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Analysis of the phenotypes in the Rett Networked Database.

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ABSTRACT

BACKGROUND: Rett spectrum disorder is a progressive neurological disease and the most common genetic cause of mental retardation in females. MECP2, CDKL5, and FOXG1 have been found to be the causative genes. Each gene and different mutations within each gene contribute to variability in clinical presentation and several groups worldwide performed genotype-phenotype correlation studies using cohorts of patients with classic and atypical forms of Rett spectrum disorder.

OBJECTIVE and METHODS: The Rett Networked Database is a unified registry of clinical and molecular data of Rett patients and it is currently one of the largest Rett registry worldwide with more than 1900 records provided by Rett expert clinicians from 13 countries.

RESULTS: Collected data revealed that the majority of MECP2 mutated patients present with the classic form, the majority of CDKL5 mutated patients with the early onset seizure variant and the majority of FOXG1 mutated patients with the congenital form. A computation of severity scores further revealed significant differences between groups of patients and correlation with mutation types.

CONCLUSIONS: The highly detailed phenotypic information contained in the Rett Networked Database allow to group patients presenting specific clinical and genetic characteristics for studies by the Rett community and beyond. These data will also serve for the development of clinical trials involving homogeneous groups of patients.

Keywords: patient registry, Rett syndrome, genotype-phenotype correlation.
INTRODUCTION

Rett syndrome (RTT, OMIM 312750) is a severe X-linked neurodevelopmental disorder that affects predominantly females with an incidence of approximately 1 in 10,000 female births. [1,2,3]. Classic RTT is infrequently observed in males because a deleterious mutation in the only copy of MECP2 typically results in severe neonatal encephalopathy and early lethality [4]. In the classic form, girls with RTT typically exhibit a relatively normal period of development for the first 6-18 months of life followed by a regression phase where patients lose acquired language and motor skill and exhibit intellectual disability and hand stereotypies. The hand stereotypies are typical in RTT and appear to be continuous, located predominantly over the anterior body midline [5]. Beyond the classic form of RTT, a number of atypical forms with different degrees of severity have been described: the Zappella variant (formerly known as preserved speech variant) [6,7], the infantile seizure onset type [8], the congenital form [9] and the ‘forme fruste’[10]. The Rett Networked Database (RND) is a registry of clinical and molecular data for patients affected by RTT [11]. Although it was initially targeting the European population of patients with RTT, it is now open to countries outside of Europe. RND is different from existing repositories for RTT clinical and molecular data [2,12,13]. First, RND records are updated by clinicians with experience in RTT. This is important to exclude potential bias existing when clinical data are gathered using questionnaires sent out to families by mail. Second, RND is among the largest RTT registries worldwide with more than 1900 patients on file. Third, RND is an open access initiative and data can be retrieved directly through a web-based search engine by interested professionals upon the submission of a research proposal to the Scientific Review Board [11] Public has access to general information and to content
description while the individual patient file can be granted only upon registration of physicians and clinical researchers in charge of specific patients. Here, we describe the first 1925 records contained in the registry and discuss the content of RND on the basis of the published guidelines for RTT clinical diagnosis. We analyzed the phenotype of patients with MECP2, CDKL5 and FOXG1 mutation to better understand the typical and atypical forms of RTT and provided information of RTT cohorts for the development of clinical trials.

MATERIALS AND METHODS

RND data

RND contains clinical files for 1958 patients affected by classic or atypical RTT (numbers are given as of March 1st, 2017). Clinical data originates from Croatia (29 patients), Denmark (64 patients), France (252 patients), Hungary (82 patients), India (3 patients), Israel (93 patients), Italy (605 patients), Romania (15 patients), Serbia (50 patients), Spain (398 patients), United Kingdom (255 patients), USA (96 patients) and Russia (16 patients).

Statistical Analysis

Differences in clinical characteristics between groups of patients were tested by Fisher's exact test or by chi-squared analysis when the normal approximation was appropriate.

