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FULL-LENGTH ORIGINAL RESEARCH

Early epileptiform EEG activity in infants with tuberous sclerosis complex predicts epilepsy and neurodevelopmental outcomes

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Summary

Objective: To study the association between timing and characteristics of the first electroencephalography (EEG) with epileptiform discharges (ED-EEG) and epilepsy and neurodevelopment at 24 months in infants with tuberous sclerosis complex (TSC).

Methods: Patients enrolled in the prospective Epileptogenesis in a genetic model of epilepsy – Tuberous sclerosis complex (EPISTOP) trial, had serial EEG monitoring

until the age of 24 months. The timing and characteristics of the first ED-EEG were studied in relation to clinical outcome. Epilepsy-related outcomes were analyzed separately in a conventionally followed group (initiation of vigabatrin after seizure onset) and a preventive group (initiation of vigabatrin before seizures, but after appearance of interictal epileptiform discharges [IEDs]).

Results: Eighty-three infants with TSC were enrolled at a median age of 28 days (interquartile range [IQR] 14–54). Seventy-nine of 83 patients (95%) developed epileptiform discharges at a median age of 77 days (IQR 23–111). Patients with a pathogenic *TSC2* variant were significantly younger (*P*-value .009) at first ED-EEG and more frequently had multifocal IED (*P*-value .042) than patients with a pathogenic *TSC1* variant. A younger age at first ED-EEG was significantly associated with lower cognitive (*P*-value .010), language (*P*-value .001), and motor (*P*-value .013) developmental quotients at 24 months.

In the conventional group, 48 of 60 developed seizures. In this group, the presence of focal slowing on the first ED-EEG was predictive of earlier seizure onset (*P*-value .030). Earlier recording of epileptiform discharges (*P*-value .019), especially when multifocal (*P*-value .026) was associated with higher risk of drug-resistant epilepsy.

In the preventive group, timing, distribution of IED, or focal slowing, was not associated with the epilepsy outcomes. However, when multifocal IEDs were present on the first ED-EEG, preventive treatment delayed the onset of seizures significantly (*P*-value <.001).

Significance: Early EEG findings help to identify TSC infants at risk of severe epilepsy and neurodevelopmental delay and those who may benefit from preventive treatment with vigabatrin.

KEYWORDS

EEG, epilepsy, epileptogenesis, neurodevelopment, tuberous sclerosis complex

Key Points

- In infants with tuberous sclerosis complex (TSC), epileptiform discharges on electroencephalography (EEG) typically appears before the age of 3 months and often with a multifocal distribution.
- Early appearance of epileptiform discharges is associated with worse developmental outcome.
- First EEG with epileptiform discharges does not predict autism spectrum disorder risk at 24 months of age.
- In the group of conventionally treated TSC infants, early onset of IED as well as focal slowing are associated with earlier seizure onset.
- Infants with TSC presenting with multifocal IED benefit more from preventive vigabatrin treatment in delaying seizure onset.

1 | INTRODUCTION

Tuberous sclerosis complex (TSC) is a rare autosomal-dominant multisystem disorder.[1] Although the clinical phenotype is both age dependent and highly variable, central nervous system manifestations form the major burden of disease in young children with TSC.[1,2] Epilepsy affects 80%–90% of TSC patients, of whom two thirds develop the first seizure during their first year of life.[3,4] Up to 60% of TSC patients develop drug-resistant epilepsy.[3,5] Intellectual disability, behavioral problems, and autism spectrum disorder (ASD) are reported to affect half of TSC patients.[2,4,6,7]

Early diagnosis, before onset of clinical seizures, enables prospective study of the process of epileptogenesis and its relation to neurodevelopment. Serial electroencephalography (EEG) is a feasible, noninvasive investigation that provides information on both brain maturation and epileptogenesis.[8,9] Domanska-Pakiela et al. reported that interictal epileptiform discharges (IEDs) preceded clinical seizure onset by 1–8 days.[10] In the study of Wu et al., the appearance of IEDs correctly identified 77% of infants who subsequently developed clinical seizures. The presence of IEDs preceded the onset of clinical seizures by an average interval of 3.6 months.[11] Both studies imply that there is a time window between the emergence of IEDs on EEG and the development of clinical seizures, providing an opportunity for preventive treatment.[11] Several studies have reported that preventive treatment with the antiepileptic drug vigabatrin after the appearance of IEDs on EEG but before seizure onset was associated with improved outcome compared to vigabatrin initiated after the appearance of seizures.[12–14]

The pathogenic TSC mutations causing mammalian target of rapamycin complex 1 overactivation, cause a cascade of events resulting in both early epileptogenesis and neurodevelopmental comorbidities. In addition to a genetic predisposition, early onset of seizures further worsens neurodevelopmental outcomes.[15] Because a relationship between epilepsy and neurodevelopment has been established, it is of interest to study whether early epileptiform EEG features, recorded before the onset of clinical seizures in infants with TSC are associated with neurodevelopmental outcome.

In this exploratory, prospective cohort study in infants with TSC we study the relation between the timing and characteristics of the first EEG with epileptiform discharges and epilepsy and neurodevelopmental outcome. We hypothesized that the early appearance of epileptiform discharges or specific EEG patterns is associated with worse epilepsy and neurodevelopmental outcome.

