

***MECP2* and *CDKL5* gene mutation analysis in Chinese patients with Rett syndrome**

Mei-rong Li · Hong Pan · Xin-Hua Bao ·
Yu-Zhi Zhang · Xi-Ru Wu

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Abstract Rett syndrome (RTT) is a progressive neurodevelopmental disorder that is caused by mutations in the X-linked methyl-CpG-binding protein2 (*MECP2*) gene. In this study, the *MECP2* sequences in 121 unrelated Chinese patients with classical or atypical RTT were screened for deletions and mutations. In all, we identified 45 different *MECP2* mutations in 102 of these RTT patients. The p. T158M mutation (15.7%) was the most common, followed in order of frequency by p. R168X (11.8%), p. R133C (6.9%), p. R270X (6.9%), p. G269fs (6.9%), p. R255X (4.9%), and p. R306C (3.9%). In addition, we identified five novel *MECP2* mutations: three missense (p. K305E, p. V122M, p. A358T), one insertion (c.45-46ins-GGAGGA), and one 22 bp deletion (c.881-902del122). Large deletions represented 10.5% of all identified *MECP2* mutations. Conversely, mutations in exon 1 appeared to be rare (0.9%). The remaining cases without *MECP2* mutations were screened for the cyclin-dependent kinase-like 5 (*CDKL5*) gene using denaturing high-performance liquid chromatography (DHPLC). One synonymous mutation (p. I72I) was found in exon 5, suggesting that *CDKL5* is a rare cause of RTT. The overall *MECP2* mutation detection rate for this patient series was 84.3:87.9% in 107 classical

RTT cases and 57.1% in 14 atypical RTT cases. Moreover, there were two patients with homozygous mutations and normal female karyotypes. However, we did not pinpoint a significant relationship between genotype and phenotype in these cases.

Keywords Rett syndrome · *MECP2* · *CDKL5* · Mutation analysis · Recurrent mutations

Introduction

Rett syndrome (RTT, MIM 312750) is a progressive neurodevelopmental disorder that affects females almost exclusively, with an estimated prevalence of approximately 1 in 10,000–15,000 females. RTT is often considered an X-linked dominant condition with male lethality (Hagberg 1985) characterized by a progressive loss of intellectual function, fine gross motor skills, and communicative abilities; deceleration of head growth; and the development of stereotypic hand movements, all occurring after a period of normal development.

In 1999, Amir et al. (1999) determined that RTT is caused by mutations in the X-linked methyl-CpG-binding protein2 (*MECP2*) gene on chromosome Xq28. Prior to the identification of the *MECP2B* isoform, mutation detection efforts focused on using polymerase-chain-reaction (PCR)-based approaches to screen exons 2, 3, and 4. The recent identification of yet another *MeCP2* isoform, *MeCP2A*, has enhanced speculation that some individuals may have mutations in exon 1 (Mnatzakanian et al. 2004).

To date, mutations have been identified in the *MECP2* sequences of approximately 80% of all RTT

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M.-r. Li · H. Pan (✉) · X.-H. Bao · Y.-Z. Zhang ·
X.-R. Wu
Department of Pediatrics, Peking University First Hospital,
No.1 of Xi An Men Street, Xi Cheng District,
Beijing 100034, People's Republic of China
e-mail: panmuren@yahoo.com

patients; the remaining 20% may possess noncoding regions of this gene, or they may harbor a second RTT-inducing gene or locus. Several reports have identified large gene deletions in RTT patients that escaped detection by PCR-based screening strategies (Laccone et al. 2004; Schollen et al. 2003). Others have identified mutations in a novel *MeCP2* isoform and in *CDKL5* (Archer et al. 2006a, b; Evans et al. 2005a; Kriaucionis et al. 2004; Mari et al. 2005; Scala et al. 2005; Tao et al. 2004; Weaving et al. 2004). This study reports the results of the mutation analysis of *MECP2* and *CDKL5* in 121 unrelated Chinese patients with classic or atypical RTT, conducted to obtain a genotypic representation of the mutational spectrum in this population.

Materials and methods

Patients

A total of 121 sporadic RTT patient cases, consisting of 107 classic and 14 atypical patients, were involved in this study. The age of RTT onset ranged from 3–36 months, and the oldest patient living with RTT was 24 years of age. The cases, all female, were distributed across 27 Chinese provinces. All patients fulfilled the classic or atypical diagnostic criteria proposed by The Rett Syndrome Diagnostic Criteria Work Group (Hagberg et al. 2002) and in 14 atypical patients, six had preserved speech variant, two early onset seizures, one forme fruste, and five were unclassified. Informed parental consent was obtained for all patients.

