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Molecular characteristics of Chinese patients with Rett syndrome

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ABSTRACT

Objective: Rett syndrome (RTT) is a neurodevelopmental disorder which affects 1/10,000 girls. The aim of this study is to delineate the molecular characteristics of Rett syndrome in China based on the largest group of Chinese patients ever studied.
Methods: In all, 365 Chinese patients with Rett syndrome were recruited. Clinical information including the family reproductive history was collected through interviewing patients and their parents as well as questionnaires. *MECP2*, *CDKL5*, *FOXG1* mutational analysis was performed using polymerase chain reaction (PCR), direct sequencing and multiplex ligation-dependent probe amplification (MLPA). The parental origin of mutated *MECP2* gene, the *MECP2* gene mutation rate in the patients' mothers, and the X-chromosome inactivation pattern of the mothers who carry the mutation were also analyzed.
Results: Almost all of the patients were sporadic cases except one pair of twins. The pregnancy loss in probands' mothers and sex ratio of offspring in probands' generation were available in 352 families and were comparable to the general population. Out of the 365 cases, 315 had *MECP2* gene mutations and 3 had de novo *CDKL5* gene mutations. No patients had *FOXG1* mutation. Among the 315 cases with *MECP2* mutations, 274 were typical cases and 41 were atypical cases. All the 3 cases with *CDKL5* gene mutations were atypical RTT with early-onset seizures. The analysis of parental origin of mutated *MECP2* gene were performed on 139 cases, 90 (64.7%) cases were informative for the study. The result showed 94.4% cases with mutations from paternal origin and 5.6% from maternal origin. Among the cases with paternal mutation, 90.6% had point mutations. C > T was the most common one, accounting for 85.7% of the point mutations. Only one normal phenotype mother (0.41%) carried the same p.R133C mutation of *MECP2* gene as her daughter with mild phenotype. The different patterns of X-chromosome inactivation in the mother and the daughter may explain their different phenotypes.
Conclusion: The high rate of paternal origin of the mutated *MECP2* gene may explain the high occurrence of RTT in female gender. The family cases of RTT are rare and the recurrence risk of RTT is very low in China. Only 0.41% (1/244) mothers carry the pathogenic gene. *FOXG1* mutations were not found in this group of Chinese patients.

1. Introduction

Rett syndrome (RTT) is an X-linked dominant neurodevelopment disorder and was initially described by Andreas Rett in 1966. It has been recognized as one of the most common causes of mental retardation and affects females exclusively with an incidence of 1 in 10,000 female worldwide [1]. The disorder is characterized by cognitive regression, autonomic dysfunction, loss of communicative abilities and purposeful hand use with development of stereotypic hand movements; deceleration of head growth; followed by apparently normal development between 6 and 18 months [2]. Affected patients also manifest gait ataxia and apraxia, autistic features, epileptic seizures and respiratory dysfunction [2]. RTT is divided into typical and atypical RTT. Atypical RTT includes congenital variant, early-onset seizures and preserved speech according to the 2002 criteria [3]. Amir et al. determined that RTT is caused by mutations of methyl CpG binding protein 2 (*MECP2*) gene (MIM 300005) on chromosome Xq28 [4], which recently have been described with a detection rate of 60–95% [5]. Large deletions in both typical and atypical cases account for about 20% of all *MECP2* mutations [6]. The cyclin-dependent kinase-like 5(*CDKL5*) gene has been implicated in the molecular etiology of atypical RTT with early-onset seizures. So far, more than 50 deleterious alleles have been reported in the literature [7]. They share the common features of

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mental retardation, early-onset seizures, Rett-phenotype features and drug-resistant seizures. The *FOXG1* gene, located in 14q11–q13 encodes the forkhead box G1 protein has been recently implicated in the congenital variant of RTT [8].