RESULTS

Overview of RND data

One thousand nine hundred fifty-eight patients with classic or atypical RTT are actually contained in RND. Among these, 1508 patients (77.0%) carry a mutation in a disease-
causing gene while 450 patients (23%) do not show mutations in any of the gene linked to RTT syndrome. As expected, MECP2 is mutated in the majority of patients with 1404 patients carrying a MECP2 mutation (93,1%). 78 patients carrying a mutation in CDKL5 (5,2%) and 26 patients carrying a mutation in FOXG1 (1,7%), much less frequent causes of RTT syndrome. All cases are sporadic except for 2 pairs of sisters and 5 pairs of monozygotic twins affected by RTT and carrying a MECP2 mutation.

**Patients carrying a mutation in MECP2**

RND contains clinical files for 1404 patients carrying a mutation in the MECP2 gene. The mutation type and clinical variant type are available for 1141 patients (1131 females and 10 males). All mutation types are present in this population with early truncating and missense mutations being the most frequent mutations despite significant difference between classical and atypical forms (Table 1). A small percentage of patients (0,5%) has a mutation whose functional significance is unknown. This category includes synonymous mutations and intronic variants outside donor/acceptor splice sites potentially affecting the splicing of MECP2 and for which not enough functional data are available in order to support pathogenicity. These mutations are absent from the databases of variants identified in control individuals. Nine hundred twenty-one patients have the classic form of RTT (80,7%), 117 patients have the Zappella variant (10,3%), 46 patients have the congenital variant of RTT (4%), seven patients have the early onset seizure variant (0,7%). Twenty-one patients (1,8%) do not fall into any of these categories because of the lack of one or several diagnostic criteria necessary for classical or atypical form classification. In addition, 19 patients (1,7%) are too young to be clinically defined as affected by the Zappella variant or the classical form. Criteria for the clinical diagnosis of RTT were last revised in RTT Diagnostic Criteria 2010 [14] in
order to include a regression period, partial or complete loss of acquired purposeful hand
skills, stereotypic hand movements, partial or complete loss of acquired spoken language
and gait abnormalities. We mined the RND data in order to investigate their compliance
with the revised diagnostic criteria. Our analysis showed that, among the patients
carrying a mutation in \textit{MECP2}, regression occurred in 94.9\% of patients, 90.6\% lost or
never acquired purposeful hand skills, 82.1\% have stereotypic hand movements, 70.5\%
lost most or all spoken language and 74\% have gait dyspraxia (Table 2). Notably, hand
stereotypies, although considered an invariant clinical sign of classical RTT, are absent
in 17.9\% of \textit{MECP2} mutated patients included in the RND dataset. On the other hand,
the intense eye pointing phenotype of RTT patients, is present in 88\% of \textit{MECP2}
positive cases (Table 2), although not included in the necessary criteria. RND data were
further interrogated to define the most frequent clinical signs, i.e. clinical signs present in
more than a 50\% of \textit{MECP2} mutation carriers (Table 3). This analysis revealed that, in
addition to the necessary criteria for RTT diagnosis, a normal head circumference at birth
(93.5\%) and a deficient sphincter control (88.7\%) are main clinical signs in \textit{MECP2}-
mutated patients. Interestingly, although 85.2\% of the \textit{MECP2} positive patients have
feeding difficulties (sometimes requiring gastrostomy), only half of them (53.6\%) have
gastrointestinal disturbances. This would suggest that part of the feeding difficulties arise
from abnormal muscle tone and oropharyngeal dysfunction [15]. Even though breathing
mechanisms in RTT preclinical models have been heavily investigated, breathing
dysfunction "only" affects half (50\%) of the patients carrying a mutation in \textit{MECP2}.
Interestingly, although loss of acquired speech is included among RTT diagnostic
criteria, RND data show that the majority of \textit{MECP2} positive cases have never spoken
(57.1\%), as reported in Table 3. Among \textit{MECP2}-mutated patients, it was possible to
compute the total score for 584 of the 1141 individuals for which the mutation status is available. This group included subjects over 5 years of age, and the most recent examination was used to compute the score. A cumulative distribution plots of patients positive for the MECP2 mutations is illustrated in Figure 1A and it is grouped on the basis of the four common point mutations (Arg106Trp, Arg133Cys, Thr158Met, Arg306Cys), the four early truncating mutations (Arg168*, Arg255*, Arg270*, Arg294*), late truncating mutations (LTM), large deletions and all other mutations. Although there is wide variability in clinical severity, there is a clear effect of specific common MECP2 point mutations on median clinical severity (Figure 1A). We indeed reported a case of 2 pairs of sisters carrying the same MECP2 mutation but with discordant clinical signs. One individual from each pair could not speak or walk, and had a profound intellectual deficit (classical RTT), while the other individual could speak and walk, and had a moderate intellectual disability (Zappella variant) [16]. The 5 monozygotic twin pairs reported in RND, are much more concordant than the sister pairs. However, it is interesting to note that only 2 out of 5 twin pairs have an identical clinical score, indicating that at least at this level of investigation they are phenotypically identical. The 3 other twin pairs differ in specific fields such as epilepsy and weight (twin pair 1), level of speech and level of phrases (twin pair 2), or height, age of regression and voluntary hand use (twin pair 3).