Mutation detection

Genomic DNA was extracted using standard procedures from the peripheral blood leukocytes of patients with RTT (Miller et al. 1988).

DNA direct sequencing

The PCR method was used to amplify the four *MECP2* exons by using published primers (Amir et al. 1999; Mnatzakanian et al. 2004). If no mutations were identified after screening exons 2–4, then exon 1 was screened. The final volume of the PCR was 25 μ l, consisting of 50 ng of DNA, 0.005 mM of each primer pair, 2.5 mM dNTPs, 1.5 mM MgCl₂, 1 \times reaction buffer, and 1U Taq DNA polymerase (Promega or Invitrogen). To amplify exon 1, the 500 mM of betain and 50% dimethylsulfoxide were added to the PCR system, which was different with exons 2, 3, and 4. The PCR

conditions used were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52–62°C for 30 s, initial extension at 72°C for 30–60 s, and final extension at 72°C for 10 min. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). The purified products were then sequenced using a BigDye Terminator Cycle Sequencing Kit and an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). The sequencing primers were the same as those used for the PCR amplification. Mutation analyses were performed using the normal, non-RTT human genomic *MECP2* sequence as a reference (GenBank accession No. AF030876).

To confirm that alterations found in this work were novel mutations and not polymorphisms, 100 normal control alleles were examined directly using PCR-restriction fragment-length polymorphism (RFLP) techniques. If the mutation was not associated with a RFLP, then DNA sequencing or denaturing high-performance liquid chromatography (DHPLC) were conducted. All variations were verified by sequencing with the forward and reverse primers.

Multiplex ligation-dependent probe amplification

If no mutation was identified with PCR in the *MECP2* sequences of RTT patients, then we used multiplex ligation-dependent probe amplification (MLPA) kit P015C (MRC-Holland, Amsterdam, The Netherlands) to screen for large deletions. The MLPA reactions were performed following the instructions provided by the manufacturer. Finally, the PCR products were separated by capillary electrophoresis using an ABI 3100 automated sequencer and size standards (Perkin Elmer Applied Biosystems, Foster City, CA USA). We used Genescan analysis software (version 3.7) and Genotype software (version 3.6) to analyze these data, which we then exported to a Microsoft Excel spreadsheet. The resulting values were approximately 1.0 for every wild type peak, 0.5 for heterozygous deletions, and 1.5 for heterozygous duplications.

Long-range PCR and sequencing

The large deletions were verified using long-range PCR and direct sequencing methods. The PCR amplifications were performed, as described by Amir et al. (1999) with the following modifications. Exons 3 and 4 were amplified using a pair of primers, comprised of upstream (exon 3-1F: 5'-GTTCCCCCGACCCCA CCCT-3', which was localized within intron 2) and downstream (exon 4-4R: 5'-CTCCCTCCCCTCGGTG

TTTG-3') primers. This amplification generated a fragment of 2,171 bp. The PCR volume was 25 μ l with GC buffer I, 2.5 mM dNTP, 0.005 mM of each primer, 100 ng genomic DNA, and 2.5 U of TaKaRa LA Taq (TaKaRa, Tokyo, Japan). An initial denaturing step of 94°C for 2 min was performed, followed by 35 cycles at 94°C for 10 s and 68°C for 150 s. All reactions were terminated by a final elongation step at 72°C for 10 min. The PCR products were sequenced, as described above.

CDKL5 gene mutation screening by DHPLC

If no mutations were identified in the *MECP2* sequence isolated from the RTT patients using general PCR and MLPA methods, then the coding region of *CDKL5* was screened by DHPLC. The PCR amplifications were conducted, as described above. The melting temperatures (Tms) for all primer pairs and a full list of DHPLC-run temperatures are listed in Table 1. The DHPLC was performed on a WAVE Nucleic Acid Fragment Analysis System HSM (Transgenomic, Crewe, UK). The PCR products displaying abnormal chromatographic profiles on the DHPLC analysis were sequenced directly.

