Received: 20 June 2019 | Revised: 7 October 2019 | Accepted: 11 October 2019

DOI: 10.1111/epi.16379

FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Scalp EEG spikes predict impending epilepsy in TSC infants: A longitudinal observational study

Joyce Y. Wu[1] | **Monisha Goyal**[2] | **Jurriaan M. Peters**[3] | **Darcy Krueger**[4] | **Mustafa Sahin**[3] | **Hope Northrup**[5] | **Kit S. Au**[5] | **Sarah O'Kelley**[2] | **Marian Williams**[6] | **Deborah A. Pearson**[5] | **Ellen Hanson**[3] | **Anna W. Byars**[4] | **Jessica Krefting**[2] | **Mark Beasley**[2] | **Gary Cutter**[2] | **Nita Limdi**[2] | **E. Martina Bebin**[2]

[1]Division of Pediatric Neurology, UCLA Mattel Children's Hospital, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California

[2]University of Alabama Birmingham, Birmingham, Alabama

[3]Department of Neurology, Boston Children's Hospital, Boston, Massachusetts

[4]Cincinnati Children's Hospital, Cincinnati, Ohio

[5]University of Texas Health Science Center Houston, Houston, Texas

[6]Children's Hospital Los Angeles, Los Angeles, California

Correspondence
E. Martina Bebin, Department of Neurology CIRC 312, University of Alabama Birmingham, 1720 2nd Ave S, Birmingham, AL 35294-0021.
Email: ebebin@uabmc.edu

Funding information
National Institute of Neurological Diseases and Stroke, Grant/Award Number: P20 NS 080199 , U01 NS 082320 and R01 NS 82649; National Center for Advancing Translational Sciences of the National Institutes of Health grant, Grant/Award Number: UL1TR001881 and 8UL1TR000077; National Institute of Child Health and Development, Grant/Award Number: U54HD090255

Abstract

Objective: To determine if routine electroencephalography (EEG) in seizure-naive infants with tuberous sclerosis complex (TSC) can predict epilepsy and subsequent neurocognitive outcomes.

Methods: Forty infants 7 months of age or younger and meeting the genetic or clinical diagnostic criteria for tuberous sclerosis were enrolled. Exclusion criteria included prior history of seizures or treatment with antiseizure medications. At each visit, seizure history and 1-hour awake and asleep video-EEG, standardized across all sites, were obtained until 2 years of age. Developmental assessments (Mullen and Vineland-II) were completed at 6, 12, and 24 months of age.

Results: Of 40 infants enrolled (mean age of 82.4 days), 32 completed the study. Two were lost to follow-up and six were treated with antiepileptic drugs (AEDs) due to electrographic seizures and/or interictal epileptiform discharges (IEDs) on their EEG studies prior to the onset of clinical seizures. Seventeen of the 32 remaining children developed epilepsy at a mean age of 7.5 months (standard deviation [SD] = 4.4). Generalized/focal slowing, hypsarrhythmia, and generalized/focal attenuation were not predictive for the development of clinical seizures. Presence of IEDs had a 77.3% positive predictive value and absence a 70% negative predictive value for developing seizures by 2 years of age. IEDs preceded clinical seizure onset by 3.6 months (mean). Developmental testing showed significant decline, only in infants with ongoing seizures, but not infants who never developed seizures or whose seizures came under control.

Significance: IEDs identify impending epilepsy in the majority (77%) of seizure-naive infants with TSC. The use of a 1-hour awake and asleep EEG can be used as a biomarker for ongoing epileptogenesis in most, but not all, infants with TSC. Persistent seizures, but not history of interictal epileptiform activity or history of well-controlled seizures, correlated with low scores on the Vineland and Mullen tests at 2 years of age.

KEYWORDS
biomarker, epileptiform discharges, seizure outcome, tuberous sclerosis complex

1 | INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder that affects approximately one in 6000 individuals due to mutations in the *TSC1* or *TSC2* genes.[1–3] Epilepsy affects about 80% of individuals with TSC,[4] mostly starting in the first 2 years of life. Approximately 65% of those with epilepsy have medically refractory epilepsy, which increases the likelihood of comorbid developmental delay and autism.[4]

Increasingly, TSC is diagnosed at a young age before the onset of epilepsy from non-neurologic findings, such as cardiac rhabdomyomas.[5] Thus, TSC is an ideal disease model for prospectively studying epileptogenesis, well before the first clinical seizure onset. This concept provides an opportunity to implement potential antiepileptogenic therapy in infants to prevent epilepsy, and potentially positively influence developmental outcomes.