**Patients carrying a mutation in CDKL5**

The phenotype of the patients carrying a mutation in CDKL5 and classified as having atypical RTT is much less documented than the classical RTT phenotype caused by MECP2 mutations. A report in 2013 described 86 patients with a mutation in CDKL5 with data originating from family questionnaires [17]. RND contains 78 records for
CDKL5 mutation positive cases (76 females and 2 males). Similar to other records in RND, the clinical and molecular information were provided by experienced clinicians through direct patients’ evaluation. The cumulative distribution of the patients positive for CDKL5 mutations, clustered on the basis of early truncating mutations, late truncating mutations, large deletions, and missense mutations, is illustrated in Figure 1B. Forty-eight patients have the early onset seizure variant of RTT (85.7%), 1 patient has the Zappella variant (1.8%) and 1 patient has the congenital variant of RTT (1.8%). Mutation type is available for 65 patients. The most frequent mutations are truncating mutations (56.9%) followed by missense mutations (27.7%). In our cohort, the majority of patients have a normal head circumference at birth (94.9%), a deficient sphincter control (95.7%), feeding difficulties (97.4%), IQ < 40 (93.2%), presence of hand stereotypies (89.2%) and had never spoken (81.0%) (Table 3). Furthermore, epilepsy before 5 years of age is statistically significant among groups of patients (p-value <0.0001 MECP2 vs. CDKL5), since it is present in 100% of CDKL5 cases. The epilepsy started before 1 year of age in 95.7% of CDKL5 mutated patients versus 3.9% of MECP2 mutated patients and 37.5% of FOXG1 mutated patients. Epilepsy is not controlled by therapy in 74.6% of CDKL5 cases versus 21.9% of MECP2 patients and 58.8% of FOXG1 mutated patients (Table 3). Involuntary movements, mood disturbances and breathing dysfunction are statistically less frequent in CDKL5 cases rather than in MECP2 or FOXG1 patients. Others features such as normal head circumference at birth, deficient sphincter controls, hand stereotypies, feeding difficulties, height and weight below the 25th percentile and troubled night time sleeping are very similar among the three groups of patients carrying a MECP2, CDKL5 or FOXG1 mutation (Table 3). As for patients with MECP2 mutations, it was possible to compute the total score for 37 out
of 65 patients for which CDKL5 mutation status was available. This group included subjects with epilepsy onset occurring in early infancy for whom the most recent examination was used to compute the score.

