

ONLINE MUTATION REPORT

Screening of *MECP2* coding sequence in patients with phenotypes of decreasing likelihood for Rett syndrome: a cohort of 171 cases

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that almost exclusively affects females and is generally considered lethal in males during gestation. The typical form is characterised, at anamnesis, by an initial period of stagnation of development followed by regression lasting for several months and occurring between 6 months and 3 years of age. The fully developed clinical picture is dominated by mental retardation, reduction of communication skills, and loss of purposeful hand movements combined with hand stereotypes, progressive microcephaly, abnormal locomotion, and other various minor signs.¹ The prevalence of RTT has been estimated to be between 0.25 and 1 per 10 000 in girls; the figure is probably higher if variant forms are included.² In 1999, it was discovered that a significant proportion of RTT cases are caused by mutations in the *MECP2* gene.³ However, detection of mutations has been very variable from one series to another, ranging from 19%³ to 100% positive cases,⁴ presumably due to the profile of the populations studied.

Methyl CpG-binding protein 2 is encoded by a gene containing four exons. Its longest transcript is 10.1 kb and is mainly expressed in the fetal brain. This 446 residue protein acts as a transcriptional repressor. It includes a methyl binding domain (MBD, residues 78–162), a transcription repression domain (TRD, residues 207–310), a nuclear localisation signal (NLS, residues 255–271), and a 63 residue C-terminal domain (CTD) involved in binding to DNA and in protein stability (reviewed by Nomura *et al*⁵).

A number of variant phenotypes in girls was described before the molecular era and included: a form with congenital onset,^{5,6} a form with early infantile onset dominated by seizures,⁷ a form fruste,⁸ a form with late childhood regression,⁹ and a form with preservation of speech.¹⁰ Subsequently, it has been confirmed that *MECP2* mutations are responsible for some of these variant forms.^{11–13}

Given the various phenotypes that occur in relation to *MECP2* mutations and the lack of a unified concept based on genotype/phenotype correlations, recent works have considered the problem of RTT through various approaches examining: prevalence and types of *MECP2* mutations in girls with recognisable forms of RTT; genotype/phenotype correlations in patients with positive molecular findings^{14–16}; and the search for *MECP2* mutations in patients with mental disorders other than RTT, such as autism,¹⁷ and the occasional discovery of *MECP2* mutations in relation to clinical pictures different from RTT, particularly in boys.^{18–22}

In the present large series, we focused on an approach practical both for the clinician requesting *MECP2* screening and for molecular geneticists: what is the likelihood of a positive test in phenotypes with decreasing clinical likelihood of RTT? To what extent are the criteria and scores proposed by RTT study groups helpful in predicting the presence of an

Key points

- *MECP2* mutations have been described in typical cases of Rett syndrome (RTT) and in variant phenotypes. In this study we have retrospectively evaluated the likelihood of finding *MECP2* mutation in phenotypes of decreasing concordance with a diagnosis of RTT.
- A cohort of 137 girls and 34 boys was studied. Three groups of girls were identified on the basis of positive necessary criteria as defined by the Rett Syndrome Diagnostic Criteria Work Group (RSDCWG) group: group 1, with "typical RTT" and exhibiting 6/6 positive necessary criteria, group 2 which were "atypical cases" with three to five such criteria, and group 3 with "poorly specific mental retardation" and with less than three criteria. An RTT score as proposed by the Osaka group was calculated for each patient and evaluated as a tool to select candidates for *MECP2* genotyping.
- Twenty five of 28 girls (89%) from group 1, 24/55 (47%) from group 2, and 3/58 (5%) from group 3 had mutated genes. RTT scores appeared to be poorly discriminant, as non-specific negative criteria, such as normal head circumference at birth, bear the same weight as positive criteria, such as loss of hand skills.
- Concurrence of the four following criteria: severe psychomotor retardation and impaired language, loss of purposeful hand skills, stereotypic hand movements, and acquired low head circumference, was highly predictive (90%) of an *MECP2* mutation independent of any anamnestic data. The likelihood of an *MECP2* mutation can thus be evaluated adequately with a simplified RTT score based on a restricted number of positive major criteria.

