



## Original article

# Rett syndrome with and without detected MECP2 mutations: An attempt to redefine phenotypes

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## Abstract

**Background:** The diagnosis of Rett syndrome (RTT) is based on a set of clinical criteria, irrespective of mutation status. The aims of this study were (1) to define the clinical differences existing between patients with Rett syndrome with (Group I) and without a MECP2 mutation (Group II), and (2) to characterize the phenotypes associated with the more common MECP2 mutations. **Patients and methods:** We analyzed 87 patients fulfilling the clinical criteria for RTT. All were observed and videotaped by the same paediatric neurologist. Seven common mutations were considered separately, and associated clinical features analysed. **Results:** Comparing Group I and II, we found differences concerning psychomotor development prior to onset, acquisition of propositive manipulation and language, and evolving autistic traits. Based on age at observation, we found differences in eye pointing, microcephaly, growth, number of stereotypies, rigidity, ataxia and ataxic-rigid gait, and severity score. Patients with truncating differed from those with missense mutations regarding acquisition of propositive words and independent gait, before the beginning of the disease, and microcephaly, growth, foot length, dystonia, rigidity and severity score, at the time of observation. Patients with the R168X mutation had a more severe phenotype, whereas those with

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R133C showed a less severe one. Patients with R294X had a hyperactive behaviour, and those with T158M seemed to be particularly ataxic and rigid. *Conclusion:* A clear regressive period (with loss of prehension and language, deceleration of growth) and the presence of more than three different stereotypies, rigidity and ataxic-rigid gait seemed to be very helpful in differentiating Group I from Group II.

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## 1. Introduction

Rett syndrome (RTT) was first described by Andreas Rett, in 1966 [1], and rediscovered in 1983, by Bent Hagberg et al. [2]. Since 1999, after the description of mutations in the MECP2 gene as the cause of RTT [3], we know that more than 90% of the patients with classical, but only 30% with variant RTT have a MECP2 mutation. Thus, we can consider two groups of patients: Group I with clinical features of RTT and a mutation in the MECP2 gene and Group II with clinical features of RTT without a mutation in the MECP2 gene, and speculate that maybe, in addition to Group I patients, different diseases are currently under the large RTT umbrella. Widespread availability of the molecular diagnosis creates a great opportunity for this to be clarified, based on clinical and genetic grounds.

Several series of patients with genetic studies have been published, and attempts of phenotype-genotype correlations made. The results, however, were not conclusive, as different methodologies were applied in the various studies, concerning clinical and mutation classifications and severity score systems [4–6]. Nevertheless, the larger studies pointed to a broad correlation between type of mutation and phenotype, patients with truncating mutations having a more severe phenotype, than those with missense mutations [5–12].

Two different genetic factors are likely to influence the phenotype in RTT: the type and location of the mutations, and the X inactivation pattern [13]. Concerning the phenotype of specific mutations, several studies [14–17] showed that patients with the R133C and R306C mutations have better overall function. In general, the most severe outcomes were found with the R270X and R255X mutations [11,16]. Mutations toward the N terminus, including T158M and R168X, were also associated with a more severe phenotype [12,17]; however, no specific neurological or behavioural findings were identified in patients with these mutations, with the exception of an association of fear and anxiety with R133C and R306C [18], and mood abnormalities in patients with R294X [16].

The aims of this study were (1) to define the clinical differences between patients with (Group I) and without (Group II) known mutations in the MECP2 gene, in order to delineate the clinical boundaries of the disease; (2) to analyze the genotype-phenotype correlation in

Group I patients and characterize phenotypes associated with the more common MECP2 mutations; and (3) to identify sub-groups of patients with RTT that might be candidates for mutational search in other genes.

## 2. Subjects and methods

All Portuguese paediatric neurologists were asked to indicate their patients with possible RTT. Patients included in the study ( $n = 87$ ) fulfilled the revised clinical criteria for RTT [19]. They were observed and videotaped by the same paediatric neurologist (TT), and a clinical checklist for RTT was completed. Informed consent was obtained from all parents, for blood sampling and to take and use videos and photographs; 36 patients were observed and videotaped at least twice, at 6 months intervals.