X-chromosome inactivation

The analysis of X-chromosome inactivation (XCI) was performed in the RTT patients, as well as the mothers of certain patients, as described and recommended by Allen et al. (1992). In brief, the lymphocyte genomic DNA was digested with the methylation-sensitive enzyme Hpa II prior to the PCR amplification of the polymorphic CAG repeat in the androgen receptor (AR) gene. The amplicons were sequenced with an ABI 3100 automated sequencer (Applied Biosystems), and the resulting sequences were analyzed with GeneScan analysis software. The peak areas were compared before and after the Hpa II digestions (data not shown).

Results

In total, 45 different *MECP2* mutations were identified in 102 of the 121 diagnosed sporadic female patients presenting with classical or atypical RTT (Table 2). One mutation was found in exon 5 of *CDKL5*. The p. A358T mutation was also observed in the tested maternal DNA. The other mutations were not found in the parents, which indicated that these mutations arose de novo.

MECP2 mutation detection by direct sequence analysis

Mutations in *MECP2* were detected in 87.9% (94/107) of the patients presenting with classic RTT and in 57.1% (8/14) of those with atypical RTT. These aberrations consisted of 71 point mutations, 17 microdeletions, 11 large deletions, 2 insertions, and 1 splicing defect. Most of the variants were missense mutations, accounting for 43.1% (44/102), followed in order of frequency by nonsense mutations 26.5% (27/102), and frame shift mutations 17.6% (18/102). The p.T158M (c.473C > T, 15.7%, 16/102) was the most common of the *MECP2* mutations, followed by p. R168X (c.502C > T, 11.8%, 12/102), p. R133C (c.397C > T, 6.9%, 7/102), p. R270X (c.808C > T, 6.9%, 7/102), c. 806delG (6.9%, 7/102), p. R255X (c.763C > T, 4.9%, 5/102), and p. R306C (c.916C > T, 3.9%, 4/102).

We also identified five novel mutations, three of which were point mutations. The first, a G > A transition at the cDNA position 364 (c.364G > A, Fig. 1a), lead to a novel methionine to valine substitution at residue 122 (p. V122M). The second novel mutation was an A > G transition at cDNA position 916 (c.916G > A, Fig. 1b), leading to a glutamate to lysine substitution at residue 305 (p. K305E). Moreover, this mutation eliminated a *Hae* II restriction site. The third novel mutation was a G > A transition at cDNA position 1076 (c.1076G > A, Fig. 1c), which caused a substitution of alanine with a threonine at position 358 and generated an *Hpy*188 III restriction site. Using RFLP analysis and these two enzymes, we tested the parental samples and the normal female controls to distinguish between the wild-type and mutant alleles. The first and second mutations were deemed de novo, as they were not detectable in the parents and 100 normal female controls. The p. A358T mutation was present in the patient's mother and absent in all 100 control alleles. A repeat GGAGGA insertion was detected at cDNA position 45–46 in exon 1 of *MECP2* (Fig. 1d). Additionally, one novel 22-bp microdeletion at cDNA position 881–902 in *MECP2* exon 4 resulted in a frameshift (Fig. 1e).

Notably, there were two RTT patients with mutations (p. P127L, p. T158M) in an apparently homozygous condition. The two mutations were not found in the parental samples.

MECP2 mutation detection by MLPA analysis and long-range PCR

The 27 RTT patients who did not exhibit any mutations in *MECP2* after the PCR screening were

Table 1 Primer sequences and conditions for polymerase chain reactions (PCR) reactions and denaturing high-performance liquid chromatography (DHPLC) analysis of cyclin-dependent kinase-like 5 (CDKL5) amplicons

