



## Epilepsy in Rett syndrome - Lessons from the Rett networked database

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## Epilepsy in Rett Syndrome- Lessons from the Rett networked database

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## EPILEPSY IN RETT SYNDROME - LESSONS FROM THE RETT NETWORKED DATABASE

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PSV- preserved speech variant

5-hmC- 5 hydroxymethyl cytosine

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## ABSTRACT

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. Analyzing data from a large cohort should overcome this shortcoming.

Methods: Data from the Rett Networked Database on 1248 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. Age of onset of epilepsy was  $4.68 \pm 3.5$  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 (OR= 2.46, 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]), 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.

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## INTRODUCTION

Rett syndrome is an X-linked neurodevelopmental disorder affecting mostly females and caused by mutations in the MECP2 gene<sup>(1, 2)</sup>. Girls with the classical Rett phenotype are normal at birth, but during the first year of life, head-circumference growth decelerates, leading to microcephaly<sup>(2)</sup>. 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<sup>(2)</sup>. Other symptoms (breathing, gastrointestinal, and orthopedic abnormalities) are characteristic of Rett syndrome<sup>(2)</sup>. 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 more than 10 words).

Epilepsy is a core symptom of Rett syndrome, with prevalence as high as 60-90%<sup>(3, 4) (5-7)</sup>. Frequency might be overestimated since nonepileptic paroxysmal events are common and can lead to misdiagnosis<sup>(8)</sup>. Epilepsy might be more prevalent among those without a proven mutation in the MECP2 gene<sup>(8, 9)</sup>. Several predictors for epilepsy previously reported by Jian et al. (2006, 2007) and Glaze et al. (2010) were: general-symptom severity, pre-regression developmental problems, impairment of ambulation and hand use, and detection of an MECP2 mutation<sup>(5, 6, 8)</sup>.

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<sup>(5, 6, 10, 11)</sup> and the p.R306C mutation<sup>(8, 11, 12)</sup> offered protection against epilepsy, while the p.T158M had an inverse effect<sup>(8, 10, 13, 14)</sup>. According to the different reports, the mutations p.R255X<sup>(8, 10, 12, 13)</sup>, p.R294X<sup>(5, 6, 10, 12)</sup>, and large intragenic deletions<sup>(10, 11)</sup> had a contradictory effect. According to one study each, p.R133C<sup>(13)</sup> and p.R168X<sup>(5)</sup> were correlated to lower incidence of

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3 epilepsy. Several other researchers did not find statistically significant genotype–phenotype  
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5 correlations, possibly because of small sample size<sup>(4, 15, 16)</sup>.  
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9 The purpose of this study was to better characterize these controversial issues using a large  
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11 international database with physician-filled standardized questionnaires.  
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## 13 METHODS

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16 The Rett Networked Database is a physician-reported database that includes 293 clinical items  
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18 and 16 genetic items grouped into 31 domains, generated through a harmonization process of data  
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20 collected from 11 countries ([http://www .rett-databasenetwork.org/](http://www.rett-databasenetwork.org/))<sup>(14)</sup>.  
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24 Data from 1911 patients were collected from the data base: clinical type of Rett syndrome,  
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26 genotype, age of onset, severity of epilepsy, and types of seizures. Severity of epilepsy was defined by a  
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28 three-grade Likert scale (which combines frequency of seizures and response to therapy) as follows: 0-  
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30 no seizures or occasional seizures not requiring drug treatment; 1- seizures controlled by antiepileptic  
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32 drugs; and 2- seizures uncontrolled by multiple drugs (table 2). Seizure types were classified according to  
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34 the 1999 ILAE definitions. The following ordinal parameters regarding disease severity scores were  
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36 documented: speech, walking, breathing disorder, and regression (table 2). Patients with missing data  
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38 regarding epilepsy occurrence were excluded.  
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43 Patients were included if they had a mutation in the MECP2 gene, regardless of clinical  
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45 phenotype (classical or Rett variant). Patients without an MECP2 mutation (either not checked or not  
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47 proven) were included if the clinical phenotype was consistent with the classical Rett phenotype.  
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49 Patients with CDKL5 and FOX G1 mutations (genes known to cause Rett variants), male Rett patients,  
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51 and patients with MECP2 duplications, were excluded.  
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54 Statistical analysis was done using the IBM™SPSS™ Version 21 software. Kaplan-Meier survival  
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56 curves were used to calculate time to onset of epilepsy. A binomial regression model was used to  
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3 examine the effect of different genotypes or phenotypes on epilepsy. A multinomial regression model  
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5 was used to assess the effect of genotype and phenotype on the severity of epilepsy. For subpopulations  
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7 in which epilepsy was detected, the confounding effect of age of onset of epilepsy on severity was  
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9 calculated using survival analysis Cox regression. Spearman correlation was used for correlation  
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11 between age of onset and severity of epilepsy. The Mann-Whitney ranking test was used to compare  
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13 between different severity scores in patients with and without epilepsy.  
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17 The study was approved by the local Ethical Committee at the University Hospital of Siena  
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19 (30/2012).  
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## 22 23 RESULTS

