



CLINICAL REPORT

MeCP2_e2 partially compensates for lack of MeCP2_e1: A male case of Rett syndrome

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Email: takeguchi5p@asahikawa-med.ac.jp**Abstract****Background:** Rett syndrome (RTT) is a neurodevelopmental disorder that predominantly affects girls. Its causative gene is the X-linked *MECP2* encoding the methyl-CpG-binding protein 2 (MeCP2). The gene comprises four exons and generates two isoforms, namely *MECP2_e1* and *MECP2_e2*. However, it remains unclear whether both MeCP2 isoforms have similar function in the brain.**Methods:** We report a case of a boy with typical RTT. Male cases with *MECP2* variants have been considered inviable, but somatic mosaicism of the variants can cause RTT in males. Whole-exome sequencing was performed to search for the genetic background.**Results:** A novel nonsense and mosaic variant was identified in exon 1 of *MECP2*, and the variant allele fraction (VAF) was 28%. Our patient had the same level of VAF as that in reported male cases with mosaic variants in *MECP2* exon 3 or 4, but manifested RTT symptoms that were milder in severity compared to those in these patients.**Conclusion:** This is probably because the variants in *MECP2* exon 3 or 4 disrupt both isoforms of MeCP2, whereas the variant in exon 1, as presented in this study, disrupts only MeCP2_e1 but not MeCP2_e2. Therefore, our findings indicate that MeCP2_e2 may partially compensate for a deficiency in MeCP2_e1.**KEYWORDS**

male with Rett syndrome, MeCP2 isoform, MeCP2_e1, MeCP2_e2, somatic mosaicism

1 | INTRODUCTION

Rett syndrome (RTT) is a neurodevelopmental disorder primarily occurring in girls. It is caused by a loss-of-function variant in one copy of the X-linked gene *MECP2* (OMIM #300005) that encodes methyl-CpG-binding protein 2 (MeCP2). In females with typical RTT due to random

X-chromosome inactivation (XCI), approximately 50% of the cells express the variant *MECP2* and the other half express the wild-type *MECP2*. Males manifesting the symptoms of typical RTT also have an *MECP2* variant that is found in females with typical RTT. These males have either an extra X-chromosome (Klinefelter syndrome) or somatic mosaicism of the variant (Kleefstra et al., 2004; Schonewolf-Greulich

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et al., 2019; Schwartzman, Bernardino, Nishimura, Gomes, & Zatz, 2001; Topcu et al., 2002; Villard, 2007; Zhang et al., 2019). Evidence can be obtained from male RTT patients with somatic mosaicism of the *MECP2* variant to better understand the relationship between variant allele fractions (VAFs) and the clinical severity of RTT, because the effect of XCI on the phenotype does not need to be considered in male cases.

MECP2 comprises four exons and generates two isoforms: *MECP2_e1* and *MECP2_e2* as a result of the alternative splicing of exon 2. MeCP2_e1 is translated from a start site in exon 1, and exon 2 is skipped through alternative splicing, whereas MeCP2_e2 is translated from a start site in exon 2 (Mnatzakanian et al., 2004). In this study, we sought to investigate the case of a male RTT patient mosaic for nonsense variant in exon 1 of *MECP2* that disrupts only MeCP2_e1 but not MeCP2_e2. To examine whether MeCP2_e2 can ameliorate some neurological symptoms due to the affected MeCP2_e1 functions, we compared the clinical severity of the present case with those of other reported male cases carrying the mosaic variants that affect both MeCP2_e1 and MeCP2_e2.

2 | MATERIALS AND METHODS

2.1 | Patient background and informed consent

The patient was a young boy with typical RTT phenotype who fulfilled the diagnostic criteria for the disorder (Neul et al., 2010). He and his parents gave informed consent to participate in this study. The experimental protocols were approved by the Committee for Ethical issues at Asahikawa Medical University.

2.2 | Mutation analysis of the *MECP2*

For Sanger sequencing, their DNA was used as the template for polymerase chain reaction (PCR). Appropriate primers were used to yield DNA fragments spanning the entire *MECP2* coding region and the intron–exon boundaries (Takahashi et al., 2008). The PCR fragments were analyzed using automated sequencing. Whole-exome sequencing of the DNA was performed on a HiSeq2000 sequencer (Illumina) with 101 bp paired end reads and 6 bp index reads. Exome data processing, variant calling, and variant annotation were performed as previously described (Itoh et al., 2018). To confirm the variant identified in the Sanger and whole-exome sequencing, the DNA fragment encompassing the variation site was amplified by PCR using the primers 5'-CATCACAGCCAATGACGGGC-3' (forward) and

5'-CATCCGCCAGCCGTGTCGTC-3' (reverse), and it was subsequently digested with restriction endonuclease Dde I. The reaction products were then visualized through ethidium bromide staining after electrophoresis on a 2% agarose gel.

