Case Report: Prenatal Diagnosis for a Rett Syndrome Family Caused by a Novel MECP2 Deletion With Heteroduplexes of PCR Product

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Rett syndrome is an X-linked dominant, postnatal neurological disorder. Approximately 80–90% of classic Rett syndrome patients harbor mutations in the coding region of MECP2. Somatic or germline MECP2 mosaicism is not rare, and paternal germline MECP2 mosaicism occurs in especially high proportions. Here, we report the case of a Chinese girl with Rett syndrome in whom a heterozygous deletion was found in exon 4 of MECP2 using multiplex ligation-dependent probe amplification. To obtain an accurate region of deletion, we narrowed down the deletion region using real-time quantitative PCR, and subsequent long-range PCR was performed to detect the deletion breakpoints. Surprisingly, three DNA bands from long-range PCR products were observed after gel electrophoresis. To exclude somatic mosaicism, we performed T-A cloning and DNA sequencing, the middle DNA band was proved to be a heteroduplex of the PCR product in vitro. Meanwhile, a prenatal diagnosis was performed for the pregnant mother of the patient. Our study showed that the patient was heterozygous for the deletion of 713-base pairs in exon 4 of MECP2 (MECP2: c.441_1153del713), resulting in a frameshift and premature termination of the 487 amino acid protein at the 154th codon. In summary, we reported a novel heterozygous deletion in the MECP2 gene with heteroduplexes of the PCR product in vitro, which can help in the genetic counseling and prenatal diagnosis of disorders of MECP2 defects.

Keywords: Rett syndrome, MECP2, prenatal diagnosis, mosaicism, heteroduplexes

INTRODUCTION

Rett syndrome (MIM#312750) is an X-linked, dominant, postnatal neurological disorder with a prevalence of ~1 in 10,000–15,000 female births. Patients with classic Rett syndrome display delayed development between the period of 6–18 months, after a period of apparently normal development. It is characterized by a distinctive set of clinical features, including loss of motor skills and communicative abilities, acquired microcephaly, and the development of stereotypical hand movements (1).

In 1999, mutations in the X-linked gene, methyl-CpG-binding protein 2 (MECP2) at Xq28 were first reported in patients with Rett syndrome. Approximately 80–90% of classic Rett syndrome patients harbor mutations in the coding region of MECP2, and approximately up to 15% of these
mutations are gross deletions and other rearrangements, which can be detected by MLPA or southern blot (2–7).

MECP2 mutations have been reported in patients with somatic mosaicism; females or even males with somatic MECP2 mosaicism can survive to birth and exhibit clinical features similar to a full mutation in females or an atypical Rett syndrome phenotype (8–11). Paternal or maternal germline MECP2 mosaicism are also not rare (2, 12–14). Therefore, special attention should be paid to somatic and germline MECP2 mosaicism. In this study, we present a novel heterozygous deletion in the MECP2 gene in a Chinese girl with Rett syndrome. A variety of methods (including multiplex ligation-dependent probe amplification (MLPA), real-time quantitative PCR (qPCR), long-chain PCR, DNA sequencing, gel electrophoresis, and T-A cloning) were used to detect the precise mutation, excluding somatic mosaicism. We also performed prenatal diagnosis for her pregnant mother to avoid germline MECP2 mosaicism.

CASE PRESENTATION

The pedigree is a family from Zhejiang province in China. The proband was a 4-year-old girl who was diagnosed with typical Rett syndrome. This girl was born by normal delivery at 38 weeks gestation. She held up her head at 3 months and could sit at 6 months, and she was able to stand with support at 10 months. At 11 months she had said “papa” and “mama,” but after 13 months she no longer spoke these words. Emotionally she was noted to be more distant and “mama,” but after 13 months she no longer responded to her parents’ call by turning her head toward them. Subsequent loss of acquired purposeful hand skills began at 15 months, and has since developed a range of stereotypical hand movements such as frequent hand wringing. Epilepsy had previously developed at 30 months and was managed with sodium valproate and lamotrigine. However, her molecular diagnosis was unclear, and her healthy mother was 20 weeks along in her pregnancy. Considering the risk of recurrence, the affected pregnant woman requested prenatal diagnosis for her fetus who may have also been at risk of Rett syndrome. This study was approved by the Ethics Committee of Women's Hospital affiliated to the Zhejiang University School of Medicine, and informed consent was obtained from all subjects.

