

Original article

Rett syndrome in Spain: mutation analysis and clinical correlations

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Abstract

Rett syndrome (RTT) is an X-linked neurodevelopmental disease that affects girls almost exclusively. In a high proportion of patients the disease is caused by de novo mutations at the *MECP2* gene, encoding methyl-CpG-binding protein 2. With the aim to characterize the spectrum of mutations in a series of sporadic RTT patients, including an affected male, and to relate the genetic results to the clinical features of the disease, a clinical checklist and a score system were elaborated to evaluate the clinical severity of the disease. Mutation analysis of the *MECP2* coding region was done by direct sequencing. De novo mutations were found in 60% of the patients, including both classic and atypical forms. The change R133H was identified in a 13-year-old boy showing a classic RTT phenotype and normal karyotype. Significant differences were observed among missense and truncating mutations regarding disease severity, age of onset of stereotypies, and the ability of the patients to sit alone and to walk. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Rett syndrome; *MECP2*; Mutation analysis; Genotype/phenotype correlations; Male Rett syndrome

1. Objectives

The aim of this study was to characterize the spectrum of *MECP2* mutations in a series of Spanish sporadic Rett syndrome (RTT) patients and to evaluate the contribution of the type of mutation to the final phenotype. With this purpose in mind, a clinical checklist and a score system to test the clinical variables were elaborated.

We present preliminary results on the spectrum of *MECP2* mutations found in our population, including our case of a 13-year-old male with classical RTT. Clinical features that have been found to correlate with the patients' type of mutation are also reported.

2. Subjects and genetic methods

Forty-six sporadic female patients and one affected male with known *MECP2* mutations are presented. Healthy parents and unaffected sisters were also analyzed. Informed consent was obtained from all subjects. DNA was prepared from peripheral blood lymphocytes by standard methods. The coding region of the *MECP2* gene was studied by direct sequencing.

3. Clinical data management

All patients were submitted to a strict selection and were diagnosed according to the Rett Syndrome Diagnostic Criteria Work Group. We analyzed 34 females and one male with classical RTT, and ten atypical female cases (five congenital, four preserved language and one form fruste). For genotype/phenotype correlations, patients were classified according to the type of mutation. A score was assigned to each clinical variable, following the criteria summarized in Table 1. Higher scores indicate greater severity. Score frequencies from each clinical feature were compared between missense and truncated protein (PTT) mutations. A global severity score was obtained for each patient by adding individual scores. The Fisher exact test was applied to measure the significance of the observed differences.

4. Results

4.1. Spectrum and distribution of *MECP2* mutations

The 47 mutations found in our patients are summarized in Fig. 1.

4.2. *MECP2* mutation in a classical RTT male patient

We report the first described case of a male with classical RTT and normal 46, XY karyotype. At 7 years of age he

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Table 1
Clinical data management

	Score	Definition
Age of onset	3	0–12 months
	2	12–24 months
	1	>24 months
Microcephaly	0	Absent
	1	Present
Sits unsupported	0	Acquired <8 months
	1	Acquired 8–16 months
	2	Acquired >16 months
	3	Never acquired
Ambulation	+1	Lose acquisition
	0	Acquired <18 months
	1	Acquired <30 months
	2	Acquired >30 months
	3	Lose acquisition
Language	4	Never acquired
	0	Preserved and propositive
	1	Lost
	2	Never acquired
Epilepsy	0	Absent
	1	Present and controlled
	2	Uncontrolled or early epilepsy
Respiratory function	0	No dysfunction
	1	Hyperventilation and/or apnea
Hands use	0	Acquired and conserved
	1	Lose purposefulness: 2–6 years
	2	Lose purposefulness: <2 years
	3	Lose all acquisitions
Onset of stereotypies	4	Never acquired
	0	>10 years
	1	>36 months
	2	18–36 months
	3	>18 months

fulfilled 8/9 necessary criteria, 7/8 supportive criteria and no exclusion criteria. Necessary criteria include normal prenatal and perinatal period, normal neurodevelopment until 12 months of age, acquired microcephaly, loss of purposeful hand use, complete loss of language skills, stereotypic hand movements (wringing, clasping and clapping), and gait

apraxia. Diagnosed at 7 years old at his first visit, he lives in a special care institution because of the death of his parents; the head circumference at birth could not be documented. Supportive criteria include breathing dysfunction, epilepsy, abnormal EEG, spasticity, vasomotor dysfunction, scoliosis, and growth retardation. At present he is 13 years old and is still walking.

MECP2 analysis showed the presence of the nucleotide change 398G → A(R133H) (Fig. 1). Both residues belong to the basic amino acid group. The mutation affects a conserved residue within the methyl-binding domain of MeCP2 protein. Parents were not available to prove its de novo origin, but it was not found in 156 sequenced chromosomes. The pathogenicity of this change will have to be tested by means of functional studies.

4.3. Clinical correlations

We had completed or almost completed studies of clinical data from 46 out of 47 RTT genetically analyzed patients. Patients' mutations were classified into two groups: missense mutations (*n* = 18) and protein truncating mutations (PTC) (*n* = 23). The five large intragenic deletions were not included in the statistical analysis (see below). Data analysis showed the following results.

