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Ten novel insertion/deletion variants in *MECP2* identified in Japanese patients with Rett syndrome

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Abstract
Rett syndrome (RTT) is an X-linked progressive and severe neurological disorder caused by mutations in the gene encoding methyl CpG binding protein 2 (*MECP2*). Among the 49 typical RTT patients examined, we identified 10 novel and eight known insertion/deletion variants, and 31 known pathogenic variants in *MECP2*. The pathogenic variants presented here should be a useful resource for examining the correlation between the genotypes and phenotypes of RTT.

Rett syndrome (RTT) is a progressive neurodevelopmental disorder that affects brain development and function in females, with a prevalence of one in 10,000 worldwide[1]. Typical RTT is caused by mutations in the gene encoding methyl-CpG binding protein 2 (*MECP2*)[2]. A database of a large collection of *MECP2* variants was established in 2002 (RettBASE: http://mecp2.chw.edu.au/index.shtml)[3]. To date, associations between clinical phenotypes and related genetic variants for *MECP2* as well as other RTT-associated genes, including *CDKL5* and *FOXG1*, are available.

Here, we report a total of 49 RTT patients with 10 novel insertion/deletion variants, eight known insertion/deletion variants and 31 known pathological variants.

All patients were diagnosed with typical RTT by Japanese child neurology experts according to the international diagnostic criteria for RTT. Clinical information and samples from the patient and parents were obtained with written informed consent. The study was approved by the ethical committee of NCNP. Genomic DNA was extracted from peripheral blood using a standard protocol. We first used the MLPA method (MRC-Holland, DL Amsterdam, The Netherlands) to identify the structural abnormalities in the *MECP2* locus. In the patients excluded for structural abnormalities, we amplified all coding exons of *MECP2* and their exon-intron boundaries by PCR and directly sequenced the PCR products using the Applied Biosystems 3730 DNA analyzer (Thermo Fisher, USA).

The insertion/deletion mutations were detected in 18 (36.7%) of 49 patients with RTT (Table 1). Among the 18 patients, 10 (55.6%) were considered novel by a comparison of our data with the known insertions/deletions deposited in the public databases, including RettBASE, gnomAD, Human Genome Mutation Database Professional 2019.2 and ClinVar. Representative data of pedigrees and sequences of the recombination breakpoints from three families are shown in Fig. 1. Patient 470 showed the insertion/deletion variant at c.1158_1258delinsCCGAGGGTGGCTCC. Patient 488 showed the deletion at c.1168_*539del. Patient 587 showed the insertion/deletion at c.1367_*791delinsCGC. Five (Patients 187, 470, 488, 559, and 587) had lost only exon 4. In addition, Patient 269 has a complex rearrangement with a 2609 bp deletion, including exon 3 and flanking introns, accompanied by two nucleotide substitution and a 25 bp deletion in exon4, c. [27-1707_c.378-206del; 1159_1160CC>AG; 1164_1188del], occurred in *cis*. The other five showed intragenic deletion involving exon 4 in the *MECP2* locus. Hardwick et al. (2007) reported that 12 out of 21 patients (57%)[4] experienced breakpoints within the "deletion-prone region (DPR)", which is characterized by short direct repeat elements and is also known as the hotspot for the smaller deletions[5,6]. In this study, the breakpoints in seven novel insertion/deletion variants (7/10: 70%) were located within the DPR. In the breakpoints of

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Table 1 Insertion/deletion variants in *MECP2* in Japanese patients with RTT

Family ID	Sex	Age (year:month)	Substitution		Exon/Intron deleted	DPR (1057–1209)	Deleted domain	Note
			Nucleotide	Amino acid				
187	F	3 years 2 months	c.816_819del	p.(Gly273Valfs*)	Exon 4		TRD	This study
269	F	3 years	c.[27–1707_c.378-206del; 1159_1160CC > AG; 1164_1188del]	p.[Pro387Ser; Pro388fs*]	Intron 2-Exon 4	Deleted	CTD	
289	F	2 years 11 months	c.378-375_c.1193del		Intron 3-Exon 4	Deleted	MBD, ID, TRD	
451	F	3 years	c.27–7899_c.1137del		Intron 2-Exon 4	Deleted	NTD, MBD, ID, TRD	
470	F	27 years 1 month	c.1158_1258delinsCCGA GGGTGGCTCC	p.(Pro387_Pro419delinsArgGlyTrpLeu)	Exon 4	Deleted	CTD	
487	F	36 years 11 months	c.27-?_378 + ?del	?	Intron 2-Exon 4		?	
488	F	2 years 4 months	c.1168_*539del	p.(Pro390_Ser486delinsValArgSerHisPro TrpTrpLeuLysSerGlyProThrProAlaProIleGlnAsn TrpGlnGlyArgPheThrGlyGlnGluSerGlyThr CysLeuLeuGlnLeuTrpHisGly)	Exon 4	Deleted	CTD	
500	F	2 years 1 month	c.27-?_378 + ?del	?	Intron 2-Exon 4		?	
559	F	2 years 1 month	c.1038_1195delinsAGCA	p.(Ser346Argfs*)	Exon 4	Deleted	CTD	
587	F	10 years 2 months	c.1367_*791delinsCGC	p.(Gly456_Ser486delinsAlaLeuGlyGlnGlyAla GlyArgLeuAlaTrpGlyGlnAlaGlyGlnSerThrAlaGly)	Exon 4	Deleted	CTD	
288	F	2 years 6 months	c.806del	p.(Gly269Alafs*)	Exon 4		TRD	Wan M et al. (1999)
376	F	4 years 2 months	c.47–57del	p.(Gly16Glufs*)	Exon 4		NTD	Mnatzakanian GN et al. (2004)
467	F	2 years 1 month	c.696del	p.(Lys233Argfs*)	Exon 4		TRD	Obata K et al. (2000)
497	F	1 year 1 month	c.710dupG	p.(Gly238Trpfs*)	Exon 4		TRD	Hoffbuhr K et al. (2001)
511	F	4 years	c.808del	p.(Arg270Glufs*)	Exon 4		TRD	Obata K et al. (2000)
539	F	5 years 6 months	c.1157_1200del	p.(Leu386fs*)	Exon 4	Deleted	CTD	RettBASE
555	F	3 years 2 months	c.1154_1197del	p.(Pro385Hisfs*)	Exon 4	Deleted	CTD	Bienvenu T et al. (2002)
572	F	1 year 9 months	c.710del	p.(Gly237fs*)	Exon 4		TRD	Amir RE et al. (2000)

