Short Report

De novo duplication of MECP2 in a girl with mental retardation and no obvious dysmorphic features


Loss-of-function mutations of MECP2 are responsible for Rett syndrome (RTT), an X-linked neurodevelopmental disorder affecting mainly girls. The availability of MECP2 testing has led to the identification of such mutations in girls with atypical RTT features and the recognition of milder forms. Furthermore, duplication of the entire gene has recently been described in boys with mental retardation and recurrent infections. We describe a girl with a heterozygous de novo MECP2 duplication. The patient, at the age of 19, has mental retardation with no autistic features. She is friendly but gets frequently anxious. She has neither dysmorphic features nor malformations. Her motor development was delayed with walking at 20 months. Speech is fluid with good pronunciation but is simple and repetitive. Diagnosis was made after single-strand conformation analysis (SSCA) and multiplex ligation-dependent probe amplification (MLPA) analysis of MECP2. Array comparative genomic hybridization (aCGH) analysis showed a duplication of 29 kb including MECP2 and part of IRAK1. Fluorescent in situ hybridization (FISH) has revealed that the duplicated region is inserted near the telomere of the short arm of chromosome 10. X-chromosome inactivation in leukocyte DNA was not skewed. We conclude that it is likely that this MECP2 duplication is responsible for the mental retardation in this patient. This case broadens the phenotypic spectrum of MECP2 abnormalities with consequent implication in diagnosis and genetic counselling of girls with non-syndromic mental retardation.

Loss-of-function mutations of the MECP2 (OMIM #300005) are responsible for Rett syndrome (RTT) (OMIM #312750)(1), a severe neurodevelopmental disorder affecting mainly girls and characterized by acquired microcephaly, global regression with loss of purposeful hand movement and ability for social interaction, severe mental retardation and typical hand stereotypies. Such mutations are also responsible for half of the cases presenting an atypical form of RTT (2). Genetic testing of MECP2 in a clinical setting is widely available today and more than 600 different mutations have been reported [RETTBASE (3), HGMD (4)].

Missense and nonsense MECP2 mutations have also been rarely identified in boys (5–8) who suffer either from a severe neonatal encephalopathy or from a severe form of mental retardation characterized by progressive encephalopathy and one or more of abnormal movements or tone, ataxia and seizures (9). Interestingly, duplications of the gene have also been described in males showing a distinct phenotype, mainly characterized by mental retardation, recurrent infections, hypotonia and progressive encephalopathy, ataxic symptoms, as well as intestinal and bladder dysfunction (10–13). The symptoms seem to correlate with the genes...
involved in the duplication but no specific phenotype–genotype correlations have been described (12, 13).

We report a girl with mental retardation and a de novo duplication encompassing MECP2 and part of IRAK1 (OMIM #300283).

**Clinical report**

The patient, at present aged 19, is the first child of unrelated healthy parents. She was born at 38 weeks of gestation by forceps, after a pregnancy marked by threat of premature birth at 30 weeks. Apgar score was 9/10/10. Birth weight was 2550 g (p10), length 48 cm (p50) and head circumference 34.5 cm (p50–p90).

Motor development was delayed with acquisition of the sitting position at 10 months and walking at 20 months. As a toddler, she was described as having generalized hypotonia and balance/coordination problems that resulted in frequent falls. Generalized hypotonia and joint laxity persist until the present day but muscle strength is normal. Difficulties in coordination without ataxia are also still present. Recent neurologic examination is described as normal, but small difficulties in smooth eye pursuit were noted.

Language development was also slow. At the age of 3.5, the patient was using a handful of words and pronunciation was not adequate for the age. Her speech is at age 19 simple and repetitive, the patient asking several times the same question but without echolalia. She has moderate pronunciation problems. Her voice has a nasal quality.

Given her difficulties in concentrating on one particular task due to short attention span and anxiety, it was not possible to precisely assess the current level of mental abilities. After neuropsychological testing, it was estimated that the patient’s mental age corresponds at present to that of a 5- to 7-year-old girl. Retardation is homogeneous among all cognitive abilities (social behaviour, autonomy, ability to deal with abstract notions, speech development). The patient followed specialized education but is not able to read or write. Her character has always been described as kind and friendly but with high levels of anxiety, especially in the absence of her parents. No autistic traits have ever been noted. Her visual memory is excellent.

The patient does not have any dysmorphic features (Fig. 1) and is physically in good health. Head circumference is 57 cm (p97). She does not

*Fig. 1. Patient at age 11.*
show any classic RTT signs (e.g. hand stereotypies). Development at puberty was normal with menarche at 15 years, followed by secondary amenorrhea; her menstrual periods occurred only twice. Due to lack of cooperation from the patient, no further gynaecological investigation could be performed. She wears glasses for hypermetropia. Colour vision (tested by Ishihara pseudochromatic plates because of parental suspicion that there was a problem of colour vision) is normal.