Exon	Reverse primer		PCR annealing temperature (°C)	Product length (bp)	DHPLC analysis	
	Forward primer	Reverse primer			Temperature of elution (°C)	Buffer (%)
2	5'-TATTACTGTTGGTTTCTTCT-3'	5'-TAACTAACTGTTCATTGCTC-3'	50	420 bp	54.9	53.4
3	5'-TGTATGCGTGCCTTGATTTG-3'	5'-TGCCACACCGAAAGACCCAC-3'	57	157 bp	53.6	48.0
4	5'-ACACTCTGGCTTCTTGCTAC-3'	5'-CACTTCTCCACACTCTATG-3'	50	244 bp	51.8	49.8
5	5'-TTGGGATGTTTTCAGTGT-3	5'-ATAATGTGAGAAACAGCCTT-3	54	291 bp	56.6	52.5
6	5'-GAAAGTTGTAGATAGAAAGC-3'	5'-AATCTCATACTCACCTCG-3'	50	276 bp	54.6	54.6
7	5'-CAATCAGGAGAACATAGAAC-3'	5'-AAGAATGTTCTTCTACCA-3'	50	242 bp	49.7	51.4
8	5'-TAGCCCATGCGAGAACAG-3'	5'-CTCAAAGCAATGACATAG-3'	56	288 bp	55.8	53.8
9	5'-CTTGTGTTCTGATGTTTGC-3'	5'-TTCCATTTGGGTTTCAGCA-3'	55	387 bp	54.2	50.5
10	5'-GCTTATCTGCTCCCTTCTGC-3'	5'-TATTTGCCATTCACATCTC-3'	56	293 bp	55.5; 57.5	50.5
11	5'-TGATATCTGCAATGACTGT-3'	5'-CCACTCCTCCACTAC-3'	57	330 bp	52.5; 55.3	53.5; 51.5
12-1	5'-GAAAGGCACATGCTTGAGAGA-3'	5'-GGCTGCTTGCTGTAGG-3'	55	322 bp	55.1; 58.6	52.3; 50.3
12-2	5'-CTACCAAGCAAGCAGCCA-3'	5'-CAGAAAAGGTTGCTCACAGAC-3'	57	358 bp	58.4	54.2
12-3	5'-TCTCAGCAGAACCCGCACTC-3'	5'-CAGCTTCAATTCCTCCATCG-3'	61	401 bp	59.7	53.4
12-4	5'-AGACACAGTGACACGAGAAC-3'	5'-ATTTCAGTCAAAGGTGCTCAAC-3'	57	369 bp	60	53.5
13	5'-TAGGACACAGGATGCTCTTAT-3'	5'-ACTTCAACTTATTTGTGGGA-3'	52	248 bp	55; 58.4	51; 49
14	5'-CTGGGCAATAGATGAGACC-3'	5'-GACTTAGCAGGAGCAGAGGC-3'	58	351 bp	54; 60.3	56.8; 53
15	5'-CTTTGGGAGGCTCAGGC-3'	5'-AACAAACCAAAATGCTCACCC-3'	61	311 bp	56.9	52
16	5'-TTTACCCAAAGTTTIGATTTC-3'	5'-GCAAAGTGTAAAGTATCCAT-3'	52	303 bp	54.4	52.8
17	5'-CTCCTTTGGGTGTTGTTGC-3'	5'-AAGATTCACTGGGCTCACG-3'	57	373 bp	59	54.5
18	5'-CACCCAGTCACTCACCTCT-3'	5'-TTCCGTGAGCGATTTGTTCTG-3'	58	442 bp	59.9	57.8
19	5'-TCTGGTCAATGGGATGTGGG-3'	5'-CAGTGAGCCGAGATGTGTG-3'	61	330 bp	59.3	52.5
20	5'-CTGGTGTCTGGGAGCCGATG-3'	5'-CTGCCATTACAAATCACCTG-3'	60	416 bp	60.5	55.3
21	5'-CTCAACGGTGGAAAGACAG-3'	5'-AGGAAACTCAACCTCAGCG-3'	60	393 bp	61	52.9

Table 2 Summary of all X-linked methyl-CpG-binding protein2 (*MECP2*) mutations identified in the Chinese Rett syndrome (RTT) patient cohort

