

Abstract

PURPOSE Rett syndrome (RTT) is a neurodevelopmental disorder that almost exclusively affects females. Epilepsy is a major clinical feature, but its long-term course in RTT has not been sufficiently explored. This study addresses the development of the epilepsy in adults with RTT.

METHODS Available females diagnosed with RTT in Norway were asked to participate. Parents/caregivers were interviewed, the girls/women were examined and their medical records reviewed. Participants were categorized according to age, epilepsy, seizure patterns and mutation severity groups. RTT severity was assessed (epilepsy score excluded).

RESULTS 70 females with classic RTT were included. A presumed pathogenic mutation in *MECP2* was found in 96%. The presence of active epilepsy (seizures last five years) was similar in all age groups above the age of ten: 11 (65%) in adolescents (11-20 years), 9 (60%) in young adults (21-30 years) and 14 (67%) in participants above 30 years of age. Tonic-clonic seizures within the last year were present in 55, 67 and 64%, and \geq weekly seizures occurred in 27, 45 and 50% in the respective age groups. Among participants with active epilepsy, 69% had unremitting seizures, whereas 31% had experienced remissions for more than six months during the last five years. In the oldest group (>30 years), only 19% had obtained seizure control for >5 years, and 14% had never experienced seizures. Seizure activity correlated with RTT severity score, whereas the relationship to mutation type remained ambiguous.

CONCLUSION Epilepsy continues to be a major concern in adults with RTT. Two thirds of women above 30 years of age remained with active epilepsy and 50% of them had seizures at least weekly.

KEYWORDS

Rett syndrome, epilepsy, aging, adulthood, co-morbidity, prognosis

1. INTRODUCTION

Rett syndrome (RTT, OMIM 312750) is a neurodevelopmental disorder with a prevalence around 1 in 10 000 live female births (Fehr et al., 2011; Laurvick et al., 2006). In the majority of girls and women with RTT mutations in the *MECP2* gene have been identified (Amir et al., 1999). In its classical form, RTT is characterized by an apparently normal early development during the first 6-18 months of life. Then a regression of communication and motor skills follows, leaving these girls with severe cognitive and physical impairments (Neul et al., 2010).

Epilepsy is one of the main clinical features of RTT, and affects approximately 70-90% of the females during their lifetime (Nissenkorn et al., 2015; Pintaudi et al., 2010; Tarquinio et al., 2017). The seizure disorder is a major concern in many families and affects quality of life of both the girl/woman with RTT and her family members (Bahi-Buisson et al., 2008). Several studies have revealed a wide variability of epileptic features in RTT (Nissenkorn et al., 2015; Pintaudi et al., 2010), but little scientific attention has been given to the course of epilepsy into adult age.

Life expectancy in RTT has increased considerably during the last 50 years (Freilinger et al., 2010). The latest survival analysis reports greater than 70% survival at 45 years (Tarquinio et al., 2015). Thus, we are facing a growing population of aging females diagnosed with RTT. A few studies from the last decade address RTT and aging on a general basis. These studies are contradictory concerning the seizure disorder. One study reports an improvement of epilepsy in adult age (Halbach et al., 2013), while two claim that epilepsy frequently still is a major concern in adulthood (Anderson et al., 2014; Vignoli et al., 2012). Studies concerning epilepsy in relation to age usually limit their focus to adolescence and early adulthood and lump the relatively few subjects older than 20 years into one group (Bao et al., 2013; Jian et al., 2007; Pintaudi et al., 2010). The course of epilepsy in later adulthood age is thus essentially unexplored.

The aim of the present paper is to describe the diversity of epilepsy in a population of females with RTT, and to address the development of the seizure disorder in adulthood.

2. METHODS

2.1 RECRUITMENT

In this population-based cross-sectional project, recruitment took place from 2014 to 2017. Invitation to participate was distributed to families or guardians of females with RTT or a RTT-like disorder through the Norwegian Rett Syndrome Association (n=126) and Frambu, the Norwegian Resource Centre for Rare Disorders, (n=116). The rate of overlapping was high, as only 165 subjects with RTT had been reported to the Norwegian Patient Registry from the Specialist Health Services in 2013. Lists of names from these sources were not revealed to the study group. In addition, some females were referred directly from habilitation clinics and neurologists.

