

Epilepsy in Rett syndrome—Lessons from the Rett networked database

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SUMMARY

Objective: Rett syndrome is an X-linked dominant neurodevelopmental disorder caused by mutations in the *MECP2* gene, and characterized by cognitive and communicative regression, loss of hand use, and midline hand stereotypies. Epilepsy is a core symptom, but literature is controversial regarding genotype–phenotype correlation. Analysis of data from a large cohort should overcome this shortcoming.

Methods: Data from the Rett Syndrome Networked Database on 1,248 female patients were included. Data on phenotypic and genotypic parameters, age of onset, severity of epilepsy, and type of seizures were collected. Statistical analysis was done using the IBM SPSS Version 21 software, logistic regression, and Kaplan-Meier survival curves.

Results: Epilepsy was present in 68.1% of the patients, with uncontrolled seizures in 32.6% of the patients with epilepsy. Mean age of onset of epilepsy was $4.68 \pm$ (standard deviation) 3.5 years. Younger age of onset was correlated to severity of epilepsy (Spearman correlation $r = 0.668$, $p < 0.01$). Patients with late truncating deletions had lower prevalence of epilepsy. Compared to them, the *p.R133C* mutation, associated with a milder Rett phenotype, increased the risk for epilepsy (odds ratio [OR] 2.46, confidence interval [CI] 95% 1.3–4.66), but not for severe epilepsy. The *p.R255X* mutation conferred an increased risk for epilepsy (OR 2.07, CI 95% 1.2–3.59) as well as for severe epilepsy (OR 3.4, CI 95% 1.6–7.3). The *p.T158M* and *p.C306C* mutations relatively increased the risk for severe epilepsy (OR 3.09 and 2.69, CI 95% 1.48–6.4 and 1.19–6.05, respectively), but not for epilepsy occurrence.

Significance: Various mutations in the *MECP2* gene have a different influence on epilepsy, unrelated to the severity of the general Rett phenotype. This might suggest a site-specific effect of MeCp2 on epileptic pathways. Further investigation of these mechanisms should promote better understanding of epileptogenesis in Rett syndrome.

KEY WORDS: *MECP2*, Seizure, Preserved speech variant, Database.

Rett syndrome is an X-linked neurodevelopmental disorder affecting mostly females and caused by mutations in the *MECP2* gene.^{1,2} Girls with the classical Rett phenotype are normal at birth, but during the first year of life, head-circumference growth decelerates, leading to microcephaly.² During the second to third year of life, regression in communication and cognition occurs, girls lose speech and purposeful hand use, and midline hand stereotypies appear.² Other symptoms (breathing, gastrointestinal, and orthopedic abnormalities) are characteristic of Rett syndrome.² Rett variant phenotypes are also known: the early epileptic variant (with severe epileptic encephalopathy during the first year of life), the congenital variant (earlier onset of microcephaly and additional clinical symptomatology from birth), and the preserved speech variant (PSV)/Zappella variant (milder phenotype with better motor function and a vocabulary of >10 words).

Epilepsy is a core symptom of Rett syndrome, with prevalence as high as 60–90%.^{3–7} Frequency might be overestimated, since nonepileptic paroxysmal events are common and can lead to misdiagnosis.⁸ Epilepsy might be more prevalent among those without a proven mutation in the *MECP2* gene.^{8,9} Several predictors for epilepsy previously reported by Jian et al. and Glaze et al. were general-symptom severity, preregression developmental problems, impairment of ambulation and hand use, and detection of an *MECP2* mutation.^{5,6,8}

The literature is inconsistent regarding correlations between different hot spot mutations and various epilepsy parameters (prevalence, incidence, severity, and age of onset) (Table 1). Several studies found that C-terminal deletions^{5,6,10,11} and the *p.R306C* mutation^{8,11,12} offered protection against epilepsy, whereas the *p.T158M* had an inverse effect.^{8,10,13,14} According to the different reports, the mutations *p.R255X*,^{8,12,13} *p.R294X*,^{5,6,10,12} and large intragenic deletions^{10,11} had a contradictory effect. According to one study each, *p.R133C*¹³ and *p.R168X*⁵ were correlated to a lower incidence of epilepsy. Several other researchers did not find statistically significant genotype–phenotype correlations, possibly because of a small sample size.^{4,15,16}

The purpose of this study was to better characterize these controversial issues using a large international database with physician-filled standardized questionnaires.

