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The role of different X-inactivation pattern on the variable clinical phenotype with Rett syndrome

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Abstract

A gene for Methyl-CpG binding protein 2 (*MECP2*), which locates Xq28, was recently found to be responsible for Rett syndrome. Although mutational analyses of *MECP2* in Rett syndrome have been extensively analyzed, the mechanism(s) by which variable clinical phenotype occurred between affected monozygotic twins or sisters have not been clarified. We hypothesized that the difference of X-inactivation pattern might explain this phenomenon. With the method based on methylation-specific PCR, we analyzed polymorphic trinucleotide repeat in the human androgen receptor gene mapped on Xq11.2-12, using DNA samples derived from previously described monozygotic twins and sisters together with their parents. Their clinical phenotypes were reported to be significantly different between siblings. We found that 1) maternally derived allele is predominantly active than paternally derived one in three out of four patients analyzed, 2) remaining one twin patient, whose ratio of active paternal allele is almost the same level as maternal allele, showed far much severe phenotype when compared with her counterpart. Together with the finding that most of the alleles with de novo mutation are from paternal X chromosome in sporadic cases, the existence of a mechanism that suppresses mutated paternal allele activation, resulting skewed X-inactivation to make clinical phenotype milder, might be speculated. Thus, when this mechanism fails to work sufficiently by an unknown reason, severer clinical phenotype could occur.

1. Introduction

Recently, a gene encoding methyl-CpG binding protein 2 (*MECP2*) have been determined to be responsible for Rett syndrome (RTT), possibly an X-linked dominant male lethal disorder [1]. Since then, mutational analysis of the gene in RTT has been reported extensively. Cheadle et al. [2] claimed the existence of a correlation of disease severity with mutation type and location. This correlation was supported by other reports. However, we have previously described female monozygotic twins [3] and sisters [4] with RTT, whose clinical phenotype are discrepant between siblings, indicating not only mutation but also some other factor(s) must modify clinical phenotypes. In fact, Amir et al. [5] claimed the importance of X chromosome inactivation on RTT phenotypes. We hypothesized that the difference of X-chromosome inactivation pattern could have some influence on severity. Here, we describe the data of mutational analysis of *MECP2* of the patients, and thus, inactivation pattern analysis in all four patients and their parents. We found in the monozygotic twins, severity of symptoms seems to depend on the ratio of inactivation of paternally derived X chromosome; symptoms tended to be severe when ratio of active paternally derived X chromosome increased.

2. Materials and methods

2.1. Patients

Japanese monozygotic twins and sisters with RTT analyzed in this report were previously described (Fig. 1) [3-4]. In the monozygotic twins, twin B is clinically severer than twin A as described in Ogawa et al. [3]. At present, they are 32 years old, the twin B became unable to walk since two years ago, on the other hand, the twin A

can walk with other's support. In the sisters, clinical symptoms in the younger one is far severer than the elder one [4]; the younger one has never walked and has seizures since 5 years old, on the other hand, the elder one has started walking since at age of 12 months and has no seizures.

2.2. DNA sequencing and analysis of X-inactivation status

Sequencing exons of *MECP2* was performed with amplified DNA as templates with ABI 310. X-chromosome inactivation pattern was analyzed with the method based on methylation-specific PCR described by Kubota et al. [6]. We employed trinucleotide repeat polymorphism located in the first exon of the human androgen receptor gene (*HUMARA*), mapped Xq11.2-12, to study X-chromosome inactivation of each allele. As all individuals analyzed were revealed to be heterozygous for this polymorphism, we can determine parental origin of each allele. The ratio of maternally derived allele versus paternally derived allele was calculated using equation described in Kubota et al. [6].

3. Results

3.1. Mutations in *MECP2*

Sequencing analysis of the twin patients revealed C to T substitution at nucleotide number 880, leading 294th arginine to stop codon (R294X). Also, G to A substitution at nucleotide number 317 was found in both sisters, that changes 106th arginine to glutamine (R106Q) (Fig. 1).

3.2. X-chromosome inactivation status

In the twin patients, inactivation pattern was totally different each other. In the twin A, most of the active allele are derived from her mother (88%), on the other hand, in twin

B, the ratio of active maternal allele was 52%, similar level as paternal allele (Fig. 2). However, in the sister cases, the ratio of active maternal allele are almost similar level (82% versus 80%) (Fig. 3).