Consent to participate was given on behalf of 93 subjects. Ascertainment of the diagnosis of the identified subjects was based on key clinical features independent of molecular findings, according to the latest consensus criteria (Neul et al., 2010). *CDKL5*- and *FOXP1*-disorders as well as conditions with RTT-like features and *MECP2* mutations not fulfilling the RTT criteria were defined as RTT-like disorders. Of the 93 subjects, 74 had classic RTT, ten had atypical RTT, seven had RTT-like disorders and two did neither have RTT nor a RTT-like disorder. Exclusion of two individuals with classic RTT due to mutations in *SCN1A*, which might influence the epilepsy, and missing clinical data for two subjects, left 70 individuals available for analysis.

2.2 CLINICAL DATA

Parents/caregivers were asked to complete a questionnaire covering information on the demographic background and the development of motor skills. We then met the families at their local hospital or in their homes. A clinical examination, including growth parameters, level of contact, presence of stereotypies and respiration

abnormalities as well as assessment of muscle tone, deep tendon reflexes, coordination and scoliosis, was performed mainly by the first author. In addition a semi-structured interview with parents/caregivers took place. Pregnancy and birth, development, communication skills, other clinical symptoms and results of previous genetic testing were addressed. Epilepsy-specific information covered the ascertainment of epileptic seizures, age of seizure onset, the history of seizure types, seizure frequency, antiepileptic drug (AED) treatment and any remissions. The potential pitfall of inaccurate reporting received particular attention. A review of medical records was thus carried out to complete the data sets. If information from interviews and records did not completely correspond, details recorded in writing at the time of the event were considered more reliable.

2.3 GENETIC ANALYSES

In participants without known mutations prior to inclusion (due to either negative or no testing), genetic sequencing ad modum Sanger and multiplex ligation-dependent probe amplification (MLPA) of *MECP2* were performed. If the results of these tests were negative, exome-based high throughput sequencing analysis with bioinformatic filtering of a panel of genes known to cause intellectual disability and/or epileptic encephalopathies was performed, using an Illumina hiseq 2500 platform. During the research study, the number of genes in the panel analyses available from the laboratory increased from 45 to 1400. Single patient analysis of 45 genes was performed for three participants and a trio (patient, mother, father) analysis of 1400 genes was performed for one participant. Samples with negative findings in the 45 gene panel were not reanalyzed with a larger panel.

2.4 DATA CATEGORIZATION

MECP2 mutations were classified into two groups, according to expected phenotypic severity based on previous reports (Cuddapah et al., 2014); severe (T158M, R168X, R255X, R270X, large deletions) and mild (R133C, R294X, R306C, other point mutations, c-terminal truncations). Age was partly used in the analyses as a continuous variable, and partly categorized into four subgroups: 1-10 years, 11-20 years, 21-30 years, and above 30 years. Head circumference was categorized using

normative z-scores (Rollins et al., 2010). Disease severity was quantified according to the Rett syndrome Severity Scale with scoring of seven parameters from 0 (absent/normal) to 3 (severe) (Kaufmann et al., 2012). When analyzing RTT severity versus epilepsy, the seizure sub-score was subtracted.

Seizure categorization was based on semiological features. According to the recently revised ILAE seizure classification (Fisher et al, 2017), seizure types were identified as either focal onset motor seizures or unknown onset tonic-clonic or other motor seizures, comprising myoclonic, tonic or atonic elements. EEG findings could not be systematically assessed in this study. Dubious epileptic symptoms with low symptom burden and little or no impact on quality of life, including discrete episodes with behavior arrest only, had to be disregarded. Care was taken not to interpret non-epileptic events as epileptic seizures (i.e. unspecific twitching, jerking, head turning, trembling, staring, laughing and respiratory abnormalities)(Glaze et al., 1998).

Active epilepsy was defined as seizures within the last five years (ILAE Commission Report, 1997). Seizure frequency within the last year was categorized as \geq daily; $<$ daily \geq weekly; $<$ weekly \geq monthly; $<$ monthly $>$ yearly; or seizure free.

