Short Report

Skewed X chromosome inactivation failed to explain the normal phenotype of a carrier female with MECP2 mutation resulting in Rett syndrome


Mutations in the X-linked MECP2 gene cause Rett syndrome, a neurodevelopmental disorder that exclusively affects girls. Females with the MECP2 mutations exhibit a broad spectrum of clinical presentations ranging from classical Rett syndrome to asymptomatic carriers, which can be explained by differences in X chromosome inactivation (XCI). Here, we report a family with a girl with Rett syndrome in whom a novel missense mutation in the MECP2 gene was transmitted through the maternal germ line. The carrier mother was asymptomatic and presented non-random XCI in the peripheral blood cells, which resulted in the X chromosome harboring the mutant allele that was predominantly active. Thus, the presence of non-random XCI in the peripheral blood cells did not provide an explanation for the normal phenotype of the carrier mother. This result suggests that mechanisms other than XCI may contribute to the phenotypic heterogeneity associated with MECP2 mutations.

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Rett syndrome is a neurodevelopmental disorder that exclusively affects girls. Currently, it is considered an X-linked dominant disorder because of mutations in the MECP2 gene encoding methyl-CpG binding protein 2 (MECP2) (1). Most cases of Rett syndrome are sporadic (approximately 99.5% of all cases), with the majority being caused by a de novo mutation in the paternal copy of the MECP2 gene (2). Simultaneous incidence of Rett syndrome in sisters has been reported in families with mothers who are either germ-line mosaics for the MECP2 mutation or heterozygotes with favorably skewed X chromosome inactivation (XCI) patterns (1, 3, 4). Thus, with regard to females with MECP2 mutations, a broad spectrum of clinical presentations ranging from classical Rett syndrome to asymptomatic carriers has been explained by differences in the XCI pattern. However, such an idea was not supported by the presence of asymptomatic carrier females exhibiting a random XCI pattern (5). Here, we report a family with a girl with Rett syndrome; the MECP2 mutation was inherited through the maternal germ line. The obligate carrier mother was asymptomatic and possessed a non-random XCI pattern, which resulted in the X chromosome harboring the mutant allele that was predominantly active. This suggests that mechanisms other than XCI may play a role in modifying the clinical phenotype associated with the MECP2 mutation.
Materials and methods

Case report

The patient, a girl aged 1 year and 6 months, was the first child of healthy unrelated parents. The patient was born at full term after an uneventful pregnancy. Her weight was 3146 g with a head circumference (34.0 cm) at the 75th percentile. Her parents were of normal intelligence. The mother did not present any of the clinical symptoms observed in Rett syndrome. Early motor development of the patient was normal; she was able to hold her head at 4 months and could sit unaided at 7 months. Developmental abnormalities were first noted at the age of 10 months when she showed inconsolable crying and stopped smiling. The parents also noticed that her face lacked expression. At 11 months of age, she lost her ability to sit unaided and became less interested in her toys. Physical examination showed hypotonia, strabismus, and intention tremor of her upper limbs. Her head circumference at the age of 18 months was 43.7 cm (third percentile), indicating that microcephaly appeared postnatally. At 13 months of age, she developed her first seizures characterized by tonic movement of the upper limbs and loss of consciousness. At that time, electroencephalography and brain magnetic resonance imaging did not reveal any abnormalities. Thus, she was diagnosed with Rett syndrome, which was further confirmed by a mutation in the MECP2 gene (Fig. 1). At this point, the parents requested genetic testing to assess the risk of having more affected children. The father and mother provided their informed consent for this study; all the studies were performed in compliance with the guidelines of Asahikawa Medical College.