2.3 | RNA isolation and RT-PCR

To examine the expression levels of *MECP2_e1* and *MECP2_e2* isoforms, total RNA was extracted from the peripheral blood cells using the PAXgene Blood RNA Kit (QIAGEN GmbH). Reverse transcription (RT) was performed using the SuperScript First-Strand Synthesis System (Invitrogen Corporation) for generation of cDNA using 1 µg of total RNA in a 20 µl reaction. Primers were designed for simultaneous amplification of both isoforms: a forward primer in exon 1 (exon1F, 5'-GAGAGGGCTGTGGTAAAAGC-3') and a reverse primer in exon 3 (exon3R, 5'-GATGGAGCGCCGCTGTTTGG-3'), which generated a 328-bp product for *MECP2_e1* and a 452-bp product for *MECP2_e2*. As an internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as described (Itoh et al., 2012). The PCR products were visualized by ethidium bromide staining, following electrophoresis on 2% agarose gels. The optical densities of the bands were quantified using an image analysis system and ImageJ software (National Institutes of Health; Bethesda, MD). The obtained PCR products were purified from an agarose gel and directly sequenced on an ABI 3130 Genetic Analyzer (Applied Biosystems).

3 | RESULTS

3.1 | Case report

The 9-year-old male patient was born after 39 weeks of uneventful pregnancy without asphyxia. His birth weight and head circumference were 2,865 g (−0.7 SD) and 33.0 cm (−0.4 SD), respectively. He acquired head control at 3 months of age, walked alone and spoke meaningful words at 9 months, and had no apparent developmental delay until he was about 2 years old. Thereafter, his development stagnated, and it was followed by a period of regression. He became less interested in his toys and hardly had any speech. At 3 years and 6 months, he was diagnosed with autism spectrum disorder and intellectual disability. At 4 years, his purposeful hand skills began to regress, and stereotypic movements such as hand wringing appeared. At 6 years, he lost his minimal spoken language, his gait became unsteady and wide based, and his head circumference was only 47.8 cm (−2.2 SD), making his postnatal microcephaly more evident. He developed epilepsy at 7 years, but his seizures were eventually controlled following treatment with carbamazepine and topiramate. The chromosomal

analysis revealed normal 46,XY karyotype. No abnormal findings were observed in brain MRI and various tests related to congenital metabolic disorders.

3.2 | Molecular studies

We performed the Sanger sequencing of *MECP2* for genetic diagnosis, but the initial analysis failed to identify the pathogenic variant of this gene. Subsequently, we performed whole-exome sequencing and identified a novel nonsense and mosaic variant in exon 1 of *MECP2*, NM_001110792.1: c.31G>T; p.(Gly11*). Deep sequencing confirmed the presence of the T allele uniquely in the patient sample at a ratio of 25 mutant T allele reads to 63 G allele reads, a VAF of 28%. The reexamination of the *MECP2* variant using Sanger sequencing confirmed the wild-type sequence, with only a small amount of the variant allele, suggesting somatic mosaicism (Figure 1a). This nonsense variant created a new Dde I restriction site. Consequently, PCR-restriction digestion analysis of the DNA obtained from the patient and his parents revealed novel fragments in addition to the estimated wild-type fragment only in the patient sample, further confirming that the mosaic variant occurred de novo (Figure 1b). RT-PCR results revealed that the variant did not

affect the expression levels of both *MECP2_e1* and *MECP2_e2* (Figure 2a). Analysis of cDNA showed the presence of an abnormal transcript with the nonsense variant of exon 1 in a mosaic state (Figure 2b).

3.3 | Compensatory role of MeCP2_e2 for lack of MeCP2_e1

The nonsense variant identified in the present case affected the coding sequence of *MECP2_e1* but not of *MECP2_e2*. Based on this finding, we further examined whether MeCP2_e2 is able to ameliorate the affected MeCP2_e1 function by comparing the clinical severity of the present case with those of six previously reported RTT males carrying *MECP2* mosaic variants that affect both MeCP2_e1 and MeCP2_e2 (Table 1). This comparison revealed that the median VAFs and the age at onset of regression of the reported male patients were 25% (range 9–36) and 13 months (range 8–18), respectively, whereas in the present case with 28% VAF, the developmental problems were not noticed until the patient was 2 years old. At 9 years, he was already able to walk independently, although his balance was poor. Among the six previously reported cases, only three were able to walk with or without support.

