

Deletion screening by fluorescence in situ hybridization in Rett syndrome patients

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Abstract – Mutations in the X-linked methyl-CpG-binding protein 2 (MECP2) gene have been found to be a cause of Rett syndrome (RTT). Mutation screening was based on various techniques including denaturing gradient gel electrophoresis, single-strand conformation polymorphism analysis, heteroduplex analysis, DNA sequencing and recently Southern Blot analysis. Mutation detection was achieved in 80% of typical RTT with a high prevalence of recurrent mutations. In order to provide further insights into the spectrum of MECP2 rearrangements in patients without any point mutation or small deletion/insertion in the coding region MECP2 gene, we screened 25 classical RTT females using fluorescence *in situ* hybridization analysis. No deletion were found in our group, suggesting that MECP2 gross rearrangements are a rare cause of Rett syndrome. © 2001 Éditions scientifiques et médicales Elsevier SAS

Rett syndrome / MECP2 gene / deletion / FISH

1. Introduction

Rett syndrome (RTT, MIM 312750) is a childhood neurodevelopmental disorder which almost affects girls, with an estimated prevalence of 1 in 10 000–15 000 [21, 11]. Patients with classic RTT appear to develop normally until age 6–18 months, at which time they enter a period of developmental regression. The affected girls suffer from gradual loss of speech and purposeful hand use, development of microcephaly, seizures, ataxia, autistic features, intermittent hyperventilation and stereotypic hand movements. Although most cases are sporadic, linkage studies in the rare familial RTT cases pointed to Xq28 as the most likely candidate region [25]. By systematic mutation analysis of genes in Xq28, Amir et al. [2] identified the RTT gene as MECP2, the methyl-CpG-binding protein 2, that is normally involved in transcriptional silencing [17]. Numerous studies have since found various mutations (missense, nonsense,

frameshift) in the coding region of the MECP2 gene, in as many as 80% of patients [1–9, 12, 14–16, 18, 19, 23–26]. Most mutations occur within the two functional domains, the methyl-CpG-binding domain (MBD) and the transcriptional repression domain (TRD) of MeCP2. Moreover, small deletions (about 20–80 bp) focused on a limited region localised after the second functional domain (TRD).

The failure to detect MECP2 mutations in the remaining 20% patients may indicate the presence of mutations in unexplored regions of the MECP2 gene, such as regulatory elements or noncoding regions. Most MECP2 mutations reported so far lead to inactive MeCP2 proteins (premature stop codons or amino-acid changes in the functional domains) arguing for an haplo-insufficiency of the MECP2 gene in RTT. Therefore, the complete loss of one allele might be involved in RTT. In this study, we looked for MECP2 deletion by fluorescence in situ hybridization (FISH) on a panel of 25 girls with classic RTT.

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2. Materials and methods

2.1. Patients

We studied 25 female patients with classical sporadic RTT without any mutations identified in the MECP2 coding region after complete sequencing and Southern blot analysis [6]. They were referred through the French Rett Syndrome Association and were diagnosed according to the Rett Syndrome Diagnostic Criteria Work Group [22]. Blood samples were obtained after informed consent.

2.2. Fluorescence *in situ* hybridization

Metaphase spreads from control females and RTT patient lymphoblastoid cell lines were prepared according to standard procedures. A slide pretreatment by pepsin digest during 10 min (10 µg/mL) was made to eliminate cytoplasm. Chromosomal DNA denaturation was obtained in 70% formamide, 2X SSC, pH 7 at 73°C during 2 minutes. The probe used was the PAC clone 671D9 containing only the MECP2 gene [accession number: AF030876, 20]. 1 µg was labelled with biotin-16-dUTP by nick translation using Bio-Nick kit (Gibco-BRL, Germany). For each experiment, the labelled PAC DNA was used together with a CEP X Spectrum Aqua probe (Vysis, USA) specific to the X chromosome α -satellite region. The probe mixture was denatured for 10 minutes at 73°C and directly added to the slide. Slides were incubated overnight at 37°C, and washed for 2 min with 0,4XSSC/0,3% Nonidet P40, pH 7, and for 15 s with 2XSSC/0,1% Nonidet P40, pH 7. Biotin-labelled probe was detected according to the protocol of the fluorescein detection kit (Oncor-Appligene, France). Finally, slides were counterstained with DAPI I (4'6 diamino-2 phenylindole) (Vysis, USA) and analysed on a Zeiss epifluorescence microscope (Jena, Germany) with a Sensys CCD camera (Photometrics, USA) and IPLab Spectrum Imaging Software (Vysis, USA).