(i) No differences were observed among missense and PTC for age of onset of the first symptoms, presence or absence of acquired microcephaly, presence or absence of respiratory dysfunction, presence or absence of seizures (although the three patients with epilepsy not controlled with anti-epileptic drugs carried PTC mutations), hand use and language acquisition and preservation.

(ii) Significant differences are summarized in Table 2.

(iii) PTC mutations are associated with a more severe disease while missense mutations lead to milder RTT clinical forms (Fig. 2).

(iv) Large deletions upstream of the transcriptional repression domain-associated variable phenotypes: two

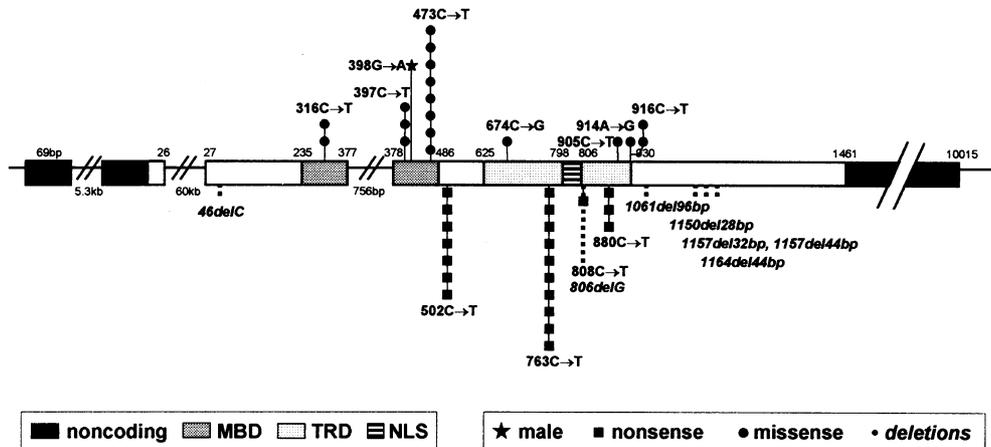


Fig. 1. Gene structure and mutations detected in the *MECP2* coding region. Forty-seven mutations are shown, corresponding to 21 nonsense mutations (upper squares), 18 missense mutations (lower circles) and eight deletions (italics). The 398G → A mutation found in the classical RTT boy is represented by a star.

Table 2
Significant differences

	Missense	Truncating protein	Fisher test (<i>P</i> value)
Sits unsupported	72% (13/18) sat before 8 months 100% (18/18) conserve	27% (6/22) sat before 8 months 18% (4/22) loose	0.01
Ambulation	61% (11/18) walked before 18 months 94% (16/17) conserve	0% (0/22) walked before 18 months 45% (10/22) never walked	< 0.0001
Stereotypies	37% (6/16) onset after 36 months	5% (1/22) onset after 36 months	0.01
Severity (global score)	6% (1/15) score >15 47% (7/15) mild RTT, score <10	48% (10/21) score >15 48% (10/21) severe or congenital, score >15	< 0.01

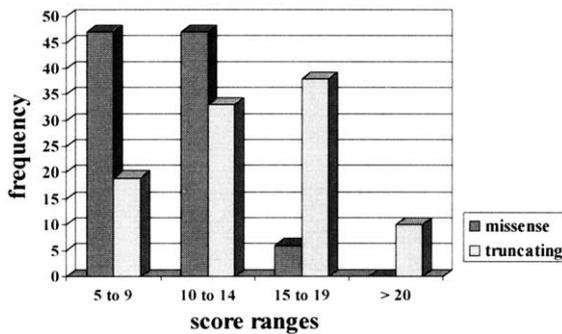


Fig. 2. Global severity scores by mutation type. Missense mutations are preferentially associated with lower severity scores, which correspond to milder RTT clinical forms, while truncating mutations lead to more severe phenotypes, represented by higher severity scores.

were found in severely affected patients (global scores of 19 and 16, respectively); one was found in a classical patient (global score of 11); and two were found in very mild forms (global scores of 5 and 6).

5. Conclusions

From the present work we can conclude that

- Mutations at the *MECP2* coding region cause the broad spectrum of classical and atypical forms of RTT.
- Males can be affected by classical RTT.
- Significant differences can be found between missense and truncating mutations in relation to the following clinical features: sitting alone (age of acquisition and conservation); ambulation (age of acquisition and conservation); age of onset of stereotypies.
- Missense mutations are associated with milder forms of RTT.
- Truncating mutations are associated with a more severe disease.
- Large deletions are found both in very mild and in severe classical patients.

Acknowledgements

We thank the Asociación Valenciana and Catalana de Síndrome de Rett for their support, all RTT patients and families for their enthusiastic participation, and M. Naudó for technical assistance. This work was supported by Fondo de Investigación Sanitaria FIS99/0235 and by the Asociación Valenciana y Catalana de Síndrome de Rett. J.A. is the recipient of a fellowship from Fondo de Investigación Sanitaria FIS99/0235.