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RESEARCH ARTICLE

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# Familial cases and male cases with *MECP2* mutations

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This is the first report of Chinese familial cases with Rett syndrome (RTT) or X-linked mental retardation (XLMR). RTT is a neurodevelopmental disorder that almost exclusively affects females. Most RTT cases are sporadic. We have studied eight cases with *MECP2* mutations in six Chinese families, including three females and five males with RTT or XLMR. All shared identical *MECP2* mutations with their mothers. The three females fulfilled the diagnostic criteria for RTT, while the five males were XLMR. A random X-chromosome inactive (XCI) pattern was seen in all the three female patients and two mothers while a skewed XCI in the rest four mothers. The clinical manifestations and pathogenic gene spectrum between male and female patients were different. The different *MECP2* mutations and different XCI pattern may be the determinants of the phenotypic heterogeneity between the family members.

**KEYWORDS**
clinical manifestations, pathogenic gene spectrum, phenotypic heterogeneity, Rett syndrome, X-linked mental retardation

## 1 | INTRODUCTION

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder, and one of the most common causes of mental retardation in girls. Usually the sufferers initially grow normally before the age of 6–18 months, followed by mental and physical regression, including loss of acquired speech ability and the skillful dexterity of the hands (Francesco et al., 2014). More than 99% of RTT cases are sporadic while the family cases are rare.

Mutations of the gene encoding methyl-CpG-binding protein 2 (*MeCP2*), located at Xq28, are responsible for causing most cases of RTT (Amir et al., 1999). *MeCP2* is a key protein in the brain, acting as a transcriptional repressor and an activator for genes associated with normal nerve cell function (Chahrour et al., 2008). Interestingly, either deficiency or overexpression of *MeCP2* results in X-linked mental retardation (XLMR). Currently, more than 500 mutations in *MECP2* have been identified (RettBasewww.chw.edu.au). *MECP2* mutations cause a wide spectrum of phenotypes, including classical RTT and RTT-variants such as congenital form, early-onset seizure type to preserved speech variant (PSV), and the forms frustes (FF); and other kinds of intellectual disabilities, such as autism, nonspecific XLMR, schizophrenia, Fragile-X-like Syndrome (FXS), learning disability, and Angelman-like syndrome (Gomot et al., 2003; Zoghbi, 2005). However, the elaborate pathogenic mechanism of *MECP2* leading to RTT phenotype remains unclear (Chahrour & Zoghbi, 2007; Williamson & Christodoulou, 2006; Zhang, Bao, Zhang, et al., 2012).

An X-linked dominant mutation mode of RTT was previously thought to be lethal in hemizygous male during the early gestational time (Franco & Ballabio, 2006).However, this hypothesis has been challenged. An observation of two boys with Rett-like phenotype and stereotypic hand movements with mental and developmental regression suggested that male RTT may survive (Schanen et al., 1998). Laurent V reported the frequency of *MECP2* mutations causing mental retardation in males was estimated between 1.3% and 1.7% (Villard, 2007). *MECP2* mutations in females often cause classical or variant phenotypes of RTT, while in males more severe phenotypes, including moderate to severe MR and congenital encephalopathy or early dearth, occur (Hitchins et al., 2004).

Familial cases, particularly males, with *MECP2* mutations are rare. To our knowledge, anecdotal reports over the past decades accumulated only approximately 28 reports in literature (Augenstein, Lane, Horton, Schanen, & Percy, 2009; Couvert et al., 2001; Dayer et al., 2007; Evans, Archer, Whatley, & Clarke, 2006; Kankirawatana et al., 2006; Khajuria et al., 2012; Klauck et al., 2002; Lambert et al., 2016; Meloni et al., 2000; Moog et al., 2006; Orrico et al., 2000; Ravn et al., 2011; Villard, 2007; Yntema et al., 2002). In this paper, we report Chinese familial cases of eight children and their six mothers with *MECP2* mutations, and address their clinical and molecular characteristics and its possibly underlying genetic mechanisms.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

The parents and their children with either RTT or XLMR cases were recruited from January 2001 to December 2015. A questionnaire including gender, age, clinical manifestation, and family history, was filled by the parents after signing an informed consent. Electroencephalogram (EEG), magnetic resonance imaging (MRI) were reviewed and recorded from the charts. The study was approved by Clinical Research Ethics Committee, Peking University. Informed consent was obtained from parents.