METHODS

The Rett Networked Database is a physician-reported database that includes 293 clinical items and 16 genetic items grouped into 31 domains, generated through a harmonization process of data collected from 11 countries (<http://www.rettbasenetwork.org/>).¹⁴

Data from 1,911 patients were collected from the database: clinical type of Rett syndrome, genotype, age of onset, severity of epilepsy, and types of seizures. Severity of epilepsy was defined by a three-grade Likert scale (which combines frequency of seizures and response to therapy) as follows: 0, no seizures or occasional seizures not requiring drug treatment; 1, seizures controlled by antiepileptic drugs; and 2, seizures uncontrolled by multiple drugs (Table 2). Seizure types were classified according to the 1999 International League Against Epilepsy (ILAE) definitions. The following ordinal parameters regarding disease severity scores were documented: speech, walking, breathing disorder, and regression (Table 2). Patients with missing data regarding epilepsy occurrence were excluded.

Patients were included if they had a mutation in the *MECP2* gene, regardless of clinical phenotype (classical or Rett variant). Patients without an *MECP2* mutation (either not checked or not proven) were included if the clinical phenotype was consistent with the classical Rett phenotype. Patients with *CDKL5* and *FOXG1* mutations (genes known to cause Rett variants), male Rett patients, and patients with *MECP2* duplications were excluded.

Statistical analysis was done using the IBM SPSS version 21 (Armonk, NY, U.S.A.). Kaplan-Meier survival curves were used to calculate time to onset of epilepsy. A binomial regression model was used to examine the effect of different genotypes or phenotypes on epilepsy. A multinomial regression model was used to assess the effect of genotype and phenotype on the severity of epilepsy. For subpopulations in which epilepsy was detected, the confounding effect of age of onset of epilepsy on severity was calculated using survival analysis Cox regression. Spearman correlation was used for correlation between age of onset and severity of epilepsy. The Mann-Whitney ranking test was used to compare between different severity scores in patients with and without epilepsy.

The study was approved by the local ethical committee at the University Hospital of Siena (30/2012).

Table 1. Genotype–phenotype correlation for epilepsy in RETT syndrome

| Study | Cohort | Data acquisition | Statistical method | CTD | LD | R133C | R255X | T158M | R106W | R306C | R168X | R294X |
|-------------------------------|------------|--|--|-----|----|-------|-------|-------|-------|-------|-------|-------|
| Present study | 1248 pts. | Physician questionnaire (RETT networked database) | Logistic regression (binomial and multinomial) | ↕ | ↕ | ↕ | | | | | | |
| Cuddapah et al. ¹² | 1,052 pts. | Physician assessment | Poisson regression, Tukey multiple comparisons | | | | | | | | | |
| Bao et al. ¹³ | 685 pts. | Family questionnaire (InterRETT database) | Logistic regression, Kaplan-Meier curve | | | | | | | | | |
| Glaze et al. ⁸ | 602 pts. | Physician assessment (American RETT Natural History) | Logistic regression | | | | | | | | | |
| Pintaudi et al. ¹⁰ | 165 pts. | Physician questionnaire | Chi-square | | | | | | | | | |
| Nectoux et al. ¹¹ | 81 pts. | Physician assessment | Kruskal-Wallis | | | | | | | | | |
| Jian et al. ⁶ | 162 pts. | Family questionnaire | Binomial regression | | | | | | | | | |
| Jian et al. ⁵ | 275 pts. | Family questionnaire | Cox regression | | | | | | | | | |

CTD, C-terminal deletion; LD, large intragenic deletion; R133C-p, R133C, R255X-p, R255X, T158M-p, T158M, -p, R106W, R306C-p, R306C, R168X-p, R168X, p, R294X. ↕ -associated with lower prevalence or incidence of epilepsy, milder severity, or later age of onset of epilepsy. ↗ -associated with higher prevalence or incidence of epilepsy, more severe course, or younger age of onset of epilepsy.