Seizure patterns were divided into four categories. Group 1: never seizures; group 2: diagnosed with epilepsy, but seizure free for more than five years; group 3: active epilepsy with remissions more than six months within last five years; group 4: persistent seizures without remissions.

2.5 STATISTICAL ANALYSIS

The descriptive analyses include mean and standard deviations or median and inter quartile range for continuous data, and absolute and relative frequencies for categorical data. Independent samples t- test or multiple linear regression were used to compare groups with continuous variables. Chi Square or Fisher's Exact Test were used for categorical variables. To assess the frequency of seizures, both cross-sectional and retrospective longitudinal data were analyzed. Significance level is ≤ 0.05 . Statistical analyses were performed using SPSS for windows version 23.

Ethics approval was obtained from the Regional Committee for Medical Research Ethics. Parental/guardian consent was obtained prior to inclusion.

3 RESULTS

3.1 EPILEPSY IN CLASSIC RTT

At inclusion median age was 21 years, ranging from 1 to 66 years (IQR 14-34 years). Epilepsy had been diagnosed at some point in 70% of the participants.

Median age of first seizure was 4 years (range 7 months – 40 years, IQR 3-7 years). Seizure onset occurred in four participants between 11 and 20 years of age, and in one participant above 20 years. Table 1 shows the distribution of the seizure patterns among the 70 participants. All individuals with active epilepsy received antiepileptic drugs (AEDs); five individuals with epileptiform EEG activity never diagnosed with epilepsy also used AEDs.

Figure 1 illustrates the relationship between age and seizure pattern. Active epilepsy (group 3 and 4), occurred in 65% of adolescents (11-20 years), 60% in young adults (21-30 years) and 67% in older adults (>30 years). Among the children (1-10 years), only five participants (29%) had developed epilepsy. None of the children had had epilepsy for as long as five years; three had experienced remissions for more than six months. The distribution of seizure patterns did not differ much with age in participants above ten years of age (Figure 1). Ten participants with previously diagnosed epilepsy had been seizure-free for more than five years. Two of them had discontinued AED treatment (Table 1), and had been seizure free for at least ten years and off medication for 23 and six years, respectively. The seizure disorders of the five participants with seizure onset after ten years of age varied considerably and did not seem to be essentially different from those with earlier onset.

3.2 SEIZURE FREQUENCY

Seizure frequency within the last year prior to inclusion did not differ notably between the age groups, but \geq weekly seizures tended to occur more often in children below 10 years (60%) compared to adolescents (27%). However, the frequency of seizures showed a tendency to increase again in adults (45-50%) (Table 2). Figure 2 neatly

illustrates the mean seizure frequency at different ages according to the retrospective longitudinal data. Seizures were more frequent in the early age groups, but remained relatively stable from early adolescence through adulthood, although with a slight increase in the oldest participants, in line with the findings in Table 2.

3.3 SEIZURE TYPES

The presence of tonic-clonic seizures tended to increase slightly with age. In the oldest group, 64% of participants with active epilepsy had tonic-clonic seizures during the last year prior to inclusion, whereas less than 50% of individuals below 20 years had this type of seizures (Table 2). Other seizure types were more equally distributed among the age groups. There was no correlation between seizure type and seizure patterns. The proportion of participants having more than one seizure type was close to 40% in the three oldest age groups; in the youngest group only 10% had multiple seizures types (Table 2).

3.4 MUTATIONS

Mutation analyses were completed for 68 of the 70 participants, and of these, 67 (99%) had a *MECP2* mutation. One had negative test for *MECP2* as well as for the applied gene panel. Three of the mutations in *MECP2* could not be classified into either of the two groups of expected phenotypic severity (Cuddapah et al., 2014). Age at inclusion differed between mutation groups (Table 3). In participants below 20 years of age, mean severity score was significantly lower in those with “mild” mutations compared to those with “severe” mutations (9.5 vs 13.3). In participants above 20 years there was no such trend (15.6 vs 14.9). The same pattern was found for epilepsy characteristics; participants under 20 years with mild mutations had a tendency to a lower prevalence of active epilepsy and a lower seizure frequency compared to the severe mutation group, whereas in participants above 20 years, the results were inverse (Table 3).