In a smaller open-label study, vigabatrin initiation upon electroencephalography (EEG) abnormalities, but prior to the onset of epilepsy, improved eventual epilepsy and developmental outcomes in children with TSC, as compared to the standard of care of treating seizures when they occur.[6] We previously described a subset of the patients in this study and showed feasibility of enrolling TSC infants prior to epilepsy onset and the use of serial EEG as a feasible strategy to identify TSC infants at risk for epilepsy in those monitored at minimum until 12 months of age.[7] Here we describe the cohort's clinical and developmental outcomes at 24 months of age.

Key Points

- Presence of interictal epileptiform discharges had the greatest predictive value in determining risk for developing seizures in infants with tuberous sclerosis complex TSC
- Routine awake and sleep video–electroencephalography (EEG) can be used as a biomarker for ongoing epileptogenesis in the majority of infants with TSC
- This study demonstrates that the decline in developmental outcome in infants with TSC is clearly linked to the persistence of seizures

2 | MATERIALS AND METHODS

2.1 | Study design and participant recruitment

This longitudinal cohort study enrolled participants across five TSC centers: University of Alabama at Birmingham, University of California Los Angeles, Boston Children's Hospital, Cincinnati Children's Hospital Medical Center, and University of Texas Health Science Center at Houston. All five sites recruited from their respective TSC clinics, with each site's principal investigator also being the TSC clinic director for that site. Each site's principal investigator reached out to his/her local and regional networks of physicians, including geneticists, pediatric cardiologists, and maternal fetal medicine specialists. In addition, the Tuberous Sclerosis Alliance helped recruit nationally by advertising the study. Enrollment goal was 40 infants, and inclusionary/exclusionary criteria as well as visit time points and testing modalities are summarized in Table 1. Participants referred for this initial screening and enrollment were seen within 2 weeks. As part of our research protocol, to help parents identify seizures, a seizure recognition educational video was shown to the parents at the time of enrollment. Enrolled participants were followed until the age of 24 months. Linear mixed models were used to evaluate longitudinal outcomes. These models included categorizations of participants repeated measures (ie, 6, 12, 24 months), and their interactions as fixed effects with no other covariates included.

The study protocol was approved by the institutional review board at each site, with direction from the leading administrative site at the University of Alabama at Birmingham. Written informed consent was obtained from the parents or legal guardians of all participants. The trial was conducted in accordance with Good Clinical Practice guidelines. Data from each study site were entered into a web-based, distributed data-management system meeting HIPAA privacy regulations.

2.2 | Video-EEG recording and interpretation

A 1-hour awake and asleep video-EEG acquisition protocol was standardized across all five sites, with 2000 Hz sampling rate, 500 Hz high-frequency filter, no low-frequency filter (although default low-frequency filter settings ranged between 0 and 0.05 Hz among the various EEG manufacturers), and 24 electrodes including ground and reference. EEG studies were performed at the baseline enrollment study visit and then every 6 weeks until the participant was 6 months of age, then every 3 months until 12 months of age, and then every 6 months until 24 months of age. Each

TABLE 1 Study protocol and design

Study inclusion criteria (meet all 3)	Study exclusion criteria (meet any 1)
1. Meets genetic or clinical diagnostic criteria for TSC1/2 citation 2. 7 mo of age or younger at the time of enrollment 3. Seizure-free at the time of enrollment	1. Gestational age <30 wk at time of delivery 2. Taking vigabatrin or mammalian target of rapamycin (mTOR) inhibitor prior to or at time of enrollment 3. History of central nervous system infection, hypoxic 4. Ischemic encephalopathy, intraventricular hemorrhage, history of clinical seizures, including infantile

	Age in months								
Tests	**Birth**	**1.5**	**3**	**4.5**	**6**	**9**	**12**	**18**	**24**
History/exam[a]	X	x	x	X	x	X	x	x	x
Video-EEG[b]	X	x	x	X	x	X	x	x	x
Developmental Testing[c]					x		x		x