RESULTS

A total of 1,248 patients were included in this study; 1,135 of them (90.1%) were mutation positive. Eight hot spot mutations, as well as C-terminal–deletion mutations and large intragenic deletions were present in 860 patients. A known clinical phenotype was available in 1,098 patients. Detailed data regarding clinical phenotype, *MECP2* mutation status, and hot spot mutations are provided in Tables 3 and 4, and supplementary Table S1.

Epilepsy was present in 850 patients (68.1%). It was more prevalent (78.8%) among *MECP2*-negative patients (Fisher's exact test), but other parameters related to epilepsy, such as age of onset, severity, and type of seizures, were not influenced by the *MECP2* status (Table S1).

Data on epilepsy severity were present in 736 patients: 27 (3.2%) experienced occasional seizures not requiring treatment (grade 0), 469 (55.2%) had well-controlled seizures (grade 1), and 240 (32.6%) had uncontrolled seizures (grade 2). Data on seizure types were present in 298 patients: 138 (46.3%) had generalized tonic–clonic seizures, 81 (26.8%) had partial-onset seizures, 43 (14.4%) had absence, 36 (12.1%) myoclonic, 27 (9.1%) tonic, 12 (4.02%) atonic. Twenty-six percent of the patients experienced two seizure types, whereas 3.4% had more than two.

Age of onset of epilepsy was $4.68 \pm$ (standard deviation) 3.5 years (range of 3 months to 21 years of age). Epilepsy appeared slightly later than the other core symptoms of Rett syndrome, that is, hand stereotypies (2.43 ± 1.67), speech regression (2.09 ± 1.1), and loss of hand use (2.28 ± 1.4), but this difference did not reach statistical significance (analysis of variance, ANOVA). Epilepsy onset was most prevalent between 3 and 5 years of age, and it appeared before the age of 8 years in >80% of the patients (Fig. 1A; Table 4). Different hot spot mutations did not influence age of onset of epilepsy, but clinical phenotype did. In analysis of the subgroups according to clinical phenotype, it was found that epilepsy appeared earlier in patients with the congenital variant (3.15 ± 0.53) compared to the classical phenotype (4.78 ± 0.2) (survival analysis – Cox regression, $\chi^2 = 5.75$, $p < 0.05$, HR 1.8, CI 95% 1.07–3.05 (Fig. 1B)). Age of onset of epilepsy in patients with the preserved speech variant (PSV) was not significantly different from patients with the classical phenotype. Earlier age of onset of epilepsy was correlated with a more severe epilepsy score (Spearman correlation, $r = 0.688$, $p < 0.01$).

The prevalence and severity of epilepsy differed between the various phenotypes. A total of 72.5% of patients with the classical phenotype had epilepsy compared to 52.3% of patients with PSV (Table 3). Using binary logistic regression, we found that PSV patients had a lower chance to develop epilepsy than patients with the classical phenotype (odds ratio [OR] 0.43, CI 95%

Table 2. Scoring of severity for core symptoms related to Rett syndrome

| Severity score | 0 | 1 | 2 |
|-------------------------------|---|--|--|
| Epilepsy | None or occasional seizures, no drug treatment needed | Seizures controlled by antiepileptic drugs | Seizures uncontrolled, multiple drug treatment |
| Hand stereotypies | None | Mild or intermittent | Dominant or constant |
| Speech | 0–10 words by 10 years of age | Loss of ability to speak | Never spoke |
| Walking | Walking unsupported | Loss of ability to walk | Never walked |
| Breathing disorder | Absent | Mild | Severe |
| Regression | After 3 years of age | 18 months to 3 years | Younger than 18 months |
| Cold extremities | Absent | Mild | Severe |
| Gastrointestinal disturbances | Absent | Mild | Severe |

