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Original Article

# Clinical Electroencephalographic Biomarker for Impending Epilepsy in Asymptomatic Tuberous Sclerosis Complex Infants

**Joyce Y. Wu MD [a], Jurriaan M. Peters MD, PhD [b], Monisha Goyal MD [c], Darcy Krueger MD, PhD [d], Mustafa Sahin MD, PhD [b], Hope Northrup MD [e], Kit Sing Au MD [e], Gary Cutter PhD [c], E. Martina Bebin MD, MPA [c,*]**

[a] *Division of Pediatric Neurology, Mattel Children's Hospital at UCLA, Los Angeles, California*
[b] *Department of Neurology, Boston Children's Hospital, Boston, Massachusetts*
[c] *Department of Neurology, University of Alabama Birmingham, Birmingham, Alabama*
[d] *Cincinnati Children's Hospital, Cincinnati, Ohio*
[e] *University of Texas Houston, Houston, Texas*

ABSTRACT

**BACKGROUND:** We assessed the clinical utility of routine electroencephalography (EEG) in the prediction of epilepsy onset in asymptomatic infants with tuberous sclerosis complex. **METHODS:** This multicenter prospective observational study recruited infants younger than 7 months, seizure-free and on no antiepileptic drugs at enrollment, who all underwent serial physical examinations and video EEGs throughout the study. Parental education on seizure recognition was completed at the time of initial enrollment. Once seizure onset occurred, standard of care was applied, and subjects were followed up until 24 months. **RESULTS:** Forty patients were enrolled, 28 older than 12 months with completed EEG evaluation at the time of this interim analysis. Of those, 19 (67.8%) developed seizures. Epileptic spasms occurred in 10 (52.6%), focal seizures in five (26.3%), generalized tonic-clonic seizure in one (5.3%), and a combination of epileptic spasms and focal seizures in three (15.7%). Fourteen infants (73.6%) had the first emergence of epileptiform abnormalities on EEG at an average age 4.2 months, preceding seizure onset by a median of 1.9 months. Hypsarrhythmia or modified hypsarrhythmia was not found in any infant before onset of epileptic spasms. All children with epileptiform discharges subsequently developed epilepsy (100% positive predictive value), and the negative predictive value for not developing epilepsy after a normal EEG was 64%. **CONCLUSIONS:** Serial routine EEGs in infants with tuberous sclerosis complex is a feasible strategy to identify individuals at high risk for epilepsy. The most frequent clinical presentation was epileptic spasms followed by focal seizures, and then a combination of both seizure types.

***Keywords:*** epileptic spasms, EEG, video EEG use in epilepsy, partial seizures

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## Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disease that affects approximately one in 6000 people, represents one of the most common genetic causes of epilepsy,[1-3] and is caused by *TSC1* or *TSC2* mutation. The neurological manifestations in TSC are common and in children represent the most disabling problems of the disease, including epilepsy, intellectual disabilities, psychiatric problems, and autism. Epilepsy is particularly prevalent, affecting about 80% of individuals with TSC[4-6] with over 60% having seizures that are severe and refractory.[4,7,8] Almost half of infants with TSC develop epileptic spasms, which is associated with poor neurological prognosis.[4]

Increasingly TSC is diagnosed at a young age before the onset of epilepsy from non-neurological findings, such as

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* Communications should be addressed to: Dr. Bebin; Department of Neurology; CIRC 312; 1530 3rd Ave S; Birmingham, AL 35294-3280.
*E-mail address:* ebebin@uab.edu


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cardiac rhabdomyomas.[9] The earlier diagnosis of TSC provides a unique opportunity to identify and validate a biomarker for epilepsy. A predictive biomarker would allow earlier intervention that may alter or curtail epileptogenesis and its adverse effects. A recent open-label study suggests that treating patients with TSC with an abnormal electroencephalography (EEG) before onset of epileptic spasms with vigabatrin may improve neurological outcome.[10] An earlier retrospective study reported similar benefit with early treatment.[11] Nonetheless, the use of clinical EEG as a reliable biomarker of epilepsy has not been rigorously validated and has been limited to retrospective analyses subject to referral, recording, and recall biases.[4,12] Our prospective study provides a unique opportunity to document the evolution of epileptogenesis, development of clinical seizures, and the utility of EEG as an early biomarker for epilepsy in TSC.