3. Results and discussion

Preliminary studies of the MECP2 coding region using a mutation screening strategy based on conformation-sensitive gel electrophoresis, sequence analysis and Southern blot analysis did not reveal any mutation in 25 classic RTT patients. Therefore, further analysis by FISH was performed in order to exclude large deletions of the RTT region in Xq28 in this

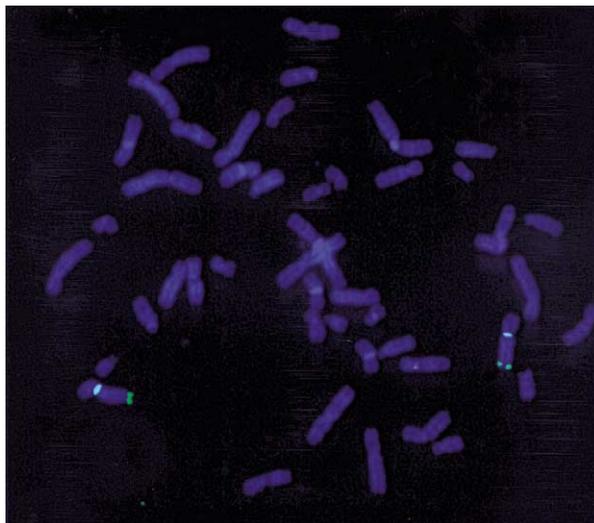


Figure 1. Metaphase chromosome spread shows the 2 control aqua spots on the X centromeres, and the 2 green spots due to the PAC 671d9 probe hybridisation on the 2 MECP2 alleles in Xq28 region. No deletion was detected in any of the 25 RTT patients studied.

cohort of 25 patients. All patients have a normal female karyotype. The 671d9 PAC clone which contains the entire MECP2 gene, gave signals on the distal Xq-region on both X chromosomes in more than 80% of the metaphases from both normal control females, and the 25 MECP2 mutation free patients (*figure 1*). In addition, no duplication of the MECP2 locus was identified in any of the 25 RTT patients analysed. Recently, Nielsen et al. [18] did not find any deletion of the entire MECP2 gene in 3 classical RTT patients, supporting our findings.

Repeated sequences are very common in deletion prone regions [27]. As an illustration the steroid sulfatase gene (STS) is completely deleted in up to 90% of patients with X-linked-ichthyosis [13], or conversely, the phenylalanine hydroxylase gene, poor in long highly homologous segments, is deleted in only 5% of patients with phenylalanine hydroxylase deficiency [10]. Based on the data presented here, the MECP2 gene seems to belong to the second gene category; indeed complete loss (or duplication) of the MECP2 gene seems to be a rare event in typical RTT cases. However, more severe phenotype associated with congenital malformations could be caused by contiguous gene syndrome due to microdeletion in Xq28 region encompassing the MECP2 gene.

In conclusion, gross rearrangements seem not to be a MECP2 gene mutational mechanism in RTT; another way to illustrate a possible haplo-insufficiency

of the MECP2 gene in RTT could be an expression study by RNA quantification. A decreased expression of the MECP2 gene would reveal mutations in regulatory elements. Finally, if any additional mutations in MECP2 non-coding regions are identified in RTT patients, a second disease locus might be involved in this neurodevelopmental disorder.

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