[a]First visit: demographics, medical history including seizures, medications, family history, physical/neurologic exam; at subsequent visits: seizure types and frequency, interval medical history, medications, physical/neurologic exam.
[b]Standardized protocol across all five sites, 1-h awake and asleep video-EEG, 2000 Hz sampling rate.
[c]Mullen and Vineland II.

video-EEG was interpreted locally at each site to ensure that families were notified of all ictal events in a timely manner. Each EEG was deidentified and uploaded to a secure server at the UCLA site, and independently interpreted by two board-certified pediatric electroencephalographers (M.G. and J.M.P.) with the National Institutes of Health/National Institute of Neurological Disorders and Stroke (NIH/NINDS) Scalp EEG Common Data Elements form. Should any of the five major categories differ between the two central readers—namely, the presence or absence of interictal epileptiform discharges (IEDs), hypsarrhythmia, ictal events, generalized or focal slowing, and generalized or focal attenuation—a third board-certified pediatric electroencephalographer (J.Y.W.) adjudicated the item(s) in dispute. Except for age, necessary for appropriate pediatric EEG interpretation, all three readers were blinded to the participant's clinical history, including epilepsy onset, seizure type, and anticonvulsant treatment. The consensus between the two central readers, or the adjudicated results, was then the final EEG interpretation for analysis.

If the infant at any point in the study developed clinical or electrographic seizures, additional medical history and EEG or video-EEG of varying duration were completed for clinical purposes at the discretion of the treating neurologist, as well as the choice and dosing of anticonvulsant drug initiation. This clinical information was recorded, as were all medical therapies throughout the duration of the study. The research video-EEG studies continued to be collected at the designated time points as outlined in the protocol, even after clinical seizure onset.

2.3 | Developmental testing

Developmental assessments with Mullen Scales of Early Learning[8] and Vineland Adaptive Behavior Scales, Second Edition (Vineland-II)[9] were obtained at 6, 12, and 24 months by research-certified pediatric psychologists at each of the five participating sites. The Mullen Scales of Early Learning, a well-validated and widely used measure to assess developmental status in infants and preschoolers was chosen to assess overall development. The Mullen includes scales measuring fine and gross motor skills, expressive and receptive language skills, and visual reception. The Vineland-II was used to evaluate each child for the presence of adaptive functional delays, that is, delays in everyday living skills in the areas of communication, daily living skills, socialization, and fine and gross motor function.

2.4 | Data analysis

Categorical outcomes, which were summarized by percentage and between-group differences, were evaluated with chi-square tests. Continuous outcomes were summarized by means, medians, standard deviations, and ranges; between-group differences were evaluation with analysis of variance (ANOVA) models. Linear mixed models were used to evaluate longitudinal outcomes. All analyses were performed in SAS version 9.4.

2.5 | Data availability

The deidentified data for this study are retained with the Data Coordinating Center at the University of Alabama at Birmingham. The data as well as the study protocol and statistical analysis will be shared upon request. A formal request through the TSC-Clinical Research Consortium (TSC-CRC) is required to access data, and a formal project proposal should be submitted before the data will be released. The primary contact for data access is through the TSC-CRC project manager at Boston Children's Hospital.