Blood samples from patients and their parents were received at the laboratory, and genomic DNA was extracted using the Puregene DNA isolation kit (Gentra, Minneapolis, MN). The coding region and exon–intron boundaries of the MECP2 gene were amplified by PCR and sequenced. The RD-PCR method was used, as described [20], for the detection of large rearrangements in the MECP2 gene. Primers and PCR conditions are available upon request.

X-chromosome inactivation (XCI) assays were performed with genomic DNA isolated from leukocytes of peripheral blood. The assay was based on a previously described method [21], which allows the determination of the X-inactivation status, using a trinucleotide repeat polymorphism in the androgen receptor gene (*AR*, RefSeq ID: NM\_000044.2), flanked by two methylation-sensitive restriction enzyme sites. The restriction enzyme (*HhaI*) will hydrolyze only the unmethylated alleles. Scoring of the XCI pattern was made by densitometry of the amplified DNA bands.

MECP2 mutations were categorized and grouped for analysis according to: (1) type of sequence change – missense or truncating (including nonsense and frameshift, but also large deletions) and (2) location of the mutation (affected domain) – TRD, MBD. The seven most common mutations were considered individually, and their associated features analysed.

For statistical analysis, patients were divided into two groups: those with (Group I) and without a molecular

diagnosis (Group II). Within the mutation-positive group, comparisons were made between patients carrying missense and truncating mutations. Cases were scored using the Pineda severity scale [6]. The SPSS (v.14) statistical package was used to analyse the data. Proportions were compared using Chi-square and Fisher's exact test. Quantitative variables were compared using the Mann–Whitney test and were presented as median (25–75th percentile).

### 3. Results

In this study we analyzed the entire coding sequence of the MECP2 gene in 87 patients, who fulfilled the revised clinical criteria for RTT [19]. Cases were classified as classical (58.6%) and variant (41.4%) forms (congenital, preserved speech, *forme fruste*, early epileptic encephalopathy and male patients) [22].

#### 3.1. Clinical differences between MECP2 positive and negative RTT patients

Mutations in MECP2 were found in 67.8% ( $n = 59$ ) of all patients, corresponding to 96.0% of classical forms and of 27.8% variants (seven congenital forms; three preserved speech). In 28 patients MECP2 mutations were not found. Among these patients 60.7% were congenital, 10.7% males, 10.7% early epileptic encephalopathies, 7.1% classical, 7.1% *forme fruste* and 3.6% preserved speech forms.

We identified a total of 29 different mutations in MECP2 including two large deletions and a mutation in exon 1; most mutations were located in exons 3 and 4 (Fig. 1). Nine of these mutations were never described before: A7fsX37, K39fsX43, Q110X, S113P, T184fsX

185, R253fsX275, I303fsX399, V380M and L386fsX399. All were *de novo* mutations, except V380M which was present in the unaffected mother (who had a balanced XCI pattern) and in 4 X chromosomes in a control population of Portuguese origin including 230 X chromosomes. We assumed the truncating mutations as causative of the disease; the new missense mutation S113P was excluded in the parents and in a control population of Portuguese origin including 230 X chromosomes.

XCI patterns were analysed for all patients carrying a MECP2 mutation, though data was not informative for 14; among the 46 patients remaining, only two showed a skewed XCI pattern. Of these, one patient with a R168X mutation had a less severe form (and a lower severity score) compared with the group of patients with the same mutation: she acquired and maintains independent gait, had neither epilepsy nor microcephaly. The other patient carried the T158M mutation, and had a phenotype similar to those carrying the same mutation. Median age at first observation and median duration of the disease were not significantly different between Group I and II, neither between groups with missense and truncating mutations. A significant difference was found when comparing clinical stage: more patients with Group I, especially those with truncating mutations, were in stage IV of the disease, which may mean that their rate of evolution was faster.

