SHORT REPORT

MECP2 gene mutation analysis in Chinese patients with Rett syndrome

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Rett syndrome (RTT) is a progressive neurodevelopmental disorder that affects almost exclusively girls. Mutations in the X-linked methyl-CpG-binding protein 2 gene (*MECP2*) have been found to be a cause. In order to study the spectrum of *MECP2* mutations in Chinese patients, we employed PCR and sequencing of the coding region of *MECP2* gene in 31 Chinese cases of classical sporadic RTT. Mutations in *MECP2* were found in about 55%. Twelve different mutations in exon 3 were identified in 17 of these 31 patients; two of these are novel. A novel missense variant was detected in the C-terminal region in a patient and her father who was normal. In addition, there was a single nucleotide variant in the 3'UTR.

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Introduction

Rett syndrome (RTT, MIM 312750) is a progressive neurodevelopmental disorder that affects girls almost exclusively, with an estimated prevalence of approximately 1 in 10 000–15 000 females.

Recently Amir *et al*¹ found that some cases of RTT were caused by mutations in the *MECP2* gene, located on chromosome Xq28. *MECP2* encodes the X-linked methyl-CpG-binding protein 2 (MeCP2), which is a global transcriptional repressor and abundant in the brain. MeCP2 specifically binds with its methyl-binding domain (MBD) to symmetrically positioned methylated CpG dinucleotides. Its transcriptional repression domain (TRD) recruits a co-repressor complex containing SIN3A and histone deacetylases and results in the repression of the target gene.² Decreased MeCP2 activity will lead to derepression of transcription at multiple CNS loci and overexpression of some genes that may be detrimental to brain development.

In this study, we report *MECP2* mutations in 31 Chinese RTT patients in order to study the spectrum of mutations in Chinese patients.

Materials and methods

Patients

Thirty-one sporadic cases of classical RTT have been followed up. They were found in nine provinces of China. All of the patients fulfilled the diagnostic criteria proposed by The Rett Syndrome Diagnostic Criteria Work Group.³

Genomic DNA was extracted using standard procedures from the peripheral blood leukocytes of patients.

PCR amplification and cloning

PCR was performed to amplify all the three exons of *MECP2* gene, using published primers and conditions¹ with some modifications. PCR products were purified and cloned into pGEM[®]-T vector (Promega). Four random clones of each fragment were selected. The plasmid DNA were extracted and purified.

Sequencing

The inserts in the plasmids were sequenced with the ABI 377 DNA sequencer (Perkin-Elmer, Applied Biosystems, CA, USA). Sequencing results were compared with reference

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to the normal human genomic *MECP2* sequence and were analysed using the CONSED software. When differences from the normal were found, purified PCR products from these patients were sequenced directly to confirm the original results.

Results

We found 12 different mutations in the *MECP2* gene in 17 of our 31 sporadic RTT patients (about 55%). The characterized mutations were all in the third exon and were heterozygous. There were four different missense mutations, three different nonsense mutations, five frameshift mutations (Table 1). Two of these are novel: 875insA in patient 25 (Figure 1A) and an adjacent double deletion of 26 bp in patient 9 (Figure 1B). In patient 5, a G→A transition at nt 21830 (GenBank accession No. AF030876) was detected. This variant in the 3'UTR has been reported previously.⁴ In patient 18, a C→G transversion at nucleotide 1141 leads to a novel Proline to Alanine substitution at residue 381 (P381A). Familial analysis in this case indicated that this P381A allele is a benign polymorphism, because the unaffected father carried the same variant.

Discussion

Codons 168, 255 and 294 are hot spots for truncating mutations. R168X is located upstream of but close to the TRD, but R255X and R294X are within the TRD (Figure 2). Patients 12 and 17, carrying R168X, died at 12 and 4 years of age respectively. Patient 17 was quite unusual. She started to show regression at 5 months of age and deteriorated rapidly, with death at 4 years of age. Patients 4, 8,

Figure 1 Nucleotide sequence of two novel mutations of the *MECP2* gene (by sequencing the PCR products). (A) patient 25 (antisense strand, nt875insT); nt 875insA. (B) Patient 9 (sense strand); 1158–1167/1171–1186 del (Because of the large size of the deletion, two different bands could be separated. We show the shorter band).