TABLE 2 Demographics per participant

Participant	Race[a]	Gender[b]	Genetic testing[c,d]	Age enrolled (mo)	Age first EEG abnormal (mo)	Type of EEG spike	Age at clinical Sz onset (mo)	Clinical Sz type[c,d]
1	C	M	*TSC1*	4.6	NA	NA	NA	NA
2	C	F	*TSC1*	7.1	NA	NA	NA	NA
3	C	M	*TSC2*	1.4	9.6	Focal	NA	NA
4	C	M	*TSC2*	2.1	NA	NA	NA	NA
5	C	F	*TSC1*	7.3	NA	NA	NA	NA
6	C	M	*TSC1*	0.4	NA	NA	NA	NA
7[e]	C	F	*TSC2*	2.7	2.7	Regional	NA	NA
8	C	M	ND	2.1	NA	NA	NA	NA
9	C	M	*TSC2*	3.8	18.6	Regional	NA	NA
10	C	F	*TSC2*	1.7	NA	NA	NA	NA
11	C	M	ND	3.9	18.2	Regional	NA	NA
12	C	M	NMI	5.6	24.7	NA	NA	NA
13	C	M	*TSC1*	0.8	0.8	Bilateral	NA	NA
14	AA	F	*TSC2*	0.6	NA	Focal	11.1	Focal
15	C	F	*TSC2*	1.5	NA	Regional	2.2	Focal
16[e]	C	M	*TSC2*	0.7	0.7	Regional	12.1	Focal
17	C	M	*TSC2*	1.2	1.2	Focal	6.1	Focal + ES
18	C	M	*TSC2*	1.1	4.0	Regional	6.9	ES
19[e]	C	M	*TSC2*	0.7	0.7	Bilateral	1.1	ES + Focal
20	C	M	*TSC2*	1.2	6.1	Bilateral	6.3	ES
21	C	M	*TSC2*	1.3	1.3	Bilateral	1.3	Focal
22	A	M	*TSC2*	1.4	1.4	Focal	3.9	Focal
23	C	M	*TSC2*	4.4	4.4	Bilateral	6.1	ES
24	H	F	*TSC2*	6.2	6.2	Focal	20.1	Focal
25	AI	M	*TSC2*	1.6	1.6	Bilateral	3.4	Focal
26	C	F	*TSC2*	1.6	NA	Focal	3.5	ES + Focal
27	C	F	*TSC2*	2.6	4.2	Bilateral	6.1	Focal + ES
28	C	M	*TSC2*	4.0	4.0	Regional	4.8	ES
29	C	F	*TSC1*	2.7	NA	Bilateral	5.5	GTC + Focal
30[e]	C	M	*TSC2*	0.7	0.7	Focal	11.3	Focal
31	C	F	*TSC2*	3.9	4.2	Bilateral	5.8	ES

(Continues)

TABLE 2 (Continued)

Participant	Race[a]	Gender[b]	Genetic testing[c,d]	Age enrolled (mo)	Age first EEG abnormal (mo)	Type of EEG spike	Age at clinical Sz onset (mo)	Clinical Sz type[c,d]
32	C	F	*TSC2*	2.4	4.5	Bilateral	5.9	ES + GTC
33	C	M	*TSC2*	6.0	9.1	Regional	12.7	ES
34	C	F	*TSC1*	3.5	3.5	Regional	4.6	ES
35	C	F	*TSC2*	6.6	6.6	Regional	8.3	ES
36[e]	C	M	ND	2.1	4.4	Bilateral	15.1	ES
37	NR	F	NMI	2.3	3.7	Focal	11.7	Focal
38[e]	C	F	*TSC2*	1.0	1.0	Bilateral	3.3	Focal + ES

[a]A, Asian; AA, African American; AI, American Indian; C, Caucasian; H, Hispanic; NR, not reported.
[b]M, male; F, female.
[c]NMI, no mutation identified; ND, not done.
[d]ES, epileptic spasms; GTC, generalized tonic-clonic; NA, not applicable.
[e]Excluded for pretreatment.

3 | RESULTS

3.1 | Cohort characteristics

A total of 40 participants enrolled in the study over approximately 17 months from February 2013 to June 2014. Their demographic, genetic, and pertinent EEG and seizure information are summarized in Table 2.

Of the 40 participants, 2 were lost to follow-up (5%) and 32 were analyzed for both seizure outcome and developmental outcome. The remaining six were pretreated with vigabatrin for electrographic seizures before clinical seizure onset or for IEDs before clinical seizure onset and excluded from analysis.

For the 38 participants, 22 boys and 16 girls were enrolled in this study with a mean age of 82.4 days ± 59.8 days. Genetic testing was performed for the majority of participants (n = 35). Mutations of *TSC1* were identified for 7, *TSC2* mutations for 26, and no mutation identified for 2 participants.

3.2 | Video-EEG findings and seizure outcome

A total of 268 of 280 anticipated EEG recordings (96%) were acquired on the 38 participants enrolled. A total of 132 visits (49%) were subject to scheduling changes, typically limited to several days outside of the 2-week window in year 1, and greater variability allowed in year 2, due to more time between visits. The interreader reliability/kappa scores were calculated between the two central readers for five major findings on the EEG, namely IEDs, focal or generalized slowing, focal or generalized attenuation, hypsarrhythmia, and ictal events. It is important to note that less than 5% of the EEG recordings were deemed to show any variation of hypsarrhythmia (modified vs classic hypsarrhythmia). IEDs seen as part of hypsarrhythmia were classified into the category of hypsarrhythmia.