Comparison between Group I and II showed some important clinical differences since birth (Table 1). Dystocic delivery (caesarean or forceps) seemed to be more common in Group I than Group II patients, though this did not reach significance. Additionally, none of the Group II patients presented microcephaly at birth, but 6 (10.2%) of Group I had an occipito-

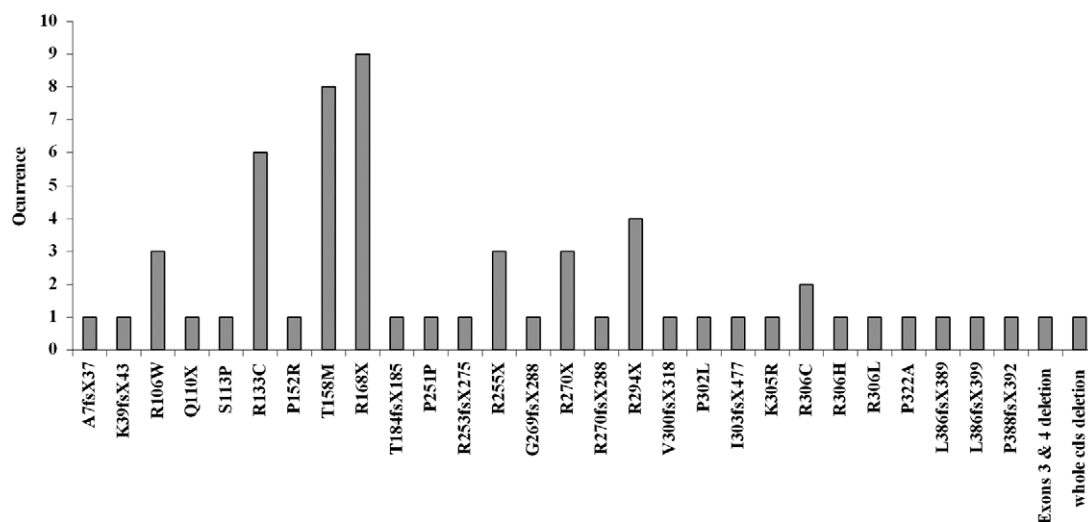


Fig. 1. Different mutations found in MECP2 in 87 Portuguese RTT patients.

Table 1  
Clinical data of Group I and Group II patients.