MECP2 mutation? Finally, which patients warrant additional screening beyond the *MECP2* coding sequence to other types of mutations, whether in *MECP2* non-coding sequence or in other genes?

Abbreviations: CTD, C-terminal domain; MBD, methyl binding domain; NLS, nuclear localisation signal; RTT, Rett syndrome; RSDCWG, Rett Syndrome Diagnostic Criteria Work Group; TRD, transcription repression domain

METHODS

Population

This retrospective study studied a cohort of 171 patients (137 girls, 34 boys) from June 2000 to September 2002. We included patients for whom a search for an *MECP2* mutation had been performed, with the exception of a few girls who had acquired perinatal brain lesions, progressive CNS disease, or the presence of a major malformation which was clear at the retrospective analysis. Fetal hypotrophy and microcephaly at birth were not a priori cause for exclusion.

MECP2 genotyping was requested through various medical channels: departments of paediatric neurology and paediatrics, both academic and non-academic; paediatricians working in institutions for children with developmental difficulties; and an association of families in which one child had been diagnosed with RTT.

Clinical items

Information was collected via questionnaire. In addition to general information, criteria of inclusion (necessary criteria), criteria of exclusion, and supportive criteria indicated in the literature (RTT Diagnostic Criteria Work Group 1988²³ (appendix A) were examined. Some questions were closed, others open. Though most physicians were informed of the *MECP2* genotype before the inquiry, they were asked for their motivation in requesting the test, as well as to which of the three groups mentioned below they would have attributed their patient prior to the test. Open questions were also asked about clinical items revisited after the results of a positive molecular test and free comments were solicited.

Grouping of girls with decreasing likelihood for RTT syndrome

Three groups were defined in girls:

- 1) A group of “typical cases”. Group 1 included girls with the six positive necessary criteria defined by the RSDCWG group²⁴ (appendix A), regardless of possible necessary negative criteria (normotrophism and normal head circumference at birth) or minor supportive criteria.
- 2) A group of “atypical cases”. Members of group 2 lacked at least one positive necessary criteria but had at least 3/6 positive necessary criteria and possibly other minor criteria supportive of RTT as defined by Hagberg *et al.*²⁴ (appendix A).
- 3) A group of girls with non-progressive, non-syndromic mental retardation, including the possibility of autistic features, who presented less than three RSDCWG positive necessary criteria.

In the three groups, no specific aetiology was identified by MRI or metabolic examination. For most patients, standard cytogenetic investigation was found to be normal prior to *MECP2* genotyping or after a negative result. Some of the non-mutated patients were tested for common micro deletion syndromes by FISH, with negative results.

Evaluation of a specific RTT score

Each case was given a score reflecting the concordance of the phenotype with the typical RTT syndrome. From the several criteria proposed by Hagberg²³, Inui *et al.*²⁵ constructed a score that we used with a slight modification: we suppressed the item “diagnosis tentative until 2–5 years of age” according to the revision of RTT criteria suggested by Hagberg *et al.*²³ However, as anamnesis was often unclear, we changed the item “apparently normal psychomotor development through the first 6 months” to “apparently regular psychomotor...”. Each item receives a value of 1 and the maximum score is 8/8.

The score is termed the “Osaka Modified Score” (OMS)²⁵ (appendix B).

In boys *MECP2* screening was considered for a cohort of patients with encephalopathy that met the following criteria: congenital, non-progressive encephalopathy with or without epilepsy; severe mental retardation; no evidence of specific cerebral lesion or disease by MRI, metabolic and infectiologic investigations; and normal standard karyotype. Most patients were also screened for FRAXA-FRAXE mutations. Besides general information, the questionnaire was directed toward dominant clinical symptoms and signs.