For the 32 participants in the EEG and seizure outcome analysis, 17 showed IEDs on EEG studies performed prior to their clinical seizure onset (true positives), and 3 did not have IEDs on their EEG recordings prior to their clinical seizure onset (false negatives). Throughout the study, seven participants maintained normal EEG studies and never developed clinical seizure (true negatives), and five had IEDs but never had clinical seizures (false positives). The positive predictive value, or how often IEDs can predict subsequent epilepsy, is 77.3%. The negative predictive value, or how often the absence of IEDs predicted no subsequent epilepsy, is 70%. The sensitivity of detecting IEDs before ensuing epilepsy is 85%. Finally, the specificity of lack of IEDs predicted no epilepsy up to 2 years of age is 58.3%. The age at the first emergence of IEDs

averaged 4.5 months ± 4.0 standard deviation (SD), with a median age of 4.0 months for those infants who went on to develop seizures. The age at seizure onset of any type, for those with antecedent epileptiform activity, averaged 7.5 months ± 4.4 SD, with a median age of 6.0 months. The interval between the onset of IEDs and clinical epilepsy onset averaged 3.6 months ± 3.4 SD.

Twelve of the 32 participants (37.5%) remained seizure-free throughout the study with no antiepileptic drug (AED) treatment; seven maintained normal EEG findings and never developed seizures, and five had evidence of interictal epileptiform activity on only one EEG by the time the study was completed at age 24 months. Twenty of the 32 infants developed seizures (62.5%). The seizure types consisted of focal seizures in seven (35%), epileptic spasms in six (30%), focal seizures and generalized tonic-clonic seizures in one (5%), and focal seizures with epileptic spasms in six (30%). No other seizure types were reported.

After seizure onset, 76% were treated within 2 days, and 90% within 1 week of seizure onset. At the completion of the study at 24 months, 8 of the 20 (40%) continued to have clinical seizures, and 9 (45%) were reported to be seizure-free, defined as seizure freedom of 3 months or longer. Only one of three participants (15%) who was a false negative (normal EEG but developed clinical seizures) was seizure-free at 24 months. Two of the three underwent epilepsy surgery after their seizure onset: one is seizure-free at 24 months and the other had reduction in their seizure severity but continued to have occasional breakthrough seizures requiring emergency rescue medication.

For the six infants who were pretreated with vigabatrin before the onset of clinical seizures, only one infant did not go on to develop clinical seizures. Of the five who did have seizures while on vigabatrin, three had focal seizures as their sole seizure type, one had focal seizures then epileptic spasms, and one had an unclassified seizure type. Two of the five had been seizure-free for 3 months or longer at the time of study completion of 24 months, and three continued to have seizures. These six participants were not included in the developmental outcome analysis because of the protocol deviation during the study.

3.3 | Developmental outcome

For the Mullen Scales of Early Learning composite scores and Vineland-II scores obtained at the three time points of 6, 12, and 24 months of age (Figure 1), the results were analyzed among three subgroups of the following: (a) true positive infants with IEDs on EEG prior to onset of clinically refractory epilepsy (ongoing seizures despite AED treatment, n = 8); (b) true positive infants with IEDs on EEG prior to onset of clinically controlled epilepsy (control for at least 3 months or longer with AED treatment, n = 9); and (c) infants who never had seizures, regardless of their EEG findings. This third group included true negative infants (no IEDs in any EEG and no seizures, n = 7) and false-positive infants (IEDs in any EEG but no subsequent seizures, n = 5). The rationale for analyzing the five false-positive patients together with the true negative patients is that this study was observational in nature, and no epilepsy treatment was started in patients with an abnormal EEG only. Moreover, these patients had only a single abnormal EEG.

