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A novel mutation in the mutations in the methyl-CpG-binding protein 2 (MECP2) gene in a Chinese patient with typical Rett syndrome and subsequent prenatal diagnosis

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Summary

Rett syndrome (RTT), which is a progressive neurodevelopmental disorder characterized by early neurological regression, severely affects cognitive function, as well as motor and language skills. Mutations in the methyl-CpG-binding protein 2 (MECP2) gene have been found in most patients with typical RTT. In this study, a female patient with severe symptoms was diagnosed as typical RTT according to the revised diagnostic criteria. Genetic analysis reveals that the child had a novel frameshift mutation, c.368delA (p.Tyr123PhefsX2), in exon 3 of the MECP2 gene. After genetic counseling, the parents were referred to the present clinic for prenatal diagnosis during their second pregnancy. The mutation was not detected in this fetus and was predicted to be unaffected by RTT. Here, the authors report a novel mutation in the MECP2 gene of a patient with typical RTT, which provides accurate information for genetic counseling and prenatal diagnosis.

Key words: Rett syndrome; MECP2; Novel mutation; Prenatal diagnosis.

Introduction

Rett syndrome (RTT, OMIM# 312750), which was first reported by Andreas Rett in 1966, is a severe and progressive neurodevelopmental disorder [1, 2]. RTT primarily affects young females and is relatively common, with an estimated prevalence of approximately 1:10,000 live female births and 1:8,500 by the age of 15 [3]. RTT exhibits a broad clinical phenotype, which is characterized by apparently normal development between six and 18 months in most patients, followed by developmental stagnation and eventual symbolic rapid regression, including the loss of acquired cognitive function, and motor and language skills [4]. Affected individuals also show characteristic clinical symptoms, including stereotypic hand movements, microcephaly, growth retardation, intellectual disability, autistic features, hypotonia, ataxia, breathing abnormalities, seizures, and scoliosis [2, 5]. The variation in the clinical phenotypes of RTT make clinical diagnosis difficult. In 2010, revised diagnostic criteria were established based on the consensus that affected individuals are divided into two categories for typical RTT and atypical RTT [6]. In 1999, RTT was found to be caused by mutations in the MECP2 gene encoding the methyl-CpG-binding protein 2 (MeCP2) [7]. Using sequencing techniques and multiplex ligation-dependent probe amplification (MLPA), mutations in the MECP2 gene have been identified in more than 95% of individuals with typical RTT, but only 50-70% of individuals with atypical RTT [8]. In this study, the authors performed MLPA analysis and Sanger sequencing to identify causative mutations in the MECP2 gene of a Chinese girl with typical RTT. The family were offered genetic counseling and prenatal diagnosis during their second pregnancy.

Materials and Methods

A pregnant, non-consanguineous couple was referred to the Department of Prenatal Diagnosis, Nanjing Maternity and Child Health Care Hospital for genetic counseling because their first-born child, a daughter (proband) was diagnosed with typical RTT by according to the recently revised diagnostic criteria for RTT [6]. At the time of genetic counseling, the child was three-and-a-half years old. Genetic analysis was performed in the present center. The present study was conducted in accordance with the Declaration of Helsinki, and received approval from the Ethics Committee of Nanjing Maternity and Child Health Care Hospital affiliated to Nanjing Medical University (China). Written informed consent to genetic analysis of the MECP2 gene in the family was also obtained from the child's parents.

Peripheral blood samples from the child and her parents were collected into K_2EDTA-containing VACUETTE tubes. Genomic DNA (gDNA) was extracted using a nucleic acid extraction kit according to the manufacturer's instructions.

The couple was offered prenatal diagnosis during their second pregnancy. Amniotic fluid was obtained for amniocentesis at 20

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Table 1. — *PCR primers used for the MECP2 gene*