**Patients carrying a mutation in FOXG1**

The phenotype of the patients carrying a mutation in FOXG1 and classified as having atypical RTT is even less documented than the phenotype caused by CDKL5 mutations. RND contains 27 records for FOXG1 mutation positive cases (19 females and 8 males). The full score is available for 26 patients. Twenty-three patients have the congenital form of RTT (88.5%), 1 patient has the classical form (3.8%), two do not fall into any of these categories (7.7%) because one or several necessary criteria for the diagnosis of classical or atypical forms were not present at the last examination (included in RND as "Rett-like"). The cumulative distribution of the patients positive for FOXG1 mutations was obtained on the basis of early truncating mutations, late truncating mutations, gene deletions and missense mutations and it is illustrated in Figure 1C. In our cohort, all patients carrying a FOXG1 mutation had IQ < 40, microcephaly and no speech at examination (Table 3). Similarly, there is a significant overlap between the phenotype of patients with FOXG1 mutation and the phenotype of patients with MECP2 or CDKL5 mutation. Eye pointing capability is reduced from 88% in MECP2 to 42% in CDKL5 and 13.3% in FOXG1 patients. The percentage of patients having epilepsy before 5 years of age is 65.5%, 100% and 87.5% in the MECP2, CDKL5 and FOXG1 group of patients, respectively. However, only MECP2 versus CDKL5 and FOXG1 versus CDKL5 comparisons were statistically significant. The percentage of patients that had a period of regression, IQ < 40, no speech at examination, scoliosis, breathing dysfunction and epilepsy not controlled by therapy is closer to the percentage observed for CDKL5
mutated patients rather than to the percentage observed for MECP2 patients. The major difference in the FOXG1 group is the higher percentage of patients that never learned to walk or speak or sit. About 92.3% of patients carrying a FOXG1 mutation has never spoken compared to the 57.1% of MECP2 and 81.0% of CDKL5 mutated patients. Moreover, FOXG1 mutated patients have never learned to sit (78.3%) and walk (91.3%) compared to MECP2 (12.9% and 31.7%) and CDKL5 (29.3% and 61.5%) respectively. The overall cumulative distribution plot of patients carrying a mutation in the MECP2, CDKL5 or FOXG1 genes is illustrated in figure 2.

Patients without identified mutation
Two hundred sixty-three patients (258 females and 5 males) have no identified mutation. Among these, 247 patients (242 females and 5 males) are negative after mutation screening of genes known to cause RTT phenotypes, 6 cases were not screened and the information is lacking for the remaining 10 patients. Although 120 patients are described as having classical RTT (46.7%) caution is needed in the consideration of such numbers. Indeed, although a majority of patients (89.2%) had a normal head circumference at birth and 88.6% have gone through a period of regression, only 46.8% of patients without mutation had hand stereotypies and 56.7% had never spoken. A third of these without a genetic aetiology (35%) had moderate to mild intellectual disability, when most MECP2 or CDKL5 positive cases had an IQ below 40. The most frequent clinical characteristics of patients tested negative for MECP2, CDKL5 or FOXG1 mutation are presented in Table 4. Obviously, this is a most heterogeneous group and efforts are currently being done to add clinical data to the corresponding files and to increase the molecular screening output.
DISCUSSION

Globally, the majority of RND patients do fulfill necessary criteria for the diagnosis of RTT with >70% of patients complying with the revised criteria [14]. A survey of the Rett syndrome Rare Disease Research Center (RDCRC) encompassing a population of 819 patients diagnosed as having RTT and carrying a mutation in the MECP2 gene was published [18]. The mutation types in RND and RDCRC cannot be compared because the latter only presents the most frequent mutations and a global percentage of missense versus nonsense mutations is not provided. However, it is interesting to observe that both cohorts have a similar proportion of large deletions (8.7% in RDCRC and 8.5% in RND) in patient with classic RTT. However, a different percentage of classic RTT patients does not carry a mutation in MECP2 gene (5% in RDCRC and 11.2% in RND). Indeed, the RND population appears to be more clinically heterogeneous than the RDCRC cohort. For example, in RDCRC, 100% of the patients comply with the main criteria of RTT Diagnostic Criteria 2010 [14] necessary for typical or classic RTT (loss of hand use, loss of communication, hand stereotypies and gait abnormalities). In RND, the same criteria are present in 70.5% to 90.6% of classic RTT patients. Surprisingly, RND patients more frequently comply with the revised supportive criteria (all criteria present in > 45% patients) while one third supportive criteria are not found in the majority of RDCRC patients. RND is more "open" than RDCRC and patients can be included even if they do not meet one of the necessary criteria for the diagnosis of "classic" RTT. Based on our experience with more than 1900 patients originating from many different countries, we observed that the diagnosis of "classic RTT", made by the physicians at the moment of clinical files submission to the RND, does not perfectly comply with the RTT Diagnostic Criteria 2010. The RTT Diagnostic Criteria 2010 do not totally account for clinical
observations made by many specialists and these criteria will probably need to be adapted again in a near future. A large cohort (n=86) of patients with a mutation in CDKL5 was previously described based on the questionnaires collected by the InterRETT database [17]. RND provides the second largest cohort of patients harbouring a mutation in CDKL5 with 78 cases. Expectedly for the early seizure variant of RTT caused by CDKL5 mutations, the majority of patients experienced at least one episode of epilepsy (>90% in both cohorts). The proportion of patients with a mutation in CDKL5 that never learned to walk in the two cohorts is also very similar (67.4% in InterRETT and 61.5% in RND), together with the proportion of patients displaying hand stereotypies (80.3% of females in InterRETT and 89.2% of patients positive for a mutation in CDKL5 in RND). There is a difference between the two cohorts concerning the speech skills, since 30 out of 76 females with CDKL5 mutation acquired early speech skills in the InterRETT cohort while only 5/42 females harbouring a CDKL5 mutation have acquired >10 words at age 5 in RND. In addition, another discrepancy concerns patients that had deceleration of head growth (59.5% in InterRETT and 29% in RND).