RTT affects females almost exclusively. Previous research did not support the hypothesis that RTT is lethal in male embryos with RTT [9]. The data showed proportions of spontaneous abortion and stillbirth of probands' mothers in case families are similar to the control families, and the Sex skewing of offspring in cases families was not significantly different from that in normal families [9]. Another hypothesis from Thomas to explain the high female to male ratio in RTT is that the de novo mutations occur mainly on the paternal X chromosome, which is inherited only by the female offspring [10]. In some researches with small samples, paternal origin *MECP2* gene mutations were found in the majority of RTT patients, which could partially explain the high female to male ratio [11,12]. Familial cases in RTT are rare and are thought to be inherited from a carrier mother or a germline mosaicism. The pattern of X-chromosome inactivation (XCI) may modify the phenotype of Rett syndrome [13–15]. Identifying the parental origin of mutation and analysis of the *MECP2* mutation in the mother may give genetic counseling whether the recurrence risk is higher or not in the families with a RTT patient.

The molecular characteristics of RTT in China are unknown. This study is based on the largest cohort of Chinese RTT cases ever studied and should draw the outline of the genetic features of Chinese RTT.

2. Material and methods

2.1. Patients

A total of 365 Chinese patients with RTT, including 307 typical patients and 58 atypical patients were enrolled since 1989. All the patients were interviewed and diagnosed by Dr. Bao and Dr. Wu (co-authors of this study). A questionnaire was filled by the parents after signing an informed consent. Ethical approval was obtained from the hospital research ethic board. All patients were female with median age of 3 years (ranged from 5 months to 28 years). Patients were distributed in all provinces of mainland China. All patients met the diagnostic criteria [3]. Among 58 atypical patients, 18 were preserved speech variant, 14 were early-onset seizures, and 26 were congenital variant.

Clinical information of the patients was obtained via family questionnaires and clinician interviews. The family reproductive history included the pregnancy loss of probands' mothers and sex ratio of probands' generation.

2.2. MECP2, CDKL5 and FOXG1 mutational analysis

Genomic DNA was extracted using standard methods from the peripheral blood leukocytes of patients with RTT and their parents. *MECP2* gene was sequenced as previously described [4]. If no mutations were identified, the MLPA-P015 probe (SALSA MLPA kit P015 *MECP2*, MRC-Holland, Amsterdam, Holland) was used for detecting large deletions or duplications. MLPA products were separated and analyzed using the ABI Prism 3100 Genetic Analyzer and Genescan software according to manufacturer's recommendations. *CDKL5* gene was screened in patients without *MECP2* mutation by PCR and MLPA (SALSA MLPA kit P189 *CDKL5*) using the method reported previously [7,16]. *FOXG1* gene mutations were analyzed by PCR and sequencing in patients without mutations in the two genes described above [8].

2.3. Parental origin of the mutated MECP2 gene

In order to identify the parental origin of mutated *MECP2* gene, 139 RTT cases with pathogenic *MECP2* mutations were analyzed using allele specific PCR method as described previously [11,12].

2.4. MECP2 mutation in mothers of RTT patients and the XCI pattern in the mother and the daughter with the same pathogenic MECP2 mutation

MECP2 mutations were screened in 244 mothers with normal phenotype whose daughters had disease-causing *MECP2* mutation by the method described above. If the mother had the same *MECP2* gene mutation as her daughter, the XCI pattern of the mother and the daughter were analyzed according to the procedures described by Allen et al. [17]. XCI was considered skewed if the ratio was $\geq$65:35, and was considered extremely skewed if the ratio was $\geq$80:20.

3. Results

3.1. Family history, pregnancy loss and sex ratio of offspring of the mothers

Almost all the cases were sporadic except one pair of twins with one twin being atypical case and the other being a congenital variant. The parents of the 365 RTT patients all had normal phenotype. Information of family reproductive history was available in 352 families. Among them, 39 mothers(11.1%, 39/352) experienced miscarriages. Thirty-six mothers had one miscarriage and 3 mothers had 2 times. The miscarriage rate was 8.4% (42/502, 42 miscarriages, 352 cases and 108 live-born siblings, totally 502 pregnancies). In these families, the *MECP2* mutation types of the probands were 17 (43.6%) with nonsense mutations, 14 (35.9%) with missense mutations, and 8 (20.5%) with other mutations. Among the point mutations, p.R255X was the most common (17.9%, 7/39) (Table 1). Most of the probands were typical RTT (97.4%, 38/39). Perinatal deaths occurred in 8 families, 3 were neonatal deaths and 5 were stillbirths. Five of them were males. There were 108 live-born case siblings in 352 families, 56 (52%) were males and 52 (48%) were females. None of the RTT patients had descendants.