Table 3. Demographic data of patients with and without epilepsy

| | Patients with epilepsy | Patients without epilepsy | Total | Comments |
|------------------------------|--------------------------|---------------------------|--------------|--|
| Total number of patients | 850 (68.1%) | 398 (31.9%) | 1,248 (100%) | |
| Clinical phenotype | | | | PSV lowered the chance to develop epilepsy compared to classical phenotype (logistic regression, OR 0.43, 95% CI 0.25–0.72) |
| Classical Rett | 653 (72.5%) | 248 (27.5%) | 901 (100%) | |
| Preserved speech variant PSV | 33 (52.3%) | 29 (46.8%) ^a | 62 (100%) | |
| Early epileptic variant | 4 (100%) | 0 (0%) | 4 (100%) | |
| Congenital variant | 21 (84%) | 4 (16%) | 25 (100%) | |
| Hot spot mutations | | | | <i>p.R133C</i> increased the risk for epilepsy compared to C-terminal deletion (logistic regression, OR 2.46, 95% CI 1.3–4.66) |
| Large deletions | 41 (61.2%) | 26 (38.8%) | 67 (100%) | |
| C-terminal deletions | 79 (58.5%) | 56 (41.5%) ^a | 135 (100%) | |
| <i>p.R106W</i> | 27 (67.5%) | 13 (32.5%) | 40 (100%) | <i>p.R255X</i> increased the risk for epilepsy compared to C-terminal deletion (logistic regression, OR 2.07, 95% CI 1.2–3.59) |
| <i>p.R133C</i> | 59 (77.6%) ^a | 17 (22.4%) | 76 (100%) | |
| <i>p.R168X</i> | 67 (65.5%) | 36 (35%) | 103 (100%) | |
| <i>p.R255X</i> | 82 (74.5%) ^a | 28 (25.5%) | 110 (100%) | |
| <i>p.R270X</i> | 51 (69.9%) | 22 (30.1%) | 73 (100%) | |
| <i>p.R294X</i> | 42 (62.7%) | 25 (37.3%) | 67 (100%) | |
| <i>p.R306C</i> | 46 (64.8%) | 25 (35.2%) | 71 (100%) | |
| <i>p.T158M</i> | 83 (70.3%) | 35 (29.5%) | 118 (100%) | |
| Speech severity score | | | | Patients with epilepsy had higher speech severity score (Mann-Whitney U ranking test, $p < 0.05$) |
| 0 | 208 (66%) | 107 (34%) | 315 (32.8%) | |
| 1 | 51 (62.2%) | 31 (37.8%) | 82 (8.5%) | |
| 2 | 409 (72.5%) ^a | 155 (27.5%) | 564 (58.7%) | |
| Walking severity score | | | | Patients with epilepsy had higher walking severity score (Mann-Whitney U ranking test, $p < 0.01$) |
| 0 | 332 (57.5%) | 245 (42.5%) | 577 (51.4%) | |
| 1 | 161 (81.7%) ^a | 36 (18.3%) | 197 (17.5%) | |
| 2 | 276 (76.5%) | 82 (23.5%) | 349 (31.1%) | |
| Breathing disorder score | | | | Patients with epilepsy had higher breathing disorder score (Mann-Whitney U ranking test, $p < 0.01$) |
| 0 | 248 (61.2%) | 157 (38.8%) | 405 (45.5%) | |
| 1 | 171 (71.8%) | 67 (28.8%) | 238 (26.7%) | |
| 2 | 205 (83%) ^a | 42 (17%) | 247 (27.8%) | |
| Regression score | | | | Patients with epilepsy had higher regression score (Mann-Whitney U ranking test, $p < 0.01$) |
| 0 | 50 (63.3%) | 29 (36.7%) | 79 (7.6%) | |
| 1 | 261 (66.1%) | 134 (33.9%) | 395 (37.8%) | |
| 2 | 426 (74.5%) ^a | 146 (25.5%) | 572 (54.7%) | |

^aStatistically significant.

0.25–0.72, $p < 0.01$). PSV patients had also a lesser risk for developing severe epilepsy (multinomial logistic regression, OR 0.129, CI 95% 0.04–0.36, $p < 0.01$). Age of onset of epilepsy did not have an additional influence on this result (survival analysis Cox regression). Characteristics of epilepsy in patients with PSV were similar among patients with the *R133C* mutation versus other mutations (supplementary Table S2).