An earlier study of the North American RTT Database relying on 914 patients with a mutation in MECP2 was published [13]. However, similar to the Australian database, the data relies on questionnaires sent out to families and even if the questionnaires were analyzed by experienced clinicians, the patients were not all directly examined by the contributors. Available results mainly concern molecular data with the distribution and nature of reported mutations. It does not contain CDKL5 or FOXG1 molecular data and does not provide details concerning the major phenotypic traits present in the studied population. In the Percy report [13], 85.5% of patients with MECP2 mutation have the typical form and 13.4% have the atypical form of RTT. Similarly, the percentage of
typical RTT patients with a MECP2 mutation in the RND is 80.7% when males and mutations of unknown significance are excluded. The cumulative distribution in Figure 1A illustrates that there is a wide clinical variability within the same MECP2 mutation. However, the “mildest” mutations are Arg133Cys and late truncating mutations in accordance with previous reports [19]. The missense mutations Arg306, Thr158 and Arg106 (arginine or threonine can be replaced by any amino acid) and the early truncating mutation Arg294* belong to the intermediate severity phenotype. The remaining early truncating mutations (Arg168*, Arg255* and Arg270*) and large deletions are among the “most severe” form of RTT syndrome. It is interesting to note that the plot of each mutation is not always parallel. For example, Thr158Met and Arg294* move more vertical, suggesting that the phenotype of patients who have these mutations is less influenced by other genetic or environmental factors. The cumulative distribution in Figure 1B illustrates that a genotype-phenotype correlation for CDKL5 may be barely recognized and the lines move together. It is interesting to note that late truncating mutations move less vertical, suggesting that the phenotype of patients who have these mutations is more variable. The cumulative distribution in Figure 1C shows a clear trend toward a less severe phenotype for FOXG1 late truncating mutations. The cumulative overall distribution in Figure 2 nicely illustrates the progressive severity going from MECP2 to CDKL5 and FOXG1 mutation. CDKL5 patients lie in the most severe range in comparison to MECP2 patients with FOXG1 patients even more shifted than CDKL5 patients towards a worse clinical phenotype and a very minimum overlap with MECP2 patients.

In conclusion, the Rett Networked Database is a registry for patients with RTT where clinical data are validated by experienced clinicians upon direct examination of
the affected individuals. One of the unique features of this database is its ability to collect a huge amount of clinical details, being almost 300 the collected clinical items with different levels of completeness, and genetic data [11]. It provides a unique resource in order to perform genotype-phenotype correlations. Overall, observation of RND data highlights clinical characteristics which occur more frequently in patients with a specific mutation (Table 3). For example, presence of regression and gait dyspraxia are statistically more frequent in MECP2 mutated patients; epilepsy and reduction in eye pointing capability are statistically more frequent in CDKL5 mutated patients, while the large majority of FOXG1 patients have never learned to walk, sit and speak. Moreover, we observed that the majority of MECP2 mutated patients have the classical form of RTT, the majority of CDKL5 mutated patients have the early onset variant and the majority of FOXG1 mutated patients have the congenital form, with some exceptions. The overlap between the different phenotypes is presented in Figure 3. RND provides an open structure, available to all interested professionals and a searchable web interface made available for registered users. These characteristics should prove useful to perform additional phenotype-genotype correlations, to better understand the typical and atypical forms of RTT, and to select adequate patient populations for future clinical trials.