|  | Group I (n = 59)  | Group II (n = 28) | p                | Missense (n = 26) | Truncating (n = 33) | p             |
|--|-------------------|-------------------|------------------|-------------------|---------------------|---------------|
| Median age (years) <sup>a</sup>                  | 7.6 (4.1–14.3)    | 7.8 (5.1–12.7)    | 0.730            | 7.0 (3.7–10.6)    | 11.3 (4.5–14.8)     | 0.275         |
| Median duration of disease (months) <sup>a</sup> | 92 (55–140)       | 82 (35–139)       | 0.411            | 72 (26–111)       | 124 (44–157)        | 0.240         |
| <i>Stage (%)</i>                                 |                   |                   |                  |                   |                     |               |
| Stage II   | 8.5               | 3.6               | 0.153            | 7.7               | 9.0                 | <b>0.008</b>  |
| Stage III  | 61.0              | 82.1              |                  | 80.8              | 45.5                |               |
| Stage IV A and IV B                              | 30.5              | 14.3              |                  | 11.5              | 45.5                |               |
| <i>Perinatal data (%)</i>                        |                   |                   |                  |                   |                     |               |
| Abnormal delivery                                | 28.8              | 21.4              | 0.466            | 23.1              | 33.3                | 0.566         |
| <i>Microcephaly at birth</i>                     |                   |                   |                  |                   |                     |               |
| Yes  | 10.2              | 0.0               | 0.263            | 11.5              | 9.1                 | 0.999         |
| Missing information                              | 16.9              | 21.4              |                  | 15.4              | 18.2                |               |
| <i>Reported psychomotor development (%)</i>      |                   |                   |                  |                   |                     |               |
| Normal until 12 months of age                    | 59.3              | 17.9              | <b>&lt;0.001</b> | 69.2              | 51.5                | 0.268         |
| Stagnation                                       | 93.2              | 92.9              | 0.999            | 92.3              | 93.9                | 0.999         |
| Median age of stagnation (months) <sup>a</sup>   | 12.0 (10.0–18.0)  | 6.0 (4.0–11.3)    | <b>&lt;0.001</b> | 16.0 (10.0–18.0)  | 12.0 (9.0–18.0)     | 0.172         |
| Regression                                       | 86.4              | 25.0              | <b>&lt;0.001</b> | 84.6              | 87.9                | 0.999         |
| Median age of regression (months) <sup>a</sup>   | 18.00 (14.0–24.0) | 14.00 (11.0–24.0) | 0.202            | 18.0 (18.0–24.5)  | 18.0 (13.0–23.0)    | 0.160         |
| Acquisition of autistic traits                   | 83.1              | 50.0              | <b>0.002</b>     | 76.9              | 87.9                | 0.222         |
| Median age of acquisition of autistic traits     | 18.0 (12.5–18.0)  | 7.5 (3.75–18.0)   | <b>0.005</b>     | 18.0 (14.0–21.0)  | 15.0 (12.0–18.0)    | 0.175         |
| Propositive manipulation                         | 91.5              | 57.1              | <b>&lt;0.001</b> | 92.3              | 93.8                | 0.999         |
| Median age of acquisition of stereotypies        | 20.0 (14.5–25.0)  | 24.0 (14.5–36.0)  | 0.425            | 24.0 (18.0–28.5)  | 18.00 (13.3–24.0)   | 0.086         |
| Propositive words                                | 71.2              | 17.9              | <b>&lt;0.001</b> | 88.5              | 57.6                | <b>0.021</b>  |
| Acquisition of independent gait                  | 63.3              | 50.00             | 0.200            | 84.6              | 48.5                | <b>0.009</b>  |
| <i>Data at observation</i>                       |                   |                   |                  |                   |                     |               |
| Eye pointing                                     | 96.6              | 57.1              | <b>&lt;0.001</b> | 92.3              | 100.0               | 0.190         |
| Microcephaly at time of observation              | 45.8              | 39.3              | 0.569            | 19.2              | 66.7                | <b>0.001</b>  |
| Low weight (<5th percentile)                     | 43.3              | 18.5              | <b>0.031</b>     | 23.1              | 57.6                | <b>0.009</b>  |
| Low height (<5th percentile)                     | 55.2              | 30.8              | <b>0.038</b>     | 38.5              | 68.8                | <b>0.033</b>  |
| Low foot length (<3th percentile)                | 67.9              | 45.8              | 0.112            | 47.8              | 83.3                | <b>0.008</b>  |
| Agitation  | 56.7              | 67.9              | 0.497            | 50.0              | 63.6                | 0.426         |
| Laughing spells                                  | 69.5              | 53.6              | 0.227            | 61.5              | 75.8                | 0.372         |
| Abnormal sleep pattern                           | 55.9              | 53.6              | 0.836            | 46.2              | 63.6                | 0.199         |
| Hyperpnea/Apnea                                  | 78.0              | 82.1              | 0.868            | 69.2              | 84.8                | 0.209         |
| Epilepsy   | 57.6              | 75.0              | 0.183            | 46.2              | 66.7                | 0.184         |
| Controlled                                       | 58.8              | 76.2              | 0.306            | 69.2              | 52.4                | 0.477         |
| Vaso-motor disturbances                          | 71.2              | 78.6              | 0.639            | 53.8              | 84.8                | <b>0.019</b>  |
| Neurogenic muscle atrophy                        | 49.2              | 28.6              | 0.114            | 42.3              | 54.5                | 0.435         |
| Chewing difficulties                             | 78.0              | 71.4              | 0.691            | 65.4              | 87.9                | 0.080         |
| Pyramidal signs                                  | 28.8              | 39.3              | 0.3465           | 23.1              | 33.3                | 0.566         |
| Ataxia   | 35.6              | 10.7              | <b>0.030</b>     | 46.2              | 27.3                | 0.219         |
| Number of stereotypies per patient <sup>a</sup>  | 4.00 (3.0–6.0)    | 3.00 (2.2–4.0)    | <b>0.050</b>     | 3.50 (2.8–7.0)    | 4.00 (3.0–6.0)      | 0.999         |
| Dystonia   | 64.6              | 75.0              | 0.458            | 46.2              | 78.8                | <b>0.0209</b> |
| Rigidity   | 49.2              | 7.1               | <b>&lt;0.001</b> | 34.6              | 60.6                | <b>0.046</b>  |
| Tremor   | 49.2              | 35.7              | 0.344            | 50.0              | 48.5                | 0.999         |
| Ataxic/rigid gait                                | 43.6              | 0.0               | <b>0.001</b>     | 36.8              | 50.0                | 0.523         |
| Scoliosis  | 72.9              | 67.9              | 0.818            | 69.2              | 75.8                | 0.791         |
| <i>Gastro-intestinal (%)</i>                     |                   |                   |                  |                   |                     |               |
| Gastro-esophageal reflux                         | 11.9              | 14.3              | 0.999            | 11.5              | 12.1                | 0.999         |
| Pineda's scale <sup>a</sup>                      | 13 (9–15)         | 14 (12–16)        | <b>0.034</b>     | 11 (8–13)         | 14 (11–16)          | <b>0.001</b>  |