### 2.2 | *MECP2* mutational analysis

Genomic DNA was extracted from the peripheral blood leukocytes of patients with RTT or XLMR and their parents (Miller, Dykes, & Polesky, 1988). *MECP2* gene was sequenced as previously described (Amir et al., 1999). Evaluations on large deletions and duplications were performed using the MLPA-P015 probe (SALSA MLPA kitP015 *MECP2*, MRC-Holland, Amsterdam, Holland). The obtained *MECP2* gene products were separated and analyzed using the ABI Prism 3100 Genetic Analyzer and Gene scan soft according to the manufacturer's instructions. We also exclude the possibility of mosaic mutation in all these cases by digital PCR and amplicon resequencing on personal genome machine (PGM) (Huang et al., 2014).

### 2.3 | X chromosome inactivation patterns (XCI) evaluations

The patterns of XCI were analyzed in all *MECP2* mutated females including the female RTT patients and their mothers using the X-linked androgen receptor (AR) locus (Allen, Zoghbi, Moseley, Rosenblatt, & Belmont, 1992). XCI was considered extremely skewed if the ratio was ≥80:20.

## 3 | RESULTS

### 3.1 | Familial cases and *MECP2* mutations

Four hundred and twenty nine children with either RTT or XLMR and their parents were recruited from 427 Chinese families. Of them, eight children, three girls, and five boys, in six families were found to have *MECP2* mutations (Figure 1). Five families had missense mutations and the remaining one had two micro-deletions (Table 1). The *MECP2* mutations were c.397C > T (p.R133C) in family A and C, c.916C > T (p.R306C) in family B, c.1164-1207 del 44bp and c.1225-1227 del AGC (p.P389X) in family D, c.1409G > A (p.R470H) in Family E, and c.441C > G (p.D147E) in family F. All the *MECP2* mutations were identified inherited from their mothers as no mutation was detected in their fathers. The *MECP2* gene analysis was also performed on a maternal grandmother (I:1) and a maternal aunt (II:2) in a family (family C, Figure 1). Neither the grandmother nor the aunt had an *MECP2* mutation. None of mosaic mutations was detected in all these familial cases.

### 3.2 | XCI of the female patients and their mothers

XCI studies showed that four mothers had skewed XCI, while all the three female patients and two mothers (Family C, II:1 and Family E, I:1) had random XCI (Table 1).

### 3.3 | Medical history

#### 3.3.1 | Family A

In family A (Figure 1A), the mother (family A, I:1), her daughter (family A, II:1), and her son (family A, II:2) had identical *MECP2* mutation c.397C > T (p.R133C).

II:1, the proband, an 8 years 8 months old girl, was the first product of the non-consanguineous Chinese parents. She was born after an uneventful pregnancy. She was referred to us at the age of 3 years 7 months because of developmental delay. Her head circumference was 49 cm. Reportedly, she was able to sit independently at age of 8 months, speak simple single words, such as "mom and dad" at 12 months, and walk at 18 months. She could recite some simple ancient Tang poetries in the guidance of her parents at age of 2 years but, then, she developed stereotypic hand movements and marked teeth grinding and lost her ability to use her fingers to pick up objects and transfer them from one hand to another. Language development also slowed down. She had difficulty in language arrangement logically. Poor eye-contact and no-interest in holding things were noticed before age of 6 months. However, she had no respiratory irregularity, and no kyphosis. EEG at 3 years 2 months showed focal spike and slow waves discharged in the left anterior and posterior temporal. Brain CT and MRI were unremarkable. Her clinical manifestations met the diagnostic criteria for PSV.

