

**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 in 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 Supple-

ment 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 Mecip 1 to -3) were amplified together in a triplex format, whereas the 3' end of exon 4 (primer Mecip 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

Minisequencing primers	MSMecip11	MSMecip4	MSMecip5	MSMecip1	MSMecip2	MSMecip3	MSMecip6	MSMecip7	MSMecip8	MSMecip10
Amino acid alteration	A140V	R270X	R294X	R168X	T158M	R255X	R306C	R133C	R106W	G269fs
Expected/alterred nucleotide	C T	C T	C T	C T	C T	C T	C T	C T	C T	G -
Expected/alterred peak	19 nt 37 nt	23 nt 24 nt	28 nt 29 nt	32 nt 35 nt	39 nt 41 nt	43 nt 51 nt	48 nt 55 nt	58 nt 63 nt	66 nt 68 nt	72 nt 74 nt

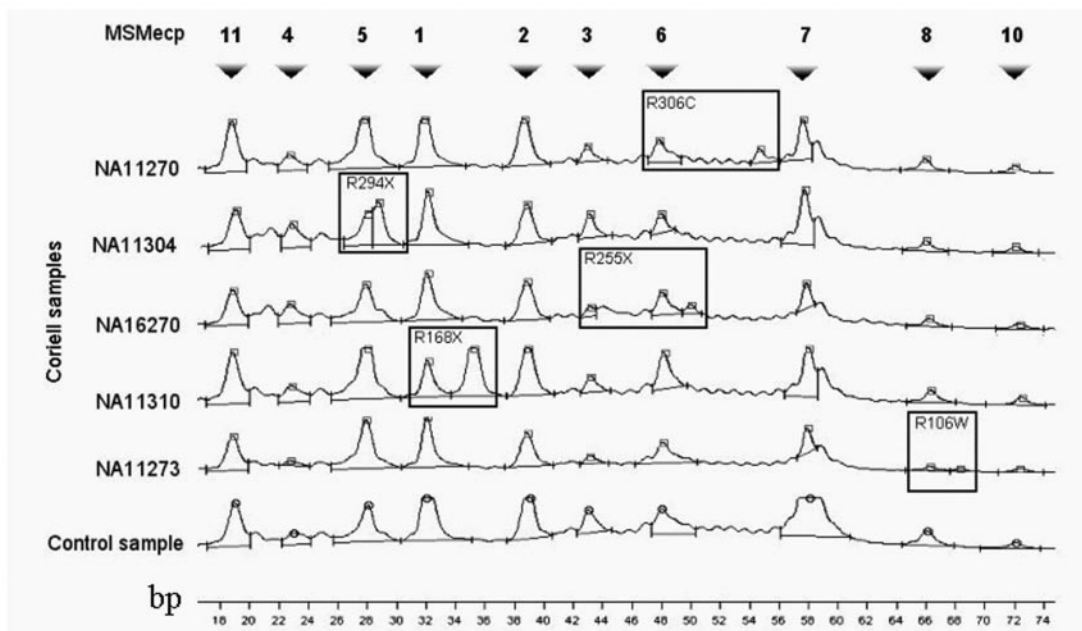


Fig. 1. Multiplex minisequencing results for DNA samples from RTT patients, obtained with 10 primers used to detect mutations in the *MECP2* gene.

The samples were obtained from the Coriell Institute. The primers for minisequencing were named, respectively, MSMecip1 to -8 and MSMecip10 to -11. The altered peaks are *highlighted*. The mutations tested by each primer are shown in the *table* at the top. Note that there is some background noise between peaks 6 and 7.

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.

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 targeted in our minisequencing procedure. In addition, Fukuda et al. (15) sequenced exons 1, 3, and 4 from 219 patients with classic or atypical RTT and found that 145 had *MECP2* 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  $\geq 2.5\%$  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.

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