<sup>a</sup> Data presented as median (25–75th percentile).

frontal circumference (OFC) below the 5th percentile (3 truncating, 3 missense mutations). Also, 63.6% of Group II patients presented an OFC at birth above the 25th percentile, against 26.6% of Group I patients. Stagnation of OFC growth occurred in 71.7% of Group I and 53.6% of Group II, at a median age of 6 months in both groups. Later, microcephaly was

acquired in 45.8% of Group I (67.6% among truncating, 19.2% for missense mutations) and 39.3% of Group II patients. Interestingly, 13.3% of Group I patients presented an OFC above the 25th percentile (3% above the 75th percentile); 26.9% of patients with missense mutations presented an OFC above the 25th percentile, against only 2.9% of those with truncating

mutations. Differences were also found for height and weight, comparing the two groups, and for head size, height, weight and foot length, comparing the group of patients with missense *versus* truncating mutations (Table 1).

Clinical classification in these groups was also different: 83.0% of Group I were classified as classical, against only 7.1% of Group II patients. Significant differences were found when comparing psychomotor development before the onset of the disease: in a higher percentage of Group II patients, this was not considered to be normal, by the parents, before the age of 12 months; a clear regression period was evident in 86.4% of Group I, but only 25.0% of Group II patients; acquisition of propositive words was more common in patients with Group I, specially those with missense mutations; evolving of autistic traits (loss of social communication) was described in 83.1% of Group I and 50% of Group II, and it occurred earlier in life in Group II; in 42.9% of Group II, propositive manipulation was never acquired (against 8.5% in Group I); independent gait was acquired by 84.6% of patients with missense, but only in 48.5% of those with truncating mutations (Table 1).

Besides growth failure, we found other important differences concerning clinical data of the patients at observation: eye pointing was present in 96.6% of Group I, but only in 57% of Group II patients; rigidity and ataxia were more frequent in Group I, and an ataxic-rigid gait was observed only in them. They also displayed a greater number of stereotypies. Dystonia, rigidity, vaso-motor disturbances and oral motor difficulties were more frequent in patients with truncating mutations (Table 1). We found no significant differences between the group I and II concerning pyramidal signs, dystonia, neurogenic muscle atrophy, vaso-motor disturbances, scoliosis, epilepsy or gastro-intestinal disturbances. Abnormal sleep pattern, agitation, laughing spells and hyperpnea/apnea were also not more frequent in patients with known mutations.

The Pineda's severity score showed that the median severity was greater in the Group I group and, within this group, in those with truncating mutations (Table 1).