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TABLES

Table 1. MECP2 patients classified as classical or atypical RTT reported in RND (total number of patients 1101). Cases below age 4 (n=19) are not taken into account here. Twenty-one patients do not fall into any of these categories because one or several necessary criteria for the diagnosis of classical or atypical forms were not present at last examination. In RND, early truncations are the mutations altering the MECP2 protein before amino acid 310. N represents the number of cases for which the corresponding item is present in the patient file, percentage is provided in brackets.

| Mutation Type     | Classic RTT N (%classical) | Atypical RTT N (%atypical) | Males N | P-value classical vs. atypical RTT | Total (%) |
|-------------------|-----------------------------|-----------------------------|---------|-----------------------------------|-----------|
| Early truncating  | 404 (43,9)                  | 36 (22)                     | 3       | <0,0001                           | 443 (40,2) |
| Missense          | 328 (35,7)                  | 86 (50)                     | 2       | 0,0002                            | 416 (37,8) |
| Late truncating   | 107 (11,6)                  | 40 (23,2)                   | 2       | <0,0001                           | 149 (13,5) |
| Gene deletion     | 78 (8,5)                    | 6 (3,6)                     | 1       | 0,0264                            | 85 (7,7)  |
| Gene duplication  | -                           | 1 (0,6)                     | 2       | 0,0199                            | 3 (0,3)   |
| Unknown significance | 4 (0,3)                    | 1 (0,6)                     | -       | 0,7848                            | 5 (0,5)   |
Table 2. Compliance of the RND data with the revised diagnostic criteria [14] for patients positive for a mutation in MECP2. Peripheral vasomotor disturbances are accounted for in the "Small cold hands and feet" score. The item "Diminished response to pain" is not present in RND data. N represents the number of cases for which the corresponding item is present in the patient file. N+ represents the number of cases positive for the clinical signs, percentage is provided in brackets.

| Clinical Sign                                      | N  | N+ (%) |
|---------------------------------------------------|----|--------|
| A period of regression                            | 1189 | 1129 (94,9) |
| NECESSARY CRITERIA                                |    |        |
| Partial or complete loss of acquired purposeful hand skills | 961 | 871 (90,6) |
| Stereotypic hand movements                        | 1185 | 973 (82,1) |
| Partial or complete loss of acquired spoken language | 1039 | 733 (70,5) |
| Gait abnormalities                                | 700 | 518 (74,0) |
| SUPPORTIVE CRITERIA                               |    |        |
| Breathing disturbances when awake                 | 1003 | 502 (50,0) |
| Bruxism when awake                                | 829 | 515 (62,1) |
| Impaired sleep pattern                            | 926 | 419 (45,2) |
| Abnormal muscle tone                              | -   | -      |
| Peripheral vasomotor disturbances                 | -   | -      |
| Scoliosis or kyphosis                             | 1189 | 754 (63,4) |
| Condition                                      | Count | Percentile |
|-----------------------------------------------|-------|------------|
| Growth retardation*                           | 1062  | 608 (57.3) |
| Small cold hands and feet                     | 1042  | 642 (61.6) |
| Inappropriate laughing or screaming spells    | 560   | 171 (30.5) |
| Diminished response to pain                   | -     | -          |
| Eye pointing                                  | 958   | 843 (88.0) |

*Growth retardation was considered to be present when weight was below the 25th percentile. When height is considered, 61.6% (n=635) of MECP2 positive patients are below the 25th percentile.
Table 3. Main clinical characteristics in patients positive for a mutation in MECP2, CDKL5, and FOXG1. Clinical characteristics are listed in descending order of percentage of patients harbouring a mutation in MECP2. N represents the number of cases for which the corresponding item is present in the patient file. N+ represents the number of positive for the clinical sign, percentage is provided in brackets. P-value of significance is provided for comparison.

