Case report

MECP2 mutant allele in a boy with Rett syndrome and his unaffected heterozygous mother

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Abstract

Rett syndrome is a severe neurodevelopmental disorder affecting principally females and characterized by a normal postnatal development followed by stagnation and regression of acquired skills. We report a 4-year-old boy with a Rett syndrome phenotype and his unaffected mother both carrying a 44 bp truncating deletion mutation (c.1158del44 or p.388X) in the MECP2 gene. The presence of a skewed X inactivation in the mother provides a possible explanation for the absence of penetrance. The finding of a MECP2 mutation in an unaffected female complicates genetic counseling and further confirms that it is essential to look for mutations in the mothers of all patients with MECP2 mutations.

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1. Introduction

Rett syndrome (RTT, MIM #312750) is a severe neurodevelopmental disorder affecting almost exclusively females and classically characterized by a normal postnatal development followed by stagnation and regression of acquired skills such as language and purposeful movement of the hands [1]. Mutations in the gene encoding methyl-CpG binding protein 2 (MECP2) were first identified in female RTT patients [2]. Recently MECP2 mutations to have been found in males presenting a wide phenotypic spectrum: some died in early infancy consequent to severe encephalopathies [3,4], others displayed developmental delay associated with seizures and neurological disorders [5–9], while others showed non-specific mental retardation [10].

We report a 4-year-old boy with a Rett syndrome phenotype and his unaffected mother both carrying a 44 bp truncating deletion mutation (c.1158del44 or p.388X) in the MECP2 gene. The significance of these results is discussed in the context of an emerging genetic and clinical understanding of MECP2 mutations in males.

2. Material and methods

2.1. Clinical description

The patient was born at term after a pregnancy marked by diminished foetal movements. Delivery and neonatal period were unremarkable; birth weight (2700 g) and OFC (31.5 cm) were below the 10th centile, while length (47 cm) was at the 10th centile; Apgar scores were 9/10/10. The parents were 24 (mother) and 26 years old (father), healthy, and of normal intelli-
gence. The mother did not present any of the clinical symptoms observed in Rett syndrome. There was no consanguinity.

At 4 months, the baby was placid and hypotonic. At 8 months convergent alternating strabismus and hypermetropia (+4.75 dioptres) were noted. Sitting was achieved at 10 months. At 15 months the boy could only stand with help and unstably. First words were said at 20 months. At this age, EEG, brain CT scan and blood karyotype did not reveal any abnormality.

At 26–27 months, the patient could only walk with the help of a frame, but could turn over and crawl unaided. He could pronounce very few words (“mama”, “baba”). Intention tremor of the upper limbs and saccadic eye movements were observed. Clinical examination revealed a mentally retarded, sociable, happily disposed boy; height and OFC were below the 3rd centile (80 and 46 cm respectively). There was marked brachycephaly, intermittent convergent strabismus, low-set anteverted ears, a prominent forehead, a prominent brow and hypertelorism (+4.75 dioptres) were noted. Seizures (up to 20 episodes/day) characterized by tonic upper limb movement were observed. At this time he also developed gastro-oesophageal reflux. EEG showed bilateral frontal paroxysmal discharges. At that time he also developed gastro-oesophageal reflux.

Clinical examination at 47 months revealed: height 90 cm, weight 12.3 kg, OFC 47 cm (all < P3), involuntarily stereotypic hand movements and frequent hand-to-mouth movements, intention tremor, permanent head nodding, intermittent convergent strabismus, multidirectional nystagmus, bruxism, absent speech with nonverbal sounds, spasticity of the lower limbs with hyperreflexia and a bilateral Babinski sign. Brain MRI was normal. At this time, the diagnosis of “male Rett syndrome” was considered and mutation analysis of the MECP2 gene performed.

2.2. Mutation analysis

Genomic DNA was prepared from blood leukocytes by standard techniques (Puregene, Gentra). The three coding exons and the flanking intronic sequences of MECP2-A isoform were separately PCR-amplified from genomic DNA in 8 fragments, using the primer pairs shown in Table 1. For mutation identification, PCR products were first verified by agarose electrophoresis and then sequenced for sequence variants by non-radioactive single-stranded conformation analysis (SSCA) using the Genephor system (Amersham Biotech). PCR products were sequenced directly with Big Dye terminator version 3 on an ABI 3100 (Applied Biosystems, Rotkreuz, Switzerland).

2.3. Parental origin

The parental origin of the mutation was determined by microsatellite analysis of markers linked to the FMR1 and F8C loci (respectively centromeric and telomeric to MECP2), using standard techniques.