## Methods

### *Subject recruitment*

Infants with TSC in this multicenter prospective observation study were enrolled from the neonatal nursery, pediatric cardiology, general pediatrics, genetics, pediatric neurology, and obstetrics/perinatology/maternal–fetal medicine clinics. TSC diagnosis was based on clinical features (i.e., cardiac rhabdomyomas, intracranial tubers or subependymal nodules or giant cell astrocytomas, characteristic skin findings, and/or other evidence on prenatal or perinatal cardiac echocardiography, neuroimaging, and skin examinations) or genetic diagnosis.[13]

Each infant with TSC enrolled met all the following inclusion criteria: (1) age <7 months, (2) seizure-free at enrollment, and (3) the genetic or clinical diagnosis for TSC.[13] Infants were excluded if any one of the following criteria was present: (1) age ≥7 months, (2) history of seizures of any type, or (3) current or past treatment with vigabatrin or inhibitors of the mammalian target of rapamycin, before study enrollment. Prematurely born infants with TSC as young as 32 weeks' gestation could participate only if there were no medical complications from prematurity, involving the brain or other major organs, such as hypoxic-ischemic encephalopathy, any intracranial hemorrhage, necrotizing enterocolitis, any respiratory diagnoses requiring ventilator support, or cardiovascular compromise. The earliest time of enrollment for these premature infants was when they reached term (37 weeks' gestation).

Infants with TSC were recruited from the TSC centers at each of the five sites (University of Alabama at Birmingham, University of California at Los Angeles, Boston Children's Hospital, Cincinnati Children's Hospital Medical Center, and University of Texas Medical School at Houston).

### *Study design*

This study was approved by the institutional review boards of all five institutions. Parental consent was obtained for all subjects. At designated time points following enrollment (1.5, 3, 4.5, 6, 9, 12, 18, and 24 months chronological age), physical and neurological examination and a 1-hour research video EEG (to include both sleep and wakefulness) were performed. The 1 hour duration of the video EEG was chosen to maximize capturing both wakefulness and sleep during the same study, yet sufficiently brief to use in and extend to the clinical setting, as well as taking into consideration families' time commitment and staying within funding constraints.

Subjects referred for initial screening and enrollment were seen within 2 weeks. Initial evaluation included physical and neurological examination and baseline video EEG (1 hour wakefulness and sleep). As part of our research protocol, a seizure recognition educational video was shown to the parents or caregivers at the time of enrollment. Enrolled subjects were followed up until age 2 years.

If the infant or child at any point in the study developed seizures, history and additional clinical video EEG(s) of varying duration were completed to confirm epilepsy onset, and antiepileptic drug (AED) treatment was initiated at the managing neurologist's discretion as dictated by individual clinical scenarios, but this clinical information was recorded, as were all medical therapies throughout the duration of the study. The research video EEGs continued at the designated time points stated above, even after clinical seizure onset.

In addition to the scheduled serial research 1-hour video-EEG studies to monitor for the development and evolution of EEG abnormalities, the parent or caregiver maintained a seizure log throughout the study. Once a diagnosis of seizures was made, the subject continued in the study to monitor developmental progress, seizure control, and response to AEDs.

### *Video-EEG acquisition and interpretation*

Video EEGs were uniformly acquired across all five TSC centers, with standard 23 electrodes placed according to the 10-20 international placement system. All video-EEG studies were recorded for 1 hour, incorporating both sleep and wakefulness, at a high sampling rate of 2000 Hz, with a high-frequency and low-pass filter of 500 Hz. All video EEGs from all five sites were anonymized, then uploaded to a secure central server and located and maintained at the University of California at Los Angeles. To view the video EEGs from all five sites, which collectively used three different video-EEG vendors, video-EEG analysis was viewed digitally with Persyst software (San Diego, CA), in the standard timescale of 30 mm/sec and standard filter settings of 1-Hz low-frequency filter (high pass) and 70 Hz high frequency (low pass), along with a 60-Hz notched filter.