| Clinical Sign                        | MECP2 | CDKL5 | FOXG1 | P-value MECP2 vs. CDKL5 | P-value MECP2 vs. FOXG1 | P-value CDKL5 vs. FOXG1 |
|--------------------------------------|-------|-------|-------|-------------------------|-------------------------|------------------------|
| A period of regression               | 1189  | 1129  | 61    | 25                      | 13                      | <0,0001                |
|                                      | (94,9)| (44,3)| (94,9)| (52)                    | (52)                    | 0,5136                 |
| Normal head circumference at birth   | 900   | 842   | 39    | 17                      | 14                      | 0,7421                 |
|                                      | (93,5)| (94,9)| (82,4)| (82,4)                  | (82,4)                  | 0,0663                 |
| Deficient sphincter control         | 974   | 864   | 46    | 23                      | 22                      | 0,1409                 |
|                                      | (88,7)| (95,7)| (95,7)| (95,7)                  | (95,7)                  | 0,2952                 |
| Eye pointing                         | 958   | 843   | 50    | 15                      | 2                       | <0,0001                |
|                                      | (88,0)| (42)  | (13,30)| (13,30)                | (13,30)                 | 0,0417                 |
| Condition                                      | Count | Percent | Count | Percent | Count | Percent | Count | Percent | Count | Percent | Count | Percent |
|------------------------------------------------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|
| Feeding difficulties                           | 813   | (85,2)  | 38    | (97,4)  | 15    | (86,7)  |       |         | 0,0364|         | 0,8772|         |
| Presence of hand stereotypies                  | 1185  | (82,1)  | 65    | (89,2)  | 24    | (95,8)  |       |         | 0,1414|         | 0,0806|         |
| IQ < 40                                        | 916   | (76,6)  | 59    | (93,2)  | 24    | (100)   |       |         | 0,0029|         | 0,0069|         |
| Microcephaly or deceleration of head growth    | 1110  | (75,4)  | 69    | (55,1)  | 26    | (100)   |       |         | <0,0001|         | 0,0037| <0,0001 |
| Gait dyspraxia                                 | 700   | (74,0)  | 25    | (52,0)  | 21    | (33,3)  |       |         | 0,0146| <0,0001 | 0,0022|         |
| No speech at examination                      | 1039  | (70,5)  | 42    | (88,1)  | 24    | (100)   |       |         | 0,0138|         | 0,0016|         |
| Epilepsy before 5 years of age                 | 689   | (65,5)  | 64    | (100)   | 16    | (87,5)  |       |         | <0,0001|         | 0,0658|         |
| Scoliosis                                      | 1189  | (63,4)  | 40    | (22,5)  | 22    | (27,3)  |       |         | <0,0001|         | 0,0033|         |
| Bruxism                                        | 829   | (62,1)  | 49    | (42,9)  | 17    | (64,7)  |       |         | 0,0072|         | 0,0782|         |

Percentages in parentheses.
| Condition                              | Cases | Min (25th) | 1st     | 3rd     | Max (75th) | Mean | 1st SD | 3rd SD | Max SD |
|----------------------------------------|-------|------------|---------|---------|------------|------|--------|--------|--------|
| Height below the 25th percentile       | 1031  | 61,6       | 20,42   | 25,17   | 68,00      | 0.0687 | 0.5147 | 0.1047 |        |
| Cold extremities                      | 1042  | 61,6       | 39,41   | 45,5,10 | 68,25      | 0.0037 | 0.1236 | 0.6212 |        |
| Weight below the 25th percentile      | 1062  | 57,3       | 47,22   | 68,17   | 68,25      | 0.2064 | 0.2825 | 0.1027 |        |
| Has never spoken                      | 1039  | 57,1       | 81,42   | 92,26   | 92,34      | 0.0021 | 0.0003 | <0.0001 |        |
| Involuntary movements                 | 781   | 54,9       | 16,24   | 66,12   | 66,8       | 0.0002 | 0.4172 | 0.0027 |        |
| Gastrointestinal disturbances         | 714   | 53,6       | 43,37   | 72,22   | 72,16      | 0.2165 | 0.0768 | 0.0279 |        |
| Mood disturbance                      | 787   | 50,6       | 28,39   | 70,12   | 70,17      | 0.0064 | 0.1024 | 0.003  |        |
| Breathing dysfunction                 | 1003  | 50,0       | 13,43   | 28,25   | 28,7       | <0.0001 | 0.0294 | 0.1555 |        |
| Troubled night time sleeping          | 964   | 44,7       | 43,62   | 61,18   | 61,11      | 0.8585 | 0.1658 | 0.189  |        |
| Never learned to walk                 | 1180  | 65         | 40,23   | 21       | <0.0001    | <0.0001 | 0.0078 |        |
|                                      | (31,7) | (61,5) | (91,3) |         |         |         |
|--------------------------------------|--------|--------|--------|---------|---------|---------|
| Epilepsy not controlled by therapy   | 1018   | 223    | 71     | 53      | 17      | <0.0001 |
|                                       | (21.9) | (74.6) | (58.8) |         | 0.0003  | 0.1938  |
| Never learned to sit                 | 1095   | 141    | 41     | 12      | 23      | 0.0025  |
|                                       | (12.9) | (29.3) | (78.3) |         | <0.0001 | <0.0002 |
| Epilepsy before 1 years of age       | 689    | 27     | 69     | 66      | 16      | <0.0001 |
|                                       | (3.9)  | (95.7) | (37.5) |         | <0.0001 | <0.0001 |
|                                       |         |        |        |         |         | <0.0001 |
Table 4. Clinical characteristics in patients negative for mutation in *MECP2*, *CDKL5* or *FOXG1*. The table is limited to the clinical characteristics present in more than 50% of these patients. N represents the number of cases for which the corresponding item is present in the patient file. N+ represents the number of positive for that clinical sign, percentage is provided in brackets.