Mutation analysis of the MECP2 gene

Genomic DNA was extracted from the peripheral blood leukocytes of the patient and her parents and used as the template for polymerase chain reaction (PCR). Appropriate primers were used to yield DNA fragments spanning the entire MECP2 coding region and intron–exon boundaries (1). The PCR fragments were analyzed using automated sequencing. To further confirm the mutation identified by direct DNA sequencing, the DNA fragment encompassing the mutation site was amplified by PCR using the following primers: forward, 5'-GGAAAAGCAGGGAAGAGAC-3', and reverse, 5'-TCGGGAAAGCTTGTGACGC-3'; subsequently, it was digested with restriction endonuclease Eag I. The reaction products were then visualized by ethidium bromide staining following electrophoresis on a 2% agarose gel.

X chromosome inactivation

XCI patterns were determined using the method of Allen (6), as previously described. Briefly, aliquots of DNA obtained from the peripheral blood leukocytes were digested overnight using the methylation-sensitive restriction endonuclease Hpa II. PCR was used to amplify 100 ng of either digested or undigested DNA using fluorescent PCR primers (7). The amplified PCR fragment contains an Hpa II site, as well as the highly polymorphic trinucleotide repeat of the androgen receptor gene. As the Hpa II sites on the inactive X chromosome are methylated, only this allele is amplified when the Hpa II-digested genomic DNA is used as the template. Further, because the methylation site is adjacent to the polymorphic trinucleotide repeat, the alleles amplified by PCR can be distinguished by their length, which allows determination of the relative ratio of XCI. The allele peak areas were analyzed using an ABI 310 automated sequencer and GENESCAN software (Applied Biosystems, Foster City, CA).

RNA isolation and RT-PCR

Total RNA was extracted from the peripheral blood cells using the PAXgene Blood RNA Kit (Qiagen GmbH, Hilden, Germany). Reverse transcription (RT) was performed using the SuperScript First-Strand Synthesis System (Invitrogen Corporation, Carlsbad, CA) for generation of cDNA using 1 μg of total RNA in a 20 μl reaction. To examine the expression level of the mutant MECP2, RT-PCR was first performed with the same primers used in the PCR-restriction digestion analysis of DNA; subsequently, the RT-PCR products were digested with restriction endonuclease Eag I. The reaction products were then separated on 2% agarose gels containing ethidium bromide. As an internal control, glyceraldehyde-3-phosphate dehydrogenase was used as described (8).

Results

Identification of a mutation in the MECP2 gene

A missense mutation, i.e. a G-to-A nucleotide change resulting in p.A447T, was detected in the girl with Rett syndrome and her obligate
mother (Fig. 1a). This missense mutation eliminated an Eag I restriction site. Consequently, PCR-restriction digestion analysis of DNA obtained from the patient and her mother revealed a novel fragment in addition to the estimated wild-type fragments, further confirming the presence of this heterozygous mutation (Fig. 1b). This additional fragment is observed together with the wild-type fragments (304 bp and 221 bp), confirming the heterozygous mutation. Amino acid residue at position 447 in MECP2 is conserved among different mammalian MECP2 (c).

Fig. 1. Maternal origin of a MECP2 mutation associated with Rett syndrome. Automated DNA sequencing using the polymerase chain reaction (PCR) product from the girl with Rett syndrome and her asymptomatic mother exhibits a G-to-A transition at nucleotide 1506 (c.1506G>A, numbering according to Genbank accession no. NM_004992) in exon 3 that results in an alanine-to-threonine substitution at amino acid position 447 (p.A447T), as indicated by the arrows (a). Eag I-digestion of the PCR product encompassing the mutation site shows an additional fragment (525 bp) in the patient and her mother, which resulted from the G-to-A transition eliminating an Eag I restriction site (b). This additional fragment is observed together with the wild-type fragments (304 bp and 221 bp), confirming the heterozygous mutation. Amino acid sequence alignments showed the changed amino acid (p.A447) is conserved among four different mammalian MECP2 including human, cat, rabbit and mouse (Fig. 1c).