Genotyping

Genomic DNA was extracted from peripheral blood using standard methods. PCR amplification of exons 2, 3, and 4 was performed as described by Amir *et al.*³ with the following modifications. Exon 4 was split into two fragments for PCR amplification. The primer 4.1 TTTGTCAGAGCGTTGTCACC localised within intron 3 was used with the reverse primer 4.4 CTGCACAGATCGGATAGAAGAC to amplify the 5' portion of exon 4. The 3' portion of this exon 4 was amplified with the forward primer 4.3, AACCACTAAGAAGCCCAA and the reverse primer 4.10 CGGTAAGAAAAACATCCCCAA. The annealing temperatures were 58 and 60°C, respectively. For some patients, exon 3 and the 5' portion of exon 4 was amplified with one primer located within exon 3 AGCCCGTCAGCCATCAGCC and primer 4.4. This amplification generated a fragment of 1539 nucleotides and was employed in response to a point mutation IVS3, –89C>T found in primer 3.1 sequence in one affected boy. The mutation inhibits PCR amplification of the *MECP2* gene with primers 4.1 and 4.4; it was then tested for in 30 cases of Rett syndrome and not found. DNA sequencing was performed by using a BigDye dideoxy sequencing kit (Applied Biosystems) with the same primers or with the internal primers described by Amir *et al.*³ DNA sequences were analysed and compared to the reference sequence (GenBank, NM_004992) by using SeqScape V1.1. Mutations or deletions were considered as new when they were not referenced in the RettBASE (<http://MECP2.chw.edu.au>).

RESULTS

Girls

Twenty-eight girls fell into group 1 (table 1), 25 of whom (89%) were found to have mutated genes, being affected by 15 different mutations or deletions (table 1). Three missense mutations involved the MBD. Two nonsense mutations were located within the NLS, one missense was located in another part of the TRD, and one missense mutation was located within the CTD. Five point or small deletions located within exon 4 led to a frameshift of the coding sequence in the CTD. One complex rearrangement located within exon 4 was not characterised.

Fifty one girls fell into group 2 (table 2), 27 of whom were found to not have mutated genes (median age at test: 7 years), while 24 (47%) had mutated genes (median age at test: 6 years), a proportion significantly lower than in group 1 (χ^2 , $p = 0.0002$). Sixteen different mutations or deletions were found with five missense mutations involving the MBD. Three nonsense mutations were located within the TRD and two within the NLS. Four point or small deletions resulted in frameshift of the coding sequence within the TRD; two complex rearrangements were not characterised. One mutation substituted a guanine for an alanine (IVS3, –2A>G) in the acceptor site of intron 3. A deletion of exon 2 and 3 was found in one patient.

In group 2, the OMS extended from 3 to 7. The median score was 6 in both mutated (min–max = 5–7) and non-mutated patients (min–max = 3–7). Eight cases exhibited

Table 1 Girls with typical/highly suggestive RTT phenotype

Patient	Age of diagnosis (years)	Age of test (years)	OMS score	Nucleotide change	Amino acid substitution
1	4	5	8	215 ins C	Frameshift
2	2	20	8	397 C>T	Arg133Cys
3	3	36	8	Idem	Idem
4	5	5	8	Idem	Idem
5	Nd	10	8	403 A>G	Lys135Glu
6	3	15	8	473 C>T	Thr158Met
7	4	11	8	Idem	Idem
8	Nd	31	8	Idem	Idem
9	3	3	8	502 C>T	Arg168X
10	4	8	8	Idem	Idem
11	3	14	8	763 C>T	Arg255X
12	Nd	14	8	808 C>T	Arg270X
13	4	4	8	Idem	Idem
14	3	22	8	Idem	Idem
15	3	7.5	8	880 C>T	Arg270X
16	Nd	15	8	916 C>T	Arg306Cys
17	6, 5	8	8	Idem	Idem
18	3	3	8	Idem	Idem
19	4	4	8	964 G>C	Pro322Ala
20	Nd	nd	8	1056 del 163	Frameshift
21	2	27	8	1153 del 44	Frameshift
22	Nd	21	8	1162 Del 30	Frameshift
23	5	5	8	1160 del 21	Frameshift
24	Nd	15	8	1194 Ins T	
25	2	7	8	Complex rearrangement	
26	Nd	9	8	No mutation	
27	4	8.5	8	No mutation	
28	4	10	8	No mutation	

Nd, not determined.

microcephaly at birth, four of whom had a mutation. Eight cases exhibited fetal hypotrophy, two of whom had a mutation.