Materials and methods

DNA from ethylenediaminetetraacetic acid blood was extracted by a manual salting out procedure (Gentra PureGene, QIAGEN, Hombrechtikon, Switzerland). The patient was screened by several methods. Single-strand conformation analysis (SSCA) was performed first, followed by multiplex ligation-dependent probe amplification (MLPA) for the MECP2 (MLPA Kit, P015C, MRCHolland, Amsterdam, Netherlands). Capillary electrophoresis was performed on an ABI 3100XL Genetic Analyzer (Applied Biosystems, Rotkreuz, Switzerland); data were analysed with Genemarker v1.51 (Softgenetics, State College, PA, USA), with normalization against five normal female samples.

The entire coding region of MECP2 (exons 1–4) was amplified (primers and conditions available upon request); Polymerase chain reaction (PCR) fragments were purified with Microclean (Web Scientific, Crewe, United Kingdom). Direct cycle sequencing of the purified products was performed on both strands using Big Dye Terminator v3.1 (Applied Biosystems) and run on a 3100 Genetic Analyzer (Applied Biosystems). Sequences were analysed using gensearch software (v3.6.3b, PhenoSystems, Lillois, Belgium).

X-chromosome inactivation was studied using PCR analysis of the AR locus, which contains a methylation-sensitive restriction site (HpaII) and a highly polymorphic small tandem repeat 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 pre-digested to completion with HpaII and capillary electrophoresis.

Array comparative genomic hybridization (aCGH) was additionally performed on the proband using the Human Genome CGH Microarray Kit 244A (Agilent Technologies, Palo Alto, CA, USA) with 8.9 kb overall median probe spacing giving a resolution of 40 kb. Briefly, 1 μg of patient and a sex-matched pooled reference DNAs were processed according to the manufacturer’s protocol. Fluorescence was scanned in a dual-laser scanner (Agilent DNA microarray scanner G2565CA, Agilent Technologies) and the images were extracted and analysed with AGILENT FEATURE EXTRACTION software (v9.5.3.1) and CGH ANALYTICS software (v3.5.14), respectively. Changes in test DNA copy number at a specific locus are observed as the deviation of the log ratio value from a modal value of 0.

Fluorescent in situ hybridization (FISH) analysis was performed on metaphase chromosome spreads in the patient and her parents. Bacterial artificial chromosome clones, selected from the University of Colombo School of Computing Human Genome Database (http://genome.ucsc.edu/cgi-bin/hgGateway), were obtained from Bluegnome (Cytocell, Cambridge, United Kingdom) and hybridized according to the manufacturer’s protocol. Clone RP11-119A22 (Xq28) (chrX: 152,909,026–153,061,110) was selected inside the duplicated segment of the patient and clone RP11-446J19 (Xq13) (chrX: 68,510,619–68,634,148) was chosen as probe control. FISH analysis with whole chromosome painting (WCP) of chromosome 10 (WCP10 Aquarius, Cytocell) was co-hybridized with RP11-119A22 to identify the chromosome in which the insertion occurred.

Results

SSCA and subsequent sequencing of the coding area of MECP2 gave a normal result. Screening by MLPA of the patient’s DNA sample revealed a duplication of the entire MECP2 extending centromerically to IRAK1. The duplication was absent in the patient’s parents samples (data not shown).

To further characterize the rearrangement extent and breakpoints, aCGH was performed. It showed that the duplicated chromosomal segment spans 129 kb on Xq28 from probe A_16_P03795726 (152,936,728 Mb) to probe A_16_P21637578 (153,049,456 Mb) (Fig. 2) according to National Center for Biotechnology Information build 36.1. No putative benign copy number variations (CNVs) have been found to overlap with this region in the Database of Genomic Variants (http://projects.tcag.ca/variation, build 36, March 2006). Additionally, eight more known benign CNVs were identified, all paternally or maternally inherited (Table 1). Only MECP2 and part of IRAK1 are included within the duplicated region and this region coincides with the smallest region of overlap that was identified in patients with Xq28 duplication (12, 14). No skewing of X-chromosome inactivation was found.
Fig. 2. Array comparative genomic hybridization (CGH) showing the Xq28 duplication. Ideogram and array CGH profile of chromosome X (a) and enlargement showing the duplicated segment (b).

Table 1. List of the additional copy number variations (CNVs) identified in the patient. They are all found in the Database of Genomic Variants and they have all been either paternally or maternally inherited.