II-2, a 2 years old boy, is the younger brother of II–1. He was developmentally normal until 6 months of age. His developmental arrest was realized at 1 year when he could not sit steadily. Clapping hands was observed at 18 months. Stereotypic hand wringing started at age 20 months. He could hold things in hands but unstably with jitter-like movement. He was referred to us at 2 years old when his head circumference was measured 46.5 cm. He could only speak sparse simple words, such as "mom and dad." However, his eye-contact was good and he clung to his mother. He could not walk due to severe whole body tremor but no seizures. Brain CT and MRI scans were normal. Because he had no autistics symptoms but the same

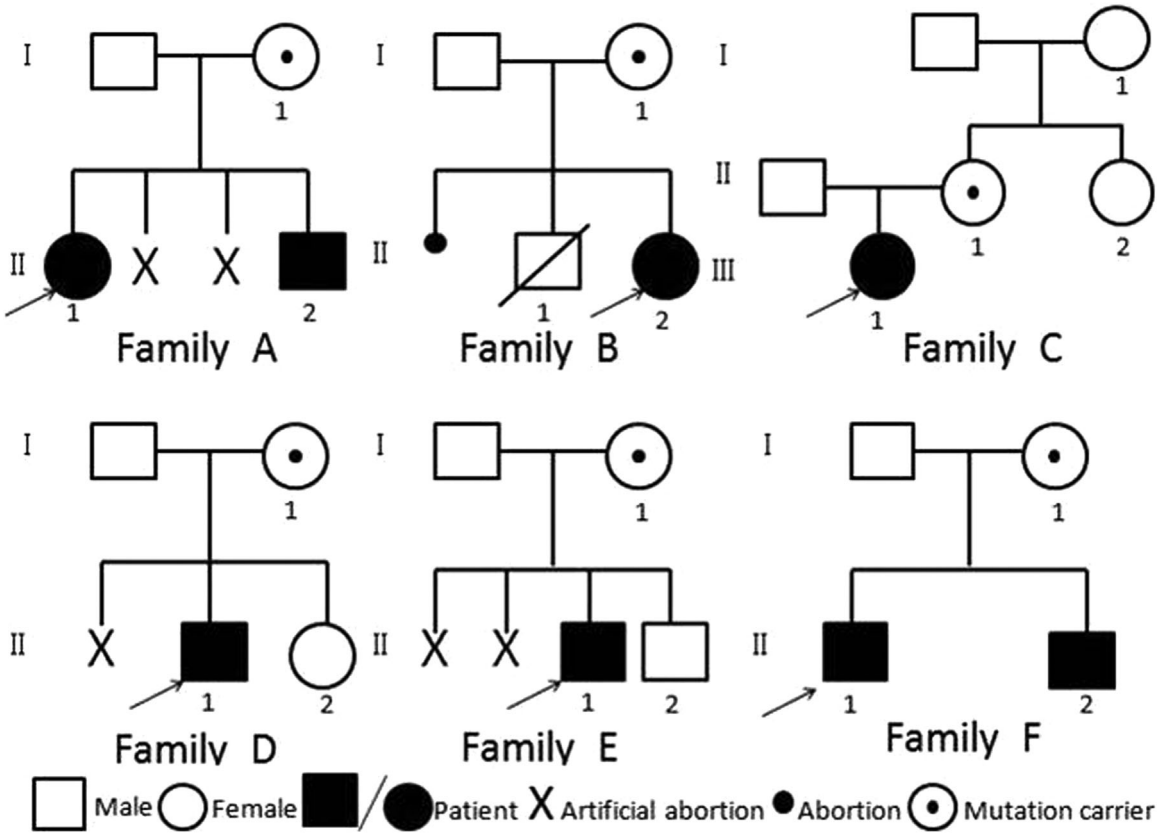


**FIGURE 1** Pedigrees of the families

mutation in *MECP2* as his sister and mother, he did not meet the diagnostic criteria for RTT but XLMR.