Mutations	Location	Domain	Nucleotide changer (c.)	Aa change (p.)	Number	
Missense	Exon 3	MBD	316C > T	R106W	2	
	Exon 3	MBD	317 G > A	R106Q	1	
	Exon 3	MBD	364G > A	V122M ^a	1	
	Exon 4	MBD	380C > T	P127L	1	
	Exon 4	MBD	358G > T	G129V	1	
	Exon 4	MBD	397C > T	R133C	7	
	Exon 4	MBD	3106G > A	R133H	2	
	Exon 4	MBD	401C > G	S134C	1	
	Exon 4	MBD	423C > G	Y141X	1	
	Exon 4	MBD	455C > G	P152R	2	
	Exon 4	MBD	473C > T	T158M	16	
	Exon 4	TRD	674C > G	P225R	1	
	Exon 4	TRD	916 G > A	K305E ^a	1	
	Exon 4	TRD	916C > T	R306C	4	
	Exon 4		965C > T	P322L	1	
	Exon 4	CTS	1005G > A	R335C	1	
	Exon 4	CTS	1076G > A	A358T ^a	1	
	Nosense	Exon 4	CRIR	502C > T	R168X	12
		Exon 4	NLS	763C > T	R255X	5
		Exon 4	NLS	808C > T	R270X	7
Exon 4		TRD	880C > T	R294X	3	
Splicing	Intron 3		IVS3-2A > T		1	
Deletions	Exon 1	NTS	45_46insGGAGGA ^a		1	
Insertions	Exon 3	NTS	119delAG		1	
	Exon 4	TRD	705delG		1	
	Exon 4	NLS	806delG		7	
	Exon 4	TRD	881_902del22 ^a		1	
	Exon 4	TRD	875insA		1	
	Exon 4	CTS	1005delG		1	
	Exon 4	CTS	1151-1183del33		1 ^c	
	Exon 4	CTS	1127_1179del53		1	
	Exon 4	CTS	1152_1195del44		1	
	Exon 4	CTS	1155_1210del26		1	
	Exon 4	CTS	1157_1197del41		1	
	Exon 4	CTS	1164_1207del44		1	
	Large deletion	Exon 1-2				1
Exon 3-4.1					1	
Exon 3-4.2					2	
Exon 3-4.4					3	
Exon 4.1			905_1168del234insCAC		1	
Exon 4.1-4.2			1047_11106del152		1	
Exon 4.1-4.2					1	
Exon 4.2-4.4					1	

Nucleotides were numbered from the first base of the translation initiation ATG codon of *MECP2A* (GenBank No. AF030876), with the exception of exon 1 *MBD* methyl-CpG binding domain, *TRD* transcription repression domain, *CRIR* corepressor interaction region, *CTS* C-terminal segment, *NLS* nuclear localization signal, *NTS* N-terminal segment

^a Novel mutation

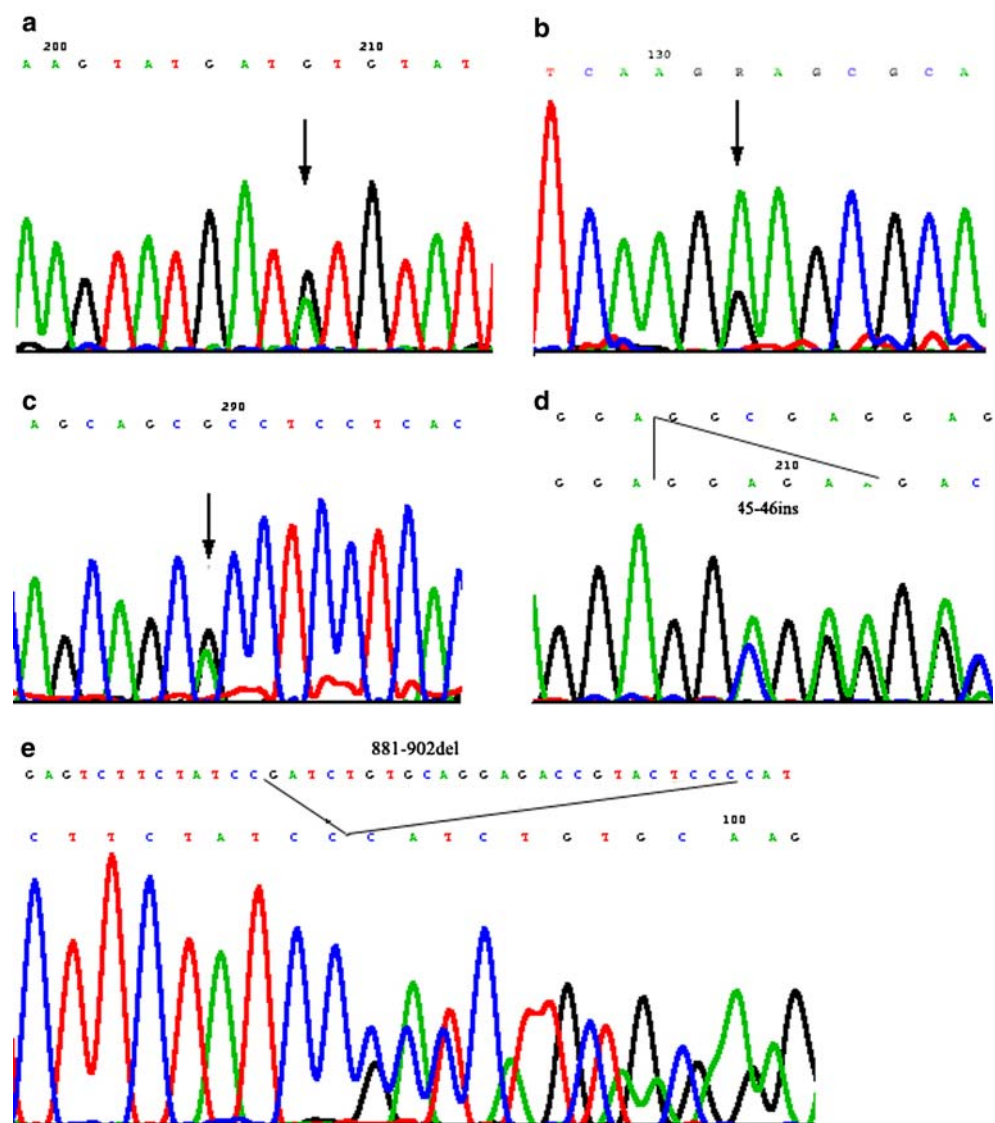
examined with MLPA. Using this alternate method, we identified large single-exon and multi-exon deletions in 9.1% (11/121) of all *MECP2* mutations. More than 90% of these deletions involved exons 3 and/or 4. Seven of the 11 large deletions involved two exons: six in exons 3 and 4, and one in exons 1 and 2 of *MECP2*. Four deletions involved only part of exon 4. In one case, the flanking *IRAK1* gene was deleted along with exons 3 and 4 of *MECP2* (Pan et al. 2006, submitted). The breakpoints were identified in two large deletions in exon 4 using long-range PCR followed by direct sequencing of the amplicons. In one case, the upstream breakpoint was located at cDNA position 905 whereas the downstream breakpoint was located at cDNA po-