4. Discussion

Our data of monozygotic twins with RTT, whose clinical phenotype were significantly different, show the possibility that different X-inactivation pattern could have some influence to clinical phenotype. They must have the same genomic component including the same *MECP2* mutation (R294X). Thus, the cause of significant amount of discrepancy of clinical phenotype cannot attribute to genomic factor nor mutation type. Epigenetic factor could be another possible mechanism. In this report, we analyzed X-inactivation pattern and found the more active paternal allele exists in twin B with severer phenotype. Our finding in the monozygotic twins with RTT is consistent with the data reported by Amir et al. [5], who analyzed correlation between *MECP2* mutation and X chromosome inactivation pattern. Recently, parental origin of *MECP2* mutations in sporadic cases with RTT is studied [7-8]. They found a high predominance of mutations of paternal origin. Together with this finding, possibility that *MECP2* mutation in monozygotic twins occurred on paternal allele is high. If a mechanism that suppress mutated paternal allele activation exists to make clinical phenotype milder, severer phenotype in twin B might be able to explain by the failure of this mechanism. On the other hand, we could not find significant difference in X-inactivation pattern in sister cases. The fact that both mutation and X-inactivation pattern have no significant difference, other unknown genomic factor(s) have to be considered to explain the phenotypic difference. As shown in Fig. 3, the allele of

healthy brother is the same as those of sister's, possibly indicating that maternal allele has no *MECP2* mutation. In this family, paternal germ-line mosaicism of the G317A mutation is the most plausible cause for recurrence of the same mutation. Detection of paternal sperm for the mutation as a mosaic form could further clarify the pathogenesis of RTT.

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Figure legends

Fig. 1

a. Cases of monozygotic twins. Twin B showed severer clinical phenotype than twin A. Both twins have the same nonsense mutation of *MECP2* gene, R294X, which locates on the transcription repression domain.

b. Cases of sisters. The younger sister showed severer symptoms when compared with her elder sister. Both sisters have the same missense mutation, R106Q, which locates on the methyl-CpG-binding domain.

Fig. 2

Result of the X-chromosome inactivation pattern assay in monozygotic twins. The ratio of the active allele is maternally derived allele versus paternally derived allele.

Fig. 3

Result of the X-chromosome inactivation pattern assay in sisters. The ratio of the active allele is maternally derived allele versus paternally derived allele.