Fifty five girls fell into group 3. Three (6%) had mutated genes, significantly fewer than in group 2 ($p < 0.0001$). Two were girls, 2 and 4 years old, respectively, and had psychomotor delay with poor speech development and autistic features. They had gait apraxia, but no RTT anamnestic profile, no deceleration of head growth, and no supportive criteria. The younger girl carried the highly prevalent 916 C>T missense mutation. The older child, who carried a 50 bp deletion starting in exon 4, had various non-specific stereotyped movements but purposeful hand skills were preserved. Her brother had slight mental retardation with behavioural difficulties, but without *MECP2* mutation. The third girl, who carried a de novo missense mutation 909 C>G (Ile303Met), had an atypical phenotype: psychomotor acquisition was judged normal in infancy, and she first consulted at 3 years of age for an upper limb tremor. Examination at 12 years of age revealed a tremor of the “benign essential tremor” type with moderate handicap. She had slight mental retardation (IQ evaluated at 70, homogenous for verbal and performance abilities). Behaviour was dominated by striking shyness, with inhibition and blushing face and increasing diffuse vasomotor disturbances at each approach. Her mother had a slight, non-handicapping tremor.

The non-mutated girls of group 3 could be further separated into three subgroups:

- The first subgroup consisted of 29 girls with both mental retardation (IQ<70, among whom 10 cases were <50) and autistic traits. Nine girls had stereotypes including hand movements, but hand skills were in accordance with mental status. Twelve cases exhibited progressive microcephaly (normal head circumference at birth, but then between -2 and -4 SD), while one patient had microcephaly at birth. In this subgroup, the OMS score was 5 or 6 in 17 girls and <5 in the remaining patients.

- The second subgroup consisted of 11 girls who had no specific mental retardation or clear autistic behaviour, but seven of whom had epilepsy. OMS score in this group was <5. Three cases had both microcephaly at birth and fetal hypotrophy.
- The third subgroup consisted of 15 girls with severe congenital encephalopathy (two familial and 13 sporadic). In five cases, clinical presentation was associated with cryptogenic infantile spasms. Presentation was severe infantile encephalopathy without recognisable etiology in the remaining 10 cases, five of whom exhibited progressive worsening epilepsy. Two cases in this group had microcephaly at birth and one also had fetal hypotrophy.

The median age at the test was 10 years (mean (SD) = 12.5(8.2)), 6 years (mean(SD) = 7.3 (5.2)), and 5 years (mean(SD) = 6.2 (4.8)) in groups 1, 2 and 3, respectively (Kruskal-Wallis test, $p = 0.0006$). Compared with group 1, age was significantly lower in groups 2 ($p = 0.005$) and 3 ($p = 0.0001$).

Predictive value of restricted “necessary criteria”

As our study did not aim at revisiting all the clinical criteria necessary to, or compatible with, a diagnosis of Rett syndrome, but rather at determining the best criteria for justifying a molecular test, we further analysed the predictive value of concurrence of different OMS items for mutation (predictive value indicates the proportion of patients with the criteria who had an *MECP2* mutation). We observed that some criteria are less relevant than others and can be omitted without significant loss of prediction. Whereas concurrence of the eight OMS items had a predictive value of 89% (group 1), concurrence of the following four items, simplified from the OMS score, had a similar or even higher positive predictive value (90.6%, as all patients of group 1, and four mutated patients of group 2 corresponded to this profile): (1) apparent severe psychomotor retardation, severely impaired expressive and receptor language; (2) loss of acquired purposeful hand skills associated with communication