To render a more balanced interpretation, each video-EEG study was reviewed by two independent central EEG reviewers (JMP, MG), who are both board-certified pediatric electroencephalographers and blinded to all clinical information except the age of the subject necessary for the EEG interpretation. Differences in the EEG interpretation, when present, were adjudicated by a third, blinded board-certified pediatric electroencephalographer (JYW).

The EEG results were classified based on age-appropriate norms, as either normal or abnormal. EEG abnormalities were evaluated in terms of the presence or absence of background abnormalities, such as generalized or focal slowing, epileptiform discharges (focal, regional, bilateral, or generalized spike or spike and wave discharges), (modified) hypsarrhythmia, voltage attenuation, as well as clinical and/or electrographic seizures, in accordance with the National Institutes of Neurological Disorders and Stroke Common Data Element Tools for Epilepsy.

### *Statistical analysis*

The study design made the following assumptions for statistical sample size. In a population of patients with TSC the study assumed the incidence of epilepsy to be 85% and that the overall frequency of epileptiform discharges is 50%. Based on the statistical analysis of the preliminary data from the University of Alabama Birmingham and the University of California Los Angeles, enrolling 30 patients results in >80% power at a nondirectional alpha of 0.05 assuming that the ratio of patients with normal EEG versus EEG with epileptiform discharges will vary from 0.2 to 0.5. The association between epileptiform discharges and epilepsy was analyzed by multiple methods, performed by a statistician at the University of Alabama Birmingham (GC): (1) time-to-event survival analysis to determine the temporal relationship between epileptiform discharges and seizure onset, (2) multivariable Cox proportional hazard analysis, to assess the contribution of multiple variables to development of epilepsy, and (3) logistical regression analysis to determine the strength of association between epileptiform discharges and epilepsy at the end of the 24-month follow-up period.

## Results

### *Cohort and clinical characteristics*

A total of 40 subjects were prospectively enrolled into the study (Table 1). At the time of the February 1, 2015 data cut-off for this interim analysis, 28 infants were older than

**TABLE 1.**
Summary of Infants With TSC Over Age 12 Months

| Subject | Race | Gender | TSC1/TSC2 | Age Enrolled (months) | Age EEG Abnormal (months) | EEG Abnormality | Age Clinical Seizure Onset (months) | Clinical Seizure Type(s) | Age at Data Cut-off (months) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Caucasian | Male | NMI | 5.4 | | | | | 16 |
| 2 | Caucasian | Male | Not tested | 3.8 | | | | | 15 |
| 3 | Caucasian | Female | Not tested | 2.1 | | | | | 22 |
| 4 | Caucasian | Female | TSC2 | 1.4 | | | | | 18 |
| 5 | Caucasian | Female | TSC1 | 7.2 | | | | | 20 |
| 6 | Caucasian | Male | TSC2 | 2.1 | | | | | 25 |
| 7 | Caucasian | Male | TSC2 | 1.5 | | | | | 24 |
| 8 | Caucasian | Female | TSC1 | 7 | | | | | 27 |
| 9 | Caucasian | Male | TSC1 | 4.4 | | | | | 26 |
| 10 | Caucasian | Female | TSC2 | 2.3 | | | 6 | ES | 13 |
| 11 | Caucasian | Female | TSC1 | 2.6 | | | 5.5 | GTC | 15 |
| 12 | Caucasian | Female | TSC2 | 1.5 | | | 2 | Focal | 14 |
| 13 | African American | Female | TSC2 | 0.4 | | | 11 | Focal | 14 |
| 14 | Caucasian | Female | TSC2 | 1.6 | | | 3.5 | ES | 20 |
| 15 | Caucasian | Male | TSC2 | 0.7 | 4.2 | Focal spikes | 6 | Focal + ES | 15 |
| 16 | Caucasian | Female | TSC1 | 3.3 | 3.4 | Regional spikes | 4 | ES | 12 |
| 17 | Caucasian | Female | TSC2 | 6.5 | 6.5 | Regional spikes | 8 | ES | 17 |
| 18 | Caucasian | Male | TSC2 | 6 | 9 | Regional spikes | 12.5 | ES | 16 |
| 19 | Caucasian | Male | TSC2 | 1.1 | 4 | Regional spikes | 7 | ES | 17 |
| 20 | Caucasian | Female | TSC2 | 4 | 4 | Bilateral spikes | 5.5 | ES | 15 |
| 21 | Caucasian | Male | TSC2 | 3.9 | 4 | Regional spikes | 5 | ES | 18 |
| 22 | Caucasian | Male | TSC2 | 1.1 | 6 | Bilateral spikes | 6.2 | ES | 22 |
| 23 | Asian | Male | TSC2 | 1.4 | 1.5 | Focal spikes | 3.5 | Focal | 21 |
| 24 | Caucasian | Female | TSC2 | 2.5 | 4 | Bilateral spikes | 6 | Focal + ES | 20 |
| 25 | American Indian | Male | TSC2 | 1.5 | 1.6 | Bilateral spikes | 3.5 | Focal | 20 |
| 26 | Hispanic | Female | TSC2 | 6 | 6 | Focal spikes | 20 | Focal | 29 |
| 27 | Caucasian | Male | TSC2 | 4.2 | 4.2 | Bilateral spikes | 6 | ES | 28 |
| 28 | Caucasian | Male | TSC2 | 1.1 | 1.2 | Bilateral spikes | 6 | ES + Focal | 25 |