| Clinical Sign                                      | MECP2 negative cases |
|---------------------------------------------------|----------------------|
|                                                   | N        | N+ (%)    |
| Deficient sphincter control                      | 228      | 205 (90,0)|
| Normal head circumference at birth                | 167      | 149 (89,2)|
| A period of regression                            | 245      | 217 (88,6)|
| Regression of hand use                            | 229      | 187 (81,7)|
| Presence of epilepsy before age 5                 | 216      | 166 (76,9)|
| Microcephaly or deceleration of head growth       | 229      | 159 (69,4)|
| Bruxism                                           | 234      | 154 (65,8)|
| IQ < 40                                            | 203      | 132 (65,0)|
| Has never spoken                                  | 240      | 136 (56,7)|
Figure 1 A. Cumulative distribution plots of the patients positive for a MECP2 mutation.

Mutations were grouped based on the four common point mutations (Arg106Trp, Arg133Cys, Thr158Met, Arg306Cys), the four early truncating mutations (Arg168*, Arg255*, Arg270*, Arg294*), the late truncating mutations, the large deletion, and the other mutations.
Arg255*, Arg270*, Arg294*), late truncating mutations (LTM), large deletions and all other mutations. Early truncating mutations correspond to mutations interrupting the protein before amino acid 310. Large deletions correspond to deletions including either single exon or the entire gene. B. Cumulative distribution of the patients positive for a CDKL5 mutation. Mutations were grouped based as early truncating mutations (mutations interrupting protein before amino acid 297), late truncating mutations, large deletions (deletions involving either single exons or the entire gene), and missense mutations. C. Cumulative distribution of the patients positive for a FOXG1 mutation. Mutations were grouped based as early truncating mutations (mutations interrupting protein before amino acid 275), late truncating mutations, gene deletions (deletions involving either single exons or the entire gene), and missense mutations.

**Figure 2** Combined graph illustrating the different clinical severities between MECP2, CDKL5, and FOXG1 mutated patients.
Figure 3 Genotypes and phenotypes in RND. The majority of MECP2 mutated patients (light blue) have the classical form (triangles), the majority of CDKL5 mutated patients (green) have the early onset seizure variant (stars) and the majority of FOXG1 mutated patients (pink) have the congenital form (crosses). Several exceptions to this rule are present: among the MECP2 mutated patients (light blue) about 6% have the Zappella variant (dots), about 2% has the congenital variant of RTT (crosses), and about 0.4% have the early onset seizure variant (stars); among the CDKL5 mutated patients (green) about 1.8% patients have the Zappella variant (dots) and another 1.8% have the congenital form (crosses); among the FOXG1 mutated patients (pink) about 5% of patients has the classical form (triangles) [2].