I:1 was the mother of the two patients (I:1 and I:2). Though she carried the same *MECP2* mutation as her children, she did not have any symptoms. Her husband was mentally normal and physically healthy.

### 3.3.2 | Family B

In family B (Figure 1B), the mother (family B, I:1) and her daughter (family B, II:2) had an identical *MECP2* mutation at c.916C > T (p.R306C).

II:2, the proband was a 5 years 10 months old girl and her head circumference was 46 cm at the first visit. The pregnancy and delivery of the girl were reportedly uneventful. Her delayed psychomotor development was noticed as she was not to sit independently until the age of 8 months and walkable at 2 years. She started speaking simple words, sounds as "mama and dada" at 8 months. She expressed her demand with one or two words, for example, "want; no; eating; and drinking" at age of 2 years. After that time, her language skill stagnated. She had poor eye contact and clumsy hand functions. She could hold things at age 7 months, transfer at 10 months, but just held them for not more than seconds before dropping. Her dexterity was sluggish. Picking up things with fingers was a challenging task for her. Stereotypic hand movements and teeth grinding were noticed at age of 1 year. Respiratory irregularity, such as holding breath, was noticed at 2 years. She had no kyphosis or seizure. Brain CT and MRI scans were normal. Findings fulfilled the diagnostic criteria for atypical RTT of PSV.

II:1, a boy, was noticed to have severe psychomotor developmental delay after his birth. He could not raise his head at age 12 months. He died of a convulsion and severe pulmonary infection at age of 2 years and 6 months. Brain CT and MRI were reportedly abnormal but the detailed reading unknown. No study on his DNA for *MECP2* was performed.

**TABLE 1** The *MECP2* mutations and X chromosome inactive patterns in familial cases

| | Family A | Family B | Family C | Family D | Family E | Family F |
|---|---|---|---|---|---|---|
| Mutation | c.397C > T | c.916C > T | c.397C > T | c.1164-1207 del 44bp, c.1225-1227 del AGC | c.1409G > A | c.441C > G |
| AA change | p.R133C | p.R306C | p.R133C | p.P389X | p.R470H | p.D147E |
| XCI of mothers | 86:14 | 80:20 | 53:47 | 78:22 | 44:56 | 80:20 |
| XCI of daughters | 59:41 | 54:46 | 55:45 | –[a] | –[a] | –[a] |

AA: amino acid.
[a]No female patients in these families.

I:1, the proband's mother, had mild MR. Her IQ was 75. She completed study from a junior high school. She was currently employed as an assembly line worker in a local textile factory. She had almost no communication with her peers or strangers, only a little communication with her family members. She could write simple sentences, read magazines, and perform simple arithmetic. She was poor in understanding complicated issues and had difficulty in learning new skills. She had no financial ability to manage her house hold expenditure. She married with non-consanguineous healthy man and had three pregnancies. The first pregnancy was miscarried at 14 gestational weeks with unknown cause. The second one was II:1, a boy and the third one was II:2, the proband. She had a sister and a brother; both and their children were mentally normal and healthy. She has mild mental retardation but did not meet the diagnostic criteria of RTT.

### 3.3.3 | Family C

In Family C (Figure 1C), the proband (Family C, III:1) and her mother (family C, II:1) had the same *MECP2* mutation c.397C > T (p.R133C). The maternal grandmother (family C, I: 1) and the maternal aunt (family C, II:2) had no mutation.

III:1, the proband was a 7 years old girl who presented at age of 43 months with head circumference 47.5 cm. She could grasp things since 7 months but just for seconds. She could sit steadily at age of 8 months, say simple words, such as "mom and dad" at 1 year, and walk independently at 18 months. Stereotypic hand movements appeared at the age of 1 year. She became unable to speak at 2 years and 1 month. Seizures began at age 4 and half years. She had poor eye contact and limited hand-use. She had no respiratory irregularity, teeth grinding, or kyphosis. EEG showed sharp and spike wave in bilateral central, parietal, and temporal areas. Brain CT and MRI were normal. She fulfilled the diagnostic criteria of RTT.