Known variants in *TSC1* and *TSC2* genes did not statistically influence the Vineland-II or Mullen Scales of Early Learning; therefore, they were not retained in the final model. The result of the linear mixed models showed significant overall differences between the three classifications for the Mullen ($P = .002$) and the Vineland-II ($P = .04$). Overall changes (all groups combined) over the three time points were not statistically significant for either scale. The group-by-time interaction was significant for the Vineland-II score ($P = .04$) but not significant for the Mullen composite score. Follow-up analyses showed that of these three subgroups, the group with refractory epilepsy scored progressively lower with advancing age on both the Vineland-II ($P = .018$) and

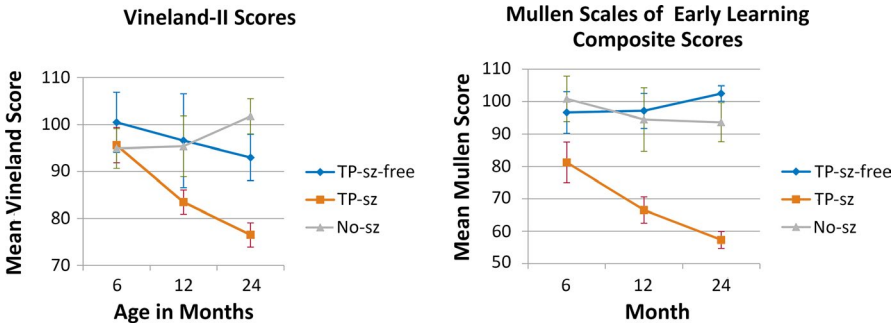

FIGURE 1 Results of developmental assessments (Vineland-II and Mullen Scales of Early Learning Composite scores) from linear mixed models evaluating longitudinal developmental outcomes. Developmental assessments were given at 6, 12, and 24 months of age. (TP-sz-free, True-positive seizure free; TP-sz, True-positive seizure; No-sz, No seizure). Vineland-II Standard Scores: <70 Well Below Average, 70-84 Below Average, 85-115 Average range, 116-130 Above Average, >130 Well Above Average. Mullen Composite standard scores: 49-70 very low, 71-84 below average, 85-115 average, 116-129 above average, 130-155 very high

TABLE 3 Research participants, locations, roles, and contributions

Name	Location	Role	Contribution
Joyce Y. Wu, MD	University of California Los Angeles Los Angeles, CA	Author	Designed and conceptualized study; drafted the manuscript for intellectual content
Darcy Krueger, MD, PhD	Cincinnati Children's Hospital Cincinnati, Ohio	Author	Site principal investigator; development of study design; drafted manuscript
Mustafa Sahin, MD, PhD	Boston Children's Hospital Boston, Massachusetts	Author	Site principal investigator; development of study design; drafted manuscript
Hope Northrup, MD	University of Texas—Houston Houston, Texas	Author	Site principal investigator; development of study design; drafted manuscript
Monisha Goyal, MD	University of Alabama Birmingham Birmingham, Alabama	Author	Interpreted the data; revised the manuscript for intellectual content
Jurriaan M. Peters, MD, PhD	Boston Children's Hospital Boston, Massachusetts	Author	Interpreted the data; revised the manuscript for intellectual content
Kit Sing Au, MD	University of Texas—Houston Houston, Texas	Author	Analyzed genetic samples, revised the manuscript for intellectual content
Sarah O'Kelley, PhD	University of Alabama Birmingham Birmingham, Alabama	Author	Analyzed neurodevelopmental assessments; developed manuscript
Marian Williams, PhD	Children's Hospital Los Angeles Los Angeles, CA	Author	Analyzed neurodevelopmental assessments; developed manuscript
Deborah A. Pearson, PhD	University of Texas—Houston Houston, Texas	Author	Analyzed neurodevelopmental assessments; developed manuscript
Ellen Hanson, PhD	Boston Children's Hospital Boston, Massachusetts	Author	Analyzed neurodevelopmental assessments; developed manuscript
Anna Weber Byars, PhD	Cincinnati Children's Hospital Cincinnati, Ohio	Author	Analyzed neurodevelopmental assessments; developed manuscript
Jessica Krefting, RN	University of Alabama Birmingham Birmingham, Alabama	Author	Designed, developed and executed study; Analyzed data; revised manuscript
Mark Beasley, PhD	University of Alabama Birmingham Birmingham, Alabama	Author	Performed bio statistical analysis; revised manuscript
Gary Cutter, PhD	University of Alabama Birmingham Birmingham, Alabama	Author	Designed study; statistical analysis; revised manuscript
Nita Limdi PharmD, PhD, MSPH	University of Alabama Birmingham Birmingham, Alabama	Author	Statistical analysis; revised manuscript
E. Martina Bebin, MD, MPA	University of Alabama Birmingham Birmingham, Alabama	Author	Designed and conceptualized study; analyzed data; revised manuscript

the Mullen Scale of Early Learning ($P = .03$). There were no differences between patients who never developed seizures and those who gained seizure freedom.