Fig. 1

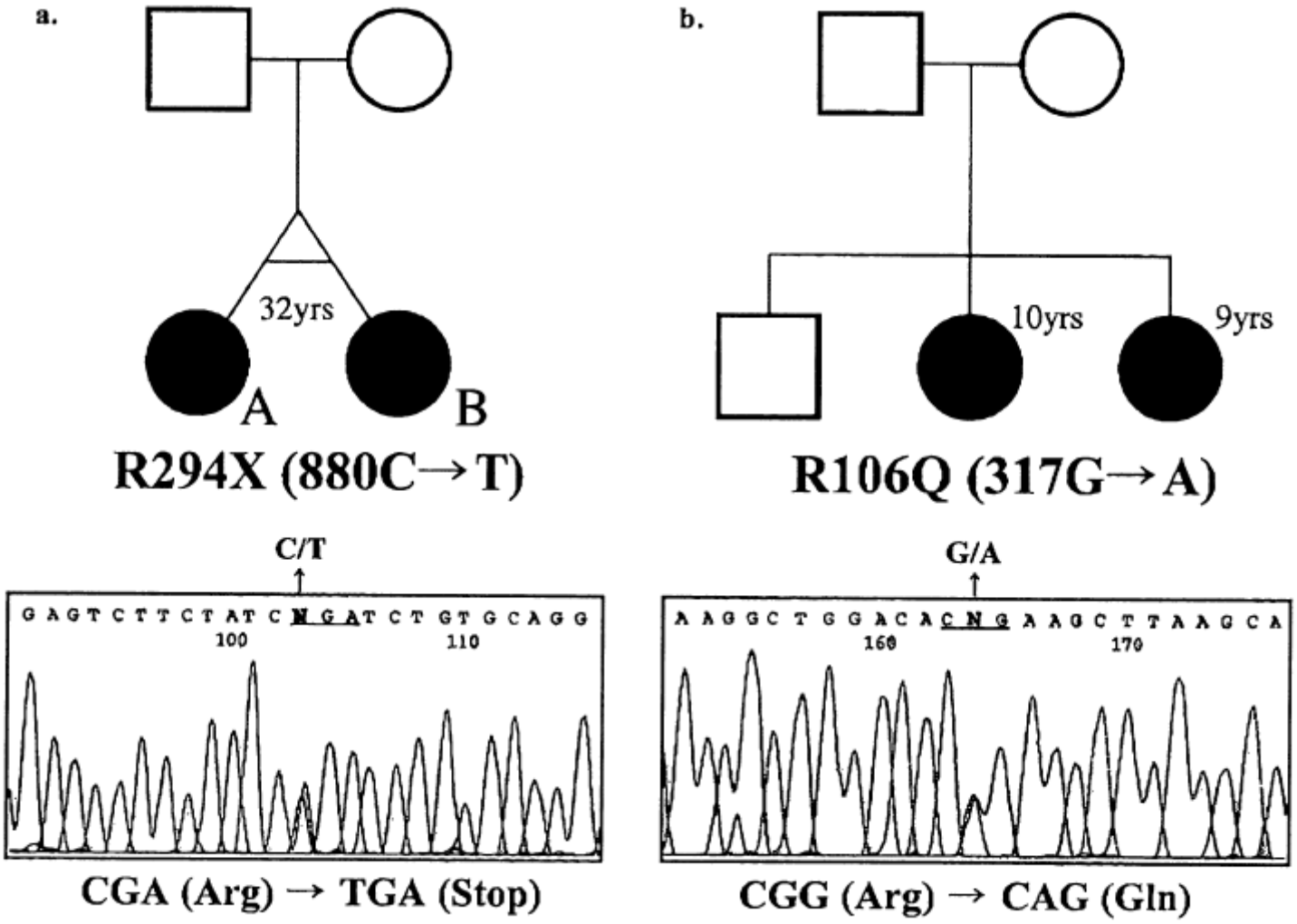

a.
32yrs
A
B
R294X (880C→T)
C/T
G A G T C T T C T A T C N G A T C T G T G C A G G
100
110
CGA (Arg) → TGA (Stop)
b.
10yrs
9yrs
R106Q (317G→A)
G/A
A A G G C T G G A C A C N G A A G C T T A A G C A
160
170
CGG (Arg) → CAG (Gln)