II:1, was the proband's mother, who also carried the *MECP2* mutation. She was noticed abnormal at 5 years old of difficulty in learning. She could only understand simple sentences. She always bit her nails when she was free. As an adult, she could take care of herself in daily living, ride a bicycle, and do housework though in tardiness. She had few hobbies including sleeping. She had some autistic behavior with no friends. She was slightly overweighed (70 kg, height of 153 cm, BMI 29.9). She was currently employed as housekeeping in a factory. She married with a non-consanguineous healthy man.

### 3.3.4 | Family D

In family D (Figure 1D), the mother (family D, I:1) and her son, the proband (family D,II:1) had *MECP2* mutation c.1164–1207 del 44bp and c.1225–1227 del AGC (p.P389X).

II:1, the proband was a 4 years 9 months old boy. His mother had threatened abortion symptoms during the first trimester of the pregnancy. The boy was born with polyhydramnios and amniotic fluid aspiration. Apgar score at 5 min was 7. He could raise his head up steadily at 3 months, sit unaided at 6 months, and speak "mom and dad" at 1 year. However, developmental regression was noticed at 1 year and 2 months. He began to speak less and less since then. He could walk only 1–2 meters at 2 years. At the age of 3 years and 5 months, he began to have seizures frequently manifesting as severe trembling and epileptic convulsions. EEG showed frequent spike-waves in bilateral Rolandic areas. The discharge index in NREM stage was about 80%. He partially responded to antiepileptic medication treatment (valproic acid and levetiracetam) as his epileptic convulsions reduced but trembling worsened. Eventually he lost his ability to stand and speak completely. His head circumference was 48 cm and was not increased in the following 2 years of follow-ups. Brain CT scan was normal. He did not fulfill the diagnostic criteria for RTT, though he had some RTT-like features such as stereotypic hand movements. He was diagnosed as XLMR.

I:1, the mother of the proband was an asymptomatic *MECP2* mutation carrier. She had three pregnancies. The first pregnancy ended by a selected abortion at one gestational month. The second pregnancy was the proband, and the third one a healthy girl.

### 3.3.5 | Family E

In family E (Figure 1 E), the mother (family E, I:1) and one son (family E, II:1) had an identical *MECP2* mutation c.1409G > A (p.R470H).

II:1, a 4 years 9 months old boy was the first child of the healthy parents. He could sit independently at 6 months, speak several simple words, and walk independently at age 1 year and 4 months. Language regression started at 2 years and he could only speak two words, no long sentences at the visit. His hand function remained relatively good. He could feed himself with a spoon and drink with a cup. He gradually developed stereotypic hand movements, such as clapping and sucking fingers at age 4 years and avoided eye contact and could not walk steadily. He had respiratory irregularity and only slept about 5 hr per day. He had no seizure. He was able to follow simple instructions, but unable to understand long sentences. Brain CT and MRI were normal. He did not fulfill the diagnostic criteria for RTT, though he had some RTT-like features. He was diagnosed as XLMR.

I:1 was the proband's mother. She was an asymptomatic *MECP2* mutation carrier. She had four pregnancies. The first two were aborted due to her personal choice. II:2 was a healthy boy.

### 3.3.6 | Family F

In family F (Figure 1F), the mother (family F, I: 1) and two sons (family F, II:1 and family F, II:2) all had an identical *MECP2* mutation c.441C > G (p.D147E).

II:2, the proband was a 2 years 8 months old boy with head circumference of 48 cm. He could raise his head up at age 3 months, sit unsupported at 6 months, and walk with aid at 1 year, but psychomotor regression followed. He could not sit steadily at 18 months. Swallowing problem appeared gradually. He had severe constipation. No respiratory irregularity, teeth grinding, stereotypic hand movements, kyphosis, or seizures manifested. He never obtained any language. Brain MRI showed cerebellar atrophy. The karyotype was 46, XY. He was diagnosed with XLMR.