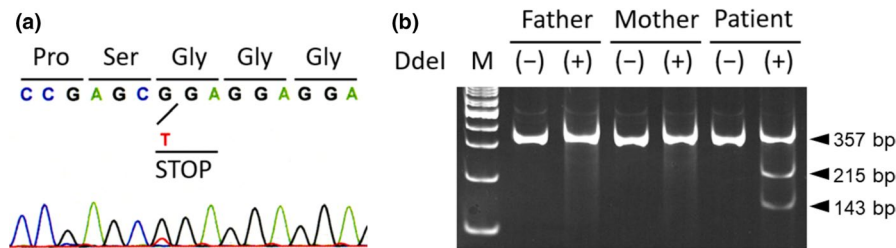


FIGURE 1 A novel mosaic variant of *MECP2* in a male patient with typical Rett syndrome. Electropherogram shows the nonsense variant in exon 1 of *MECP2* [NM_001110792.1: c.31G>T; p.(Gly11*)] in mosaicism (a). Dde I digestion of the PCR product encompassing the variation site shows additional fragments (143 and 215 bp), which resulted from the G-to-T transition creating a new Dde I restriction site in the patient but not in his parents (b). These additional fragments are observed together with the 358 bp wild-type fragment, confirming the mosaic variant in the patient

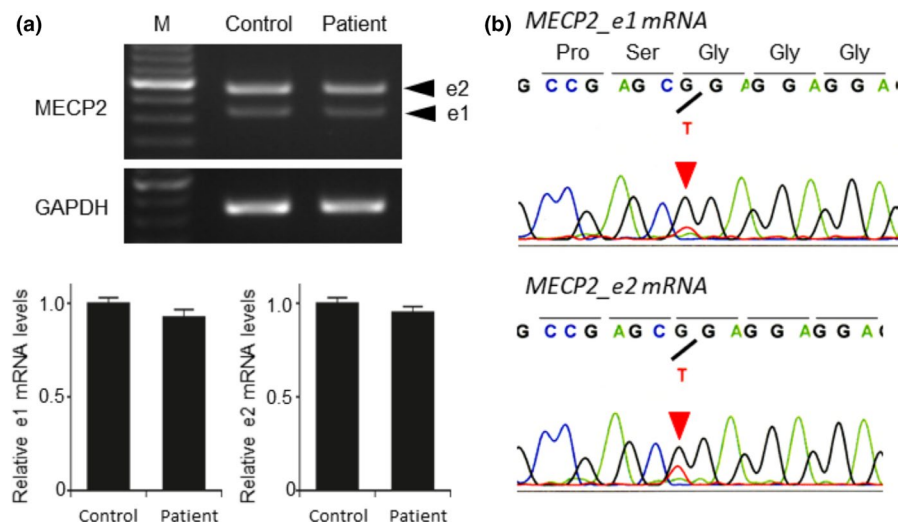


FIGURE 2 Equal amounts of *MECP2_e1* and *MECP2_e2* mRNA and abnormal transcript with the nonsense variant in a mosaic state. RT-PCR results reveal that both *MECP2_e1* and *MECP2_e2* mRNA amounts are unaffected in the patient. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (a). Sequencing analysis of the obtained PCR products shows the presence of abnormal transcript with the nonsense variant in a mosaic state (b)

TABLE 1 Relationship between variant allele fractions and the clinical severity in males with typical Rett syndrome associated with mosaic *MECP2* variants

Age at evaluation (Authors)	Variants			Onset of regression	Hand skills	Language	Gait	Stereotypy
	Nucleotide change	Predicted effect on protein sequence	VAFs ^a					
9 years (Present case)	c.31G>T	p.(Gly11*)	28%	24 months	Poor	Lost	Poor	Present
12 years (Topcu et al., 2002)	c.808C>T	p.(Arg270*)	36%	11 months	Lost	Never	Never	Present
11 years (Kleefstra et al., 2004)	c.473G>T	p.(Thr158Met)	25%	13 months	Lost	Lost	Lost	Present
2 years (Zhang et al., 2019)	c.316C>T	p.(Arg106Trp)	26%	18 months	Never	Never	Never	Present
2 years (Zhang et al., 2019)	c.353G>A	p.(Gly118Val)	20%	13 months	Poor	Lost	Poor	Present
8 years (Schonewolf-Greulich et al., 2019)	c.1308dupT	p.(Gln437Serfs*50)	9% (15%)	18 months	Poor	Lost	Poor	Present
9 years (Schonewolf-Greulich et al., 2019)	c.808C>T	p.(Arg270*)	36% (45%)	8 months	Lost	Never	Poor	Present

^aVAFs, variant allele fractions in blood lymphocytes (in fibroblasts).