3.5 SEIZURE PATTERNS AND CLINICAL SEVERITY

Mean score on the Rett Syndrome Severity Scale was 9.9 in seizure pattern group 1 (never seizures), 12.6 in group 2 (seizure-free last five years), 12.2 in group 3 (active seizures with remissions) and 13.8 in group 4 (active seizures without remissions).

To control for age and mutation type confounders, multiple regression analysis was performed; the adjusted mean global severity increased by 2.9 from seizure pattern group 1 to 4 ($p=0.001$, Table 4).

4 DISCUSSION

4.1 AGE, EPILEPSY AND SEIZURE PATTERNS

The present study includes a considerable proportion of females with RTT in Norway. More than half the participants were older than 20 years, and almost one third were above 30 years. No other study with a main focus on epilepsy has included such a large proportion of adults and aging females with RTT. Thus, this cross-sectional sample provides a unique opportunity to study the impact of epilepsy in adulthood.

The prevalence of active epilepsy was similar across the age groups after the age of ten. Approximately two thirds of these participants had experienced seizures within the last five years. The percentage of seizure-free participants during the last year did not increase after the age of 30 years. This is in contrast to the common notion of an improvement and sometimes a remission of epilepsy in adult age that has prevailed ever since the first reports on the natural history of RTT (Naidu et al., 1986; Steffenburg et al., 2001). However, some recent studies have demonstrated results adhering to this notion (Glaze et al., 2010; Halbach et al., 2013), others have found, like the present paper, that epilepsy is a major concern in adulthood (Anderson et al., 2014; Vignoli et al., 2012).

In a large multicenter prospective study on the longitudinal course of epilepsy based on data from the Rett Syndrome Natural History Consortium, three distinct seizure patterns emerged: a) no seizures, b) frequent remissions and relapses, and c) unremitting and persistent seizures (Tarquinio et al., 2017). In that study, information on seizure activity the last six months was collected at annual or semi-annual visits to the clinic. The remitting-relapsing pattern was identified in 41%, whereas only 16% had never experienced remission. In the present cross-sectional retrospective study, we applied the same seizure pattern categories, but extended the observation periods to the last five years. For only 17%, remissions for more than six months

were reported, while 39% had not had remissions. Unsurprisingly, more children had never had seizures compared to adults. The discrepancies in the two studies are probably for the most part due to different methodologies: retrospective recall and medical records in the present study and prospective follow-up in the American study. The term remission was used for absence of seizures exceeding six months at completion in the American study, whereas in the present study terminal remission was conventionally defined as absence of active epilepsy (5 years seizure-free) (Sillanpaa et al., 2017). Hence, the two studies cannot be compared in these respects.

Seizure frequency tended to differ with age; it was highest in young children with recent seizure onset, although the number of young children with epilepsy was low. Seizure frequency decreased in adolescence and early adulthood, but there was a trend towards a slight increase later in adulthood, in contrast to previous ideas. This tendency was also apparent in the retrospective longitudinal data (Fig 2). Half of the women above 30 years had seizures at least weekly. More adults had tonic-clonic seizures compared to children and adolescents and more women above 30 years had multiple seizure types.

Seizure types and episodic behavioral abnormalities are multiple in RTT and are often difficult to differentiate on a clinical basis. Very few participants in this sample had undergone ictal video-EEG recordings due to spells of uncertain significance, but only seizure types clearly identified from the current operational ILAE seizure classification (Fisher et al., 2017) were acknowledged in the present study. Seizure semiologies and EEG characteristics in RTT are consistent with both focal and generalized seizures (Dolce et al., 2013; Steffenburg et al, 2001), and often fall within the category of unknown onset (Fisher et al., 2017). Importantly, the epilepsy of RTT is an example of “combined generalized and focal epilepsies”, along with some other genetic epilepsies, such as Dravet Syndrome. This particular type of epilepsy has only recently been acknowledged as a separate entity by the International League Against Epilepsy (Scheffer et al., 2017).