2 | MATERIAL AND METHODS

2.1 | Patients

This prospective, exploratory cohort study analyzed EEG studies that were collected as part of the long-term, prospective multicenter Epileptogenesis in a genetic model of epilepsy – Tuberous sclerosis complex (EPISTOP) project (NCT02098759). The primary objective of the EPISTOP project was to identify clinical and molecular biomarkers of epileptogenesis in a prospective clinical study of infants with TSC. A secondary objective of the EPISTOP project was to investigate the potential benefit of preventive treatment with vigabatrin after the appearance of IED on the EEG vs conventional treatment, in which vigabatrin was initiated only after the onset of clinical or electrographic seizures.[14] In the EPISTOP trial, two cohorts of infants with TSC were followed: a conventional cohort (clinical and EEG follow-up and start of vigabatrin after seizure onset) and a preventive group (identical follow-up and start of vigabatrin after reaching specific EEG criteria—focal IED for >10% of the recording time, multifocal IED, generalized IED, or hypsarrhythmia—and before seizure onset).[14] Results and methodology, including the assignment to the preventive and conventional group, addressing the secondary objective of the EPISTOP project are published elsewhere.[14,16]

Enrollment of infants for this study took place from November 2013 to August 2016 at 10 sites. Inclusion criteria were male or female infants aged up to 4 months with a definite diagnosis of TSC,[17] without previous clinical seizures seen by caregivers or recorded at baseline EEG, and written informed consent of their caregivers in accordance with the Declaration of Helsinki. Exclusion criteria were infants with observed clinical seizures before and at the baseline visit, antiepileptic treatment at or prior to study entry, contraindications for magnetic resonance imaging (MRI), or any severe or uncontrolled medical condition that was considered as possibly affecting the EPISTOP analyses or procedures. The EPISTOP study was approved by local ethical committees at all study sites.

For the present EEG study, 83 EPISTOP children were included in whom a full EEG set was available. One hundred one patients gave informed consent for the EPISTOP trial.[14] Seven patients were misdiagnosed with TSC, reducing the number to 94 patients in the EPISTOP trial.[14] For one patient, only local EEG scoring was available but no full EEG sets to perform our analysis. In 10 patients, there was a discrepancy between local EEG scoring and central EEG interpretation during follow-up. Those 10 patients were excluded from the current EEG analysis. Two of those 10 patients were in the preventive treatment cohort, reducing the number in the preventive treatment group in our cohort to 23. Our cohort combines the randomized and observational arms of the EPISTOP trial. The "conventional group" of our EEG study included all children with serial video-EEG follow-up, but without preventive treatment. Therefore, the conventional group included infants treated with vigabatrin after seizure onset and children without seizures and without vigabatrin during follow-up.

2.2 | EEG

Video EEG was conducted at inclusion and then every 4 weeks until the age of 6 months, every 6 weeks until the age of 12 months, and every 8 weeks until the age of 24 months. EEG recordings included wake and sleep to at least stage two and had a minimum duration of 1 hour. A minimum of 19

electrodes in the 10–20 position were used. In infants younger than 3 months of corrected age, a reduced array with nine electrodes was allowed (Fp1, Fp2, C3, C4, T4, T3, O1, O2, Cz). EEG assessment was performed using BrainRT software version 3.5 (OSG BVBA, Rumst, Belgium). For this EEG study, the first EEG showing epileptiform discharges was analyzed by three experienced clinical EEG readers at the University of Leuven in Belgium. When there was disconcordance, consensus was reached after discussion between the readers. Epileptiform discharges included both IEDs and electrographic seizures. IEDs included sharp waves, sharp and slow wave complexes, slow sharp waves, spikes, spike and slow wave complexes, polyspikes, slow wave complexes, generalized IEDs, and hypsarrhythmia. IEDs were categorized into focal and multifocal. Electrographic seizures were defined as paroxysmal EEG patterns, with clear distinction from normal background activity, evolving both in frequency, amplitude, and morphology; without clinical correlate; and lasting for at least 10 seconds.[18] Besides the appearance of epileptiform discharges, we also assessed the presence of focal slowing.

2.3 | Outcome measures

The following epilepsy-related outcome measures were included:

- Age at the first electrographic or clinical seizure, whichever occurred first (for those without electrographic seizures on the first EEG with epileptiform discharges)
- Drug-resistant epilepsy for those with complete follow-up[19]

The neurodevelopmental outcomes were studied at the age of 24 months for those with complete follow-up:

- ASD risk based on the Autism Diagnostic Observation Scale 2 (ADOS-2) score or Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) clinical criteria.[20]
- Cognitive, language, and motor developmental quotients (DQs) based on the Bayley Scales of Infant and Toddler Development III (BSID-III).[20]

2.4 | Statistical analysis

Continuous non-normally distributed variables between two independent groups were assessed by the Mann-Whitney *U* test (MWU). The Kendall tau test was used to correlate non-normally distributed continuous variables. Because the EPISTOP trial demonstrated that preventive treatment with vigabatrin successfully delays the onset of seizures and significantly reduces the risk of drug-resistant epilepsy, we analyzed epilepsy-related outcomes separately for the conventional and preventive group. The positive predictive value (PPV), and corresponding negative predictive value (NPV), of IEDs in the prediction of seizures were calculated (Table S2). Prediction, based on EEG characteristics, of the interval between the first appearance of epileptiform discharges and the first seizure was done with Kaplan-Meier survival method with Breslow test, since the assumption of nonproportional hazards, assessed with log-minus-log plots, was not consistently met. The multivariable cox-regression model could not be performed for the same reason. The mean seizure-free interval was calculated and indicates the length of time that a patient can be expected to remain seizure-free.[21] Missing data were censored in the survival analysis. We also assessed subgroups of patients based on EEG characteristics, including focal slowing and the distribution of IED, which had more benefit from preventive antiepileptic treatment in terms of delaying seizure onset than the conventional group. Associations between discrete variables and discrete outcome measures were analyzed using univariable and multivariable logistic regression analyses. Multivariable linear models were used to assess the relation between continuous and discrete variables and the DQs at 24 months. Most important non-EEG characteristics considered in multivariable analyses were the mutation and the treatment strategy (conventional vs preventive).[22] Multicollinearity was assessed for each multivariable regression model. Tolerance values were greater than 0.1 and VIF values were less than 10, indicating no collinearity between the variables. A two-sided *P*-value below .05 was considered statistically significant. Analyses were performed using the Statistical Package for the Social Sciences (SPSS version 26.0 Armonk, NY: IBM Corp).