NM_004992.3(MECP2_i001)
ID Interdomain, *CTD* C-terminal domain, *MBD* methyl CpG binding domain, *NTD* N-terminal domain, *TRD* transcriptional repression domain

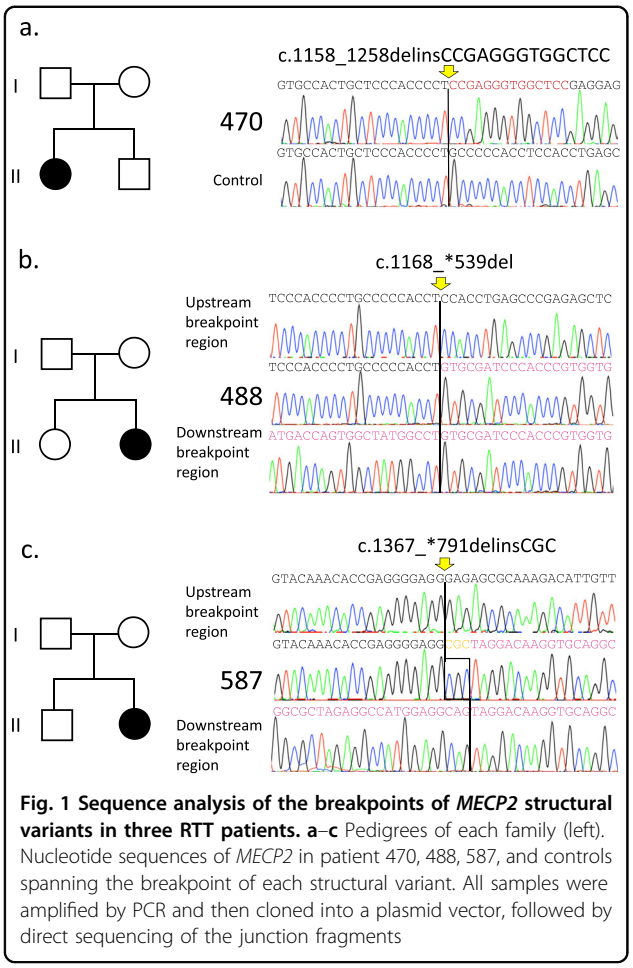

Fig. 1 Sequence analysis of the breakpoints of *MECP2* structural variants in three RTT patients. a–c Pedigrees of each family (left). Nucleotide sequences of *MECP2* in patient 470, 488, 587, and controls spanning the breakpoint of each structural variant. All samples were amplified by PCR and then cloned into a plasmid vector, followed by direct sequencing of the junction fragments

Patients 269, 289 and 451, no repetitive sequences were found adjacent to the breakpoint. These findings suggest that the de novo deletion events involving *MECP2* can be unique to families and that homology-mediated mechanisms are unlikely to be associated with these events.

In addition, we identified known pathogenic variants in 31 patients (Supplementary Table 1). No novel change was identified, suggesting that the molecular basis for recurrent de novo nucleotide substitutions in *MECP2* is common among the different populations.

The list of *MECP2* variants found in 49 Japanese patients with RTT should provide a useful resource to further examine the correlation between genotypes and disease phenotypes.

HGV Database

The relevant data from this Data Report are hosted at the Human Genome Variation Database at

https://doi.org/10.6084/m9.figshare.hgv.2648
https://doi.org/10.6084/m9.figshare.hgv.2651
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https://doi.org/10.6084/m9.figshare.hgv.2657
https://doi.org/10.6084/m9.figshare.hgv.2660
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https://doi.org/10.6084/m9.figshare.hgv.2705
https://doi.org/10.6084/m9.figshare.hgv.2708
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Conflict of interest

The authors declare that they have no conflict of interest.

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