Abbreviations:
EEG = Electroencephalogram
ES = Epileptic spasms
GTC = Generalized tonic clonic seizure
TSC = Tuberous sclerosis complex

1 year and were included in the current analysis (Fig 1). Three additional subjects older than 12 months were excluded since most recent EEG analysis by the blinded reviewers was not completed at the time of cut-off. Of the remaining subjects, three had either electrographic seizures or epileptiform discharges on EEG and were excluded because they were treated with AEDs before the onset of clinical seizures. These subjects could not be included in the statistical calculation for positive and negative predictive values because they had received a therapeutic intervention. The remaining six subjects were excluded from this interim analysis because they were still younger than 12 months and therefore needed longer follow-up time.

Gender was distributed evenly among the 28 infants included for the interim analysis, and the average age at time of enrollment was 2.7 ± 2.0 months. Of the 28 subjects enrolled, 26 underwent TSC genetic testing, 20 (76.9%) had a pathologic mutation in *TSC2*. Mutations in *TSC1* were identified in five (19.2%). In 1 (3.8%), no mutation in either *TSC1* or *TSC2* was found.

In this cohort of 28 infants older than 12 months, 19 (67.9%) developed clinical seizures during the observation period. The average age at time of seizure onset was 6.7 ± 4.1 months, the youngest within age 2.0 months and the oldest at age 20 months. Epileptic spasms were the most common seizure type and occurred in 10 infants (52.6%). Focal seizures occurred either as the sole seizure type (five subjects, 26.3%) or with epileptic spasms (three subjects, 15.8%). In contrast, seizures with generalized onset were rare, with generalized tonic-clonic seizures occurring only in one (5.3%) subject, and no other clinical seizure types were reported (Table 2).

Of these EEG interictal and ictal findings, the presence of epileptiform discharges preceded the onset of first clinical seizure in 14 of 19 infants (73.7%), which occurred between ages 1.2 and 9.0 months (Table 2). The interval between the first EEG with epileptiform discharges and the first clinical seizure was an average of 2.8 ± 3.4 months, median of 1.9 months (interquartile range 1.5, 3.0 months). No epileptiform discharges were detected with any of the video EEGs in five subjects (26.3%) before the onset of clinical seizures. Seizure type in this latter group included focal seizures, epileptic spasms, and generalized tonic-clonic seizure (Fig 2).