3 | RESULTS

3.1 | First epileptiform discharges on EEG

For the present EEG study, 83 EPISTOP children were included for whom a full EEG set was available. Nine patients had incomplete follow-up. These nine patients were included in the analysis of age at first seizure but were not included in the assessment of outcomes at 24 months of age (Figure 1). The median age at enrollment was 28 days (IQR 14–54 days). Serial EEG studies never showed epileptiform discharges in four patients, and therefore these four patients could not be included in our prediction models (Table S1). However, one of these four patients developed a seizure during follow-up. The clinical and EEG characteristics of the 79 patients who had epileptiform discharges during follow-up are shown in Table 1. The median age at first epileptiform discharge was 77 days (IQR 23–111). In 34 of 83 patients (41%), the first recorded EEG at inclusion already showed

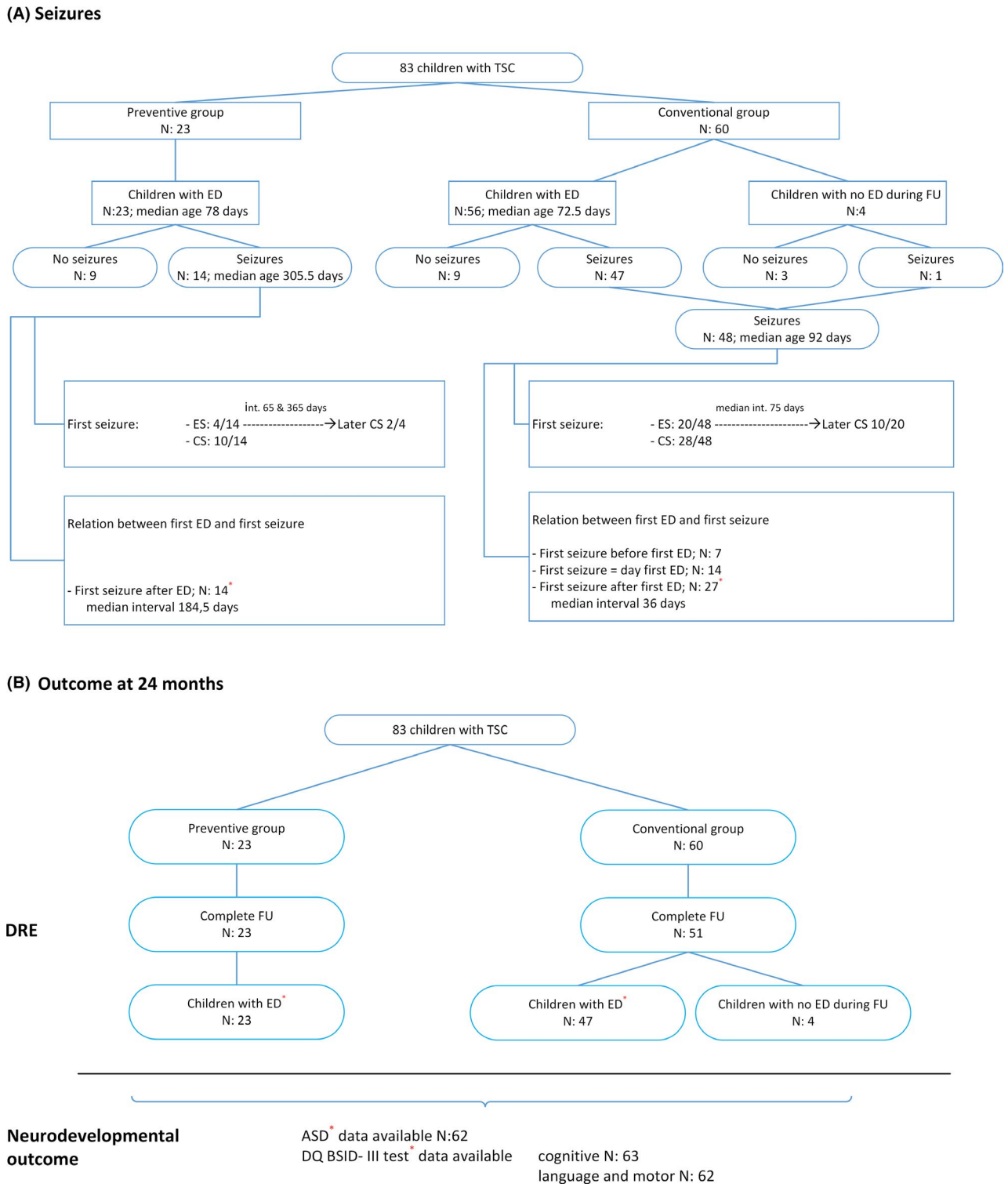


FIGURE 1 Flow-chart showing patients' disposition. A, clinical and electrographic seizure. B, Drug-resistant epilepsy and neurodevelopmental outcome at the age of 24 months. A red star (*) indicates the patients that were included in the prediction model. ASD, autism spectrum disorder risk; BSID-III, Bayley Scales of Infant and Toddler Development III; CS, clinical seizure; DQ, developmental quotient; DRE, drug-resistant epilepsy; ED, epileptiform discharge; ES, electrographic seizure; FU, follow-up; Int., interval; NS, no seizure

epileptiform discharges. Figure 2A shows the cumulative increase in number of patients with epileptiform discharges during the study: At the age of 4 months 78% (65/83) had at least one EEG with epileptiform discharges; at 5 months 83% (69/83); at 6 months 87% (72/83); at 12 months 93% (77/83); and at 18 months 95% (79/83).