Primer names	Sequence (5′-3′)	AT[a]	Fragment size[b]	Location[c]
Exon 1-F	GGAGAGAGGGCTGTGGTAAAAG	63°C	207bp	e1-CDS1
Exon 1-R	CATCCGCCAGCCGTGTCGTCCG			
Exon 2-F	CAATGGGGGCTTTCAACTTA	56°C	219bp	e2-CDS1
Exon 2-R	AAAACAGATGGCCAAACCAG			
Exon 3-1F	CCTGCCTCTGCTCACTTGTT	63°C	340bp	e1-CDS2 or e2-CDS2
Exon 3-1R	GGGGTCATCATACATGGGTC			
Exon 3-2F	AGCCCGTGCAGCCATCAGCC	65°C	350bp	
Exon 3-2R	GTTCCCCCCGACCCCACCCT			
Exon 4-1F	TTTGTCAGAGCGTTGTCACC	61°C	380bp	e1-CDS3 or e2-CDS3
Exon 4-1R	CTTCCCAGGACTTTTCTCCA			
Exon 4-2F	AACCACCTAAGAAGCCCAAA	61°C	380bp	
Exon 4-2R	CTGCACAGATCGGATAGAAGAC			
Exon 4-3F	GGCAGGAAGCGAAAAGCTGAG	65°C	366bp	
Exon 4-3R	TGAGTGGTGGTGATGGTGGTGG			
Exon 4-4F	TGGTGAAGCCCCTGCTGGT	65°C	414bp	
Exon 4-4R	CTCCCTCCCCTCGGTGTTTG			
Exon 4-5F	GGAGAAGATGCCCAGAGGAG	61°C	411bp	
Exon 4-5R	CGGTAAGAAAAACATCCCCAA			

Note: [a] Abbreviations: AT, the annealing temperature of the thermal cycling conditions; [b] Based on Reference Sequence: NG_007107.2; [c] Abbreviations: e1, the isoform E1 of MECP2; e2, the isoform E2 of MECP2; CDS, coding sequences.

weeks of gestation. Fetal gDNA was extracted from the amniotic fluid cells using a micro DNA kit. Maternal cell contamination of the amniotic fluid sample was evaluated by the short tandem repeat (STR) fingerprint. A commercial aneuploidy testing kit was used for amplification of eight STR markers (D13S628, D13S742, D13S634, D13S305, D18S1002, D18S391, D18S535, and D18S386) located on chromosomes 13 and 18. The fluorescent polymerase chain reaction (PCR) products were analyzed using a genetic analyzer and GeneMarker software (Version 1.91).

Multiplex ligation-dependent probe amplification analysis (MLPA) analysis was performed to detect copy number variations (CNVs) in the MECP2 gene using an MLPA kit. The P015-F1 probe mix contained 17 probes covering the four exons of the MECP2 gene. Hybridization, ligation, and amplification were carried out according to the manufacturer's protocol. Final products were detected using a genetic analyzer using LIZ500 as an internal size standard. The raw data were analyzed for gross deletions or duplications with Coffalyser Net software.

The exon regions of the MECP2 gene were amplified by PCR using nine pairs of primers (Table 1) [9, 10], with slight modifications to the PCR protocol. The reaction mixture (50 μL) contained 100 ng DNA template, 1× GC Buffer I, 0.4 mM dNTPs, 0.4 μM of each primer, and 2.5U LA polymerase. The thermal cycling conditions were as follows: an initial denaturation step at 95°C for five minutes, followed by 35 cycles of 95°C for 30 seconds, appropriate annealing temperature (range 56-65°C) for 30 seconds, and 72°C for 30 seconds, with a final extension incubation at 72°C for ten minutes. All PCR reactions were carried out using a thermal cycler (Applied Biosystems) according to the manufacturer's instructions. The amplified products were sequenced directly using PCR primers with a sequencing kit V3.1 ready reaction on a genetic analyzer. Sequencing data were compared with the reference sequence of the annotated MECP2gene to identify nucleotide variations. Nucleotide positions were determined according to the standard MECP2 gene reference sequence.

Results

The proband was the first child delivered to healthy, non-consanguineous parents, after an uneventful pregnancy. According to the mother, the child was born at full term by cesarean section. The baby's weight, length, and head circumference were normal at birth and she started walking unaided at the age of 16 months. Epileptic seizures began at 18 months, and the severity of this phenotype increased with age. Brain MRI at 19 months showed no evidence of structural anomalies, although slight hyperintensity in the right occipital lobe was noted. Neurologic examination showed severe retardation of growth and mental development. At the age of 34 months, the child presented significantly stereotypic hand movements, glazed eyes, and hyperpnea. At the same age, electroencephalography (EEG) during normal sleep showed epileptiform discharges in the left temporal lobe region, including spike wave, spike-slow wave, and polyspike-slow wave complexes. Due to a lack of patient cooperation, no EEG examinations were performed while the child was awake. At the age of three-and-a-half years, the child was unable to walk and crawl independently, and showed partial loss of the ability to speak, only speaking a few simple words, such as "Mama" and "Baba". The frequency of myoclonic seizures had increased. Blood and urine analysis by tandem mass spectrometry and gas chromatography-mass spectrometry, respectively, showed that the child's blood amino acids and organic acids in her urine were normal. Furthermore, conventional G-banding analysis confirmed a normal karyotype. The child was diagnosed as typical RTT according to the revised clinical diagnostic criteria (Table 2).