II:1, a 7 years old boy was the proband's older brother. He was born after an uneventful pregnancy. Similar to his younger brother his developmental milestone was initially normal before the age of 1 year, (family F, II:1). He could sit independently at 6 months, grasp things at 7 months, and walk with support at 1 year. However, his hand dexterity was poor. He could say only two words at 16 months.

Psychomotor regression was noticed at 1 and a half years. He was unable to purposely use his hands at that time without development of stereotypic hand movements. Subsequently he lost his ability to sit independently, recognize his parents, and speak at 2 years. He had dysphagia, constipation, and poor appetite. He could only be fed with liquid food. Nystagmus and poor eye contact were noticed. He has been bedridden with no reaction to the outside world after the age of 7 years. He had joint contracture (elbow, knee, and ankle), hypotonia but no seizures. Brain MRI showed cerebellar atrophy. He was diagnosed with XLMR.

I:1, the mother of the two boys, was an asymptomatic *MECP2* mutation carrier.

## 4 | DISCUSSION

This is the first series of Chinese familial cases with *MECP2* mutations, including three females and five males, in six families. The calculated ratio of our familial cases was 1.86% (8/429). All cases were confirmed in a maternally inherited pattern sharing an identical mutation with their mothers while no mutations in their father. Additionally, we confirmed no existence of a mosaic mutation in their parents using digital PCR and PGM (Xu et al., 2015). The phenotypes of the three female patients were consistent with those of RTT but the five males not. To our knowledge, only about 28 familial cases with *MECP2* mutations have been reported (Augenstein et al., 2009; Couvert et al., 2001; Dayer et al., 2007; Evans et al., 2006; Kankirawatanaet al., 2006; Khajuria et al., 2012; Klauck et al., 2002; Lambert et al., 2016; Meloni et al., 2000; Moog et al., 2006; Orrico et al., 2000; Ravn et al., 2011; Villard, 2007; Yntema et al., 2002), with genotypic mutations including missense, frameshift, and nonsense mutations, intragenic deletions and copy number variations (CNV). The phenotypes of those mutation carriers manifested from classical RTT congenital encephalopathy, nonspecific XLMR, and to clinically asymptomatic. Paolo M et al. classified male cases with *MECP2* mutations into three categories: (1) a 47,XXY karyotype, or somatic mosaic carrying the same *MECP2* mutations that cause classic RTT in females and (2) *MECP2* mutation via disrupt DNA binding or nuclear localization; and no mutations found in females with RTT or mild symptoms in heterozygosity (Moretti & Zoghbi, 2006). Similarly, Laurent V categorized males with *MECP2* mutations into three groups: (1) patients have a mutation seen in the typical cases of RTT. These males may have severe neonatal encephalopathy and early death. If the boy has a somatic mosaic or XXY karyotype, milder phenotype may manifest; (2) the males with different degree of mental retardation have mutations that are not found in their mothers with RTT or other disorders; and (3) males have a variable copies of mutated *MECP2*, such as *MECP2* duplication syndrome, or *MECP2* DS (Villard, 2007).

In our series, Patient II:2, the boy in family A had the mutation c.397C > T (p.R133C) which was one of the hot mutation spots of *MECP2* gene (Smeets et al., 2005). This mutation is frequently seen in atypical milder RTT named PSV (Bebbington et al., 2008). This boy had severer clinical symptoms than did his sister and fulfilled the diagnostic criteria for XLMR. Our second male patient (II:1, family D) had double mutations of c.1164-1207 del44bp and c.1225-1227 del AGC (p.P389X) with clinical symptoms of epilepsy, mental retardation, and severe systemic tremor, which occurred after a period of initially normal development. His clinical manifestations did not fulfill the diagnostic criteria for RTT, though he had some RTT-like phenomenon, which was different and severer than those of a female with the same mutation. The relationship between the phenotype and the deletion mutation of c.1164_1207 del44bp (p.P389X), known as a hotspot for deletion mutations (Neul et al., 2008), has been described in RettBase (www.chw.edu.au).