4 | DISCUSSION

This prospective multicenter observational study of infants with TSC provides several important findings: interictal spikes on serial scalp video-EEG studies identified correctly nearly 80% of infants who subsequently developed epilepsy. Furthermore, antecedent spikes predicted the impending seizure onset on average about 3.5 months before seizure onset. For these initially seizure-naive infants, this critical interval between the first appearance of IEDs and the subsequent seizure onset presents a window of opportunity for a disease-modifying antiepileptogenic therapy. Clinical trials are needed to determine if such interventions can alter the overall course of epilepsy in TSC.

It is interesting to note that for the five infants who had IEDs but never developed seizures, all five had IEDs on a single EEG only, which resolved on subsequent EEG studies. This differed from the 17 infants who had persistent IEDs on serial EEG recordings before their seizure onset. Why this cortical irritability is present only transiently and culminated in seizure onset in some TSC infants but not others is not clear and deserves further attention and investigation. One possible concern is that the identification of subtle sharps or spikes was not accurate. The kappa scores between the EEG readers were 0.54 for IEDs and 0.56 for ictal events. Only IEDs predicted and correlated with eventual seizure outcome.

Although the kappa score is lower than what we had strived for, there are examples in the literature citing the difficulty in obtaining higher interrater kappa score on EEG studies. Perhaps the best example would be Hussain et al (2015), in which EEG recordings from an age group of infants similar to this study posed difficulty in assessing hypsarrhythmia. More specifically, raters from different institutions in the Hussain et al[10] study had a kappa score of 0.52 in assessing a single focus of IEDs, similar to our interinstitutional kappa score in this study.

The epilepsy incidence rate of 62.5% from this prospective cohort is consistent with, although somewhat lower than, the 77.9% epilepsy incidence rate by 24 months of age in a larger retrospective study, which may have an inherent bias given that the patients were seen at an epilepsy center or the removal of TSC patients presenting with epilepsy from our cohort.[4] The 30% incidence of epileptic spasms in our subjects is consistent with that of the large retrospective series.[4] Focal seizures in 35% of our cohort by 2 years of age is difficult to compare to the nearly 87% of patients with focal seizures in all age groups,[4] not limited to the first 2 years of life.

The refractory seizure rate of 40%, however, is lower than the 64% refractory epilepsy rate reported in one large retrospective series.[4] Potential explanation of the lower refractory epilepsy rates here include higher rates of referral to epilepsy centers of patients with difficult to control epilepsy in retrospective studies, exclusion of early onset epilepsy patients from the study, and longer-term follow-up in the retrospective study cohorts. Long term it will be interesting to see if early recognition and prompt treatment of seizures in TSC alter long-term seizure outcomes.

Perhaps, as or more important than epilepsy outcome, the developmental outcome among the subgroups of TSC infants in this study demonstrates that the progressive decline in developmental assessments is clearly linked to the persistence of seizures. Our data suggest that there is a specific association between severity of epilepsy and comorbid conditions, including developmental delay, reported previously in retrospective and prospective series.[4,11–13] We recognize that this study did not incorporate the neuroimaging findings for this cohort, which also may be a contributing factor to the cognitive decline in some of the participants.

Our study demonstrates that early TSC diagnosis and serial EEG monitoring of infants can identify those TSC infants at highest risk for developing seizures months before clinical seizures will begin, but it does not clarify the role of early therapy in long-term outcome. Our clinically available EEG biomarker, through risk-stratification, can limit vigabatrin exposure to only those at high risk for the development of epilepsy. It could be used in trials focused on preventative therapy in TSC, like the EPISTOP completed in August 2018. This randomized open-label trial of preemptive vigabatrin in TSC reported a delay in onset of clinical seizures and a reduction of drug-resistant epilepsy.[14] Further study is needed and studies like the PREVeNT trial (NCT02849457) and final results from EPISTOP (www.EPISTOP.eu) will aid in determining if early interventions prior to the development of epilepsy with antiseizure medications such as vigabatrin will be useful to prevent epilepsy, refractory epilepsy, and cognitive impairment in children with epileptiform EEGs.