sition 1,168, resulting in the deletion of 234 bp. In the other case, one of the breakpoints was located within the deletion-prone region (DPR) whereas the downstream breakpoint was located at cDNA position 1,047, resulting in a deletion of 152 bp.

CDKL5 mutation detection by DHPLC analysis

In the remaining 16 RTT patients without *MECP2* mutations, a synonymous mutation (p. I72I) was detected in exon 5 of *CDKL5*. We were unable to sample and test the parental DNA, but we did not detect this mutation in 100 of the normal controls, as determined with DHPLC.

Fig. 1a–e Nucleotide sequences of five novel mutations of X-linked methyl-CpG-binding protein2 (*MECP2*) gene in patients with Rett syndrome (RTT). The *blackened arrow* indicates the affected nucleotide. Deleted or inserted bases are indicated above. **a** c.364G > A (p.V122M); **b** c.916G > A (p.K305E); **c** c.1076G > A (p.A358T); **d** c.45_46insGGAGGA (ATG from exon1); **e** c.881-902del22



Discussion

MECP2 mutation detection

Mutations in *MECP2* as causative factors for RTT have been reported worldwide, for example, in Japan, the United States, and France (Amano et al. 2000; Amir et al. 1999; Philippe et al. 2006). The mutations are distributed along the entire *MECP2* gene and comprise all mutation categories. Mutation distribution may be considered tripartite. Missense mutations are clustered prevalently in the methyl-CpG-binding domain (MBD). Nonsense mutations are more scattered but tend to be located between the MBD and transcription repression domain (TRD) or within the TRD. This preferential location may reflect the functional importance of the domains or might be the conse-

quence of a marked sequence-specific hypermutability region (Miltenberger-Miltenyi and Laccone 2003).

The study presented herein represents mutation data from a Chinese population consisting of 121 sporadic RTT patients, 107 and 14 of whom presented with classic or atypical RTT, respectively. Mutations were detected in 87.9% (94/107) of patients with classic RTT, which is consistent with the estimated reports. Roughly 57.1% (8/14) of the patients with atypical RTT possessed mutations in their *MECP2* sequences. Recent reports described a range of 65–79% for the mutation detection rate among patients with atypical RTT (Smeets et al. 2003, 2005). Kammoun et al. recommended selecting well-defined clinical criteria while searching for *MECP2* mutations, as all individual variables did not exhibit similar weights (Kammoun et al. 2004). Thus, we hypothesized that the different

mutation frequencies of atypical RTT were correlated with different clinical information about atypical RTT. The total mutation frequency among all RTT patients was 84.3% (102/121).

