Multiplex Protocol Suitable for Screening for MECP2 Mutations in Girls with Mental Retardation

To the Editor:

In girls, mutations in the gene for methyl-CpG-binding protein 2 (MECP2) are associated with clinical presentations that include classic Rett syndrome (RTT), Angelman syndrome-like phenotype, autism, and even mild forms of mental retardation (1–7). The gene, located on chromosome Xq28, is expressed in the brain, where it is involved in the growth and maturation of neurons (8, 9). MECP2 also influences expression of the genes UBE3A and GABRB3, which may help explain the Angelman syndrome-like and/or autism phenotypes (10). In males, mutations in MECP2 can be associated with X-linked mental retardation or with severe neonatal-onset encephalopathy (11, 12).

The molecular diagnosis of MECP2 mutations has been complex and expensive, depending on mutation identification using a scanning technique followed by DNA sequencing. In classic sporadic RTT, a mutation can be detected in 70%–90% of cases. However, in atypical RTT and/or familial cases, this rate drops to 34% and 29%–45%, respectively (13–15). In Angelman-like patients, MECP2 mutations are seen in ≈10% of cases (4, 5, 16). Thus, current protocols are not cost-efficient, except in very typical circumstances. We designed a simpler strategy suitable to screen for MECP2 mutations in girls, even at the expense of some sensitivity.

We accessed RettBase (17) and identified the 10 most common mutations associated with Rett syndrome: 502C>T (R168X), 473C>T (T158M), 763C>T (R255X), 808C>T (R270X), 880C>T (R294X), 916C>T (R306X), 397C>T (R133C), 316C>T (R106W), 419C>T (A140V), and G269fs (806delG). We designed 4 primer pairs to amplify regions of exons 3 and 4, where these mutations occur (see Table 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol52/issue3/). We then used our multiplex minisequencing technique (18) to identify those 10 mutations rapidly and inexpensively (see primers in Table 2 in the online Data Supplement). Three of the amplicons from exons 3 and 4 (primers Mecp 1 to -3) were amplified together in a triplex format, whereas the 3' end of exon 4 (primer Mecp 4) was amplified separately to detect microdeletions. After inspecting the amplification products in a gel, we purified them to eliminate the excess primers and deoxynucleoside triphosphates (18) and used them as templates for multiplex minisequencing.

To validate our procedure, we obtained from the Coriell Cell Repository (19) DNA samples from patients with 5 of the 10 MECP2 mutations: R168X, R255X, R294X, R306X, and R106W. The minisequencing results (Fig. 1) were excellent, but there was...
some background in the region 45–58 bp (Fig. 1) that occasionally interfered with recognition of the R306C and R255X mutations. Because of this, we duplicated the multiplex minisequencing in 2 overlapping reactions: one encompassing all primers (Fig. 1) and the other containing only the first 7 minisequencing primers (see the online Data Supplement). In all 5 Coriell samples, the MECP2 mutations were diagnosed correctly.

In addition to base changes, recurrent small deletions, especially in the region coding for the C-terminal domain, may lead to Rett syndrome. To detect these deletions, we amplified the Mecp 4 amplicon, which extended from nucleotides 1073 to 1396 in the cDNA, and evaluated the PCR products in 6% polyacrylamide gels. Our procedure readily identified the 26-bp deletion (1160del26) in sample NA16382 from the Coriell Cell Repository.

Our procedure readily identified the products in 6% polyacrylamide gels.

Schanen et al. (13) sequenced exons 2–4 from 81 patients with classic RTT and from 4 atypical cases; 76.5% of their patients had the 10 common mutations. Among these, 104 patients (72%) would have been detected by our technique. Hence, our minisequencing protocol seems to have a sensitivity >70% in the identification of MECP2 mutations in Rett patients.

Some girls clinically diagnosed as having an Angelman syndrome-like phenotype and who do not present any abnormalities in the 15q11–13 region or methylation defects in UBE3A have been shown to carry MECP2 mutations, generally the same ones seen in Rett syndrome (4, 5, 16, 20). We used our minisequencing protocol to study 7 such patients from our clinical service. Among these, we detected 1 with the relatively common nonsense mutation 808C>T. Although our sample size was small, it demonstrated the usefulness of our procedure.

After Down syndrome, the fragile X and Rett syndromes are believed to be the most common causes of developmental delay in females (21). MECP2 mutations may be responsible for ≈2.5% of the institutionalized individuals with mental retardation (22). The exact numbers are not known because molecular testing has been a costly and slow endeavor. We hope that the availability of our simple and inexpensive screening technique will facilitate the diagnosis of patients with MECP2 mutations.

References


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DOI: 10.1373/clinchem.2005.060178