To perform the prenatal diagnosis, we first had to know the genetic basis of Rett syndrome in the proband. Peripheral blood samples were drawn from three family members, and genomic DNA was extracted using standard protocols. We carried out a routine polymerase chain reaction (PCR) to amplify the encoding exons of MECP2; exon 4 (exon 3 before the discovery of a new 5 UTR exon) was amplified as five overlapping fragments (4a, 4b, 4c, 4d, and 4e) (12), and no mutations were found in the four exons of MECP2.

We then detected the deletions using MLPA (MRC-Holland, kit P015C), which covers all four MECP2 exons and the flanking genes IRAK1, L1CAM, and SYBL1. MLPA screening suggested that the proband had a hemizygous copy number for MLPA probes 10842-L12494 and 01347-L12498. Additionally, a heterozygous deletion of exon 4 in MECP2 was found in the proband (Figure 1A).

We then used three pairs of real-time qPCR primers to narrow down and identify the deletion breakpoints (see Supplemental Information, Supplementary Figures 1A,B, Figure 1B). The product of long-range PCR (upon running a gel electrophoresis) showed three DNA bands of the proband (Figure 1C). The DNA was cut and purified using a DNA gel extraction kit, and the DNA bands were cloned into the pMD19-T vector via T/A ligation. After plasmid extraction, a PCR and a DNA sequencing were performed. The DNA sequence of the top band was the same as the wild-type. Surprisingly, not only the bottom band, but also the middle one, showed a 713-base pair deletion in exon 4 of MECP2 (c.441_1153del713) (Figure 1D).

We then identified that the middle band was a heteroduplex of the wild chain and a c.441_1153del713 mutated chain. As shown in Figure 1F, for base pairing, the mutated chain combined with the wild chain around the common parts (parts A and C), but the unpaired part (part B) of the wild chain may form a particular structure. PCR amplification of the wild chain cannot be performed; therefore, the PCR product of the middle band only showed the c.441_1153del713 mutation of MECP2. As shown in Figure 1E, by the classical method of producing heteroduplexes, untreated PCR products (left) and those treated with heating and re-annealing were the same, further proving that the middle band was heteroduplex (Figure 1E).

In summary, we found a novel heterozygous deletion in c.441_1153del713 of MECP2 (NM_004992.4) in the proband. This mutation resulted in a frame shift of MECP2, and premature termination of the 487 amino acid protein at the 154th codon, creating a large loss from the methyl-CpG binding domain to the C-terminal domain.

DISCUSSION

Approximately 80–90% of classic Rett syndrome patients harbor mutations in the coding region of MECP2. Furthermore, ~70% of Rett syndrome-causing MECP2 mutations are C to T transitions at eight CpG dinucleotide mutation hotspots located within exons 3 and 4, and small deletions in the 3’ end of exon 4 comprise ~10% of all mutated alleles (4, 15). Routine PCR-based DNA sequencing usually does not detect these deletions.

MLPA is useful for detecting large deletions in a single gene, but small deletions or polymorphisms that might affect the binding of the MLPA probe, resulting in a false positive, have occurred in Rett syndrome diagnosis (4). In addition, the mosaic could not be detected using MLPA. Therefore, it is necessary to identify MLPA results using other detection methods, such as real-time quantitative PCR and long-range PCR. Combined with the MLPA results, real-time quantitative PCR can narrow down the region of deletion. Primer sites in the dizygous regions immediately flanking the breakpoints were selected for long-range PCR amplification across the deletion junction, and DNA sequencing was performed to determine the accurate deletion region.