In our study, we determined that 88/121 (72.7%) of the Chinese RTT alleles were mutated in exon 4 (c.378_1461 of *MECP2A*), which encodes for a portion of MBD and TRD. A few mutations (occurring in five cases) were located in exon 3 (c.27_377) whereas no mutation was identified in exon 2 (c.1_26). Mutations located in *MECP2B* exon 1 are rare, as they represent just 0.9% of all *MECP2* mutations. We report here five novel *MECP2* mutations: one in exons 1 and 3 and three in exon 4. The most frequent point mutations were determined to be missense (41.1%), which tended to cluster in the MBD (aa 90–174, 35/43, 81.4%) and in the TRD (aa 219–322, 6/43, 14.0%). Nonsense mutations represented 26.7% of all examined *MECP2* mutations and tended to cluster in the MBD and TRD. Nearly half (12/27, 44.4%) of the nonsense mutations deleted a portion of the nuclear localization signal (NLS) (aa 267–283) or the MBD and are not likely to encode an actively transported nuclear protein, and they may reduce the DNA binding capacity, respectively. The seven most frequently observed and reported *MECP2* mutations represent 56.9% (58/102) of all of the identified mutations in our study. Moreover, in some reports, the most common mutation is p. R168X (Cheadle et al. 2000; Dragich et al. 2000; Wan et al. 1999). However, the p. T158M was a predominant finding herein, which is consistent with our previous report (Pan et al. 2002) and with reports on cohorts of American (Buyse et al. 2000), British-Italian (Vacca et al. 2001), Japanese (Fukuda et al. 2005), and Korean descent (Chae et al. 2002).

The DPR, which represents a highly repetitive region in *MECP2* exon 4, is located in the region that is 3' to the TRD, where many intragenic deletion breakpoints occur (C-terminal deletions) (Laccone et al. 2004). In our study, 16 different or complex deletions were located in the DPR, representing 11.0% (16/121) of the *MECP2* mutations. These deletions were similar to those recently isolated by Laccone et al. (2004). Recombination between the DPR and a second highly repetitive region located in intron 2 may be responsible for mediating the commonly identified large deletions encompassing exons 3 and 4. It is also likely that there are recombinogenic repetitive elements in the 3'UTR that give rise to the large intragenic deletions found in exon 4 (Archer et al. 2006a, b). One previous report suggested that truncated MeCP2 proteins may degrade faster in vivo, which would contribute to their loss of function (Yusufzai and

Wolffe 2000). The other report (Chandler et al. 1999), however, showed that the deletion of the 63 amino acids at the C-terminus of MeCP2 impairs its DNA binding capacity during the transcription regulation process. Each patient in 11 RTT cases presenting with large *MECP2* deletions were missing one or two exons. The four patients who had deletions in exon 4 did not experience the characteristic seizures and scoliosis whereas the six patients with deletions in exons 3 and 4 endured seizures, complete loss of hand function, no language ability, and accompanying scoliosis (Pan et al. 2006, submitted). One previous study suggested that RTT phenotype is more likely to be complete as the child grows up (Kammoun et al. 2004). In our study, the majority of patients were younger than 10 years. Thus, we intend to conduct follow-up analyses on these patients to augment our sample population. To our knowledge, only six cases, including those in our study, presented with deletions in exons 1 and/or 2 (Archer et al. 2006a, b; Erlandson et al. 2003). Therefore, exon 1 deletions or mutations are rare.

One patient had a novel *MECP2* missense mutation (c.1076G > A, p. A358T) that was inherited maternally, although the mother possessed a normal phenotype. The XCI demonstrated a preferential inactivation of the paternal allele, with a frequency of 82%. The maternal allele XCI analysis yielded no information (Fig. 2). It is possible that the mother had gonadal mosaicisms or she was spared from the disease by a favorable X-inactivation event, as reported previously (Amir et al. 1999; Wan et al. 1999). We also found a 6-bp insertion in the [GGA]₅ trinucleotide repeats in exon 1 of the patients who did not have mutations in exons 2, 3, or 4 of *MECP2*, thus the patient has a classic manifestation. This patient lost her speaking ability at 18 months, had stereotypical hand movements, and did not feed herself after the onset of developmental delay. We suggested that a 45–46ins-GGAGGA was a potential pathogenic mutation. Taken together, these findings, including our 16 cases with the earlier published finding from exon 1, revealed that nine exon 1 mutations have been detected among 245 mutation-negative RTT patients (Amir et al. 2005; Bartholdi et al. 2006; Evans et al. 2005b; Laccone 2004; Ravn et al. 2005; Saxena et al. 2006). At a practical level, the prevalence of exon 1 mutations estimated is 3.7% among mutation-negative RTT cases whereas the overall contribution of exon 1 mutation to the occurrence of RTT is less than 1%. Our findings confirm the emerging consensus that mutations in exon 1 are responsible for only a minority of RTT cases.