Table 2a Girls with atypical RTT phenotype: patients with mutation

n	Age of diagnosis (years)	Age of test (years)	Missing necessary criteria	OMS score	Nucleotide change	Amino acid substitution
1	2	6	5, 7, 8	5	397 C>T	Arg133Cys
2	4	4	4	7	Idem	Idem
3	2	6	4	7	422 A>G	Tyr141Cys
4	3	3	5	7	455 C>T	Pro152Arg
5	3	6	5	7	452 A>G	Asp151Gly
6	Nd	16	8	7	473 C>T	Thr158Met
7	5	5	6, 8	6	Idem	Idem
8	2	2	4, 8	6	Idem	Idem
9	Nd	14	5	5	Idem	Idem
10	4	4	5, 8	6	Idem	Idem
11	2	10	8	6	502 C>T	Arg168X
12	Nd	11	4	7	763 C>T	Arg255X
13	5	5	5, 8	6	Idem	Idem
14	2	2	5, 8	6	Idem	Idem
15	Nd	9	8	6	807 del C	Frameshift
16	Nd	9	8	7	808 C>T	Arg270X
17	3	8	5, 7	6	880 C>T	Arg294X
18	2	6	4	6	1156 Del 41	Frameshift
19	Nd	14	4	7	1156 Del 41	Frameshift
20	4	4	4	7	1157 Del 32	Frameshift
21	2	6	4, 5	6	IVS3, -2A>G	Frameshift
22	3	3	4, 7, 8	5	Complex rearrangement	
23	3	3	4, 5, 8	6	Complex rearrangement	
24	2	2	5, 8	6	Del exons 2-3	

dysfunction and social withdrawal; (3) stereotypic hand movements (wringing/squeezing/clapping/tapping, mouthing, and washing/rubbing automatisms); and (4) acquired low head circumference. Concurrence of other criteria had high, though less predictive values. Concurrence of the former criteria 1, 2, and 3 (the criterion “acquired low head circumference” being absent) had a predictive value of 64% that the patients would have mutated genes. The predictive value of having a *MECP2* mutation dropped to 34% when the criterion “Acquired low head circumference” replaced “Stereotypic hand movements” (this latter criterion being absent). Only one mutated and two unmutated girls were reported with loss of hand skills without hand stereotypes. The concurrence of a limited number of criteria with the exclusion of others, was either not encountered in the present population or had a low predictive value. For example, no girl had “loss of acquired purposeful hand skills associated with communication dysfunction and social withdrawal” without also having “apparent severe psychomotor retardation, severely impaired expressive and receptor language”. Concurrence of the four following exclusive criteria: (1) “Apparently normal perinatal period”, (2) “apparently regular psychomotor development through the first 6 months”, (3) “normal head circumference at birth”, and (4) “development of severely impaired expressive and receptor language, apparent severe psychomotor retardation” had a very low predictive value: only 3.7% patients from this group had an *MECP2* mutation.

Boys

MECP2 screening was performed in 34 patients. A subgroup of 23 had non-specific, sporadic congenital encephalopathy with severe or profound mental retardation. Epilepsy was present in 16 (infantile spasms in two), inferior limb spasticity in 14, dystonic movements in seven, autistic traits including hand stereotypes in 13, and an episode of respiratory failure leading to death was present in one. Eleven other patients had non-specific mental retardation or borderline mental status (IQ between 50 and 79) with associated autistic features, three of them with stereotypic

hand movements. Three cases were familial, two of them with a pedigree compatible with X-linked transmission. Neurological symptoms or signs (seizures, microcephaly, and moderate spasticity) were present in three. No patient was found to be mutated. One patient had a point mutation in intron 2.

Motivation for *MECP2* genotyping

To determine the clinical criteria used by paediatricians requesting the biological test, the clearest result was that paediatric neurologists and geneticists fell into two different groups: the geneticists used selective criteria while the generalist paediatricians had a tendency to assume that mental retardation and autistic traits in a girl was compatible with Rett syndrome (data not shown).