The remaining nine infants of the cohort of 28 have remained seizure-free, on no AED, at the time of this interval analysis, and all nine infants have had normal video EEGs, without the presence of epileptiform discharges on any of the video EEGs (Table 3).

The positive predictive value (PPV), or how often the presence of a biomarker can correctly predict the disease in a population, can be determined from the ratio of true positives (those subjects with both abnormal EEG and subsequent seizures) to the sum of true positives and false

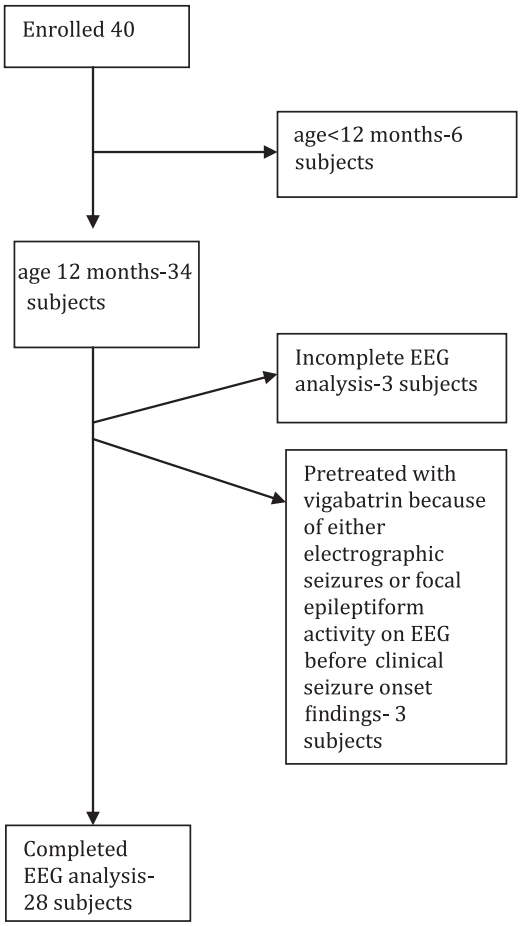


**FIGURE 1.**
Cohort of study participants with tuberous sclerosis complex. EEG, electroencephalography.

**TABLE 2.**
Clinical Seizure Semiology and EEG Characteristics

| Seizure characteristics in 19 subjects who have had a clinical seizure since enrollment, by group: | | |
|---|---|---|
| Seizure Type | Epileptiform Discharges (n = 14) | Normal EEG (n = 5) |
| Focal seizures | 3 | 2 |
| Epileptic spasms | 8 | 2 |
| Focal seizures + epileptic spasms | 3 | 0 |
| Generalized seizures | 0 | 1 |
| EEG characteristics in 19 subjects who have had a clinical seizure since enrollment: | | |
| Fourteen of 19 (73.6%) had epileptiform activity detected on EEG before the onset of clinical seizures | | |
| | Average (months) | Median (months) |
| Age at time of first epileptiform discharges | 4.2 (S.D. = 2.1) | 4.0 |
| Age at time of first clinical seizure | 6.7 (S.D. = 4.1) | 6.0 |
| Time interval between epileptiform discharges and seizure | 2.8 (S.D. = 3.4) | 1.9 |
| Five of 19 (26.3%) had no epileptiform activity detected on EEG before the onset of clinical seizures | | |
| Subject ID | Last Normal EEG (months) | Clinical Seizure Onset (months) |
| 10 | 4 | 6 |
| 11 | 4 | 5.5 |
| 12 | 1.5 | 2 |
| 13 | 9 | 11 |
| 14 | 1.5 | 3.5 |

Abbreviation:
EEG = Electroencephalography

positives (those with abnormal EEG and no subsequent seizures). The corresponding PPV (Table 3) for the presence of epileptiform activity on an EEG preceding the development of clinical seizure onset in infants with TSC is then 14/(14 + 0), or 100% CI (76.8%, 100.0%).