TABLE 1 Clinical, EEG, epilepsy, and neurodevelopmental data of children with epileptiform discharges during follow-up

	Patients with epileptiform discharges (*N* = 79)
Baseline	
Median GA at birth in weeks (IQR)	38 0/7 (37 0/7–40 0/7)
Sex	
Male	43 (54%)
Female	36 (46%)
Mutation	
Pathogenic *TSC1* variant	18 (23%)
Pathogenic *TSC2* variant	61 (77%)
Treatment	
Preventive treatment	23 (29%)
Conventional treatment	48 (61%)
No treatment	8 (10%)
EEG characteristics	
Age at first epileptiform discharges	
Median GA in weeks (IQR)	49 5/7 (42 2/7–55 1/7)
Median chronological age in days (IQR)	77 (23–111)
Presence of IED	77 (98%)
Focal IED	27 (34%)
Multifocal IED	50 (63%)
Number of foci	2 (1–3)
Electrographic seizures	12 (15%)
Focal slowing	26 (33%)
Epilepsy outcome	
Clinical or electrographic seizures	61 (77%)
Median age at seizure onset in days (IQR)	108 (57–216)
Drug-resistant epilepsy	35 (50%)[a]
Hypsarrhythmia	3 (4%)°
Epileptic spasms	10 (14%)[a]
Neurodevelopmental outcome	
ASD	19 (31%)[b]
Median cognitive DQ (IQR)	75 (60–90)[c]
Median language DQ (IQR)	66.50 (59–77)[b]
Median motor DQ (IQR)	73.00 (63.75–82.75)[b]

Four children of 83 patients had no epileptiform discharges during follow-up. Of the 79 patients with epileptiform discharges during follow-up, two had electrographic seizures, without IEDs in-between the electrographic seizures on the first EEG with epileptiform abnormalities.

Abbreviations: ASD, autism spectrum disorder; DQ, developmental quotient; GA, gestational age; IED, interictal epileptiform discharges; IQR, interquartile range.

[a]70 patients with follow-up until the age of 24 months; [b]Data available for 62 patients; [c]Data available for 63 patients.

The description of the first epileptiform discharges is shown in Table 1. In 27 of 79 infants (34%), the first epileptiform discharges were focal and in 50 of 79 infants (63%) they were multifocal. Two infants had electrographic seizures without any other IEDs. Infants displaying multifocal IEDs on the first EEG with IEDs, were younger than those with focal IEDs (median age 68 days [IQR 19–105] in 50/77 vs 93 days [IQR 40–157] in 27/77, MWU *P*-value .049). Infants with electrographic seizures on their first EEG with epileptiform discharges were significantly younger than those who did not (median age 25.5 days [IQR 5.25–75.50] vs 85 days [IQR 34–115], MWU *P*-value .034).

Study of the clinical characteristics showed that patients with a pathogenic *TSC2* variant were significantly younger at first EEG with epileptiform discharges than patients with a pathogenic *TSC1* variant (median 68 days [IQR 20–105] in *TSC2* vs 132.5 days [IQR 63.5–282.5] in *TSC1*, MWU *p*-value .009). *TSC2* patients more frequently had multifocal compared to *TSC1* patients (42/61 [69%] in *TSC2* vs 8/18 [44%] in *TSC1*—logistic regression *P*-value .042, odds ratio [OR] 3.1, 95% confidence interval [CI] 1.04–9.2).

3.2 | Epilepsy-related outcome

3.2.1 | Conventional treatment group

Sixty patients (including the four infants with no epileptiform discharges during follow-up) were included in the conventional group (Figure 1A). The median age at first observed epileptiform discharges in the 56 patients with epileptiform discharges was 72.5 days (IQR 19.50–114). Twelve patients (12/60, 20%) did not develop electrographic or clinical seizures during follow-up. Forty-eight patients (48/60, 80%) developed clinical or electrographic seizures at a median age of 92 days (IQR 43.25–159.75; Figure 2B). In 20 of these 48 (42%), the first seizure was an electrographic seizure: subsequently, 10 of these 20 patients (50%), developed clinical seizures at a median interval of 75 days (IQR 51 −355.25; Figure 1A).

In 14 of 48 patients who developed seizures, the first seizure was recognized on the same day as the first EEG with epileptiform discharges. Twenty-seven patients developed clinical or electrographic seizures after the first EEG with epileptiform discharges by a median interval of 36 days (IQR 19–110; Figure 1A). In 7 patients, the first clinical seizure occurred before epileptiform discharges were seen on EEG.

To assess the value of IEDs in the prediction of subsequent seizures, the PPV and NPV were calculated. The PPV of the presence of IEDs in predicting the subsequent development of electrographic or clinical seizures was 75%. The NPV was 12.5% (Table S2).