A majority of mutations identified herein were heterozygous; however, two that we identified were

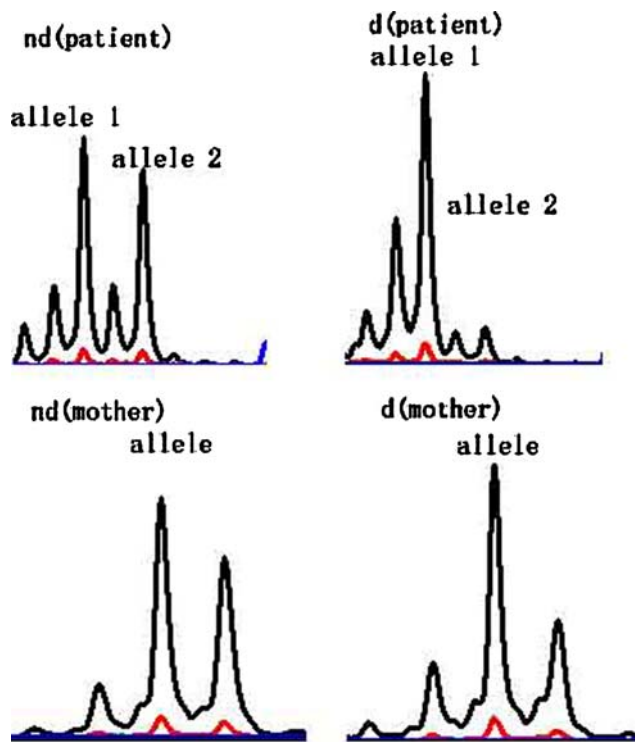


Fig. 2 A358T X-inactivation pattern of the patient and her mother. The *upper* automated sequencer traces show two alleles of the androgen receptor of the patient; the *lower* automated sequencer traces show the alleles of the androgen receptor of the patient's mother; the *left* is the trace of undigested by restriction enzymes HpaII, the *right* is the trace of digested by HpaII

homozygous. p. P322L and p. T158M (patients 119 and 120, respectively) were not present in their parents, which implicated the existence of one of these deletions on the parental alleles. One other study also identified this condition (Schollen et al. 2003). It is recommended that future *MECP2* mutation screenings attempt to avoid overlooking similar mutations in RTT patients. Nearly 77.7% (94/121) of the classic and atypical RTT individuals showed changes within the coding region of exon 4. This finding suggests that an initial analysis of exon 4 would provide the most efficient approach in a mutation protocol.

CDKL5 mutation analysis

A small handful of mutations in *CDKL5* has been identified recently in patients with an early seizure phenotype of atypical RTT (Archer et al. 2006a, b; Evans et al. 2005a; Mari et al. 2005; Scala et al. 2005; Tao et al. 2004; Weaving et al. 2004). To date, 15 different *CDKL5* mutations have been identified in 15 atypical RTT patients with early onset seizure disorder. In an attempt to identify the mutations in the

remaining 16 RTT patients without a defined *MECP2* alteration, we used DHPLC to screen the coding region of *CDKL5*. From these screenings, we identified one case with a synonymous mutation (c.216T > A, p. I72I) in exon 5. Unfortunately, the parental samples were not available for analysis; however, we did not detect the mutation in 100 of the normal control alleles. This finding suggests that this mutation is related to the RTT. This patient manifested an atypical RTT, could speak a single word at 8 months, lost her speaking capability at 12 months, and then experienced spasms. She presented with stereotypical hand rubbing and did not get scoop accompanied with grind. Presently, she can walk, has regained some hand function, but is autistic.

In conclusion, mutations in exon 4 of *MECP2* appear to be the most frequent among those found in Chinese RTT patients. The mutation in *MECP2* exon 1 and in *CDKL5* appears to be rare. Our data suggest that escape from PCR detection is a fairly common event, especially among the larger deletions, and that routine mutation screening in *MECP2* should include quantitative analysis of the *MECP2*, preferably with MLPA. We have found that MLPA, as a complement to DNA sequencing, is a useful tool for detecting whole-exon deletions.

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