Patient II:1, a boy of family E, had a mutation c.1409G > A (p.R470H) which has not previously been reported in female patients. Notably, mutation of c.1409G > A (p.R470H) is located at the downstream position of *MECP2* gene which may leave the majority of *MECP2* function undisturbed and, therefore, its pathogenicity may be weak (Scala et al., 2007), correlating a milder phenotype in this boy than the other male patients. He was diagnosed as nonspecific XLMR.

Another novel mutation of c.441C > G (p.D147E) was detected in two boys of Family F which has not been reported in female RTT patients. This mutation is located at methyl-CpG-binding domain (MBD) and may disrupt DNA binding site leading to DNA malfunctioning (Baubec et al., 2013). These two boys never obtained language skill but with an arrested brain development. The two boys did not fulfill the diagnostic criteria of RTT but were consistent with XLMR. Hypothetically, the boy II:1 of Family B, who died at 1 year old and whose DNA analysis for *MECP2* was not performed, was highly suspected carrying the same mutation of c.916C > T (p.R306C) in his sister and mother, a hot mutation spot in typical RTT cases. Nevertheless, the boy's clinical findings might be in the line with those described in the first group by Laurent V, contributing to severe neonatal encephalopathy and early death.

The *MECP2* mutations occurring in RTT are predominantly de novo but rare familial cases. Previous reports indicated that the paternal type of inheritance constitutes more than 90% sporadic RTT cases (Trappe et al., 2001; Zhang, Bao, Cao, et al., 2012; Zhang, Bao, Zhang, et al., 2012). However, in our familial cases, all mutated *MECP2* were inherited from their mothers. Among the mothers, two had MR, while the remaining were asymptomatic. The disputed phenotypes between the mothers and their children with the same *MECP2* mutation might be partially contributed by different XCI patterns and genotypes. Regarding the actions, first of all, XCI may play a very important role in modulating the phenotype. In a comparison to the three female patients (families A, B, C) diagnosed with RTT were with a random XCI, the mothers of families A, B, D and E had nonrandom XCT. Three of these mothers were asymptomatic. Notably, the phenotype of the random XCI mother of family C was severer than the non-random XCI mother of family A with the same mutation. However, not all the clinical heterogeneity could be explained by XCI pattern in variation. In family B, the mother (family B, I:1) had mild MR. In family C, both the mother and the daughter had random XCI, however, the clinical symptoms of the mother were milder than those of her daughter, indicating that the random XCI may play a major role in the disease phenotype and there may have a genetic anticipation. However, whether the onset of the phenotypes was due to the

variation of XCI patterns between the samples of brain and blood or other as yet to be determined mechanisms warrants further investigations.

Second, there are differences in the pathogenic spectrum of *MECP2* gene between male and female. The mutations of c.1409G > A (p.R470H) in family E and mutations of c.441C > G (p.D147E) in family F were never reported in female RTTs. They might be mutations only causing milder disease phenotypes in hemizygotic male. Females carrying this kind of *MECP2* mutations may be asymptomatic due to the gene dosage compensation mechanism, as seen in the mothers of families E and F. Although the mothers were asymptomatic carriers, their male offspring were significantly phenotypic of XLMR.

Furthermore, in contrast to that most RTT mutations in the sporadic cases are paternal in origin; mutations in our familial cases were maternal in origin. The occurrence of paternal and maternal mutations maybe yielded due to different processes of male and female gametes in their parents (Chandley, 1991). Spermatogenesis begins at puberty and continues throughout adult life, while oogenesis is complete at birth (Wijsman, 1991). Spermatogenesis undergoes many more cell divisions than oocytes, which increases the chances of stochastic mutations with each replication in sperm.

In summary, our study expands our knowledge on familial cases with *MECP2* gene mutations which may produce a spectrum of various clinical manifestations in females and males. In contrast to the sporadic cases with de novo mutation in a paternal inheritance pattern, familial cases with mutated *MECP2* genes were inherited in the maternal pattern.

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