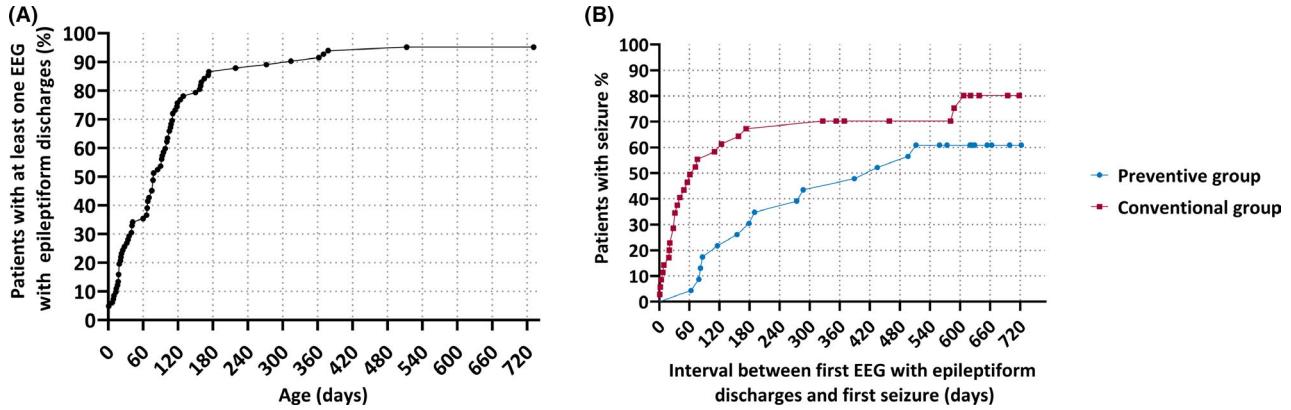
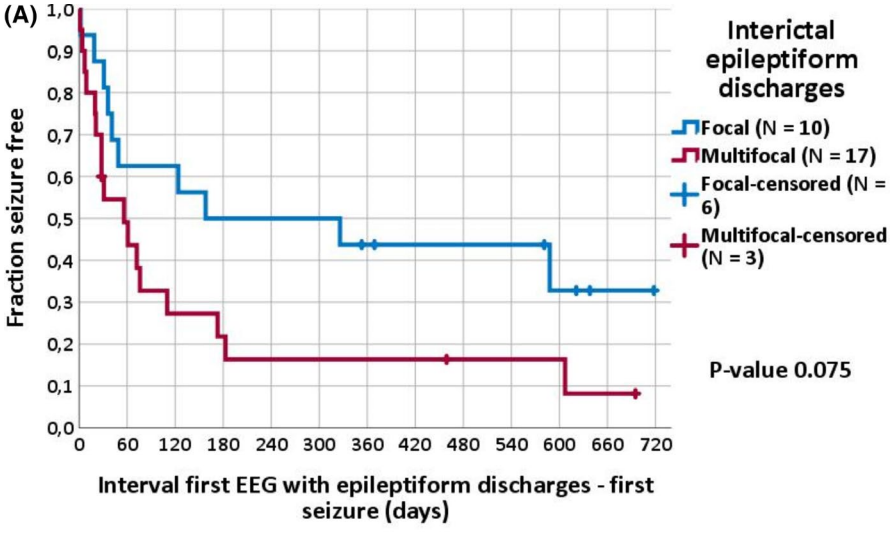


FIGURE 2 A, proportion of children with at least one EEG with epileptiform discharges in relation to the age of the child in days. B, relation of the time in days between the appearance of epileptiform discharges and the development of electrographic or clinical seizures in the preventive and conventional group



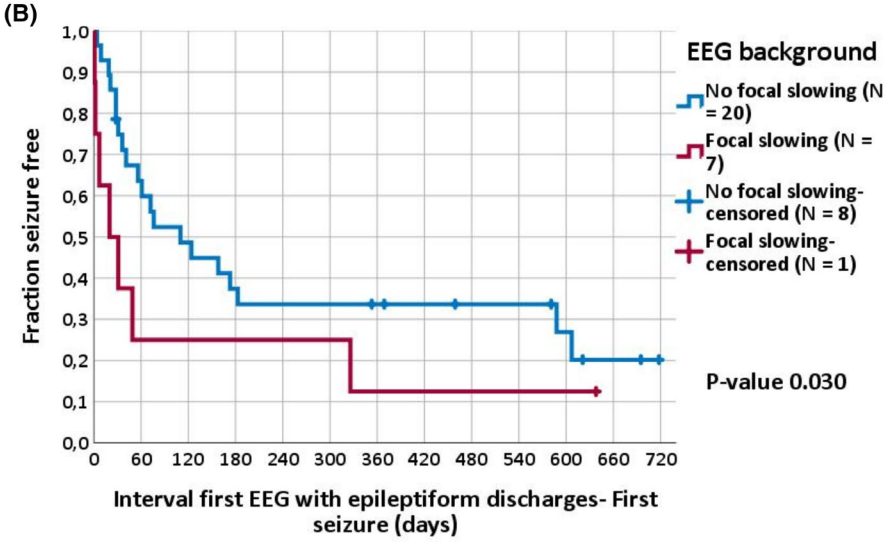


FIGURE 3 Time in days between the appearance of IEDs on EEG and the onset of electrographic or clinical seizures for different EEG characteristics present on this first EEG with epileptiform discharges. *P*-values were obtained with Breslow test. IEDs, interictal epileptiform discharges

We also assessed the predictive value of specific features of the first epileptiform discharges on the subsequent development of clinical or electrographic seizures in the 27 patients who developed seizures after the first EEG with epileptiform discharges. The mean seizure-free interval (153.9 days, 95% CI 51.3–256.7) of patients with multifocal IEDs on the first EEG with epileptiform discharges was shorter compared to the mean seizure-free interval (394 days, 95% CI 97–500.7)

of patients with focal IED. This difference, however, did not reach statistical significance (Breslow test *P*-value .075; Figure 3A). On the other hand, the presence of focal slowing on the first EEG with epileptiform discharges was associated with a significantly shorter mean seizure-free interval (134.3 days, 95% CI 0–283.9)) compared to patients without focal slowing on the first EEG with epileptiform discharges (mean 271.4 days, 95% CI 161.5–381.3, Breslow test *P*-value .030; Figure 3C).

Twenty-eight of 51 patients with complete follow-up to the age of 24 months (55%) developed drug-resistant epilepsy. Children who developed drug-resistant epilepsy were significantly younger at first epileptiform discharges (median 35 days, IQR 11.75–100.25) compared to children without evolution to drug-resistant epilepsy (median 101 days, IQR 36 −173, MWU *P*-value .019).

Children with multifocal IEDs on the first EEG with epileptiform discharges had a higher probability of developing drug-resistant epilepsy compared to children with focal IEDs (72% (21/29) vs 37.5% (6/16), OR 4.4, 95% CI 1.1–16, *P*-value .026 (four children with no epileptiform discharges and two children with electrographic seizures but no IEDs were not included in this analyses). In a multivariable model, the age at first epileptiform discharges (*P*-value .429) and the presence of multifocal IED (*P*-value .058) were no longer significant predictors of drug-resistant epilepsy (Table S3). Focal slowing was not significantly associated with the risk of drug-resistant epilepsy (65% (11/17) vs 57% (17/30) if no focal slowing, OR 1.4, 95% CI (0.4–4.8), *P*-value .590).