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25 A total of 1248 patients were included in this study; 1135 of them (90.1%) were mutation-  
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27 positive. Eight hotspot mutations, as well as C-terminal-deletion mutations and large intragenic  
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29 deletions were present in 860 patients. A known clinical phenotype was available in 1098 patients.  
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31 Detailed data regarding clinical phenotype, MECP2 mutation status, and hotspot mutations are provided  
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33 in tables 3, 4, and supplementary table 1s.  
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38 Epilepsy was present in 850 patients (68.1%). It was more prevalent (78.8%) among MECP2-  
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40 negative patients (Fisher`s exact test), but other parameters related to epilepsy, such as age of onset,  
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42 severity, and type of seizures, were not influenced by the MECP2 status (supplementary table 1s).  
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46 Data on epilepsy severity were present in 736 patients: 27 (3.2%) experienced occasional  
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48 seizures not requiring treatment (grade 0), 469 (55.2%) had well-controlled seizures (grade 1), and 240  
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50 (32.6%) had uncontrolled seizures (grade 2). Data on seizure types were present in 298 patients: 138  
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52 (46.3%) had generalized tonic-clonic seizures, 81 (26.8%) had partial onset seizures, 43 (14.4%) absence,  
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54 36 (12.1%) myoclonic, 27 (9.1%) tonic, 12 (4.02%) atonic. Twenty-six percent of the patients experienced  
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56 two seizure types, while 3.4% had more than two.  
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3 Age of onset of epilepsy was  $4.68 \pm 3.5$  years (range of 3 months to 21 years of age). Epilepsy  
4 appeared slightly later than the other core symptoms of Rett syndrome, i.e., hand stereotypies  
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Age of onset of epilepsy was  $4.68 \pm 3.5$  years (range of 3 months to 21 years of age). Epilepsy appeared slightly later than the other core symptoms of Rett syndrome, i.e., hand stereotypies ( $2.43 \pm 1.67$ ), speech regression ( $2.09 \pm 1.1$ ), and loss of hand use ( $2.28 \pm 1.4$ ), but this difference did not reach statistical significance (ANOVA). Epilepsy onset was most prevalent between 3 and 5 years of age, and it appeared before the age of 8 years in more than 80% of the patients (fig. 1a; table 4). Different hotspot 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 ( $3.15 \pm 0.53$ ) compared to the classical phenotype ( $4.78 \pm 0.2$ ) (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 to 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 (OR = 0.43, CI 95% [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 vs. other mutations (supplementary table 2s).

Among the different hotspot mutations, the lowest prevalence of epilepsy was associated with C-terminal deletions: 58.5% vs. 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

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with p. R133C mutation were more likely to have controlled epilepsy (multinomial logistic regression, OR =2.59, CI 95% [1.33-5.02],  $p < 0.01$ ), while 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 features<sup>(4)</sup> suggest an intrinsic role of MECP2 in seizure pathophysiology. Animal models of Rett syndrome using nonsense-<sup>(17)</sup> as well as missense-<sup>(18-20)</sup> 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<sup>(17, 21, 22)</sup>.

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<sup>(13, 16)</sup>. 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