Large deletions and duplications in the MECP2 gene

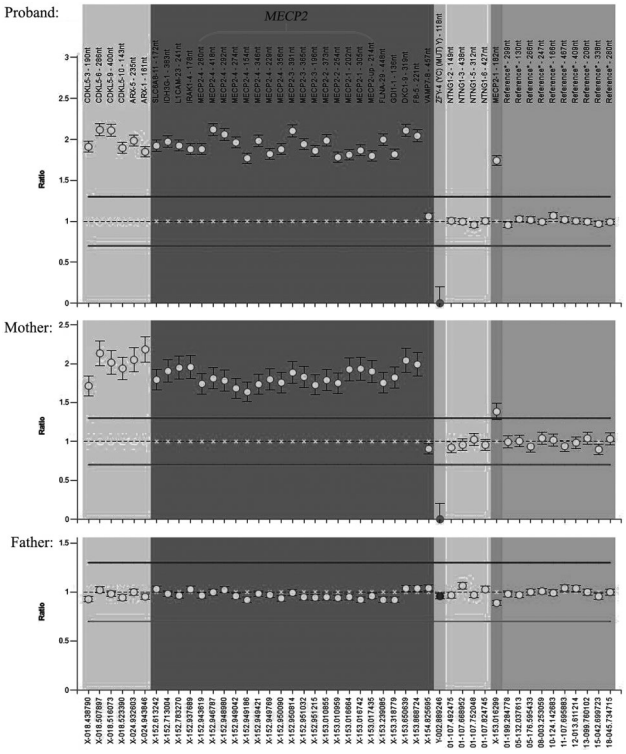

Figure 1. — Multiplex ligation-dependent probe amplification (MLPA) analysis for the detection of large deletions or duplications in the MECP2 gene of the proband and her parents. Normal copy numbers were observed in the proband and her parents.

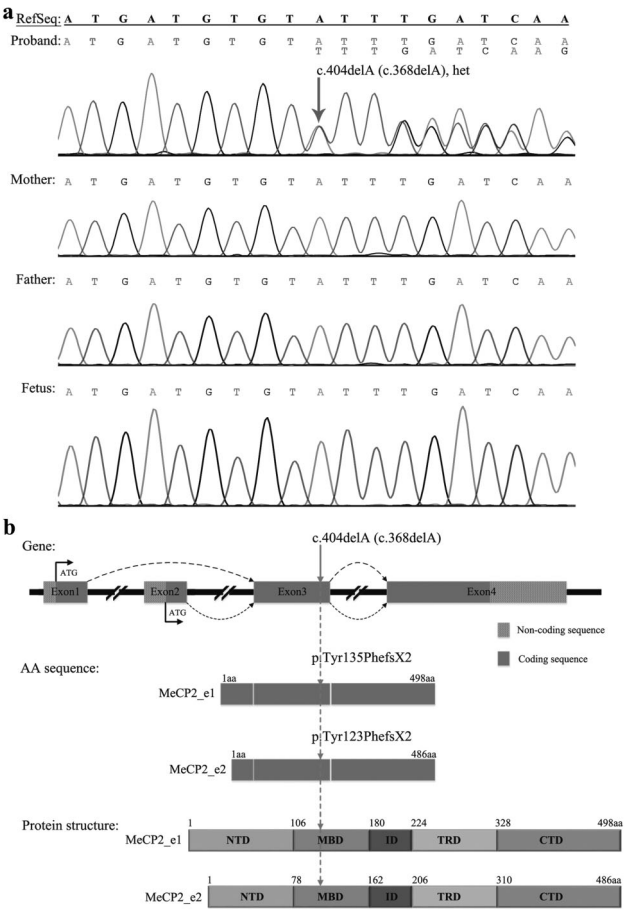

Figure 2. — Visualization of the c.368delA mutation in the MECP2 gene. (a) heterozygous mutation of c.368delA in the proband. The arrow indicates the c.368delA mutation. The mutation was not detected in the parents or the fetus;
(b) Schematic illustration of the different splices that lead to the expression of the two different isoforms, MeCP2_e1 and MeCP2_e2. The arrow indicates the mutation loci in the MeCP2 protein. Abbreviations: MBD, methyl DNA-binding domain; ID, intervening domain; TRD, transcription repression domain; CTD, C-terminal domain.