3.2.2 | Preventive treatment group

Twenty-three patients completed EPISTOP on preventive treatment. The median age at the first EEG with epileptiform discharges was 78 days (IQR 41–111). Nine patients (39%) did not develop clinical or electrographic seizures during follow-up. In four patients, the first seizure was an electrographic seizure; two of these developed clinical seizures at the age of 65 and 376 days. In 10 patients, the first seizure was a clinical seizure. In the 14 patients who developed electrographic or clinical seizures, the median age at the first seizure was 305.5 days (IQR 188.25–473.50; Figure 2B). The median interval between the first EEG with epileptiform discharges and the first clinical or electrographic seizure was 184.5 days (IQR 85–400.5). Age at first epileptiform discharges, the presence of multifocal IEDs, and focal slowing, were not predictive for the subsequent development of clinical or electrographic seizures.

Seven patients (7/23, 30%) developed drug-resistant epilepsy. None of the features of the first EEG with epileptiform discharges were associated with drug-resistant epilepsy risk (Table S4).

3.2.3 | Patients with multifocal IEDs on the first EEG with epileptiform discharges benefit more from preventive antiepileptic treatment

Of interest, of the 36 children who presented with multifocal IEDs on the first EEG with epileptiform discharges, those who were treated with preventive treatment (n = 16) had a significantly longer mean seizure-free interval (451.8 days, 95% CI 321.4–582.2) compared to those in the conventional group (n = 20; mean seizure-free interval 154 days, 95% CI 51.3–256.7, Breslow test *P*-value <.001; Figure 4A). In contrast, the mean seizure-free interval was not significantly different between the children who first presented with focal IEDs and those who were treated with preventive treatment (n = 7; mean seizure-free interval 353.7 days, 95% CI 184.4–523.1) compared to those with the same presentation and conventional treatment approach (n = 16; mean seizure-free interval 349 days, 95% CI 197.4–500.7, Breslow test *P*-value .527; Figure 4B).

3.3 | Neurodevelopmental outcome

Twenty of 66 patients (30%) who underwent neurodevelopmental assessments were diagnosed with ASD symptoms at the age of 24 months (Tables 1 and S5). We could not identify any EEG variable on the first EEG with epileptiform discharges that could predict ASD risk: neither the age at first epileptiform discharges (*P*-value .284), nor the presence of focal or multifocal IED (*P*-value .406), presence of electrographic seizures (*p*-value .462) or focal slowing (*p*-value .670) was predictive for ASD symptoms at the age of 24 months (Table S5).

On the other hand, a younger age at first epileptiform discharges was significantly associated with lower cognitive (*P*-value .007 Kendall Tau), language (*P*-value .002), and motor (*P*-value .002) DQs on the BSID-III results at 24 months (Figure 5). In a multivariable linear regression model analysis, including the *TSC1*/*TSC2* variant and the treatment group as covariables, age at first epileptiform discharges remained a significant predictor for the cognitive (*P*-value .010), language (*P*-value .001), and motor (*P*-value .013) DQs at 24 months (Table S6). No other variables could predict neurodevelopmental outcome.

4 | DISCUSSION

In this cohort of prospectively followed infants with TSC, we investigated the potential value of early EEG findings in the prediction of epilepsy and neurodevelopmental outcome. Infants with a diagnosis of TSC were followed with regular video-EEG, and in this article we focus on the first EEG