Similarly, the negative predictive value (NPV), or how often the absence of a biomarker can correctly predict the nondisease state in a population, is determined from the ratio of true negatives (subjects with both normal EEG and no seizures) to the sum of true negatives and false negatives (subjects with normal EEG but subsequently developing seizures). The corresponding NPV (Table 3) of the absence of epileptiform activity on the EEG and no subsequent epilepsy in infants with TSC is then 9/(9 + 5), or 64.3% CI (35.1%, 87.2%).

The other EEG findings of focal or generalized slowing, attenuation, hypsarrhythmia or modified hypsarrhythmia, and ictal events did not consistently precede the onset of clinical seizures, and none reached statistical significance.

## Discussion

This prospective multicenter observational study provides unique insight into the development of epilepsy in TSC and the clinical utility of serial EEGs in the identification of patients at high risk for impending seizure onset. This preliminary report is driven by compelling strength of interim analysis and provides early supportive data for risk stratification in planned prospective research on effects of pre-emptive antiepileptic treatment in TSC.

The risk for epilepsy in TSC has been previously estimated to be 80%, based on retrospective studies in patients of all ages, including older children and adults.[4,14,15] Determining prevalence in infants is more difficult as not all the studies separated their analyses into younger cohorts. Jozwiak et al.[14] reported seizures present by age 2 years in 83%, whereas Chu-Shore et al.[4] reported 63% by age 1 year and 82% by age 3 years. Using an observational study design in which infants were enrolled before seizure onset, and followed up prospectively, we calculated in this study the incidence of infants with TSC developing clinical seizures before age 2 years as 67.8 %. Although our approach overcomes recall and reporting bias inherent to retrospective studies, the estimate in this prospective study may yet underestimate the true incidence, as not all subjects have been observed past age 24 months. Continuing to follow our present cohort prospectively will allow more definitive calculation of annual and cumulative incidence of epilepsy for infants with TSC, by age, throughout infancy and childhood.

Only one prior published study has evaluated EEG findings in infants with TSC before the onset of seizures, consisting of five subjects.[16] Patients were enrolled from age

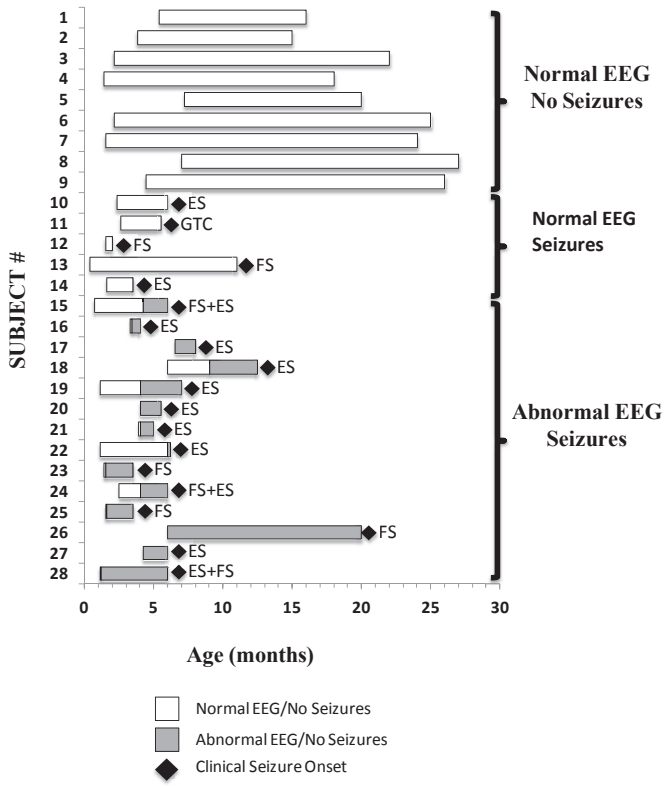


**FIGURE 2.**
Summary of electroencephalography (EEG) in relation to clinical seizure onset. ES, epileptic spasms; FS, focal seizures.