3.2. MECP2, CDKL5 and FOXG1 gene mutation

Out of 365 Chinese RTT cases, *MECP2* gene mutations were found in 315 patients (86.3%), 274 (89.3%, 274/307) were typical cases and 41 (70.7%, 41/58) were atypical ones. Mutations included 249 (79.0%) point mutations, 39 (12.4%) micro-deletions, 5 (1.6%) insertions, 1(0.3%) splicing defect and 21 large deletions. In point mutations, 127 (40.3%) were nonsense mutations and 122 (38.7%) were missense mutations. p.R168X was the most common one, accounting for 14.0% (44/315) of all the mutations, followed by p.T158M (39 cases), p.R270X (27 cases), p.R255X (27 cases), p.R306C (21 cases), p.R294X (21 cases), p.R133C (17 cases) and p.R106W (13 cases). In micro-deletions, p.G269fsX288 was the most common one, accounting for 30.8% (12/39). MLPA analysis was performed on 71 cases, large deletions were found in 21 cases accounting for 6.7% (21/315) of all the *MECP2* mutations. In large deletion mutations, exons 3 and/or 4 of *MECP2* gene were involved in 20 (95.2%) cases. The first exon was involved in only one case. The phenotypes of these cases were 20 typical and 1 atypical.

Three patients (21.4%, 3/14) with *CDKL5* gene mutations were found in the group with early-onset seizure variant. All the *CDKL5* gene mutations were de novo mutations which were not found in their parents. Two cases were splicing mutation (ISV + 1A > G) located in intron 6 and 13 respectively and the other one was nonsense mutations (c.1375C > T; p.Q459X) located in exon 12. No large deletion or duplication was found in *CDKL5* gene (Table 2).

None of the patients were found to have a mutation in *FOXG1*, even those in the congenital onset variant form.

Table 1
MECP2 gene mutations of the probands whose mother ever had miscarriages.

Mutations	Nucleotide changer (c.)	Aa change (p.)	Domain	Location	Number	RTT type		Total ($n = 39$)
						Typical	Atypical	
Nosense	763C > T	R255X	NLS	Exon 4	7	7	–	17 (43.6%)
	880C > T	R294X	TRD	Exon 4	4	3	1	
	808C > T	R270X	NLS	Exon 4	3	3	–	
	502C > T	R168X	CRIR	Exon 4	2	2	–	
	730C > T	Q244X	TRD	Exon 4	1	1	–	
Missense	316C > T	R106W	MBD	Exon 3	5	5	–	14 (35.9%)
	916C > T	R306C	TRD	Exon 4	3	3	–	
	473C > T	T158M	MBD	Exon 4	2	2	–	
	397C > T	R133C	MBD	Exon 4	1	1	–	
	455C > G	P152R	MBD	Exon 4	1	1	–	
	464T > C	F155S	MBD	Exon 4	1	1	–	
	403A > G	K135E	MBD	Exon 4	1	1	–	
Micro-deletions	808delC	R270fs	TRD–NLS	Exon 4	1	1	–	3 (7.7%)
	112–116del 5	–	NTS	Exon 3	1	1	–	
	1163–1188del26	–	CTS	Exon 4	1	1	–	
Large deletion	Exon 4	–	–	–	1	1	–	1 (2.6%)
MECP2 (–)	–	–	–	–	4	4	–	4 (10.3%)

3.3. Parental origin of the mutated MECP2

Totally, 90 out of 139 (64.7%) RTT cases presented at least one SNP, which were informative for the analysis of parental origin of mutated *MECP2* gene. Among them 94.4% (85/90) mutations were paternal. In these cases with paternal mutation, 90.6% (77/85) were point mutations, C > T was the most common one accounting for 85.7% (66/77) cases, the next was C > G mutation accounting for 7.8% (6/77) cases, followed by A > G and A > T mutation. Only 5.6% (5/90) were maternal origin. *MECP2* mutations in these patients were c.806delG (p.G269fs) in 3 cases, c.748delC (p.R250fs) in 1 case and c.502C > T (p.R168X) in 1 case. So micro-deletion was common in the maternal origin mutations, which were different from the paternal mutations. No mutation was found in the mothers of these five patients.