### 3.2. Clinical presentation among MECP2 positive RTT patients (group i)

In general, we identified three neurological presentations in Group I patients:

(1) Some had a *severe form* ( $n = 27$ ; 45,8% of all Group I patients), with major axial hypotonia, in which dystonia and rigidity were present after few years of evolution (median, 11 years). These patients either regressed earlier (median, 15.0; range, 6–36 months), or never had been normal (18.5%); 74.0% did not acquire independent gait; those who did, lost it at a median age of 66 months (range, 24–120). Epilepsy was present in 70.4% of the cases. Among patients with the severe form, 81,4% had truncat-

ing mutations, and all but one with the R168X mutation presented it; they showed a median severity score of 15 (range, 12–20).

(2) In an *intermediate form*, ataxia predominated ( $n = 17$ ; 28.8% of all Group I patients). Most acquired an independent gait (94.1%), but this was both ataxic and rigid; they regressed at a median age of 18 months (13–26), and 52.9% had epilepsy. Their median severity score was 11 (range, 8–14); 68.8% of the patients with this presentation had missense mutations, particularly T158M.

(3) A *milder form* was present in 15 (25,4%) of Group I patients, with few neurological signs except for mental retardation and autistic features. All acquired independent gait and maintained it during the period of follow-up; 40% developed focal dystonia after some years of evolution, and gait became rigid in two patients (one with the R133C mutation, older than 20 years; one six years old with the R294X mutation). Epilepsy was present in 33.3% of these patients. They regressed at a median age of 25 months (range, 13–60), and had a median severity score of 9 (range, 4–11); 60% had missense mutations. Most of our patients with the R133C mutation illustrate this presentation well. Three patients with R294X mutation also presented this phenotype.

Interestingly, the distribution of mutation types was significantly different between these clinical groups. In the milder group, 60% of the patients had missense mutations. In the intermediate group, 70,6% of the patients had missense mutations; and in the more severe group 81.5% of the cases had a truncating mutation. The frequency of missense *versus* truncating mutations was significantly different between the three clinical subtypes (Fisher's exact test,  $p = 0.001$ ) (Fig. 2).

### 3.3. Clinical presentation associated with specific MECP2 mutations

The clinical features associated with specific mutation groups found in Group I patients are presented in

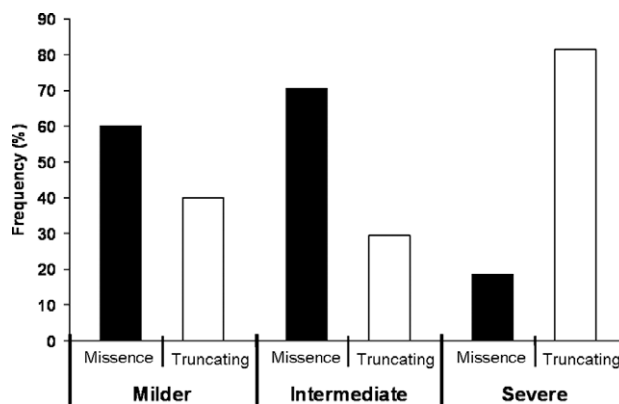


Fig. 2. Frequency of missense *versus* truncating mutations according to the three clinical subtypes.

**Table 2.** Overall, we observed that those with the *R168X* mutation presented the most severe phenotype. Only two of nine patients with this mutation acquired independent gait; one lost it one year later, while the other had a very rigid and ataxic gait. Two presented microcephaly at birth and six developed it within the 2nd year of life. The majority had low height and weight, and epilepsy. They also had continuous hands-apart stereotypies.

Group I patients with the *R294X* mutation seemed to have a peculiar phenotype with an extremely unusual hyperactive motor behaviour. They had a lot of different and exuberant hand stereotypies and with other topographies (including vocal), and all had epilepsy. All displayed focal or segmental dystonia, and, in three, gait became rigid and broad based, in the earlier stages of the disease; however, they maintained a good eye contact, and seemed less profoundly retarded than other patients with better motor function.