4.2 MUTATION GROUPS

There is a general consensus about the association between genotype and general phenotype in RTT (Cuddapah et al., 2014). In contrast, the association between genotype and epilepsy remains unclear and results have been somewhat conflicting (Bao et al., 2013; Cardoza et al., 2011; Nissenkorn et al., 2015). One recent study suggested that seizure frequency is not strongly associated with mutation type (Tarquinio et al., 2017).

In the present sample, the overall correlation was weak, and epilepsy features were almost identical in participants with so-called mild and with severe mutations. However, the age distribution in the two groups was strikingly skewed. The mean age of participants with mild mutations was significantly higher than in the severe mutation group. Children and adolescents with mild mutations had significantly lower mean global severity and less frequent seizures, compared to participants with mutations associated with more severe disease. In contrast, adults with mild mutations had a trend to higher global severity scores and more severe epilepsy. They even had earlier seizure onset than adults with mutations considered more severe.

We can only speculate on the cause of the age difference in the two mutation groups. A survival effect might be operative. Life expectancy may generally be shorter in individuals with RTT who have severe mutations and higher global severity as well as hazardous seizure disorders (Tarquinio et al., 2015). However, the trend to a milder overall phenotype (including seizure frequency) in women with RTT reaching advanced age in the group with mutations previously associated with more severe disease was striking. These trends are a surprising finding, and should be further investigated with larger samples.

4.3 EPILEPSY AND GLOBAL CLINICAL SEVERITY

The scores on the Rett Syndrome Severity Scale correlated significantly with the seizure pattern severity, with mean scores increasing from seizure pattern group 1 (without epilepsy) to group 4 (active epilepsy without remission). Due to the wide age range in the present sample, aging and deteriorating health were regarded as a potential bias (Cianfaglione et al., 2016; Cuddapah et al., 2014). When adjusted for age and mutation group, the association was still significant. This finding is in line

with other studies (Jian et al., 2007; Tarquinio et al., 2017), although these used different scales for clinical severity. Jian et al. (2007) found an association between RTT severity and parent-reported seizure rate, while Tarquinio et al. (2017) compared participants with and without epilepsy and found that global severity scores were higher in those with epilepsy.

RTT is a condition that highlights the current discussion on the differentiation between a “developmental encephalopathy” and an “epileptic encephalopathy” where the epileptic activity itself contributes to cognitive and behavioral impairments beyond what might be caused by the underlying condition alone. According to the 2017 revised ILAE epilepsy classification (Scheffer et al., 2017), the concept of epileptic encephalopathy should be applied more widely than just for some severe epilepsies of childhood with bilateral and abundant epileptiform activity. Even in the self-limited focal epilepsies of childhood, there is evidence of a widespread impact of the epileptic disease process on cognitive functions (Wickens et al., 2017). The present findings cannot determine whether the more severe overall RTT phenotype simply is associated with more severe epilepsy, or if the clinical epileptic activity itself influences the severity of the developmental disorder. Further research should endeavor to clarify whether RTT is a “developmental encephalopathy with epilepsy” or a combined “developmental and epileptic encephalopathy” where both factors play a part (Scheffer et al., 2017). If the latter is true, early intense anti-seizure treatment might have the potential to ameliorate the overall clinical consequences of RTT.

4.4 LIMITATIONS AND STRENGTHS OF THE STUDY

It is challenging to distinguish between epileptic and non-epileptic seizures in RTT. In Norway, all patients with epilepsy are routinely examined with interictal EEG recordings, but in this disorder EEG is universally abnormal, and the diagnosis of epilepsy should not rely on interictal abnormalities (Tarquinio et al., 2017). The study design with parental reports might have influenced the results by over-reporting of epileptic seizures (Glaze et al., 2010). Tarquinio et al. (2017) report that physicians diagnosed seizures in attacks that parents believed were non-epileptic in 3% of the cases, whereas parents suggested seizures in 4% of episodes that physicians considered to represent other types of spells. The problem of inappropriate seizure

recording is probably as common in adults, as caregivers in group homes are often multiple, unexperienced and may be responsible for the individuals for only shorter periods. Nevertheless, care was taken not to interpret typical episodic RTT behavior, such as midline stereotypies, hyperventilation and autistic features as epileptic seizures. On the other hand, subtle non-motor seizures with behavior arrest or impaired awareness only may not have been clinically recognized.