2.4. X chromosome inactivation

X chromosome inactivation was studied as described [11] using PCR analysis of the ZNF261 loci, which contain methylation-sensitive restriction sites (CfoI) and highly polymorphic STR (small tandem repeats) allowing differentiation of the two alleles. Inactivation bias is evaluated by comparison of relative allele intensities after PCR amplification of intact genomic DNA and genomic DNA digested with methylation-sensitive restriction enzymes (CfoI) and sequencing indicated that she was a heterozygous carrier of the same deletion (Fig. 1). DNA polymorphism analysis showed that the deletion occurred on the paternal allele (alleles for the markers FMR1 (Xq27.3) and F8C (Xq28) were respectively: grandfather 2 1; mother 1/2 1/2; patient 2 1) and was de novo in the mother.

3. Results

PCR analysis of genomic DNA revealed an apparent deletion within exon 4 identified by sequencing as a 44 bp deletion beginning at nucleotide position 1158, coded c.1158del44 (Fig. 1). This mutation was predicted to result in a frame-shift at codon 386 and subsequent premature truncation of the MeCP2 protein just two amino acids downstream (p.388X).

PCR analysis of leukocyte DNA from the mother and DNA sequencing indicated that she was a heterozygous carrier of the same deletion (Fig. 1). DNA polymorphism analysis showed that the deletion occurred on the grandpaternal allele (alleles for the markers FMR1 (Xq27.3) and F8C (Xq28) were respectively: grandfather 2 1; mother 1/2 1/2; patient 2 1) and was de novo in the mother.

Table 1

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Sequence of primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>L: 5'-GTTATGTTATTTAGTCTTTGG-3'</td>
</tr>
<tr>
<td>Exon 3.1</td>
<td>L: 5'-CTGTGTTATTTAAGCTAAATGTT-3'</td>
</tr>
<tr>
<td>Exon 3.2</td>
<td>L: 5'-GGGGTCTCATATACTATGACTGTC-3'</td>
</tr>
<tr>
<td>Exon 4.1</td>
<td>L: 5'-GCCCGAGGGAGGCTACTG-3'</td>
</tr>
<tr>
<td>Exon 4.2</td>
<td>L: 5'-GGGGCGGCAGCCAGCCACCTC-3'</td>
</tr>
<tr>
<td>Exon 4.3</td>
<td>L: 5'-TGAGCCTGACAGCGTTGCTTCTC-3'</td>
</tr>
<tr>
<td>Exon 4.4</td>
<td>L: 5'-CTCCTGTGCAAGTGAGTTAGAA-3'</td>
</tr>
<tr>
<td>Exon 4.5</td>
<td>L: 5'-GCACACCCCTCTGACGGTG-3'</td>
</tr>
</tbody>
</table>

R: 5'-GCACACCCCTCTGACGGTG-3'
Skewed inactivation of the mother’s X chromosomes in leukocyte DNA was revealed by ZNF261 analysis with a ratio of approximately 95:5 (Fig. 1).

4. Discussion

This patient with a truncating deletion mutation of MECP2 fulfills the revised diagnostic criteria for Rett syndrome [1] and confirms that males with MECP2 mutations can present a phenotype closely resembling classical female Rett syndrome.

Review of the published male MECP2 mutations suggests that in males, severe mutations responsible for typical Rett syndrome in females cause lethal neonatal encephalopathy unless attenuated by somatic mosaicism or an XXY karyotype [5]. In contrast, milder mutations (for example certain missense mutations) are present in patients with a wide range of clinical pictures from male Rett syndrome [8,9] to non-specific X-linked mental retardation and even early onset schizophrenia [10].

C-terminal truncation mutations such as the one described here are typically found in females with classical, sporadic Rett syndrome. It was therefore entirely unanticipated to find that the mother of our patient was heterozygous for the mutation while having a normal intellect and no evident neurological symptoms.

The discovery of a skewed X-chromosome inactivation (XCI) provides a possible explanation for the lack of penetrance of the mutated allele, since asymptomatic female carriers with highly skewed XCI have been occasionally described [3].

In conclusion, the finding of a MECP2 mutation in an asymptomatic female further confirms that it is essential to look for mutations in the mothers of all patients with MECP2 mutations. In families such as the one we describe, genetic counseling would be extremely difficult were the mutation to be transmitted to a female foetus.
We suggest that the X-inactivation status be examined in asymptomatic carriers, in the aim of elucidating the absence of penetrance in a minority of heterozygotes.

Acknowledgement

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References