Among the different hot spot mutations, the lowest prevalence of epilepsy was associated with C-terminal deletions: 58.5% versus 68.1% in the general population (Table 4; Fig. 2). Logistic regression using C-terminal deletions as reference revealed increased risk for epilepsy for *p.R133C* (OR 2.46, CI 95% 1.29–4.66, $p < 0.01$) and *p.R255C* (OR 2.07, CI 95% 1.2–3.6, $p < 0.01$) mutations (Table 3; Fig. 2). Patients with *p.R133C* mutation were more likely to

Table 4. Demographic data of patients with epilepsy, according to epilepsy severity

| Severity score of epilepsy | 0 | 1 | 2 | Total | Comments |
|----------------------------|------------|-------------------------|-------------------------|------------|---|
| Total number of patients | 27 (3.2%) | 469 (63.7%) | 240 (32.6%) | 736 (100%) | |
| Age of onset of epilepsy* | | | | | Earlier age of onset correlates with more severe epilepsy score (Spearman correlation, $r = 0.688$, $p < 0.01$) |
| 0<1 years | 0 (0%) | 13 (59.1%) | 9 (40.9%) | 22 (100%) | |
| 1<3 years | 1 (0.8%) | 83 (65.4%) | 43 (33.9%) | 127 (100%) | |
| 3<5 years | 24 (13.1%) | 102 (55.7%) | 57 (31.1%) | 183 (100%) | |
| 5<10 years | 1 (1.1%) | 130 (75.1%) | 57 (31.1%) | 173 (100%) | |
| ≥ 10 years | 1 (2%) | 36 (73.5%) | 12 (24.5%) | 49 (100%) | |
| Clinical phenotype | | | | | No correlation between phenotype and severity of epilepsy |
| Classical Rett | 18 (3.2%) | 367 (64.7%) | 183 (32.1%) | 567 (100%) | |
| PSV | 2 (6.3%) | 26 (81.3%) | 4 (12.5%) | 32 (100%) | |
| Early epileptic variant | 0 (0%) | 1 (33.3%) | 2 (66.7%) | 3 (100%) | |
| II categories | | | | | |
| 1-2.99, 3-4.99, 5-9.99 | | | | | |
| Congenital variant | 1 (5.9%) | 11 (64.7%) | 5 (29.4%) | 17 (100%) | |
| Hot spot mutations | | | | | p.R133C increased the chance for epilepsy severity 1 |
| Large deletions | 1 (2.5%) | 27 (67%) | 12 (30%) | 40 (100%) | |
| C-terminal truncating | 3 (4.2%) | 53 (73.6%) | 16 (22.2%) | 72 (100%) | (multinomial logistic regression, OR = 2.59, 95% CI 1.33–5.02) |
| p.R106W | 1 (4%) | 16 (64%) | 8 (32%) | 25 (100%) | p.R255X increased the risk for epilepsy severity 2 |
| p.R133C | 1 (1.9%) | 42 (80.8%) ^a | 9 (17.3%) | 52 (100%) | (multinomial logistic regression, OR = 3.4, 95% CI 1.6–7.3) |
| p.R168X | 2 (3.5%) | 36 (63.2%) | 19 (33.3%) | 57 (100%) | |
| p.R255X | 2 (3.1%) | 34 (53.1%) | 28 (43.8%) ^a | 64 (100%) | p.R306C increased the risk for epilepsy severity 2 |
| p.R270X | 0 (0%) | 31 (73.8%) | 11 (26.2%) | 42 (100%) | |
| p.R294X | 2 (5.1%) | 24 (61.5%) | 13 (33.3%) | 39 (100%) | (multinomial logistic regression, OR = 2.69, 95% CI 1.19–6.05) |
| p.R306C | 1 (2.6%) | 18 (47.4%) | 19 (50%) ^a | 38 (100%) | p.T158M increased the risk for epilepsy severity 2 |
| p.T158M | 2 (2.7%) | 42 (56%) | 31 (41.3%) ^a | 75 (100%) | (multinomial logistic regression, OR = 3.09, 95% CI 1.48–6.4) |