Analysis of XCI

The mother was a carrier with the p.A447T mutation that affected her daughter. Non-random XCI has been implicated in reducing the severity of Rett syndrome phenotype because of preferential inactivation of the X chromosome that harbors the mutant allele (3, 9, 10). Therefore, we examined if a favorably skewed XCI pattern could explain the normal phenotype of the
carrier mother. In order to assess the XCI pattern of the mother and her affected daughter, we assayed the methylation status of the androgen receptor alleles (Fig. 2a). In the carrier mother, a non-random XCI pattern was discovered; the ratio of the two alleles was 75:25. However, the more active X chromosome was transmitted to her daughter with Rett syndrome. Thus, in the carrier mother, the X chromosome harboring the mutant allele was predominantly active, which was expected to lead to a severe disease phenotype. Therefore, the difference in the XCI patterns did not provide an explanation for the normal phenotype of this carrier mother. We could not determine the XCI pattern in the girl with Rett syndrome because she was homozygous at the androgen receptor locus.

Expression of mutant MECP2 in the carrier mother

A meiotic crossover may have taken place between two X chromosomes of the mother, which results in separation of the androgen receptor alleles from the MECP2 alleles.

To exclude this possibility, we examined the expression of mutant MECP2 in the peripheral blood cells of the carrier mother. Eag I-digestion of the RT-PCR product revealed the predominant expression of mutant MECP2 in the carrier mother (Fig. 2b). This result was compatible with that of the XCI assay at the androgen receptor locus.

Discussion

We described a family with a girl with Rett syndrome in whom a novel missense mutation in the MECP2 gene was transmitted through the maternal germ line. The carrier mother was clinically asymptomatic and presented non-random XCI, resulting in the X chromosome harboring the mutant allele that was predominantly active. The p.A447T mutation may be a rare variant that does not cause the disease. Although the changed amino acid is conserved among different mammalian MECP2, further functional studies may be required to make the association clear between this mutation and Rett syndrome. Our data revealed that the carrier mother expresses the mutated MECP2 in the peripheral blood cells. If this variant is a disease-causing mutation, the non-random XCI pattern in the peripheral blood cells does not provide an explanation for the normal phenotype of this carrier mother. Notably, the XCI patterns in the peripheral tissues do not necessarily reflect the status of the brain that would be most likely the major determinant of the phenotypic severity (11–13). Considering the possibility of tissue-specific XCI variation, this mother may have an inverted XCI

Fig. 2. Non-random X chromosome inactivation (XCI) resulting in predominant expression of mutant MECP2 in the carrier mother. Patterns of XCI were determined in the peripheral blood cells (a). The polymorphic repeated sequence at the androgen receptor locus was amplified by polymerase chain reaction (PCR) using the Hpa II undigested or digested genomic DNA as the template. After digestion with the methylation-sensitive restriction endonuclease Hpa II, a non-random pattern of XCI is observed in the asymptomatic carrier mother in whom the X chromosome harboring the mutant allele is predominantly active. The Rett syndrome patient is homozygous at the androgen receptor locus. The expression of mutant MECP2 was examined by RT-PCR on RNA extracted from the peripheral blood cells of each individual in this family (b). Eag I-digestion of the RT-PCR products reveals the predominant expression of mutant MECP2 (525 bp) in the peripheral blood cells of the carrier mother. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. Negative controls without reverse transcription for each PCR reaction were performed and showed no expression.
pattern in the brain that favors the expression of the normal allele. There is another possible explanation for the normal phenotype of this carrier mother. Other genes may influence the biological consequences of a mutation in the MECP2 gene. This idea is supported by the identification of identical MECP2 mutations that have been found in males with X-linked recessive mental retardation and in females with Rett syndrome and their asymptomatic mothers with a random XCI pattern (14). Thus, a different genetic background possibly modifies the clinical phenotype associated with the MECP2 mutation, although such a second gene remains to be identified. In conclusion, it is important for genetic counseling to note that non-random XCI in the peripheral blood cells does not necessarily provide an explanation for the normal phenotype of the carrier mothers of patients with MECP2 mutations.

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References