9 days to 9 weeks and had serial EEGs at 4-week intervals. EEG abnormalities were detected in four subjects between ages 0.5 and 5.0 months, all of whom (100%) subsequently developed seizures within 1-8 days of the first abnormal EEG. The remaining subject with a normal EEG never developed clinical seizures. In this study, we found a similar high correlation between epileptiform discharges and subsequent seizures, with the average age when epileptiform discharges were first detected as 4.2 months. However, sensitivity was notably lower (73.7%) and with a much longer time interval between epileptiform discharges and clinical seizure onset that averaged 2.8 months and median of 1.9 months. Our study involved multiple centers, a larger cohort than previous studies, and multiple blinded EEG readers, adding additional patient diversity, power, and scientific rigor to the calculated lower limit of sensitivity. An unresolved variable that could contribute to the differences observed is the frequency of surveillance EEGs in the asymptomatic cohort. The every 6 weeks EEG at earlier time points in our study was chosen to balance study sensitivity with clinical feasibility for participating families. This longer time interval between scheduled EEGs, especially at later time points when expanded to 3-6–month intervals, may also explain the relatively high false-negative rate observed (and corresponding calculated NPV) in our study. The longer interval between scheduled EEGs increases the likelihood that newly emerging epileptiform abnormalities before the onset of clinical seizures may have gone undetected in the interim. However, the calculated time between epileptiform activity and seizures was an average 2.8 months, median 1.9 months. We suspect that with more frequent sampling, the interval time, here measured only in monthly increments, would likely be shorter with the possibility of showing higher sensitivity and lower NPV. A sizeable window between epileptiform discharges and clinical seizure onset is key, as such a window provides a unique and feasible opportunity to design and implement anti-epileptogenic treatment strategies that may delay or prevent clinical seizure onset.

**TABLE 3.**
Statistical Analysis Summary

| | Clinical seizure | No Clinical Seizure |
|---|---|---|
| Epileptiform discharges | 14 | 0 |
| No epileptiform discharges/Normal electroencephalography | 5 | 9 |

| Sensitivity (%) | Specificity (%) | Positive Predictive Value (%) | Negative Predictive Value (%) |
|---|---|---|---|
| 73.7 | 100 | 100 | 64 |

In our study infants with TSC are as likely to present with focal seizures, epileptic spasms, or focal seizures mixed with epileptic spasms (either concurrently or subsequently to onset of focal seizures). Furthermore, similar to results of the Domańska-Pakieła et al. study,[16] classic or modified hypsarrhythmia was not found in any infant before the onset of focal seizures or epileptic spasms. This would suggest that classic or modified hypsarrhythmia, reported to occur in up to 71% of patients with TSC and clinical epileptic spasms,[17] occurs after seizure onset and corresponds to later events in the epileptogenesis process. These observations on the evolution of epilepsy onset in infants with TSC have an important impact on clinical management, as treatment delay may adversely affect long-term epilepsy and developmental outcome.[11,17] First, parents and clinicians should know that either focal-onset seizures or epileptic spasms may be an initial seizure manifestation in infants with TSC. Second, because hypsarrhythmia may follow epileptic spasms, clinicians should not wait for hypsarrhythmia, either in the classic or modified form, to appear on EEG before initiating appropriate treatment for epileptic spasms. Finally, the earliest signs of seizures, whether focal seizures, epileptic spasms, or a mix thereof, may be very subtle and could go unrecognized or misdiagnosed without a high index of clinical suspicion on the part of parents and clinicians. We found it very useful to show videos of multiple clinical seizure types, both classic and subtle forms, to parents to increase their likelihood of recognizing and reporting to clinicians the earliest clinical events of concern. We also encouraged parents and caregivers to send video files obtained with mobile phones for clinician review and confirmation.

Although an EEG is currently recommended at the time of initial TSC diagnosis,[11,13,18] the results of this study not only support the importance of that initial EEG but also the importance of subsequent EEGs in monitoring the development of seizures and epileptiform discharges. This recommendation is consistent with the European recommendation, which suggested close EEG monitoring in the first few months of life and consideration of preventative treatment in the presence of EEG ictal discharges.[19]

In conclusion, this study is the first multicenter prospective study to evaluate serial EEGs as a biomarker for subsequent epilepsy in the infant population with TSC. Our study demonstrates the feasibility and importance of close EEG surveillance in infants with TSC, with high PPV of epileptiform discharges for predicting those who subsequently develop epilepsy. This interim analysis highlights the value of early diagnosis of infants with TSC and the value of serial EEG beginning at the time of diagnosis. Importantly, our study suggests there is a critical window of time between emergence of epileptiform discharges and clinical seizure onset, which provides a unique opportunity to investigate potentially disease-modifying antiepileptogenic treatment strategies in this population.