Table 1 Genotype and phenotype of patients carrying *MECP2* mutations

Mutation type	Patient No.	Nt. change(region)	Amino acid change	Age	Stage of disease*	Unusual features
Missense mutations	29	397C→T(MBD)	R133C	7 years	III	
	11	473C→T(MBD)	T158M	18 years	IV	
	19	473C→T(MBD)	T158M	8 years	III	
	23	473C→T(MBD)	T158M	7 years	III	
	27	473C→T(MBD)	T158M	5 years	III	
	16	674C→G(TRD)	P225R	9 years	III	
	7	916C→T(TRD)	R306C	death at 5 years		frequent seizures, some precipitated by fever
	13	916C→T(TRD)	R306C	11 years	IV	
Nonsense mutations	12	502C→T(CRIR)	R168X	death at 12 years		
	17	502C→T(CRIR)	R168X	death at 4 years		onset at 5 months
	4	763C→T(TRD)	R255X	18 years	IV	
	8	880C→T(TRD)	R294X	15 years	IV	
Frameshift mutations	22	806 delG(TRD)		3 years	II	
	25	875 insA(TRD)		6 years	III	
	26	1152del 44bp(CTS)		9 years	III	
	9	1158-1167/1171-1186del(CTS)		14 years	IV	normal head circumference, overweight
	31	1164del 44bp(CTS)		6 years	III	

*All patients presented with manifestations required by The Rett Syndrome Diagnostic Criteria Work Group.³ For diagnosis; CRIR: Co-repressor interacting region; CTS: C-terminal segment.

Figure 2 Distribution of mutations in the *MECP2* gene.

9, 22, 25, 26 and 31 carrying either truncating or frameshift mutations were in stage II, III or IV with relatively mild clinical symptoms (Table 1). Patient 9 was examined at 11 years of age. She was only mildly affected. Her head circumference was normal (54 cm) and she was overweight (60 kg, height 145 cm). So truncating mutations within or downstream of TRD or deletions in the C-terminal region are consistent with reduced clinical severity when compared with truncating mutations N-terminal to this domain.⁵

The large fragment deletions are within a hot spot for deletion or insertion. For the mutation 1152del 44 bp, both the donor and acceptor sites of the deletion are ACC. For the mutation 1164del 44 bp, they are GCCCCC. It is of interest to note that all the three large fragment deletions produce premature stop codon at the same position.

A missense variant (P381A) was detected in patient 18 and her unaffected father. Apparently it is non-pathogenic. The daughter's disease could be due to an allelic variant in the non-coding region of *MECP2* or a mutation in another gene. Hence, familial studies are important to distinguish polymorphisms from causative mutations.

In our study, direct sequencing of the PCR product frequently gave profiles with a number of background peaks which often interfered with the proper reading of the nucleotide sequence. Hence we first cloned the purified PCR product and sequenced the cloned fragment. However, since we only picked four random clones of each fragment for the initial sequencing, there is a possibility that the clones carried the normal fragment instead of the mutated

from the patient who is almost always a heterozygote. This could be the reason for our low mutation pick up rate.

Two novel mutations and a novel benign polymorphism were found. Most of the mutations found are located in mutation hot spots previously reported. It is apparent that recurrent mutations in the *MECP2* gene can occur globally. This will help in developing a rapid method of detection/screening of *MECP2* mutations. On the other hand, *MECP2* mutations had also been found in non-RTT X-linked mental retardation.⁶ This should be borne in mind in the differential diagnosis of mental retardation in females.

The pathogenesis of RTT remains unclear. Further studies should include more detailed correlation between genotype and phenotype, and the roles of *MECP2* gene and MeCP2 protein in neurodevelopment.

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