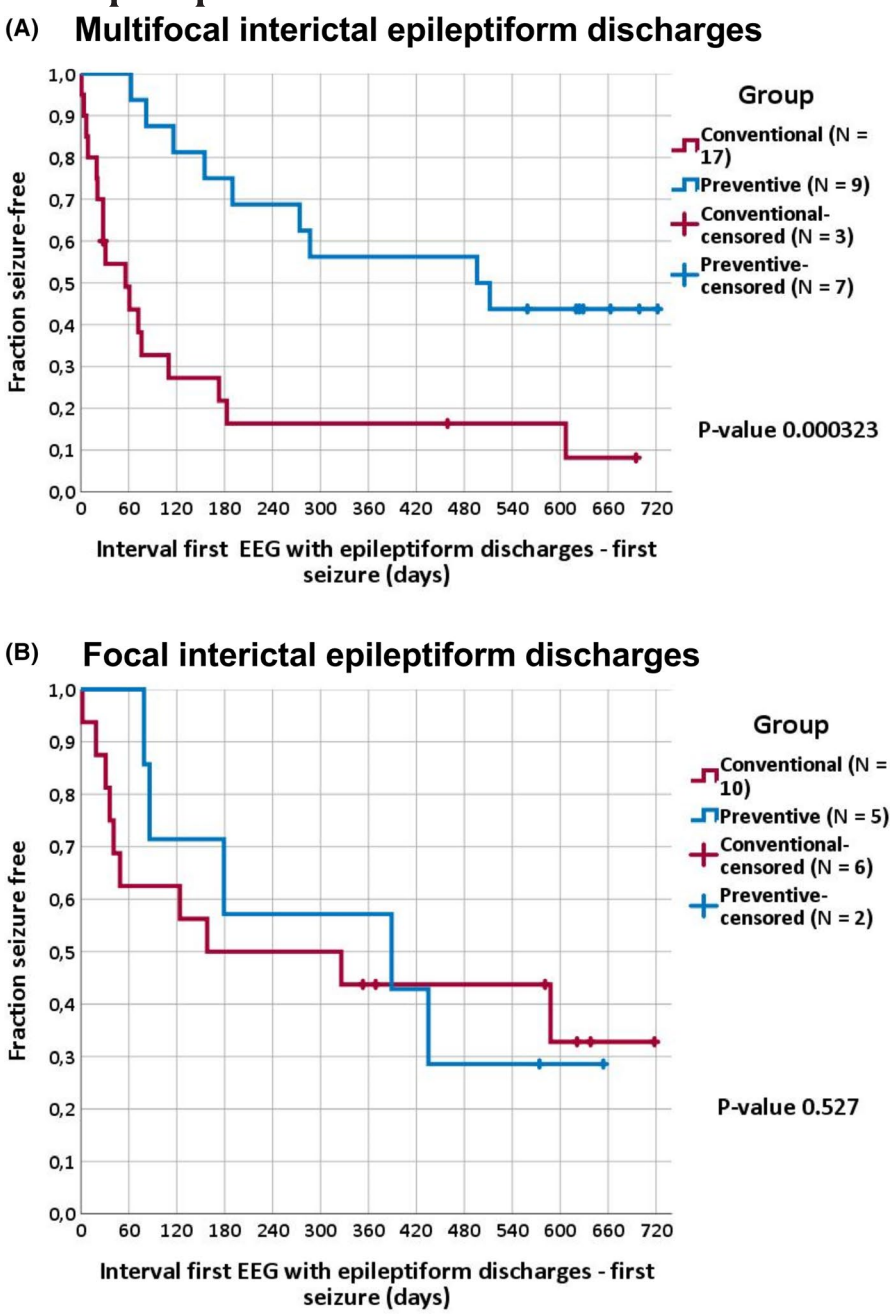


FIGURE 4 Time in days between the appearance of IEDs on EEG and the onset of electrographic or clinical seizures for the conventional and preventive group. A, Presentation with multifocal IEDs. B, Presentation with focal IEDs. *P*-values were obtained with Breslow test. IEDs, interictal epileptiform discharges

showing epileptiform discharges. This cohort is unique because infants were young at inclusion, without preceding seizures or antiepileptic drugs and were prospectively followed. In the subgroup of infants followed and treated in the conventional manner, the appearance of IED predicted seizures in 75%. It is interesting to note that focal slowing predicted an earlier onset of seizures. This finding is not entirely unexpected, as the presence of focal slowing is associated with both structural and functional abnormalities of the underlying brain region.[23] Drug resistance could not be predicted, although younger age at first epileptiform discharges or multifocal IEDs on the first EEG with epileptiform discharges each separately predicted drug resistance. In the preventive treatment group, it is worth noting that no predictive value of early EEG characteristics could be established. However, we could identify a subgroup of infants who benefit more from preventive treatment in delaying seizure onset: those who present with multifocal IED on their first EEG with epileptiform discharges. In these infants, early vigabatrin can modify the epilepsy course.

The age at which the first epileptiform discharges occurred is highly predictive for neurodevelopmental outcome: the earlier, the more severe the cognitive, language, and motor delay. When the first epileptiform discharges appear after the age of 160 days, all children had a cognitive and motor Bayley scale >70 (Figure 5). However, we did not find an association of early epileptiform discharges and ASD at 24 months. Not surprisingly, infants with a pathogenic variant in *TSC2* had