Patients with the *R133C* mutation had the mildest phenotype among all those within the Group I. None had microcephaly, and all but one acquired and maintained independent gait. Three could make phrases, albeit with a peculiar voice tone, and answered to simple questions, after a considerable delay of time. Also, only one had epilepsy (the same patient who lost independent gait), and five maintained propulsive prehension (three fed themselves with a spoon).

Some particular clinical features were also found in patients with the *T158M* mutation; the majority could walk without support, but their gait tended to be very rigid and ataxic, since the beginning of the disease; postural and intention tremor was evident, and they had many different stereotypies.

**4. Discussion**

*4.1. Clinical features: redefining MECP2 positive and negative RTT*

The diagnosis of Rett syndrome remains clinical, and this may lead clinicians to decide whether or not, to request a laborious MECP2 mutation screening. Both from a scientific and economic point of view, it is very important to define more restrictive criteria, to improve the rate of finding a MECP2 mutation, and to identify groups of patients that may have a different disease.

Comparing patients *with* and *without* a MECP2 mutation (Group I and Group II), we concluded that there are significant clinical differences between them. Among Group II patients, only a minority had a normal development during the first year of life, and a regressive period was not evident to their parents; also, autistic behaviour, when noticed, occurred very early in life, which means that these children may never have been normal.

Table 2  
Clinical characteristics of patients with the seven most frequent mutations.

|  | R168X n = 9            | T158M n = 8 | R133C n = 6      | R294X n = 4 | R270X n = 3 | R106W n = 3 | R255X n = 3 |
|--|------------------------|-------------|------------------|-------------|-------------|-------------|-------------|
| Median age (years) <sup>a</sup> (range)                | 11 (2-23)              | 6 (1-16)    | 7 (5-31)         | 6.5 (5-11)  | 7 (2-24)    | 7 (3-7)     | 15 (2-21)   |
| Median age of regression (months) <sup>a</sup> (range) | 12 (6-23)              | 18 (14-27)  | 34 (18-60)       | 33 (18-36)  | 14 (13-21)  | 18 (all 18) | 15 (13-28)  |
| Clinical classification                                | 8 classical 1 co-natal | 8 classical | 3 classical 3 PS | 4 classical | 3 classical | 3 classical | 3 classical |
| Microcephaly (%)                                       | 66.6                   | 2.5         | 0.0              | 50          | 66.6        | 66.6        | 33.3        |
| Low weight (%)   | 77.7                   | 37.5        | 16.6             | 2.5         | 33.3        | 0.0         | 66.6        |
| Low height (%)   | 66.6                   | 50          | 50               | 2.5         | 66.6        | 33.3        | 66.6        |
| Acquisition of independent gait (%)                    | 22.2*                  | 87.5*       | 100*             | 100*        | 66.6        | 33.3        | 33.3        |
| Independent ataxic-rigid gait (n)                      | 1/2                    | 5/7         | 1/5              | 2/4         | 0/2         | 0/1         | 1/1         |
| Ability to speak (%)                                   | 0.0                    | 0.0         | 50               | 0.0         | 0.0         | 0.0         | 0.0         |
| Propulsive prehension (%)                              | 11                     | 50          | 83.3             | 75          | 33.3        | 33.3        | 33.3        |
| No. of stereotypies <sup>a</sup>                       | 3 (2-8)                | 4-50 (2-11) | 3 (2-10)         | 9-50 (5-12) | 3 (9-3)     | 3 (3-4)     | 3 (3-4)     |
| Motor agitation (%)                                    | 0.0                    | 37.5        | 50               | 100**       | 66.6        | 66.6        | 66.6        |
| HPP/Apnoea (%)   | 88.8                   | 7.5         | 66.6             | 100         | 66.6        | 66.6        | 66.6        |
| Rigidity (%)   | 66.6                   | 62.5        | 16.6             | 100         | 33.3        | 33.3        | 33.3        |
| Epilepsy (%)   | 66.6                   | 37.5        | 16.6             | 100         | 66.6        | 66.6        | 33.3        |
| Scoliosis or Kyphosis (%)                              | 77.7                   | 62.5        | 66.6             | 50          | 66.6        | 66.6        | 66.6        |
| Score in Pineda's scale <sup>a</sup>                   | 16 (9-19)              | 11 (7-14)   | 7.5 (4-11)       | 10 (8-13)   | 11 (10-16)  | 13 (8-15)   | 12 (11-14)  |

PS, preserved speech.