Of course, a recall bias of historical data may be present in this kind of study. To minimize this source of error we reviewed medical records for most participants. Only large scale prospective studies will ultimately determine to what extent the validity of this study is influenced by these factors, as well as by the relatively low number of participants in some subgroups.

Nevertheless, a unique strength of the present study is its population-based character, reducing the selection bias of specialized clinics and yielding a wide age span. In spite of the high proportion of adults in this study compared to previous ones, a somewhat skewed distribution towards lower age might well be present. Families having a daughter with RTT in the younger age groups may be more active in the parent association, and parents with newly diagnosed children may make use of more services from the Resource Center for Rare Disorders. Thus, a larger proportion of families with younger girls with RTT may have received the invitation to participate. Although this was a nationwide study, the number of participants was below 60% of those registered with RTT in the Norwegian Patient Registry (n=165).

Moreover, the general awareness of the RTT phenotype is probably higher among child neurologists than among adult neurologists due to the characteristic history of RTT features in early childhood. In adult age, difficult-to-treat epilepsy is usually the symptom that brings individuals with RTT to the attention of the specialist health care, whereas individuals without seizures or with resolved and well controlled epilepsy often are treated on a less specialized health care level. Hence, RTT might more often remain unrecognized in individuals without seizures. Even if recognized, the broader and more common diagnostic categories of severe intellectual disability and autism spectrum disorder may be applied for this rare disorder for the sake of ease in a busy clinical practice.

It has been suggested that the RTT phenotype may have a broader genetic background than previously recognized which may cause an overlap with other genetic disorders (Ehrhart et al., 2018). Hence, we chose not to include two individuals with Rett features harboring SCN1A mutations and early seizure onset due to a possible link to Dravet syndrome. Nevertheless, we decided to keep three individuals with classic RTT without identified mutations according to the diagnostic criteria for RTT.

Another strong point is the fact that almost all participants in this study were personally examined by one clinical investigator (the first author), with extensive knowledge about RTT. The same person interviewed the parents or caregivers of almost all participants and organized and collected all data in a uniform manner.

5 CONCLUSIONS

In the present sample, two thirds of females with RTT still have active epilepsy in adult age. The most common seizure pattern in individuals above the age of 30 was relentlessly unremitting seizures, whereas some experienced remissions and relapses. For a minority of individuals with previously diagnosed epilepsy long-lasting seizure control was achieved, while a few never developed seizures.

Several publications convey the view that the seizure disorder in RTT usually improves or remits in adult age. This notion needs to be modified. The present results confirm that epilepsy frequently remains as a major concern in advancing age of females with RTT. Continued specialist epilepsy service is needed in these individuals.

DECLARATIONS OF INTEREST

None

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TABLES

TABLE 1 The distribution of seizure patterns in 70 patients with classic Rett syndrome

Seizure patterns	Classic RTT N (%)
Group 1: Never seizures	21 (30)
- 1a: No AEDs	16 (23)
- 1b: With AEDs	5 (7)
Group 2: Seizure free last five years	10 (14)
- 2a: AEDs discontinued	2 (3)
- 2b: With AEDs	8 (11)
Group 3: Active epilepsy with seizure remissions and relapses last five years	12 (17)
Group 4: Active epilepsy without seizure remissions and relapses	27 (39)
- 4a: Remissions, but not last five years	7 (10)
- 4b: Never remissions	20 (29)

AED: Anti-epileptic drug

TABLE 2 Seizure frequency and seizure types within last year by age in females with active epilepsy and classic RTT

Age	N	Seizure frequency N (%)			Seizure types N (%)			
		≥ Weekly	< Weekly ≥ monthly	< Monthly	Tonic-clonic	Focal motor	Other motor	>1 seizure type
1-10 years	5	3 (60)	0 (0)	2 (40)	1 (20)	3 (60)	2 (40)	1 (10)
11-20 years	11	3 (27)	2 (18)	6 (55)	6 (55)	5 (45)	2 (18)	5 (45)
21-30 years	9	4 (45)	2 (22)	3 (33)	6 (67)	3 (33)	1 (11)	3 (33)
>30 years	14	7 (50)	5 (36)	2 (14)	9 (64)	8 (57)	4 (29)	7 (50)