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3 years, but in a significant percentage of cases (7.45%), seizures appeared after 10 years of age. This  
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5 information should be taken into account when directing the follow-up of patients into adolescence.  
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8 Seizures were uncontrolled in 31.9% of patients, and seizure control was inversely correlated with the  
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10 age of onset of epilepsy. Our results are limited, since the scale used for grading epilepsy severity  
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12 includes seizure frequency as well as response to treatment. Other parameters, such as duration of  
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14 seizures, frequency of status epilepticus, and number of seizure-related hospitalizations, are missing as  
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16 well. The collection of data required a sophisticated harmonization process in order to overcome the  
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18 multilingual, multinational origins of the data. However, diverse fields of expertise among the treating  
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20 physicians, the availability of different medical services, as well as various protocols for diagnosis and  
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22 care, are potential pitfalls for the uniformity of the data.  
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27 While data on types of seizures were present for only one-third of patients, this is the first large-  
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29 scale study to characterize seizure types in Rett syndrome<sup>(3, 4, 16)</sup>. Almost half of the seizures were  
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31 generalized tonic-clonic, followed by partial seizures, myoclonic seizures, and absences. The  
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33 classification of seizures was not video-EEG based, which may raise the possibility that some focal  
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35 seizures with secondary generalization were misinterpreted as generalized tonic-clonic seizures while  
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37 complex partial seizures might have been misinterpreted as absences. However, the presence of  
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39 absence seizures is not surprising since this is the prototype of epilepsy in Rett animal models<sup>(22)</sup>.  
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44 In this study, we found several hotspot mutations that affect epilepsy, occasionally in a different  
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46 manner from which they are known to affect the general Rett phenotype. Patients with p.R133  
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48 mutation were more likely to have epilepsy, but seizures were easily controlled (tables 1, 3, 4, fig. 2).  
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50 This finding contradicts a previous report by Bao et al.<sup>(13)</sup>, and is rather surprising, because the mutation  
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52 is known to cause a milder disease, usually associated with PSV<sup>(12)</sup>. Interestingly, in our group, only  
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54 15/76 (19.7%) of the patients with the p.R133C mutation presented with the PSV phenotype. Recently,  
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56 the p.R133C mutation was found to disrupt a novel mechanism of neural chromatin structure regulation  
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3 by MeCp2, related to the binding of 5 hydroxymethyl cytosine<sup>(19)</sup>. The increased prevalence of epilepsy  
4 suggests a differential role of 5hmC binding in the epigenetics of epilepsy pathogenesis.  
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9 In addition, we found increased risk for epilepsy, as well as for more severe epilepsy, in patients  
10 with the p.R255X mutation, a truncating mutation known to cause a severe Rett phenotype<sup>(12)</sup>.  
11 Differences of opinion regarding the effect of p.R255X on epilepsy exist in the literature: decreased  
12 prevalence of epilepsy<sup>(6, 8)</sup> vs. increased prevalence<sup>(10)</sup>, incidence<sup>(13)</sup> or severity<sup>(12)</sup> (table 1). In contrast, C  
13 terminal deletions, known to cause a milder clinical phenotype, were also less likely to cause epilepsy;  
14 this was consistent with data found in the literature<sup>(5,6,10,11)</sup>. The milder phenotype, including epilepsy,  
15 might be due to retained function in the C-terminal truncated protein.  
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25 The p.T158M mutation was associated with increased risk for severe epilepsy; this was  
26 consistent with previous reports<sup>(8, 10, 11, 13)</sup>. The p.T158M mutation is known to be a clinically severe  
27 mutation<sup>(12)</sup> due to its localization within the methyl-binding domain. Using animal models, it can be  
28 seen that mutations at this site critically impair the binding of the MeCp2 protein to promoters of the  
29 BDNF gene, explaining the general severe phenotype<sup>(20)</sup>.  
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38 Another mutation found in our study to worsen epilepsy severity was p.R306C, in contradiction  
39 with previous reports in the literature<sup>(8, 11, 12)</sup>. This mutation, which is associated with a mild-to-moderate  
40 Rett phenotype<sup>(12)</sup>, has a dual mechanism of pathogenesis: it impairs the binding of the transcriptional  
41 repressor protein EnCoR to the MeCp2 protein<sup>(23)</sup>, and it abolishes the activity-induced phosphorylation  
42 of threonine at site 308<sup>(18)</sup>. In mouse models mutated at the 308 site, there was a decrease in the  
43 activity-dependent transcription of Npas4 and BDNF, leading to a lowering of the seizure threshold<sup>(18)</sup>.  
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52 In conclusion, the genotype-phenotype correlation for epilepsy still remains a controversial  
53 issue, since results differ between different series (table 1). Sample size, different data acquisition  
54 methods (physician assessment, physician-filled questionnaire, family questionnaire), or different  
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3 outcome measures (prevalence, incidence, severity, age of onset) may account for some of these  
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5 inconsistencies<sup>(4-6, 8, 11-13, 15, 16) (10, 24)</sup>. However, the activation of modifier gene pathways or epigenetic  
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7 factors, such as different BDNF polymorphism distribution<sup>(4)</sup> or X chromosome inactivation through  
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9 different populations, might be an additional explanation. Meanwhile, the existent data should be used  
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11 in clinical practice. Epilepsy in patients with p.R255X and p.T158M mutations should be treated  
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13 aggressively in contrast to patients with p.R133C and C-terminal deletions.  
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18 Future studies should return the information obtained in clinical epidemiologic studies to the  
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20 bench. Investigation of animal models with special missense mutations with propensity towards  
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22 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.