<table>
<thead>
<tr>
<th>Name of CNV</th>
<th>Known CNV coordinates</th>
<th>Patient's CNV coordinates</th>
<th>Gain/loss</th>
<th>Parental origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation_31177</td>
<td>chr4: 69,055,784–69,219,241</td>
<td>chr4: 69,114,000–69,165,000</td>
<td>Gain</td>
<td>Paternally</td>
</tr>
<tr>
<td>Variation_2614</td>
<td>chr6: 162,334–356,964</td>
<td>chr6: 204,528–284,743</td>
<td>Loss</td>
<td>Maternally</td>
</tr>
<tr>
<td>Variation_37462</td>
<td>chr8: 39,351,343–39,506,379</td>
<td>chr8: 39,356,000–39,505,000</td>
<td>Loss</td>
<td>Paternally</td>
</tr>
<tr>
<td>Variation_48051</td>
<td>chr11: 55,092,454–55,210,062</td>
<td>chr11: 55,124,000–55,207,000</td>
<td>Loss</td>
<td>Paternally</td>
</tr>
<tr>
<td>Variation_3117</td>
<td>chr16: 31,918,174–33,744,011</td>
<td>chr16: 33,280,000–33,539,000</td>
<td>Loss</td>
<td>Maternally</td>
</tr>
<tr>
<td>Variation_8850</td>
<td>chr17: 41,360,800–42,168,300</td>
<td>chr17: 41,521,000–41,566,000</td>
<td>Gain</td>
<td>Paternally</td>
</tr>
<tr>
<td>Variation_34615</td>
<td>chr18: 1,712,427–1,885,905</td>
<td>chr18: 1,715,000–1,828,000</td>
<td>Loss</td>
<td>Maternally</td>
</tr>
<tr>
<td>Variation_37014</td>
<td>chrX: 153,098,879–153,206,129</td>
<td>chrX: 153,176,000–153,211,000</td>
<td>Gain</td>
<td>Possibly maternally</td>
</tr>
</tbody>
</table>

Metaphase FISH analysis of the patient revealed that the additional copy of MECP2 is inserted in the subtelomeric short arm region of the chromosome 10; whole chromosome painting of chromosome 10 excluded a more complex rearrangement with other chromosomes (Fig. 3). The rearrangement is coded as ins(10;X)(p15;q28) (RP11-119A22+, wcp10+; RP11-119A22+, wcp 10-). FISH analysis of the parents was normal, with one and two copies of MECP2, respectively, in the father and mother.

Discussion

Our patient has a de novo duplication of MECP2 and part of IRAK1. Her phenotype does not fit the established diagnostic criteria for RTT or its variants. Male patients with duplications of the Xq28 region share a common phenotype consisting of mental retardation, infantile hypotonia, ataxia, delay in the motor milestones, absence of speech, brain magnetic resonance imaging findings (especially progressive encephalopathy), while recurrent
MECP2 duplication in girl with mental retardation

Infections are a frequent but not consistent finding (12). The minimum reported size of the duplication includes only MECP2 and IRAK1 while the larger ones can include several telomeric or centromeric genes. It has recently been shown that the inclusion of filamin A, alpha isoform 1 in the duplication may be responsible for additional intestinal and bladder dysfunction (13). The origin of the duplication of these male patients can be either de novo or inherited, in which case the mothers always exhibit complete or almost complete skewing of X-chromosome inactivation (15).

Recent data from mice have shown that MeCP2 (which is also X-linked) acts not only by repressing but also by activating a large number of genes. Furthermore, there is evidence from the same mouse models that deletion and duplication of MeCP2 have measurable and opposite effects on the expression of target genes (16). These data lead to the assumption that MeCP2 expression is very tightly regulated, at least in mice.

MECP2 exhibits functional monosomy, being completely inactivated on one X-chromosome of normal diploid females (17) and it has already been shown that MECP2 over-expression after MECP2 duplication is responsible for the pathological phenotype of boys who carry the duplication (15). Our hypothesis concerning the patient reported here is that the additional copy of MECP2 inserted on chromosome 10 is constitutionally active and leads to over-expression of the gene, causing the patient’s mental retardation.

The identification of a girl with a symptomatic MECP2 duplication broadens our knowledge of pathologies related to MECP2. We recommend considering MECP2 duplication testing in females with mental retardation (either specifically or via aCGH). Finding such a CNV also has consequences for genetic counselling and cascade testing in families in which boys have MECP2 duplications. This study also further underlines the importance of FISH analysis of patients with duplications identified by aCGH screening.

Acknowledgements

We would like to express our sincere gratitude to the family for allowing this report on their daughter. We also thank Mrs Nathalie Valenza who performed the neuropsychological evaluation of the patient and Dominique Marelli for expert technical assistance.

Conflicts of interest

The authors have no conflict of interest to declare.

References

13. Clayton-Smith J, Walters S, Hobson E et al. Xq28 duplication presenting with intestinal and bladder dysfunction and a