Table indicates data for patients with available grading scale for epilepsy severity.
^aStatistically significant.

have controlled epilepsy (multinomial logistic regression, OR 2.59, CI 95% 1.33–5.02, $p < 0.01$), whereas patients with *p.R255X* mutation had increased risk for severe epilepsy (multinomial logistic regression, OR 3.4, CI 95% 1.6–7.3, $p < 0.01$). Two additional missense mutations carry increased risk for severe epilepsy, but not for having epilepsy: *p.T158M* mutation (multinomial logistic regression, OR 3.09, 95% CI 1.48–6.4, $p < 0.01$) and *p.R306C* (multinomial logistic regression, OR = 2.69, CI 95% 1.19–6.05, $p < 0.01$) (Table 4). Survival analysis with Cox regression revealed no additional influence of age of onset of epilepsy on its severity.

Nonparametric tests (U Mann-Whitney test) comparing severity scores for Rett morbidity in patients with and without epilepsy, revealed statistically significant differences for speech score ($p < 0.05$), walking score ($p < 0.01$), breathing disorder score ($p < 0.01$), and regression score ($p < 0.01$) (Table 3).

DISCUSSION

Epilepsy is frequently comorbid in children with developmental disabilities, but its relatively higher prevalence in patients with Rett syndrome, its relative resistance to treatment, and its unique electroencephalographic features⁴ suggest an intrinsic role of *MECP2* in seizure pathophysiology. Animal models of Rett syndrome using nonsense¹⁷ as well

as missense^{18–20} mutation mice demonstrated reduced seizure threshold. Hippocampal slices isolated from mutant mice showed abnormal neuronal network activity, with hyperexcitable pyramidal cortex prone to hypersynchronization of cell oscillation and seizures, probably due to a defect of synaptic transmission secondary to the *MECP2* deficiency.^{17,21,22}

In our study, which represents the largest database cohort collection so far, we found epilepsy in 68% of patients with Rett syndrome, comparable to previous smaller-scale studies.^{13,16} The database did not include the current age of patients and was not longitudinal; therefore, we could not assess the incidence of epilepsy and its natural history. The most prevalent age range for epilepsy onset was 3–5 years, but in a significant percentage of cases (7.45%), seizures appeared after 10 years of age. This information should be taken into account when directing the follow-up of patients into adolescence. Seizures were uncontrolled in 31.9% of patients, and seizure control was inversely correlated with the age of onset of epilepsy. Our results are limited, since the scale used for grading epilepsy severity includes seizure frequency as well as response to treatment. Other parameters, such as duration of seizures, frequency of status epilepticus, and number of seizure-related hospitalizations are missing as well. The collection of data required a sophisticated harmonization process in order to overcome the multi-lingual, multinational origins of the data. However, diverse