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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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## LEGENDS

**Legend figure 1**

## FIGURE 1: TIME TO ONSET OF EPILEPSY

Kaplan-Meier survival curve revealing time to onset of epilepsy in the general Rett population (1a) and main clinical phenotypes (1b). The curve is calculated only for patients with epilepsy.

**Legend figure 2**

## FIGURE 2: PREVALENCE OF EPILEPSY ACCORDING TO HOTSPOT MUTATIONS

This figure illustrates the prevalence of epilepsy in relation to different hotspot 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).

**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, 2014 <sup>(12)</sup>	1052 pts.	Physician assessment	Poisson regression, Tukey multiple comparisons				↑			↓		↑
Bao et al, 2013 <sup>(13)</sup>	685 pts.	Family questionnaire (InterRett database)	Logistic regression, Kaplan-Meier curve			↓	↑	↑				
Glaze et al, 2010 <sup>(8)</sup>	602 pts.	Physician assessment (American Rett Natural History)	Logistic regression				↓	↑	↑	↓		
Pintaudi et al, 2009 <sup>(10)</sup>	165 pts.	Physician questionnaire	Chi square	↓	↑		↑	↑				↑
Nectoux et al, 2008 <sup>(11)</sup>	81 pts.	Physician assessment	Kruskal-Wallis	↓	↓			↑		↓		
Gian et al, 2007 <sup>(6)</sup>	162 pts.	Family questionnaire	Binomial regression	↓			↓					↓
Gian et al, 2006 <sup>(5)</sup>	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

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**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-3 years	Under 18 months
Cold extremities	Absent	Mild	Severe
Gastrointestinal disturbances	Absent	Mild	Severe

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TABLE 3: DEMOGRAPHIC DATA OF PATIENTS WITH AND WITHOUT EPILEPSY

	Patients with epilepsy	Patients without epilepsy	Total	Comments
<b>Total number of patients</b>	<b>850 (68.1%)</b>	<b>398 (31.9%)</b>	<b>1248 (100%)</b>	
<b>Clinical phenotype</b>				PSV lowered the chance to develop epilepsy compared to classical phenotype (logistic regression, OR 0.43, 95% CI 0.25-0.72 ).
<b>Classical Rett</b>	653 (72.5%)	248 (27.5%)	901 (100%)	
<b>Preserved speech variant PSV</b>	33 (52.3%)	29 (46.8%)*	62 (100%)	
<b>Early epileptic variant</b>	4 (100%)	0 (0%)	4 (100%)	
<b>Congenital variant</b>	21 (84%)	4 (16%)	25 (100%)	
<b>Hotspot mutations</b>				p.R133C increased the risk for epilepsy compared to C-terminal deletion (logistic regression, OR 2.46, 95% CI 1.3-4.66).  p.R255X increased the risk for epilepsy compared to C-terminal deletion (logistic regression, OR 2.07, 95% CI 1.2-3.59)
<b>Large deletions</b>	41 (61.2%)	26 (38.8%)	67 (100%)	
<b>C-terminal deletions</b>	79 (58.5%)	56 (41.5%)*	135 (100%)	
<b>p.R106W</b>	27 (67.5%)	13 (32.5%)	40 (100%)	
<b>p.R133C</b>	59 (77.6%)*	17 (22.4%)	76 (100%)	
<b>p. R168X</b>	67 (65.5%)	36 (35%)	103 (100%)	
<b>p. R255X</b>	82 (74.5%)*	28 (25.5%)	110 (100%)	
<b>p. R270X</b>	51 (69.9%)	22 (30.1%)	73 (100%)	
<b>p. R294X</b>	42 (62.7%)	25 (37.3%)	67 (100%)	
<b>p. R306C</b>	46 (64.8%)	25 (35.2%)	71 (100%)	
<b>p. T158M</b>	83 (70.3%)	35 (29.5%)	118 (100%)	
<b>Speech severity score</b>				Patients with epilepsy had higher speech severity score (Mann-Whitney U ranking test, p<0.05)
<b>0</b>	208 (66%)	107 (34%)	315 (32.8%)	
<b>1</b>	51 (62.2%)	31 (37.8%)	82 (8.5%)	
<b>2</b>	409 (72.5%)*	155 (27.5%)	564 (58.7%)	
<b>Walking severity score</b>				Patients with epilepsy had higher walking severity score (Mann-Whitney U ranking test, p<0.01)
<b>0</b>	332 (57.5%)	245 (42.5%)	577 (51.4%)	
<b>1</b>	161 (81.7%)*	36 (18.3%)	197 (17.5%)	
<b>2</b>	276 (76.5%)	82 (23.5%)	349 (31.1%)	
<b>Breathing disorder score</b>				Patients with epilepsy had higher breathing disorder score (Mann-Whitney U ranking test, p<0.01)
<b>0</b>	248 (61.2%)	157 (38.8%)	405 (45.5%)	
<b>1</b>	171 (71.8%)	67 (28.8%)	238 (26.7%)	
<b>2</b>	205 (83%)*	42 (17%)	247 (27.8%)	
<b>Regression score</b>				Patients with epilepsy had higher regression score (Mann-Whitney U ranking test, p<0.01)
<b>0</b>	50 (63.3%)	29 (36.7%)	79 (7.6%)	
<b>1</b>	261 (66.1%)	134 (33.9%)	395 (37.8%)	
<b>2</b>	426 (74.5%)*	146 (25.5%)	572 (54.7%)	