were first detected by MLPA analysis. Two copies of MECP2 without CNVs were identified in the proband (Figure 1). Further sequencing revealed a heterozygous variant, c.368delA, in exon 3 of the MECP2 gene (NM_004992.3) (Figure 2a). The variant was not identified in either of the proband's parents, indicating this mutation is a de novo variant. The small deletion introduces a downstream stop codon, which results in a truncated protein product (p.Tyr123PhefsX2) devoid of multiple functional domains in the MeCP2 protein (Figure 2b). The variant was not identified in any of the common population databases, including the 1,000 Genomes Project, dbSNP 144 and the Exome Aggregation Consortium (ExAC: http://exac.broadinstitute.org). According to the new guidelines for the classification of sequence variants [11], the identified variant falls within categories that provide very strong evidence of pathogenicity. The mutation was not found to be described in previous reports or the RettBASE variation database (http://mecp2.chw.edu.au), suggesting that this is a novel mutation. Although the causative mutation was absent in the lymphocytes of the parents, the couple were advised of the risk of recurrence due to the possibility of germline mosaicism. After genetic counseling, the parents requested prenatal testing for the second pregnancy and fetal DNA was obtained for amniocentesis at 20 weeks of gestation. Preliminary STR analysis invariably excluded the presence of maternal contamination (Figure 3). Sequencing analysis revealed that the mutation was not present in the fetus (Figure 2a) and chromosomal analysis revealed a 46, XY normal karyotype (data no shown). The parents were advised that the male fetus was predicted to be unaffected by RTT.

Discussion

In this study, the authors reported a novel mutation in the MECP2 gene of a patient with typical RTT, which provides accurate information for genetic counseling and prenatal diagnosis. The deletion mutation c.404delA (c.368delA) in

Table 2. — *Clinical manifestations of the child compared with Rett syndrome diagnostic criteria.*

Diagnostic criteria	Proband[a]
Required for classic RTT	
1. A period of regression followed by recovery or stabilization	Yes
2. All main criteria and all exclusive criteria	Yes
Required for variant RTT	
1. A period of regression followed by recovery or stabilization	-
2. Two of the 4 main criteria	-
3. Five of 11 supportive criteria	-
Main criteria	-
1. Partial or complete loss of acquired purposeful hand skills	Yes
2. Partial or complete loss of acquired spoken language or language skill	Yes
3. Gait abnormalities: impaired (dyspraxia) or absence of ability	Yes
4. Stereotypic hand movements including hand wringing/squeezing, clapping/tapping, mouthing, and washing/rubbing automatisms	Yes
Exclusion criteria for classic RTT	
1. Brain injury secondary to peri- or postnatal trauma, neurometabolic disease, or severe infection that cause neurological problems	No
2. Grossly abnormal psychomotor development in the first six months of life, with early milestones not being met	No
Supportive criteria for variant RTT	
1. Breathing disturbances when awake	Yes
2. Bruxism when awake	-
3. Impaired sleep pattern	-
4. Abnormal muscle tone	Yes
5. Peripheral vasomotor disturbances	-
6. Scoliosis/kyphosis	-
7. Growth retardation	Yes
8. Small cold hands and feet	-
9. Inappropriate laughing/screaming spells	-
10. Diminished sensitivity to pain	-
11. Intense eye communication – "eye pointing"	-

[a] *Did not appear in the child at this time.*

the MECP2 gene existed in the proband but not in her parents; thus the authors concluded that this is a de novo mutation in MECP2. Previous genetic studies have demonstrated that most MECP2 mutations are de novo and mainly of paternal origin. To the authors' knowledge, more than 20 familial cases have been reported so far [7, 9, 12-31]. These cases can be accounted for by germline mosaicism or by skewed X chromosome inactivation in an asymptomatic female carrier [2, 5, 32]. Due to the possibility of germline mosaicism, Mari *et al.* offered prenatal diagnosis to nine couples with a daughter affected by RTT, despite the fact that they were not MECP2 mutation carriers [23]. The first case of RTT caused by germline mosaicism identified in prenatal diagnosis was reported as a result. Although the risk of RTT recurrence is low, the investigators pointed out that prenatal diagnosis should be offered to parents with a proband with a mutation in the MECP2 gene [10, 23, 33]. Thus, the parents of the proband in this study were referred to this clinic for prenatal diagnosis during their second pregnancy.