Fig. 2

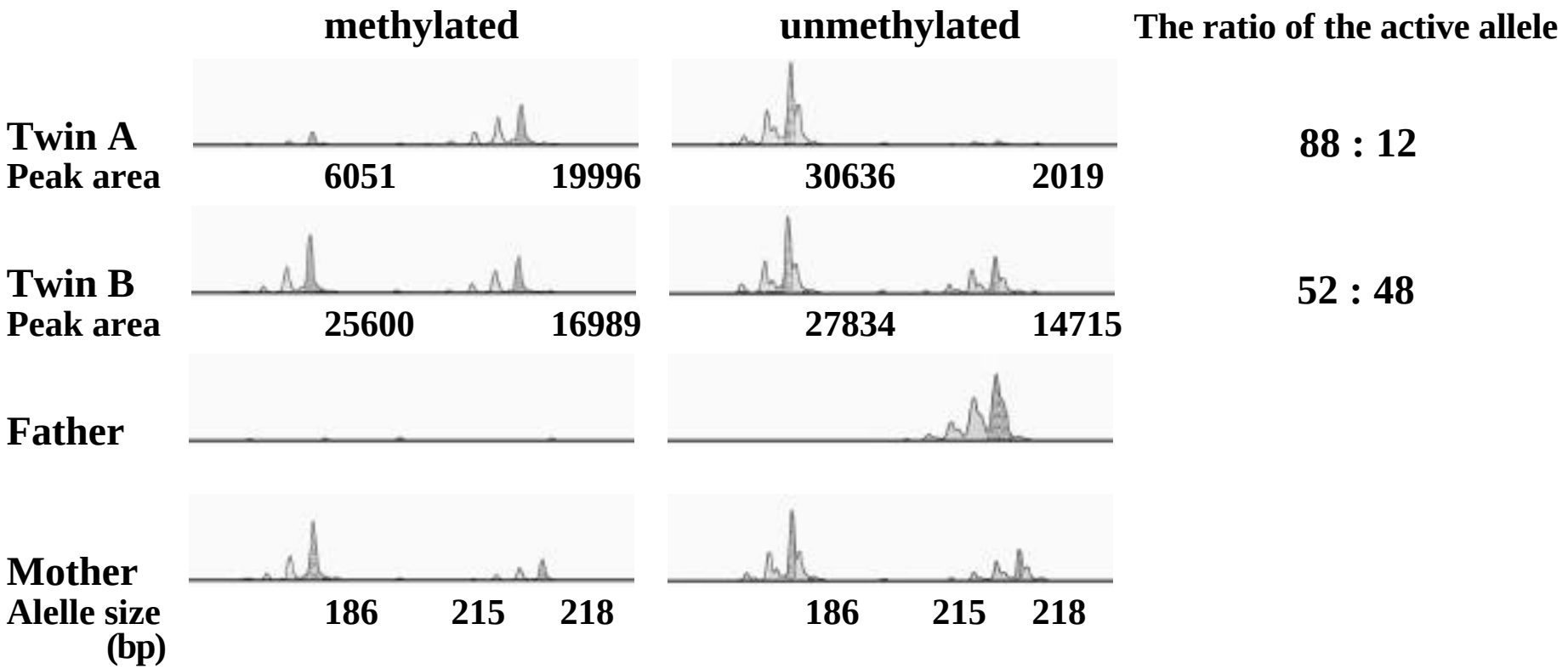
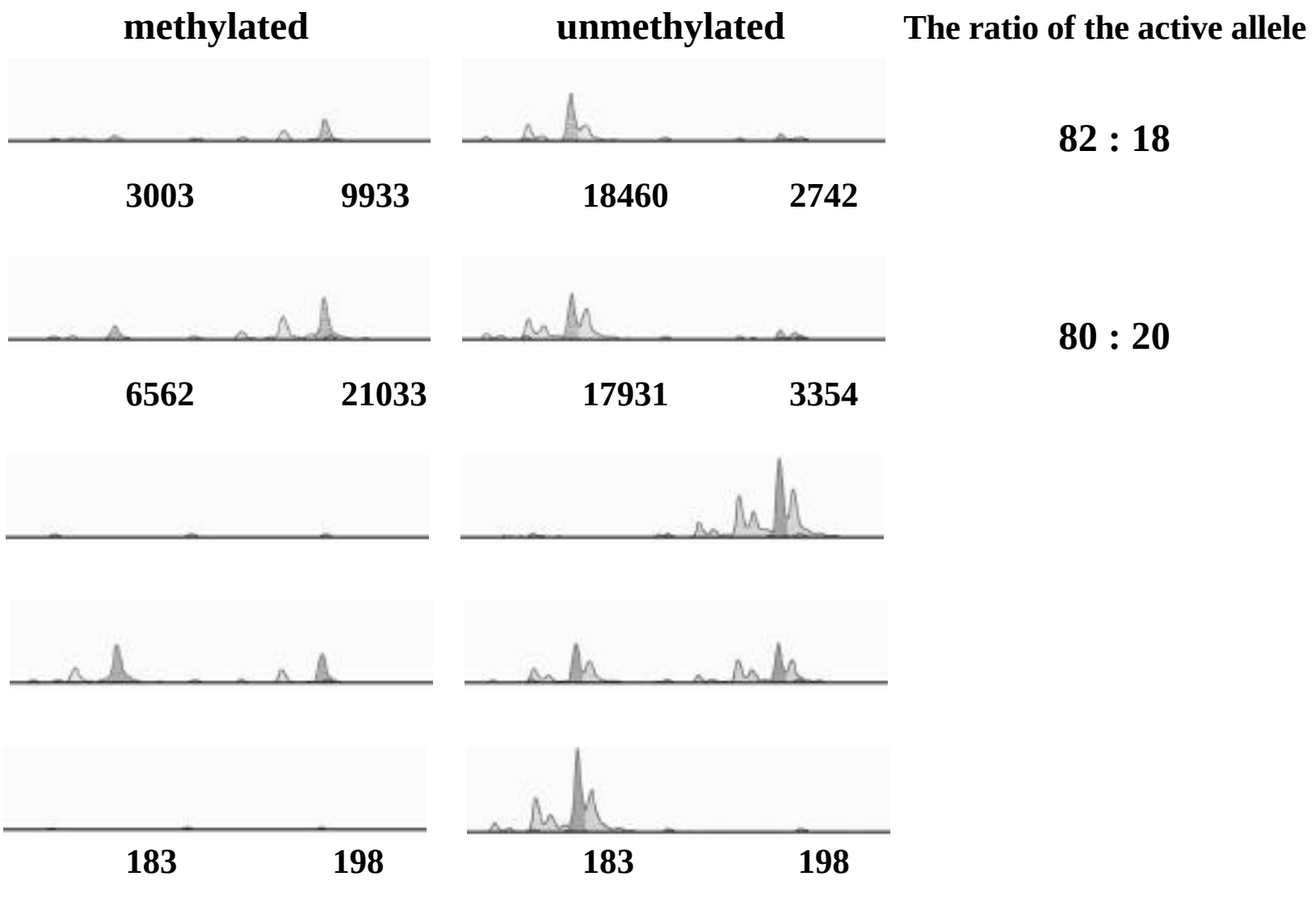

methylated
unmethylated
The ratio of the active allele
Twin A
Peak area
6051
19996
30636
2019
88 : 12
Twin B
Peak area
25600
16989
27834
14715
52 : 48
Father
Mother
Alelle size (bp)
186
215
218
186
215
218

Fig. 3


methylated
unmethylated
The ratio of the active allele
Elder sister
82 : 18
Peak area
3003
9933
18460
2742
Younger sister
80 : 20
Peak area
6562
21033
17931
3354
Father
Mother
Brother
Alelle size (bp)
183
198
183
198