TABLE 3 Mutation groups in relation to RTT severity, age groups and epilepsy characteristics

	Mild mutations		Severe mutations		p-value
		N		N	
Age ^a	27.1 ±17.0	38	17.5 ±11.5	26	0.009 ¹
RTT severity ^a					
1-20 years	9.5 ±2.7	15	13.3 ±3.6	17	0.002 ¹
>20 years	15.6 ±2.7	20	14.9 ±2.2	9	ns ¹
Active epilepsy ^b					
1-20 years	6 (38)	16	10 (59)	17	ns ²
>20 years	16 (73)	22	5 (56)	9	ns ²
Age of seizure onset ^a					
1-20 years	4.1 ±1.7	7	3.1 ±1.1	11	ns ¹
>20 years	5.1 ±2.9	19	7.3 ±6.5	6	ns ¹
≥Weekly seizures ^c					
1-20 years	0	6	6 (60)	10	0.034 ³
>20 years	10 (63)	16	0	5	0.035 ³

^a) Mean ±SD; ^b) n(%); ^c) n(% of those with active epilepsy)

¹) Independent sample t-test; ²) Chi square; ³) Fisher exact

TABLE 4 The relationship between RTT severity and seizure patterns adjusted for age and mutation groups by multiple regression analysis

Variable	Unadjusted effect	95% CI	p-value	Adjusted effect	95% CI	p-value
Seizure pattern group 2 vs 1	2.695	0.623-4.767	0.012	1.477	-0.775-3.729	0.194
Seizure pattern group 3 vs 1	1.762	-0.190-3.714	0.076	1.417	-0.509-3.343	0.146
Seizure pattern group 4 vs 1	3.364	1.782-4.947	<0.001	2.851	1.226-4.476	0.001
Age	0.074	0.029-0.118	0.002	0.076	0.028-0.123	0.002
Mutation group severe vs mild	0.958	-0.609-2.526	0.226	1.626	0.301-2.951	0.017