3.4. MECP2 mutation in the mothers and XCI patterns

The *MECP2* mutation was found only in 1/244 (0.41%) mother. She had R133C mutation which was the same as her daughter. The mother had normal phenotype and her daughter had mild phenotype: sited unaided at 8–9 months and walked independently at 18 moths, speaks 4–5 words at the time of the study, partially preserved the purposeful hand skills. No mutation was found in the father. The RTT patient had random XCI (XCI = 59:41) and her mother had extremely skewed XCI pattern (XCI = 84:16).

Table 2
MECP2 and *CDKL5* gene mutations analysis in Chinese RTT cases.

Gene	Mutations type	Aa change (p.)	Location	Domain	RTT type		Total ($n = 318$)
					Typical	Atypical	
MECP2	Nosense	R168X	Exon 4	CRIR	38	6	127
		R255X	Exon 4	NLS	24	3	
		R270X	Exon 4	NLS	22	5	
		R294X	Exon 4	TRD	15	6	
		Others	–	–	6	2	
	Missense	T158M	Exon 4	MBD	33	6	122
		R306C	Exon 4	TRD	18	3	
		R133C	Exon 4	MBD	15	2	
		R106W	Exon 3	MBD	13	0	
		Others	–	–	28	4	
	Splicing	IVS3–2A > T	Exon 3	–	1	–	1
	Micro-deletion	G269fsX288	Exon 4	NLS	11	1	39
		Others	–	–	27	–	
	Insertion	–	–	–	3	2	5
	Large deletion	Exon 1–2	–	–	1	–	21
		Exon 2–4	–	–	1	–	
		Exon 3.3	–	–	1	–	
		Exon 3–4	–	–	4	–	
		Exon 3–4.2	–	–	2	–	
		Exon3–4.3	–	–	1	–	
		Exon 3–4.4	–	–	3	–	
		Exon 4.1–4.2	–	–	2	–	
		Exon 4.2–4.4	–	–	1	–	
		Exon 4.3	–	–	1	–	
		Exon 4.3–4.4	–	–	1	1	
		Exon 4.4–4.5	–	–	1	–	
		Exon 4	–	–	1	–	
CDKL5	Nosense	Q459X	Exon 12	Catalytic domains	–	1	3
	Splicing	ISV + 1 G > A	Intron 6	–	–	1	
		ISV + 1 G > A	Intron 13	–	–	1	

The X-chromosome with R133C mutation was transferred from the mother to the daughter.

4. Discussion

RTT is a severe neurodevelopmental disorder and mutations of *MECP2* gene plays an important role in causing RTT [4]. Mutations in the *CDKL5* and *FOXG1* genes are associated with atypical RTT with early-onset seizures and congenital form [8,18].

In this group of Chinese patients, 315 (86.3%) cases had *MECP2* gene mutations, the mutations detection rate was 89.3% in 307 typical cases and 70.7% in 58 atypical ones, which is similar to previous reports [19,20]. The most frequent point mutation was p.R168X, then R255X, R270X, R294X, T158M, R306C, R133C and R106W. Most of them were located in the transcription repression domain (TRD) and nuclear localization signal (NLS). The above eight hot *MECP2* mutations represent 66.3% (209/315) of all the identified mutations and similar results from other countries were reported [21,22]. The gross rearrangement of *MECP2* gene in 20 typical cases and 1 congenital variant accounts for 6.7% (21/315) cases. MLPA is an important supplement of the direct sequencing for *MECP2* gene screening. Large deletions frequently involve either exon 4 or both exons 3 and 4. Miltenberger-Miltenyi et al. found there is a highly repetitive region (deletion prone region, DPR) locating from 3′ to the TRD, where many intragenic deletion breakpoints occur (C-terminal deletions) [23]. Part deletion of the C-terminus of *MECP2* impairs its DNA binding capacity during the transcription–regulation process. In our study, 19 large deletions involved the DPR. Only one deletion involved in exon 1 (del exon 1–2). In short, *MECP2* gene mutation is the major cause of RTT and *MECP2* gene mutational analysis is an important support for the clinical diagnosis of Rett syndrome.