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## References

1. Sparagana SP, Roach ES. Tuberous sclerosis complex. *Curr Opin Neurol*. 2000;13:115-119.
2. Kwiatkowski DJ. Rhebbing up mTOR: new insights on TSC1 and TSC2, and the pathogenesis of tuberous sclerosis. *Cancer Biol Ther*. 2003;2:471-476.
3. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med*. 2006;355:1345-1356.
4. Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia*. 2010;51:1236-1241.
5. Webb DW, Fryer AE, Osborne JP. On the incidence of fits and mental retardation in tuberous sclerosis. *J Med Genet*. 1991;28:395-397.
6. Sparagana SP, Delgado MR, Batchelor LL, Roach ES. Seizure remission and antiepileptic drug discontinuation in children with tuberous sclerosis complex. *Arch Neurol*. 2003;60:1286-1289.
7. Curatolo P. Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complex-associated epilepsy. *Pediatr Neurol*. 2015;52:281-289.
8. Aboian MS, Wong-Kisiel LC, Rank M, Wetjen NM, Wirrell EC, Witte RJ. SISCOM in children with tuberous sclerosis complex-related epilepsy. *Pediatr Neurol*. 2011;45:83-88.
9. Datta AN, Hahn CD, Sahin M. Clinical presentation and diagnosis of tuberous sclerosis complex in infancy. *J Child Neurol*. 2008;23:268-273.
10. Jozwiak S, Kotulska K, Domanska-Pakiela D, et al. Antiepileptic treatment before the onset of seizures reduces epilepsy severity and risk of mental retardation in infants with tuberous sclerosis complex. *Eur J Paediatr Neurol*. 2011;15:424-431.
11. Bombardieri R, Pinci M, Moavero R, Cerminara C, Curatolo P. Early control of seizures improves long-term outcome in children with tuberous sclerosis complex. *Eur J Paediatr Neurol*. 2010;14:146-149.
12. Jozwiak S, Goodman M, Lamm SH. Poor mental development in patients with tuberous sclerosis complex: clinical risk factors. *Arch Neurol*. 1998;55:379-384.
13. Northrup H, Krueger D, International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol*. 2013;49:243-254.
14. Jozwiak S, Schwartz RA, Janniger CK, Bielicka-Cymerman J. Usefulness of diagnostic criteria of tuberous sclerosis complex in pediatric patients. *J Child Neurol*. 2000;15:652-659.
15. Devlin LA, Shepherd CH, Crawford H, Morrison PJ. Tuberous sclerosis complex: clinical features, diagnosis, and prevalence within Northern Ireland. *Dev Med Child Neurol*. 2006;48:495-499.
16. Domańska-Pakieła D, Kaczorowska M, Jurkiewicz E, Kotulska K, Dunin-Wąsowicz D, Jóźwiak S. EEG abnormalities preceding the epilepsy onset in tuberous sclerosis complex patients - a prospective study in 5 patients. *Eur J Paediatr Neurol*. 2014;18:456-468.
17. Muzykewicz DA, Costello DJ, Halpern EF, Thiele EA. Infantile spasms in tuberous sclerosis complex: prognostic utility of EEG. *Epilepsia*. 2009;50:290-296.
18. Krueger D, Northrup H, on behalf of the International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex surveillance and management: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol*. 2013;49:255-265.
19. Curatolo P, Jóźwiak S, Nabbout R, TSC Consensus Meeting for SEGA and Epilepsy Management. Management of epilepsy associated with tuberous sclerosis complex (TSC): clinical recommendations. *Eur J Paediatr Neurol*. 2012;16:582-586.