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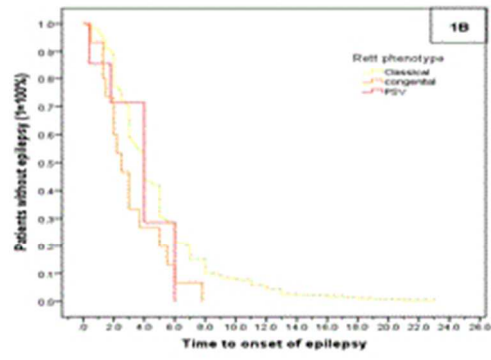
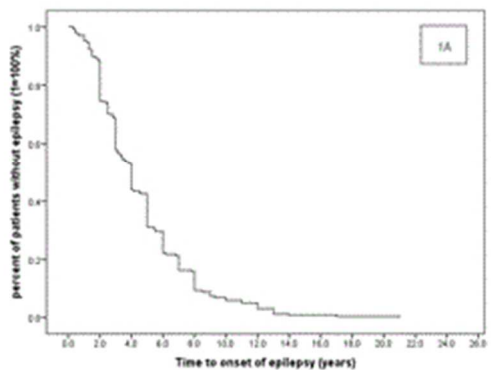
**TABLE 4: DEMOGRAPHIC DATA OF PATIENTS WITH EPILEPSY, ACCORDING TO EPILEPSY SEVERITY**

Severity score of epilepsy	0	1	2	Total	Comments
<b>Total number of patients</b>	<b>27 (3.2%)</b>	<b>469 (63.7%)</b>	<b>240 (32.6%)</b>	<b>736 (100%)</b>	
<b>Age of onset of epilepsy*</b>					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(13.1%)	130(75.1%)	57(31.1%)	173(100%)	
After 10 years	1(2%)	36(73.5%)	12(24.5%)	49(100%)	
<b>Clinical phenotype</b>					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%)	
Congenital variant	1 (5.9%)	11 (64.7%)	5(29.4%)	17 (100%)	
<b>Hotspot mutations</b>					p.R133C increased the chance for epilepsy severity 1 (multinomial logistic regression, OR =2.59, 95% [1.33-5.02]).
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%)	
p.R106W	1(4%)	16(64%)	8(32%)	25(100%)	
p.R133C	1(1.9%)	42(80.8%)*	9(17.3%)	52(100%)	p.R255X increased the risk for epilepsy severity 2 (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%)*	64(100%)	
p. R270X	0(0%)	31(73.8%)	11(26.2%)	42(100%)	p.R306C increased the risk for epilepsy severity 2 (multinomial logistic regression, OR= 2.69, 95% CI 1.19-6.05).
p. R294X	2(5.1%)	24(61.5%)	13(33.3%)	39(100%)	
p. R306C	1(2.6%)	18(47.4%)	19(50%)*	38(100%)	
p. T158M	2(2.7%)	42(56%)	31 (41.3%)*	75(100%)	p.T158M increased the risk for epilepsy severity 2 (multinomial logistic regression, OR= 3.09, 95% CI 1.48-6.4).

**Legend table4:**

Table indicates data for patients with available grading scale for epilepsy severity

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PREVALENCE OF EPILEPSY ACCORDING TO HOTSPOT MUTATIONS  
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