ACKNOWLEDGMENTS

This study was supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health (P20 NS 080199 and U01 NS 082320). Table 3 details participants' names, locations, roles, and contributions. J.Y.W. was also supported by the NINDS/NIH (R01 NS 82649), and the Today's and Tomorrow's Children Fund from UCLA Mattel Children's Hospital at the University of California Los Angeles. M.S. was also supported by the Senior Investigator award from Boston Children's Translational Research Program. This study also utilized clinical research facilities and resources supported by the National Center for Advancing Translational Sciences of the National Institutes of Health grant (UL1TR001881, 8UL1TR000077) and National Institute of Child Health and Development (U54HD090255). Author contributions: Drs. Wu, Goyal, Peters, Krueger, Sahin, Northrup, Cutter, Limdi, and Bebin all contributed equally to the study design and concept, analysis, and interpretation of the results. Drs. Au, O'Kelley, Williams, Pearson, Hanson, Byers, Beasley, and Jessica Krefting were involved in data acquisition, and analysis and interpretation of the data. The statistical analyses were completed by Dr Nita Limdi and Dr Mark Beasley, both affiliated with the University of Alabama at Birmingham.

CONFLICT OF INTERESTS

All the authors on this manuscript submission report no disclosures. 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.

ORCID

E. Martina Bebin https://orcid.org/0000-0002-6725-2814

REFERENCES

1. Caban C, Khan N, Hasbani DM, Crino PB Genetics of tuberous sclerosis complex: implications for clinical practice. (Report). Appl Clin Genet. 2017;10:1.

2. Crino PB, Nathanson KL, Henske EP. The Tuberous sclerosis complex. (Disease/Disorder overview). N Engl J Med. 2006;355(13):1345–56.
3. Sparagana PS, Roach SE. Tuberous sclerosis complex. Curr Opin Neurol. 2000;13(2):115–9.
4. Chu CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. Epilepsia. 2009;51(7):1236–41.
5. Datta AN, Hahn CD, Sahin M. Clinical presentation and diagnosis of tuberous sclerosis complex in infancy. J Child Neurol. 2008;23(3):268–73.
6. Jóźwiak S, Kotulska K, Domańska-Pakieła D, Łojszczyk B, Syczewska M, Chmielewski D, et al. Antiepileptic treatment before the onset of seizures reduces epilepsy severity and risk of mental retardation in infants with tuberous sclerosis complex. (Report). Eur J Paediatr Neurol. 2011;15(5):424–31.
7. Wu JY, Peters JM, Goyal M, Krueger D, Sahin M, Northrup H, et al. Clinical electroencephalographic biomarker for impending epilepsy in asymptomatic tuberous sclerosis complex infants. Pediatr Neurol. 2016;54:29–34.
8. Mullen E. Mullen Scales of Early Learning. Bloomington, MN: NCS Pearson; 1995.
9. Sparrow SS, Cicchetti DV, Balla DA. Vineland Adaptive Behavior Scales. 2nd ed. Circle Pines, MN: American Guidance Service; 2005.
10. Hussain SA, Kwong G, Millichap JJ, Mytinger JR, Ryan N, Matsumoto JH, et al. Hypsarrhythmia assessment exhibits poor interrater reliability: A threat to clinical trial validity. Epilepsia. 2015;56(1):77–81.
11. Capal JK, Bernardino-Cuesta B, Horn PS, Murray D, Byars AW, Bing NM, et al. Influence of seizures on early development in tuberous sclerosis complex. Epilepsy Behav. 2017;70(Pt A):245–52.
12. Davis PE, Filip-Dhima R, Sideridis G, Peters JM, Au KS, Northrup H, et al. Presentation and diagnosis of tuberous sclerosis complex in infants. Pediatrics. 2017;140(6):e20164040.
13. Northrup H, Krueger DA. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. Pediatr Neurol. 2013;49(4):243–54.
14. Moavero R, Benvenuto A, Emberti Gialloreti L, Siracusano M, Kotulska K, Weschke B, et al. Early clinical predictors of autism spectrum disorder in infants with tuberous sclerosis complex: results from the EPISTOP Study. J Clin Med. 2019;8(6):E788.

How to cite this article: Wu JY, Goyal M, Peters JM, et al. Scalp EEG spikes predict impending epilepsy in TSC infants: A longitudinal observational study. *Epilepsia*. 2019;60:2428–2436. https://doi.org/10.1111/epi.16379