DISCUSSION

High frequency of mutations in typical cases

When patients are selected according to strict clinical criteria,²⁴ the likelihood of finding a mutation in *MECP2* coding sequences appears to be very high, nearly 90% in the present large series. Most series on RTT, in contrast, have reported a frequency of mutation usually lower and highly variable, between 35 and 77% of cases.^{15 25-37} This difference is likely due to the fact that phenotypes were less strictly defined. Our results are more in accordance with other, less systematised studies where patients were grossly subdivided into “typical” or “classical” cases, reaching a positivity >80%, whereas “possible cases” or “retarded patients with Rett-like features” reached a positivity of <50%.^{4 38-40} In our series, median age when tested in typical cases was higher than that calculated in non-typical or non-specific cases. This result probably reflects the fact that the phenotype is more likely to be complete as the child grows up. The large reserve of old mostly typical patients waiting for *MECP2* genotyping may also have increased the mean age of group 1. It remains to be determined whether this figure holds true for every ethnic group. We can only observe that various ethnic groups were represented in our cohort, as is generally the case in genetic studies in France.

Table 2b Girls with atypical RTT phenotype: patients without mutation

Patients	Age of tentative diagnosis (years)	Age of test (years)	Missing necessary criteria	OMS score
1	1.5	1.5	4, 5, 7	5
2	2	7	4	7
3	Nd	14	5	7
4	Nd	10.5	5	7
5	3.5	3.5	4, 5, 6	3
6	2.5	2.5	5, 6	6
7	3	9	5, 8	6
8	2	2	5	7
9	Nd	Nd	5, 8	6
10	Nd	6.5	5, 8	6
11	6	11	4, 5	6
12	4.5	4.5	4, 5	6
13	Nd	16	5, 8	6
14	1.5	1.5	4, 5, 8	4
15	Nd	10	5	7
16	Nd	9	5, 8	6
17	1.6	1.6	5, 6	6
18	2.5	2.5	6, 8	5
19	3	3	5, 6	6
20	Nd	22	4	7
21	2	2	5, 6, 8	5
22	Nd	Nd	4, 8	4
23	4	11	5, 8	4
24	Nd	22	5, 8	6
25	2.5	2.5	5, 7	5
26	5	15	6, 8	6
27	Nd	10	4	5

The numbers of each "missing necessary criterion" correspond to those listed in appendix A.

Poor selectivity of scores in use

Whereas the selection of well-defined clinical criteria helps in the search for *MECP2* mutations, all individual criteria do not have the same weight. The most classical features, defined by Rett and revisited by Hagberg *et al.*¹ do manifest a high specificity: the stagnation–regression of the psychomotor abilities after months of regular development, the reduction of purposeful hand movements, and the presence of monotonous hand stereotypes, when present, make the compilation of other diagnostic criteria unnecessary. In particular, the criterion "typical course in infancy" is often lacking, due to inaccurate anamnestic information.^{41–42} Nevertheless, the present inquiry shows that the simple concurrence, in a young girl, of mental retardation, low head circumference, autistic traits, and striking anomalies of hand use implies the same likelihood for an *MECP2* gene defect. Our inquiry suggests two other relevant points: (1) most practitioners poorly differentiate "hand stereotypes" observed in RTT from other stereotypies observed in autistic children. This is not a serious drawback, as the hand stereotypies most characteristic of RTT are regularly associated with a loss of purposeful hand movements, the latter item being the easiest to observe; and (2) some "inclusion criteria" according to the RSDCW group, though undoubtedly relevant to RTT diagnosis, individually remain unspecific, such as "apparently normal perinatal period" or "normal head circumference at birth". Features such as early deviant communicative ability, delayed nociception, sudden laughing/screaming spells, spine deformation, and even cold impaired vasomotor regulation can also be encountered in a large array of severe cerebral and mental disorders of infancy.

All present considerations indicate that the Osaka score appears poorly discriminatory between mutated and non-mutated cases as shown in group 2, in which many patients with scores in the range of 4–7 could be mutated or non-mutated. Any score used in clinical practice requires separate analysis of the different items. The Osaka score would be more discriminatory if the classical, major abnormal criteria were weighted with a higher value. The present experience

suggests that all patients combining at least three of the six necessary positive criteria (appendix A) should receive molecular screening, the less frequent criteria being "gait apraxia" and "communication dysfunction with loss of learned words" (table 2a). As regards research prospects, such as searching for mutations in non-coding *MECP2* sequences or in other genes involved in gene transcription regulation through CpG-methylation, the priority would logically be to include cases combining all positive necessary criteria rather than those exhibiting undifferentiated elevated Osaka scores.