Mutations in the *CDKL5* gene have been associated with early-onset seizure variant of RTT. In Nemos's report, the mutation rate was 28%. Missense, splicing or truncated mutations were identified [7]. In our study, mutations of the *CDKL5* gene accounted for 21.4% (3 of 14) of this variant. One patients carried c.1375C > T, a nonsense mutation (p.Q459X) in exon 12, leading to truncation in the large C-terminal region of *CDKL5* gene, which is involved in both the regulation of its catalytic activity and its subcellular localization [24]. The other two were splicing mutations ISV + 1 G > A located in intron 6 and 13, which are conserved in all species. Archer et al. found a same mutation (IVS6 + 1G > A) in a patient and proved it was a pathogenic mutation [25]. Weaving et al. reported a similar mutation IVS13 − 1G > A in exon 13 in a patient with early-onset epilepsy overlapping-Rett syndrome [26].

RTT affects females almost exclusively, and only a few reports had described male patients with *MECP2* gene mutations. There are several hypotheses for this phenomena, which continue to be debated. Hagberg et al. hypothesized that affected male offspring do not survive hence the almost exclusive female population affected. Mutations in *MECP2* gene are considered developmentally lethal in male embryos [2]. Our observations shows that the rate of miscarriages of 352 Chinese mothers with RTT daughters were 8.4% which is lower than the range of 10–15% of the general population. In 39 cases families with miscarriages, R255X mutation in *MECP2* gene was the most common mutation accounting 17.9% of the cases. The 2.3% (8/352) of the rate of perinatal deaths was not higher than the one in the general population (3%). Similar results had been reported by Fyfe et al. [9]. Out of 108 live-born case siblings, 56 (52%) were males and 52 (48%) were females, though nearly two thirds of the perinatal deaths were males in case families. These results did not support the hypothesis that RTT is lethal in male embryos.

To explain the gender-biased occurrence of RTT and other X-linked dominant genetic diseases, Thomas et al. hypothesized that mutations occur predominantly on the male-derived X chromosome, which determines the female gender of offspring. It was suspected that the X chromosomes in oocyte is in a low methylated state, while X chromosome in sperm cells in a highly methylated state leads to a completely inactivation. During sperm cells meiosis, the spontaneous deamination of cytosine transforms into thymine (C > T mutation), which leads to a de novo mutation [10,27]. To test this hypothesis, the parental origin of *MECP2* mutation of 139 cases was analyzed in this study and showed that in 94.4% (85/90) cases the mutated *MECP2* gene were of paternal origin, only 5.6% (5/90) cases were of maternal origin, and 85.7% point mutations were C > T mutation. The high percentage of paternal origin mutation and C > T mutation supported Thomas's theory which may partially explain the high female to male ratio.

RTT familial cases are rare and account for 0.5–1% cases. The general knowledge regarding RTT families with affected females are sparse as only 10 families have been described [28, 29]. The family cases are even more sparse in China. In this group of Chinese patients, almost all the cases are sporadic, except one pair of twins. Due to the severe mental disability of RTT patients, there is little possibility for them to be married and to have children. So to date, family cases always have been explained by germline mosaicism or skewed XCI in the carrier mothers. In order to test the rate of the mother carrier, *MECP2* mutational analysis was performed on 244 patients' normal phenotype mothers, only one (0.41%) mother was found to carry the p.R133C mutation, same mutation as her daughter. The daughter had milder phenotype with preserved speech variant and manifested as other patients with this type of mutation [30]. XCI analysis using the peripheral blood showed the daughter had the X-chromosome with R133C mutation from her mother, she had random XCI pattern and her mother who was normal had extremely skewed XCI pattern. In this family, XCI may play a very important role in modulating the phenotype.

In conclusion, this is the first systematic research of the molecular characteristics of Chinese patients with RTT. *MECP2* gene mutations were found in 86.3% patients, and *CDKL5* gene mutation were found in 21.4% patients with early-onset seizure variant. Mutation detection rate was 89.3% (274/307) in typical cases and 75.9% (44/58) in atypical cases. *FOXG1* mutations were not found in this group of Chinese patients. The pregnancy loss in the mother is not higher and sex ratio of offspring is the same as that in general population, which therefore does not support the theory of male embryonic lethality. About 94.4% *MECP2* mutations were of paternal origin, which may explain the high female to male ratio in RTT. The family cases were very rare in China, and there is only one pair of twins in this group of patients. Among 244 mothers, only one carries the pathogenic mutation with extremely skewed XCI. So the recurrent risk of RTT in China is very lower.

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