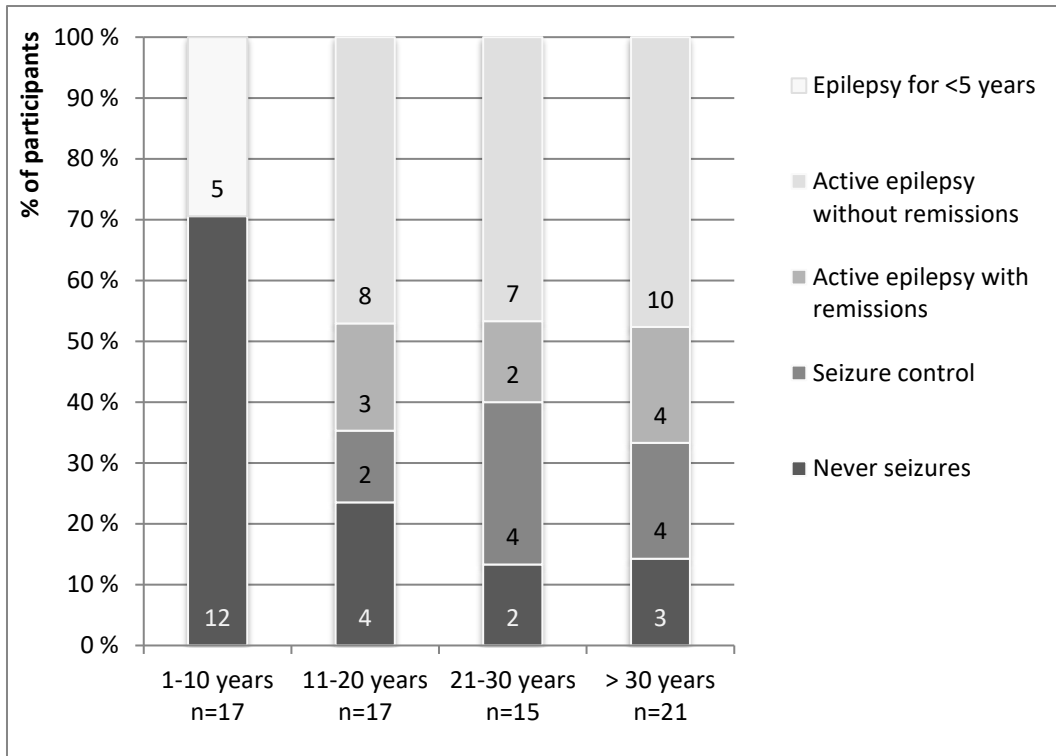


FIGURE 1 The relationship between age and seizure patterns within the last five years in females with classic RTT.

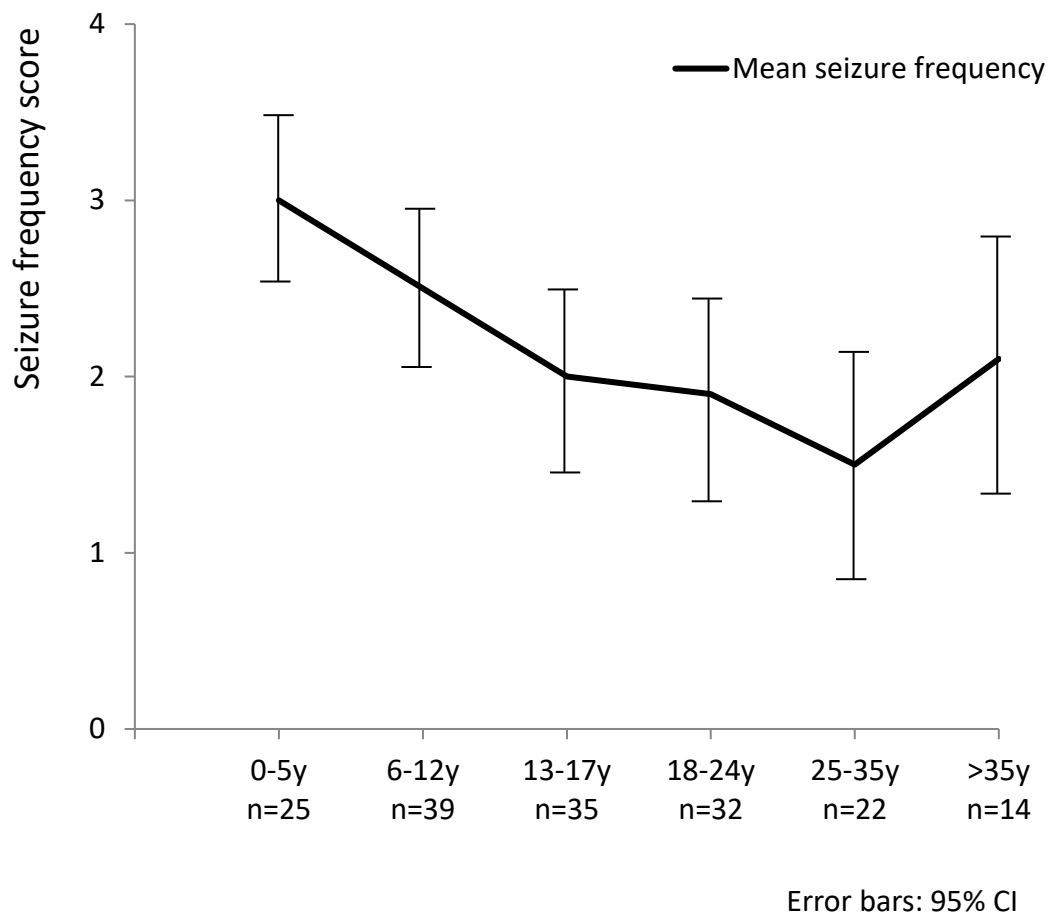


FIGURE 2 Longitudinal relationships between age and mean seizure frequency in females with classic RTT ever diagnosed with epilepsy. Seizure frequency scores: 0 = no seizures last year; 1 = \geq yearly, <monthly; 2 = \geq monthly, <weekly; 3 = \geq weekly, <daily; 4 = \geq daily.