Long-range PCR products with heterozygous deletion mutations usually showed two DNA bands after agarose gel electrophoresis. The proband in our study revealed three bands.
We first thought that the proband was a triple mosaicism for one normal MECP2 gene allele and two different deletions alleles in vivo, resulting in the formation of three DNA bands after PCR in vitro. We initially suspected that a double strand DNA break occurred in MECP2 during gene replication, and a variant of replication slippage occurred on both newly synthesized strands between the repeat motifs of microhomology, leading to the formation of the two different MECP2 gene deletions in vivo, the similar mechanism has been reported in the AR gene (15). If this was truth, the MECP2 deletions should be de novo mutations, occurring early in embryonic development of the proband. The probability of germline MECP2 mosaicism in the parents could be ruled out, there seemed be no need to perform prenatal diagnosis for the proband’s mother.

But by T-A cloning and comparing with the PCR product untreated and treated with heating and re-annealing, we found our previous assumption was wrong, the middle DNA band was proven to be heteroduplexes. Heteroduplexes are usually formed either during PCR cycling or by mixing of PCR product of mutant and wild-type DNAs in vitro, some large insertions or deletions may create stable heteroduplexes, and this stability is insensitive to variability in electrophoretic conditions (16). As far as we know, there is no evidence to show that a large number of heteroduplexes can exist in vivo. For the proband, the middle DNA band is just the heteroduplexes formed by PCR product in vitro, not in vivo. So the middle DNA band can’t inherit from her mother. But MECP2 deletion (bottom DNA band) of the proband really exists in vivo, and the deletion may not be de novo, it is probably inherited from the parent, who is germline mosaicism with MECP2 deletion.

Although Rett syndrome is usually sporadic, it is commonly accepted that pregnant women who have a family history of
Rett syndrome, should be offered an invasive prenatal diagnosis (17). Maternal and paternal MECP2 germline mosaicism have been both reported in Rett syndrome families. Recently, Zhang et al. indicated that the cohort level of paternal germline MECP2 mosaicism was 23.8% (5/21) by the detection of sperm (14). Meanwhile, maternal germline MECP2 mosaicism was also verified in many Rett syndrome families, despite lack of the direct detection (2, 12, 13). In the case, we didn’t find the MECP2 deletion in the blood of the parents, and not found the MECP2 mosaicism in the sperm of the proband’s father (data not show), but the maternal germline mosaicism can’t be ruled out. For the family, the recurrence risk of Rett syndrome still existed, it is necessary for the proband’s mother to perform prenatal diagnosis.

In summary, we reported a novel MECP2 heterozygous deletion in a Rett syndrome family. The proband was heterozygous for the deletion of 713-base pairs in exon 4 of MECP2, resulting in a frameshift and premature termination. To avoid recurrent risk, we performed prenatal diagnosis for her pregnant mother. Life is precious, which makes prenatal diagnosis a grave responsibility. It is necessary to obtain a clear molecular diagnosis, but each molecular diagnostic technique has its drawbacks. To avoid misdiagnosis, we performed prenatal diagnosis with multiple methods. In the case, we reported a novel heterozygous deletion in the MECP2 gene with heteroduplexes of the PCR product in vitro, and provided a reference for the prenatal diagnosis of genetic diseases with complicated mutations, not limited to Rett syndrome.

DATA AVAILABILITY STATEMENT
The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

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ETHICS STATEMENT
The studies involving human participants were reviewed and approved by the Ethics Committee of Women’s Hospital affiliated to the Zhejiang University School of Medicine. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)’ legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS
HZ, YS, YZ, JH, and MZ conducted experiments. YZ prepared the figures. YS and JH performed MLPA and PCR. MZ recruited samples. HZ and YZ wrote the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL
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