4 | DISCUSSION

We present clinical and molecular findings in a male RTT mosaic for a novel nonsense variant of *MECP2*. Deep sequencing with a next-generation sequencing method revealed the small amount of the variant allele c.31G>T; p.(Gly11*) with 28% VAF, which the initial Sanger sequencing failed to identify. The PCR-restriction digestion analysis further confirmed the somatic mosaicism of the variant. RT-PCR results revealed that both *MECP2_e1* and *MECP2_e2* mRNA amounts were unaffected in the patient, suggesting that the nonsense variant in exon 1 of *MECP2* likely escapes nonsense-mediated mRNA decay (NMD) and does not affect transcription of *MECP2_e2*. The level of sensitivity of a premature-termination codon (PTC) - containing mRNA to NMD is multifactorial. It has been shown that mRNAs carrying PTCs in close proximity to the translation initiation AUG codon escape NMD, called the “AUG-proximity effect” (Silva, Ribeiro, Inacio, Liebhaber, & Romao, 2008). Consequently, this variant may lead to translational reinitiation at a downstream AUG codon producing an N-terminally truncated protein functionally distinct from wild-type MeCP2_e1, but does not affect the translation of *MECP2_e2*.

The majority of the RTT-associated *MECP2* variants are located in exons 3 and 4, which simultaneously disrupt both the MeCP2_e1 and MeCP2_e2 isoforms. RTT associated with exon 1 variants of *MECP2* is rare, and its detection rate is 8.1% in patients with typical or atypical RTT (Saunders, Minassian, Chow, Zhao, & Vincent, 2009). However, exon 2 variants that exclusively affect MeCP2_e2 have never been identified in RTT, suggesting that MeCP2_e2 does not have

an essential function in the brain. Supporting this notion, a mouse model with a deletion in *MECP2* exon 2 failed to recapitulate the neurologic symptoms characteristic of RTT (Itoh et al., 2012). In this study, we presented the nonsense variant in exon 1 of *MECP2* that disrupted MeCP2_e1 but not MeCP2_e2. Notably, however, we examined the variant using DNA extracted from peripheral blood leukocytes, so that it is uncertain whether the VAF in brain will be equivalent to that observed in the present study. Nonetheless, this study demonstrated that a male carrying an *MECP2* exon 1 mosaic variant and expressing 28% less MeCP2_e1 in blood than normal individuals exhibited the typical RTT phenotype, indicating that even a mild reduction of MeCP2_e1 is sufficient to cause RTT.

In a previous study using the isoform-specific knockout mice in which MeCP2_e1 was lacking while the expression of MeCP2_e2 was preserved, the neurologic deficits of RTT were recapitulated (Yasui et al., 2014). The study implied that an RTT phenotype may occur even in the presence of MeCP2_e2, which is unable to compensate for the lack of MeCP2_e1. Nevertheless, recent animal studies have revealed the partial rescue of Rett-like symptoms in MeCP2-null mice through the reexpression of MeCP2_e2 (Jugloff et al., 2008; Kerr et al., 2012). However, there had been no clinical evidence whether MeCP2_e2 is able to ameliorate some neurological symptoms due to the affected MeCP2_e1 functions. Genotype-phenotype correlations are difficult to make in female RTT patients because of the differences in XCI. Examination of male RTT patients with somatic mosaicism of the *MECP2* variant allowed us to assess the relationship between VAFs and clinical severity. Comparison of the clinical severity of the present case involving the *MECP2_e1*-specific variant

with those of other reported male cases with mosaic variants that affect both MeCP2_e1 and MeCP2_e2 revealed that the present case had a milder phenotype even though the VAFs were almost the same (Table 1). In conclusion, this study is the first to present clinical and molecular evidence that MeCP2_e2 may partially compensate for the deficiency of MeCP2_e1, although further functional studies are needed.

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CONFLICT OF INTEREST

None of the authors declare any conflict of interest related to this study.

AUTHOR CONTRIBUTIONS

RT and ST conceived and planned the study, and drafted the manuscript. MI performed whole-exome sequencing. RT, ST, MK, RT, and NS performed genetic analysis. YT, YL, and NS acquired clinical phenotype data. All authors provided important feedback on the analysis and manuscript, and approved the final version.

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