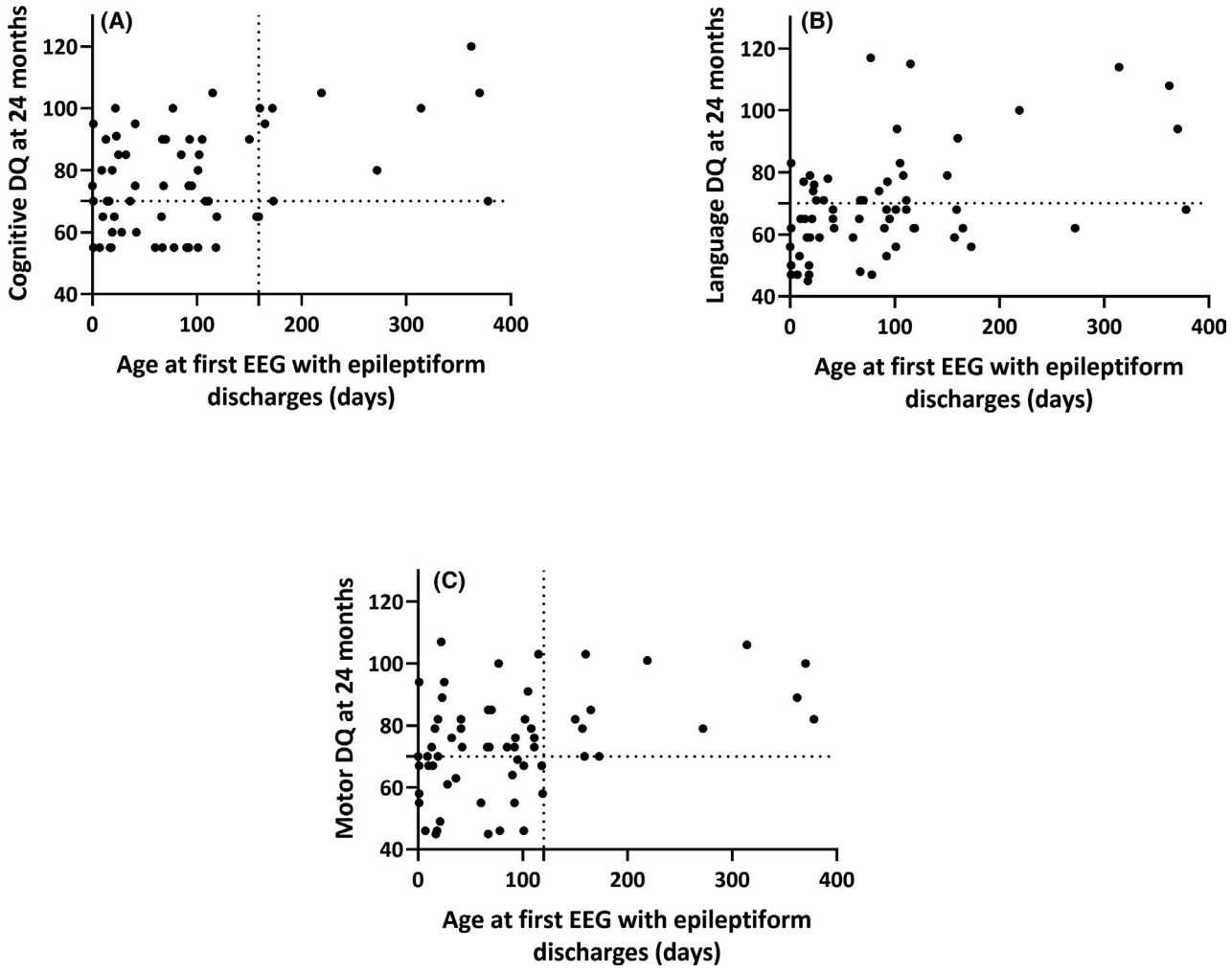


FIGURE 5 Relation between the age at first epileptiform discharges in days and the cognitive (A), language (B), and motor (C) DQ based on Bayley Scales of Infant and Toddler Development III test results at 24 months. A younger age at first epileptiform discharges was significantly associated with lower cognitive (*P*-value 0.007, correlation coefficient 0.243), language (*P*-value .002, correlation coefficient 0.277), and motor (*P*-value .002, correlation coefficient 0.278) DQs on the BSID-III results at 24 months. Horizontal dashed line: DQ of 70. Vertical dashed line: latest first appearance of epileptiform discharges in patients with a DQ <70. DQ, developmental quotients

epileptiform discharges earlier than those with a *TSC1* variant, illustrating the known more severe phenotype in *TSC2*.[24] The main findings of our study have clinical implications, as they can help to identify TSC infants at risk of developing epilepsy and intellectual disability.

In the conventional group, the seizures were not preceded by IED in 21 of the 48 patients (44%) with clinical or electrographic seizures. In the remaining, the median interval between first detectable epileptiform discharges and seizure onset was only 36 days. This suggests that the onset of seizures in TSC can be a rather an abrupt phenomenon. In the prospective serial EEG study of Wu et al., the PPV for the presence of IEDs on EEG preceding the development of clinical seizures by the age of 2 years was 77.3%.[11] Their findings are comparable with the reported PPV of 75% for clinical or electrographic seizure onset in our conventional group. The NPV in the study of Wu et al. was 70%.[11] Absence of IED had a low NPV of 12.5% for no subsequent clinical or electrographic seizures in our conventional group. The interval between IEDs and clinical seizure onset averaged 3.6 months in Wu et al.[11] Despite these similarities, there are specific differences in study designs. First, in our study, the age at onset of electrographic or clinical seizures was the outcome of interest. All detected electrographic seizures were treated in the same way as clinical seizures according to the latest recommendation, namely immediate initiation of vigabatrin.[25] The clinical significance and implications of electrographic seizures are not fully understood. The second difference in study design was the enrollment window. Our patients were younger at enrollment (average age of 1 month) compared with the patient cohort of Wu et al. (average age of 2.7 months). Because both studies excluded patients with previous seizures or antiepileptic drugs, it is possible that our study included patients with a more severe epilepsy

phenotype and a more rapid process of epileptogenesis. Finally, in all age categories, the interval between the EEG recordings was shorter in the EPISTOP study.

Ogórek et al. observed in the EPISTOP cohort that *TSC2* pathogenic variants are associated with a more severe clinical phenotype, including younger age at seizure onset and higher risk of drug-resistant epilepsy and intellectual disability.[24] This finding stresses the important prognostic role of genetic findings in the prediction of neurological comorbidities in TSC. The current study now adds early epileptiform discharges and specific EEG patterns such as focal slowing and multifocal IED to the overall predictive model.

Post factum, one of our limitations is the interval between the EEG studies, which may be too long to capture epileptogenesis that proceeds rapidly, including capturing electrographic seizures preceding clinical seizures. More information about epileptogenesis could be obtained with shorter intervals between EEG recordings. The low sample size in the subgroups could have resulted in a failure to detect a significant relation between the EEG characteristics and the outcome of interest. Moreover, the low sample size impaired the inclusion of interaction terms in the multivariable models. Because this is an exploratory study, no corrections for multiple comparisons were made.[26] Another limitation is that socioeconomic status, parental IQ, ASD features in parents, or parental education were not assessed and therefore not included as potential confounders in the prediction models of neurodevelopmental outcome. Our results were obtained from prospectively studying a cohort of infants identified early in life. Whether our results could be extrapolated to infants and children who are diagnosed with TSC later is focus of future research. More tailored prediction models, combining multiple patient, molecular, EEG, and MRI features, in a larger cohort will result in better predictions of both epilepsy and neurodevelopment, facilitating personalized follow-up and interventions.

5 | CONCLUSION

In a prospectively studied cohort of young infants with TSC, epileptiform discharges were seen on early EEG and often included multifocal IEDs. Early epileptiform discharges were associated with more severe cognitive, language, and motor delay but not with ASD. Infants with multifocal IEDs on first EEG with epileptiform discharges gained more from preventive treatment in terms of delaying seizure onset, than children with focal IED. The presence of focal slowing on the first EEG with epileptiform discharges was predictive of earlier seizure onset. Together with molecular findings, early EEG findings can provide better insight into epileptogenesis and identify infants at higher risk of worse epilepsy and neurodevelopmental outcomes.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article. Additional data are available from the corresponding author, upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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