in patients with RTT. (1) R133C mutation, (2) P152R mutation, (3) T158M mutation, (4) S306C mutation.

transversion was observed in P152R. Thus, mutations in patients with RTT are restricted to some portions of *MECP2*.

The clinical symptoms and their causes were slightly different in patients with different DNA mutations. Patients with the P152R mutation were severely handicapped; none of them can walk now. Patients with the R306C mutation had a mild form and all of them can walk and one patient can speak several significant words. In our study, the patients with R306C and R133C have the preserved speech variant. Further study of these links are necessary to confirm the genotype-phenotype correlation of RTT.

Amir *et al*[2] reported that five (23.8%) of 21 patients with RTT had DNA mutations in *MECP2* in their preliminary study. Wan *et al*[3] detected DNA mutations of *MECP2* in half of the patients with RTT. Furthermore, Zoghbi[8] later found DNA mutations of *MECP2* in approximately 70% of patients with RTT. We found 15 different DNA mutations of *MECP2* in 36 (90%) of 40 patients with RTT, which is a much higher percentage than in the previous reports.[2 3 8] We have determined the whole DNA sequence in encoding and splicing regions of *MECP2* in patients who did not show heteroduplex DNA bands by the DGGE method. Thus, the rate of mutations of *MECP2* in our study may increase compared with that in other studies.[2 3 8] However, no mutations in the coding and splicing portions of *MECP2* were detected in four of the patients with RTT screened so far. We have not analysed the 3' untranslated region (3'-UTR) of *MECP2*. The long 3'-UTR of *MECP2* is differently expressed in brain and other tissues, suggesting that both the primary sequence and the three dimensional structure of the 3'-UTR have essential roles in the post-transcriptional regulation of *MECP2* expression.[6] Therefore, DNA mutations in the 3'-UTR of *MECP2* may be responsible for the symptoms in patients with RTT. However, there is another possibility, that RTT is genetically heterogeneous and caused by other gene(s).

In conclusion, we have confirmed that mutations in *MECP2* are responsible for RTT. Fifteen different mutations in *MECP2* have been detected in 90% of patients with RTT and seven common mutations were

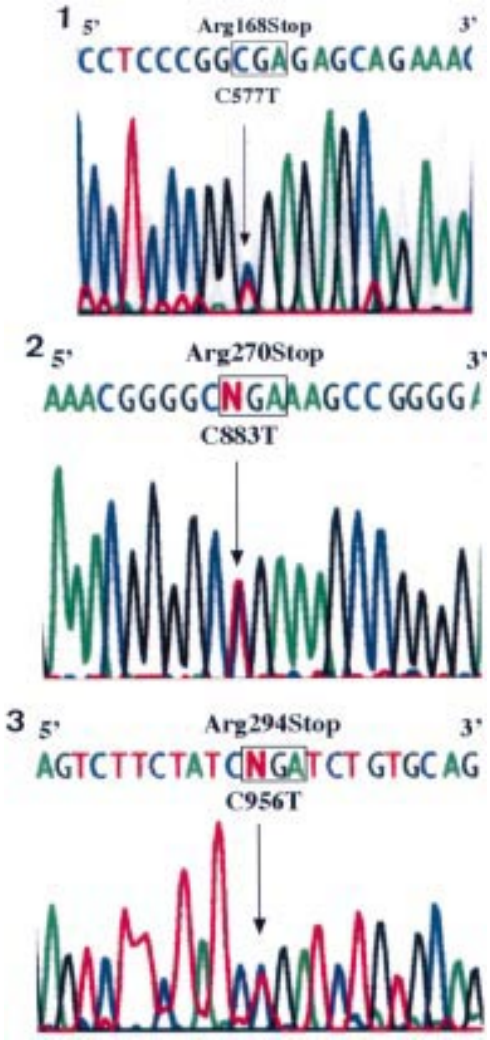

Figure 2 Nucleotide sequence of three common nonsense mutations of MECP2 in patients with RTT. (1) R168X mutation, (2) R270X mutation, (3) R294X mutation.

defined. Early diagnosis of RTT is now possible in patients with neurodevelopmental problems using DNA analysis of *MECP2*. Particular clinical symptoms were associated with DNA mutations in some patients with RTT and it may therefore be more heterogeneous than reported previously.

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Mutations in the *MECP2* gene in a cohort of girls with Rett syndrome

EDITOR—Rett syndrome is a severe, progressive, neurodevelopmental disorder which almost exclusively affects females. At first the affected girls appear to develop normally but after a year to 18 months they begin to deteriorate. Not only do they fail to progress but they lose skills already learnt until finally they have severe developmental delay with dementia and autistic behaviour, an apraxic gait, breathing dysfunction, and stereotyped hand movements, such as excessive hand wringing. Lost skills are not regained.[1] The disease, which affects ~1 in 10 000 girls, accounts for about 10% of profound handicap in females. More than 95% of cases are sporadic leading to the assumption that the syndrome must be the result of an X linked dominant gene with male lethality. Thomas[2] has also suggested that the lack of males with the syndrome could be accounted for by the increased rate of de novo germline mutations in males. This would imply that affected females arise as a consequence of de novo mutations in their fathers. Marked skewing of X inactivation has not been detected in sporadic cases of the syndrome either in affected girls or in their mothers,[3] but in one familial case the mother of three affected girls was found to have >95% skewing of inactivation in favour of the normal chromosome remaining preferentially active.[4] The few available familial cases allowed the gene to be mapped to Xq28[5 6] and in 1999 Amir *et al*[7] reported that mutations in the *MECP2* gene, located in Xq28, were associated with Rett syndrome in 5/21 of de novo cases. Amir *et al*[7] and Wan *et al*[8] reported a total of 10 mutations in the *MECP2* gene of which five were missense and five were nonsense mutations. Four out of the five missense mutations were located in the highly conserved methyl binding domain (MBD), the fifth being in the equally highly conserved transcription repression domain (TRD) of the gene. The nonsense mutations, causing truncation of the protein product, were located both within and between these two functional domains. Wan *et al*[8] also found that certain of the truncating mutations were hot spots, the R168X mutation in particular being detected seven times. Because of this, despite the identification of equal numbers of individual missense and nonsense mutations, out of a total of 18 mutations detected, 12 were found to lead to a truncated product. Several other incidences of multiple recurrence were also detected, indicating the presence of further hot spots which all involved C→T transitions at CpG dinucleotides.

We report a mutation analysis of the *MECP2* gene undertaken on a further 40 patients with Rett syndrome.

Table 1 Detection of mutations and variants in the MeCP2 gene in Rett syndrome patients

Mutation type	*Nationality*	*Domain*	*Nucleotide change*	*Amino acid change*	*Occurrence*	*Previous detection*
Missense	European	MBD	376C→G	P101R	1	No
	European	MBD	390C→T	R106W	1	Amir *et al*[7] Wan *et al*[8]
	European	MBD	547C→T	T158M	2	Amir *et al*[7]
	European	TRD	990C→T	R306C	1	Wan *et al*[8]
Protein truncation	European (2) Asian (1)		576C→T	R168X	3	Wan *et al*[8]
	European	TRD	837C→T	R255X	3	Amir *et al*[7] Wan *et al*[8]
	European (1)	TRD	882C→T	R270X	2	No
	European Asian (1)	TRD	954C→T	R294X	2	No
Variants	European		1263G→A	E397K	1	Wan *et al*[8]
	European		971C→T	T299T	1	
	European		22939ΔA	Intron	1	
	European		23668G→C	Intron	1	