As a chromatin-associated protein, the MeCP2 protein binds methylated CpGs in DNA to repress transcription of target genes. This protein plays an important role in maintaining normal neuronal and brain development [5, 34]. The MeCP2 protein exists in two different isoforms, MeCP2_e1 and MeCP2_e2, generated by alternative splicing. MECP2_e1 has been identified as the predominant isoform involved in RTT based on evidence showing that mutations in exon 1 impact MeCP2_e1 exclusively and selective deletion of MeCP2_e2 does not result in RTT-associated neurological phenotypes in vitro [35, 36]. The 1-bp deletion mutation results in the introduction of a premature stop codon, causing the generation of a truncated MeCP2 protein (MeCP2_e1: p.Tyr135PhefsX2 and MeCP2_e2: p.Tyr123PhefsX2) (Figure 2b). The truncated MeCP2 protein is devoid of the methyl DNA-binding domain (MBD) and lacks multiple functional domains, including the intervening domain (ID), transcription repression domain (TRD), and C-terminal domain (CTD). The MBD binds specifically to methylated DNA, while the ID between the MBD and TRD binds to unmethylated DNA to facilitate MBD-dependent binding [37]. The TRD recruits co-repressor complexes to bind methylated CpG sites for regulation of core histone deacetylation, which results in transcriptional repression of target genes [34, 38]. MeCP2-mediated nucleosomal array compaction and oligomerization is dependent on the CTD [39, 40]. The frameshift MECP2 mutation (c.368delA) causes marked changes in the structure of the MeCP2 protein, which seriously affect its functions. To the present authors' knowledge, this mu-

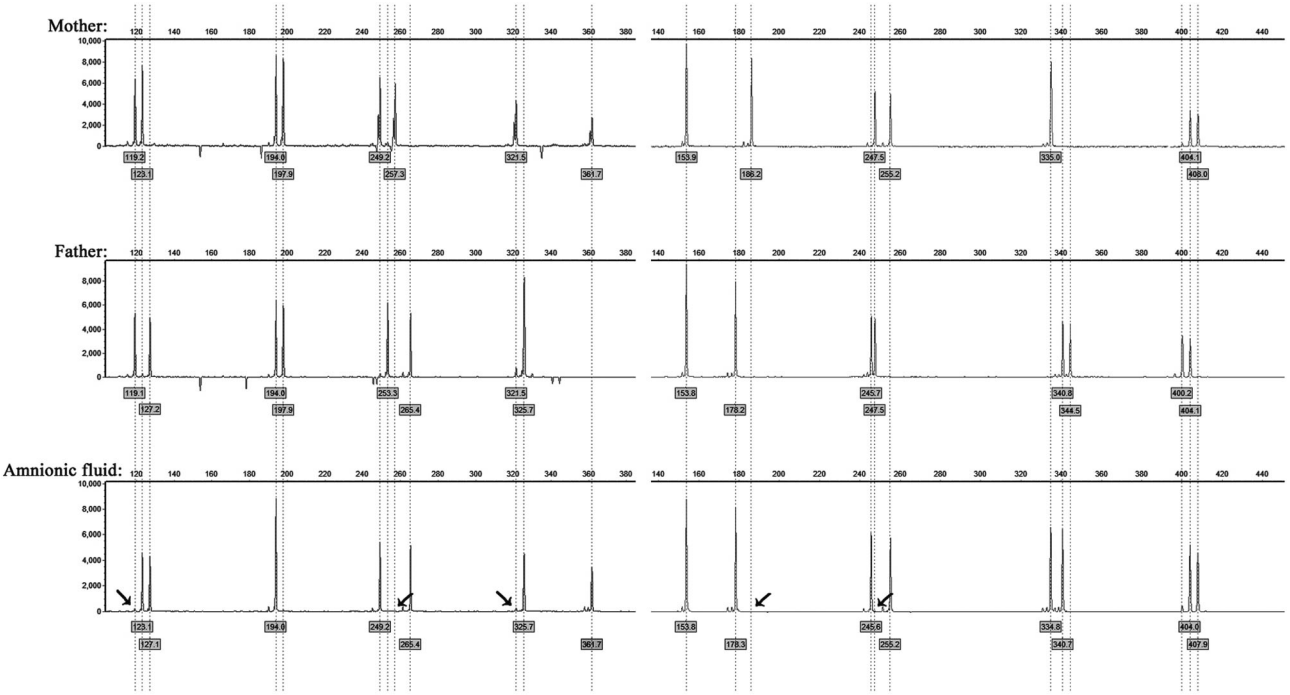

Figure 3. — Short tandem repeat (STR) analysis of the parents and fetus. Eight STR markers were detected and the results showed that there was no maternal contamination of the fetal gDNA. The black arrow indicates the absence of maternal STR signaling.

tation has not been reported previously and is not present in the RettBASE variation database (http://mecp2.chw.edu.au).

In summary, the authors identified a novel MECP2 mutation in a Chinese girl with typical RTT. As a result, the parents received genetic counseling regarding the risk of recurrence and prenatal diagnosis was offered in their second pregnancy because of the possibility of germline mosaicism. The fetus did not carry the mutation and was predicted to be unaffected by RTT. The present findings provide important information for genetic counseling and broaden the genotypic spectrum of typical RTT.

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