Genotype/phenotype correlations

In *MECP2* disorders, correlation between specific mutations and phenotypes remains uncertain. In our series as in others,^{14, 16, 35, 40, 43–45} no clear phenotypic characteristics were observed when considering the effect of the mutation (missense, non-sense, deletion) or the expression of the most frequent mutations such as 379 C>T, 397 C>T, 473 C>T, and 763 C>T. Incomplete phenotypes, such as Rett syndrome with preservation of speech, appear to be linked to modifying factors rather than specific mutations.¹² Whether the X inactivation profile significantly interferes with the phenotype remains unclear,^{14, 35, 46, 47} and was not studied in the present series. It should be mentioned that one missense mutation (909 C>G, Ile303Met) not reported in international data bases was found in an atypical phenotype.

Non-significant involvement of *MECP2* in boys

MECP2 mutations have been shown to be responsible for severe congenital encephalopathy in boys, especially in sibships including a girl with typical RTT syndrome.^{21, 48, 49} Though also possible in sporadic encephalopathies,¹⁹ our series suggests that *MECP2* mutations do not represent a significant cause in this situation. As no suggestive clinical feature is capable of orienting the diagnosis, the search for *MECP2* mutations in boys, in common practice, should be limited either to certain cases of familial X-linked mental

retardation^{18, 22} or to severe congenital encephalopathy in a family including an RTT girl.

Our study suggested that a large proportion of paediatricians continues to think that “MECP2 is highly mutated in X-linked mental retardation”,¹⁸ both in specific and non-specific mental retardation, especially in girls. Whereas widespread MECP2 screening of mental retardation cases was certainly justified in the few years following the discovery of the gene, the present experience suggests that at least three positive necessary criteria are a reasonable prerequisite for the test in common practice. Regular information on the phenotypes related to MECP2 mutations, whether typical or less typical of RTT, will remain necessary in the near future.

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APPENDIX A

Diagnosis criteria according to the Rett Syndrome Diagnostic Criteria Work Group (RSDCWG) revised by Hagberg *et al.*²³ In the present study, the six “positive necessary criteria” are numbers 2, 4, 5, 6, 7, and 8, and the two “negative necessary criteria” are numbers 1 and 3.

NECESSARY CRITERIA

1. Apparently normal prenatal and perinatal history
2. Psychomotor development largely normal through the first 6 months or may be delayed from birth (in a girl with ultimate mental deficiency)
3. Normal head circumference at birth
4. Postnatal deceleration of head growth in the majority
5. Loss of achieved purposeful hand skills between ages 6 and 30 months
6. Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing, and washing/rubbing automatisms
7. Emerging social withdrawal, communication dysfunction, loss of learned words, and cognitive impairment
8. Impaired (dyspraxic) or failing locomotion

SUPPORTIVE CRITERIA

- Awake disturbances of breathing (hyperventilation, breath-holding, forced expulsion of air or saliva, air swallowing)

- Bruxism
- Impaired sleep pattern from early infancy
- Abnormal muscle tone successively associated with muscle wasting and dystonia
- Peripheral vasomotor disturbances
- Scoliosis/kyphosis progressing through childhood
- Growth retardation
- Hypotrophic small and cold feet; small, thin hands

APPENDIX B. THE “OSAKA MODIFIED SCORE” EVALUATED IN THE PRESENT STUDY²⁵ (EACH CRITERION HAS A VALUE OF 1)

- Apparently normal perinatal period
- Apparently regular psychomotor development through the first 6 months
- Normal head circumference at birth
- Deceleration of head growth between the ages of 5 months and 6 years
- Loss of acquired purposeful hand skills between the ages of 6 months and 30 months, associated with communication dysfunction and social withdrawal
- Development of severely impaired expressive and receptor language, presence of apparent severe psychomotor retardation
- Stereotypic hand movements such as hand wringing/squeezing/clapping/tapping, mouthing, and washing/rubbing automatisms appearing after hand skills are lost
- Appearance of gait apraxia and truncal apraxia/ataxia between the ages of 1 and 4 years.

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