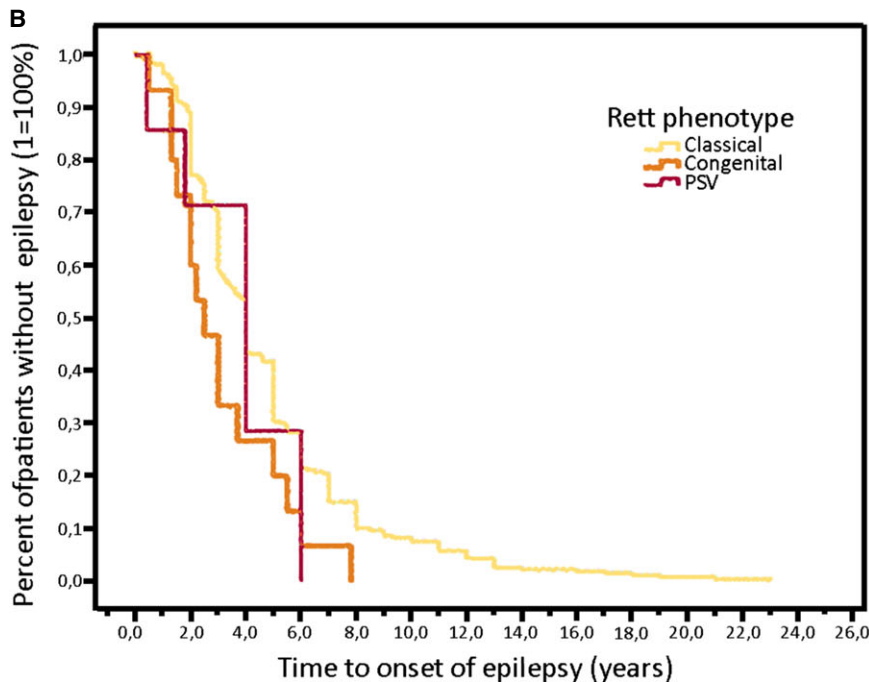
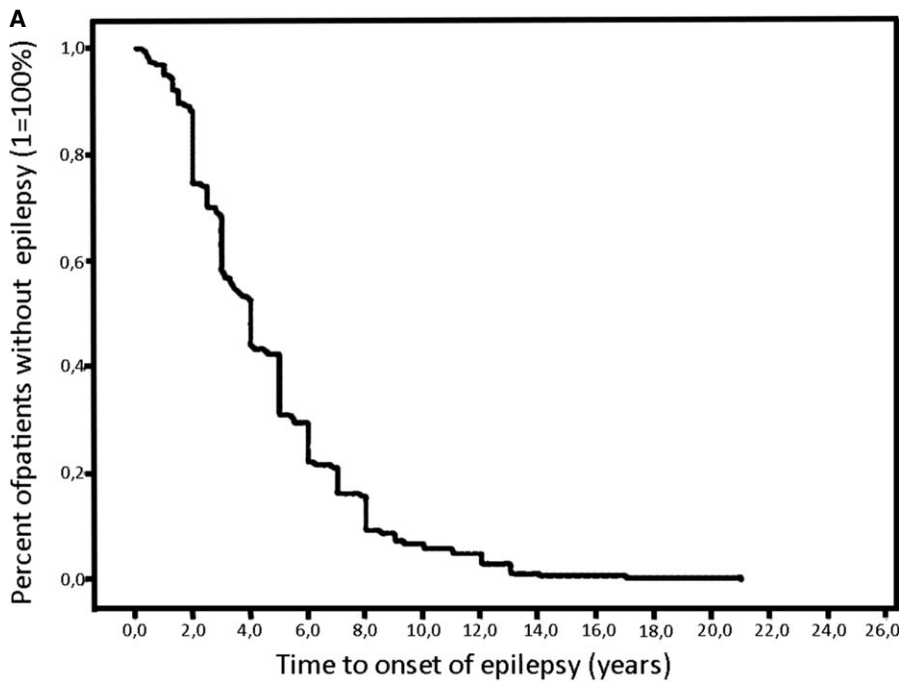


Figure 1. Time to onset of epilepsy. Kaplan-Meier survival curve revealing time to onset of epilepsy in the general Rett population (A) and main clinical phenotypes (B). The curve is calculated only for patients with epilepsy. *Epilepsia* © ILAE

fields of expertise among the treating physicians, the availability of different medical services, as well as various protocols for diagnosis and care are potential pitfalls for the uniformity of the data.

Although data on types of seizures were present for only one third of patients, this is the first large-scale study to characterize seizure types in Rett syndrome.^{3,4,16} Almost half of the seizures were generalized tonic-clonic, followed by partial seizures, myoclonic seizures, and absences. The classification of seizures was not video-electroencephalog-

raphy (EEG) based, which may raise the possibility that some focal seizures with secondary generalization were misinterpreted as generalized tonic-clonic seizures, whereas complex partial seizures might have been misinterpreted as absences. However, the presence of absence seizures is not surprising because this is the prototype of epilepsy in Rett animal models.²²

In this study, we found several hot spot mutations that affect epilepsy, occasionally in a manner different from which they are known to affect the general Rett phenotype. Patients with



Figure 2.