\* One patient lost independent gait, 1 year after acquisition.

\*\* Very agitated.

<sup>a</sup> Data presented as median (25-75th percentile).

Propositive manipulation was acquired in the vast majority of Group I, but less frequently in Group II patients. On the whole, we observed that patients with no detected MECP2 mutation had worse social contact and less stereotypies. Our results suggest that eye pointing seems to be an important sign to suspect for Group I. Rigidity, and especially the presence of an ataxic-rigid gait, seems to be very specific of Group I: none of the patients without a MECP2 mutation presented it. Growth failure was also more evident in the group with detected mutations. Surprisingly, hyperpnea/apnea were not more frequent in patients with known mutations, which suggests that this sign is not particularly helpful to differentiate group I from group II patients.

#### 4.2. Genotype-phenotype correlations in MECP2 positive RTT

At a comparable age of observation, patients with truncating mutations had a higher probability of displaying microcephaly, as well as low weight, low height, small foot length and dystonia. Rigidity and oral motor difficulties, albeit not significant, were also more frequent in the group of patients with truncating mutations. Overall, the mean severity score of the patients with truncating mutations was higher, and these patients were more likely to display a severest than a milder or intermediate phenotype as was already described by other authors [11–16].

Regarding the most common MECP2 mutations in our series, patients with the R294X mutation seemed to have an extremely unusual hyperactive motor behaviour. They had many different stereotypies, and all had epilepsy. Robertson et al. [18] described also that patients with this mutation were more likely to have mood difficulties, but they did not report hyperactive motor behaviour; they also reported that hand stereotypies were less frequent in these patients, which is in contradiction with our data.

Ambulant Group I patients may have a normal gait for decades, mainly those with less severe mutations, as is the case of R133C; however, the majority of ambulant patients have a particular wide-based and rigid gait, unique of this disease. Bradykinesia and rigidity appear at different times of evolution, some patients presenting these neurological signs very early in life. As we showed in previous work, stereotypies other than those with the hands tend to disappear, and even hand stereotypies slowed down with age, which may reflect the neurochemical evolution of this complex disease [23].

In Group I patients, motor disability was not necessarily correlated with severity of the behavioural phenotype or mental impairment. In our sample, some girls who never acquired independent gait had a good social contact and seemed to be calmer than others with very good motor function.

#### 4.3. MECP2 positive RTT: explaining the symptom clusters

It is not clear why different MECP2 mutations can produce a particular phenotype, but one hypothesis is that some CNS areas are more susceptible than others to the degree of dysfunction of this protein.

The different timing of disease onset among patients with a MECP2 mutation and the different neurological pictures – previously recognized by others, but with a different classification [24] – may be partially explained by the preferential function of MECP2 in mature, rather than immature neurons [25]. One could speculate that a less functional protein may affect more dramatically the brainstem and cerebellar structures, provoking, for instance, such a severe hypotonia that patients can never acquire an independent gait. On the other hand, a more functional (or less disruptive) protein may not affect so severely the cerebellar structures, but absence of its full functionality may still be critical for the cortical function and, thus, provoke a milder phenotype where mental retardation predominates. In the human brain, although the number of MeCP2-expressing neurons increases dramatically throughout gestation, the percentage of neurons expressing MeCP2 in the cortex continues to increase from birth to age 10 years [25]. This may also contribute to explain the progressive features of this disorder.

#### 5. Final remarks

Some important questions remain, nevertheless: why are some classical forms of RTT not associated with MECP2 mutations? How do different gene mutations produce a similar phenotype? Detailed analysis of larger series of mutation-negative RTT patients may help define clinically homogeneous sub-groups that are good candidates for the search of mutations in other genes.

#### Disclosure

The author report no conflict of interest.

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