Prevalence of epilepsy according to hot spot mutations. This figure illustrates the prevalence of epilepsy in relation to different hot spot mutations. C-terminal mutations are associated with a lower prevalence of epilepsy. *p.R133C* conferred increased risk for epilepsy [OR 2.46, CI 95% 1.3–4.66] compared to C-terminal deletions (binomial logistic regression, $p < 0.01$). *p.R255X* conferred increased risk for epilepsy [OR 2.07, CI 95% 1.2–3.59] compared to C-terminal deletions (binomial logistic regression).

Epilepsia © ILAE

p.R133 mutation were more likely to have epilepsy, but seizures were easily controlled (Tables 1, 3 and 4, Fig. 2). This finding contradicts a previous report by Bao et al.,¹³ and is rather surprising, because the mutation is known to cause a milder disease, usually associated with PSV.¹² Of interest, in our group, only 15 (19.7%) of 76 of the patients with the *p.R133C* mutation presented with the PSV phenotype. Recently, the *p.R133C* mutation was found to disrupt a novel mechanism of neural chromatin structure regulation by MeCp2, related to the binding of 5-hydroxymethyl cytosine.¹⁹ The increased prevalence of epilepsy suggests a differential role of 5hmC binding in the epigenetics of epilepsy pathogenesis.

In addition, we found increased risk for epilepsy, as well as for more severe epilepsy, in patients with the *p.R255X* mutation, a truncating mutation known to cause a severe Rett phenotype.¹² Differences of opinion regarding the effect of *p.R255X* on epilepsy exist in the literature: decreased prevalence of epilepsy^{6,8} versus increased prevalence,¹⁰ incidence,¹³ or severity¹² (Table 1). In contrast, C-terminal deletions, known to cause a milder clinical phenotype, were also less likely to cause epilepsy; this was consistent with data found in the literature.^{5,6,10,11} The milder phenotype, including epilepsy, might be due to retained function in the C-terminal truncated protein.

The *p.T158M* mutation was associated with increased risk for severe epilepsy; this was consistent with previous reports.^{8,10,11,13} The *p.T158M* mutation is known to be a clinically severe mutation¹² due to its localization within the methyl-binding domain. Using animal models, it can be seen that mutations at this site critically impair the binding of the MeCp2 protein to promoters of the *BDNF* gene, explaining the general severe phenotype.²⁰

Another mutation found in our study to worsen epilepsy severity was *p.R306C*, in contradiction with previous reports in the literature.^{8,11,12} This mutation, which is associated with a mild-to-moderate Rett phenotype,¹² has a dual

mechanism of pathogenesis: it impairs the binding of the transcriptional repressor protein EnCoR to the MeCp2 protein,²³ and it abolishes the activity-induced phosphorylation of threonine at site 308.¹⁸ In mouse models mutated at the 308 site, there was a decrease in the activity-dependent transcription of *Npas4* and *BDNF*, leading to a lowering of the seizure threshold.¹⁸

In conclusion, the genotype–phenotype correlation for epilepsy still remains a controversial issue, since results differ between different series (Table 1). Sample size, different data acquisition methods (physician assessment, physician-filled questionnaire, and family questionnaire), or different outcome measures (prevalence, incidence, severity, and age of onset) may account for some of these inconsistencies.^{4–6,8,10–13,15,16,24} However, the activation of modifier gene pathways or epigenetic factors, such as different *BDNF* polymorphism distribution⁴ or X chromosome inactivation through different populations, might be an additional explanation. Meanwhile, the existent data should be used in clinical practice. Epilepsy in patients with *p.R255X* and *p.T158M* mutations should be treated aggressively in contrast to patients with *p.R133C* and C-terminal deletions.

Future studies should return the information obtained in clinical epidemiologic studies to the bench. Investigation of animal models with special missense mutations with propensity toward epilepsy (such as *p.R133C* and *p.306C*) may shed light on the epileptogenic pathways in Rett syndrome.

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DISCLOSURE

None of the authors has any conflict of interests to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with the guidelines.

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