Original Article

Longitudinal correspondence of epilepsy and scalp EEG fast (40 – 200 Hz) oscillations in pediatric patients with tuberous sclerosis complex

Hiroki TSUCHIYA [a,*], Fumika ENDOH [a,b], Tomoyuki AKIYAMA [a], Masao MATSUHASHI [c], and Katsuhiro KOBAYASHI [a]

[a] Department of Child Neurology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital, Okayama, Japan

[b] Department of Child Neurology, NHO Minami-Okayama Medical Center, Okayama, Okayama, Japan

[c] Department of Epilepsy, Movement Disorders and Physiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

***Corresponding author at:** Department of Child Neurology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Shikatacho

2-chome 5-1, Kita-ku, Okayama 700-8558, Japan

Text: 4,373 words Abstract: 248 words References: 26

Number of tables: 2 Number of figures: 6

Supplementary Table: 1 Supplementary Figures: 3

Abstract

Introduction: Epilepsy associated with tuberous sclerosis complex (TSC) has very complex clinical characteristics. Scalp electroencephalogram (EEG) fast (40 – 200 Hz) oscillations (FOs) were recently suggested to indicate epilepsy severity. Epileptic FOs may undergo age-dependent longitudinal change in individual patients, however, and the typical pattern of such change is not yet fully clarified. We therefore investigated the age-related correspondence between clinical courses and FOs in pediatric patients with TSC-associated epilepsy. ***Subjects and Methods:*** FOs were semi-automatically detected from scalp sleep EEG data recorded from 23 children (15 boys, 8 girls; initial data obtained at < 10 years of age) with TSC-associated epilepsy. ***Results:*** The number of FOs per patient that were associated with spikes was significantly greater than that of FOs unassociated with spikes (median 145 and 5, respectively; p=0.0001 by the Wilcoxon signed-rank test). In the eight patients who had West syndrome (WS) in infancy, FOs associated with spikes were abundant during the WS period prior to adrenocorticotropic hormone therapy, with significantly greater numbers of FOs compared to the post-WS period (median 242 and 0, respectively; p=0.0078). As there was no such time-dependent difference regarding FOs unassociated with spikes, FOs associated with spikes were identified as epileptic. The detected FOs included both

gamma and ripple oscillations with no consistent age-dependent shifts in dominant frequency. There were no apparent age-related changes in FO duration. ***Conclusions:*** Epileptic scalp FOs are confirmed to correspond to severity of epileptic encephalopathy, particularly in WS, even during the long-term evolutional courses of TSC-associated epilepsy.

[248 words]

Keywords: Tuberous sclerosis complex; Fast oscillations; Scalp EEG; High-frequency oscillations

1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal-dominant genetic disorder caused by mutations in the *TSC1* or *TSC2* genes. It is observed in approximately 1 in 6000 individuals, representing one of the most significant genetic causes of epilepsy [1,2]. Epilepsy is a particularly prevalent manifestation of the disorder, affecting approximately 80% of individuals with TSC [3-5]; over 60% have seizures that tend to be severe and refractory [3,6,7]. The characteristic feature of epilepsy related to TSC is age-dependent expansion of the epilepsy network and worsening seizures. Although most TSC patients have seizures within the first year of life [8], abnormal formation of the cortical/subcortical networks causes seizures to become aggravated and more generalized with age [9], with multiple epileptogenic foci spreading widely and rapidly through both hemispheres, even in cases that had been limited to a single focus early in the clinical course [10]. Seizure types in TSC are various, including epileptic spasm, focal seizure, focal to bilateral tonic-clonic seizure, generalized tonic-clonic seizure, and atypical absence seizure; and seizure type can change all at once or shift back and forth during the clinical course [11]. Therefore, epilepsy associated with TSC has very complex clinical characteristics.

Recently, scalp electroencephalogram (EEG) fast oscillations (FOs), which include

ripple (80 – 200 Hz) and gamma (40 – 80 Hz) oscillations, have been suggested to correlate with and thereby indicate epilepsy severity [12-14]. These oscillations may correspond to intracranial high-frequency oscillations (HFOs) [15]. FOs are abundantly observed in West syndrome (WS) in infancy [16,17] as well as in epileptic encephalopathy with continuous spike-and-wave during sleep (CSWS) in childhood [14,18,19]. The age-dependent pattern of longitudinal changes in epileptic FOs in individual patients is not yet fully clarified, but improved knowledge of this pattern might provide clues concerning the pathophysiological mechanisms of epilepsy, particularly age-dependent severe epilepsies. As TSC is one of the most important etiologies of severe intractable epilepsies that tend to persist over the long term, we investigated the correspondence between complex clinical courses and FOs according to age in pediatric patients with TSC-associated epilepsy. We hypothesized that hypsarrhythmia in WS may be a unique phenomenon of epileptic encephalopathy associated with a dramatic increase in FOs throughout the long clinical course of TSC-associated epilepsy.

2. Subjects and methods

2.1. Subjects

The inclusion criteria in the present study incorporated pediatric patients with TSC who were born after January 1, 2000 and who visited Okayama University Hospital (OUH) before May 31, 2018. All patients fulfilled the clinical and/or genetic diagnostic criteria for definite TSC [20], and all of them had epilepsy. This study included only children for whom digital EEG data and sufficient clinical information had been obtained during young childhood < 10 years of age at OUH. Seizure types were classified according to the International League Against Epilepsy [21], and WS was defined as a combination of epileptic spasms in series and hypsarrhythmia on interictal EEG. Seizure freedom refers to suppression of all types of seizures for at least one year at the time of last follow-up.

This retrospective study was approved by the Okayama University Ethics Committee (approval code Ken-1911-024).

2.2. Methods

We adopted analytic methods similar to those used in our pervious study on scalp ripple oscillations [14]. We recorded EEG with a Nihon-Kohden (Tokyo, Japan) Neurofax system with a sampling rate of 500 Hz. Our EEG electrodes included Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, Fz, Cz, and Pz defined according to the

International 10-20 System in a referential montage. The reference was the average EEG of the earlobes (A1 and A2) (indicated as Aav).

A 60-second-long data section with no or minimal artifacts was manually selected from each individual's non-rapid eye movement (NREM) sleep EEG record. This data section length was adopted because it was difficult to obtain an uninterrupted clean section longer than 1 minute from every EEG record taken during a nap. First, candidate FOs within the selected data section were automatically detected with a program written by von Ellenrieder et al. [22] for MATLAB (version 9.1.0; MathWorks Inc., Natick, MA, USA). This program was designed to detect HFOs as localized increments of signal power with a duration of at least four cycles in narrow frequency bands based on a finite impulse response (FIR) filter. The whole frequency band of analysis, which included the gamma (40 – 80 Hz) and ripple (80 – 200 Hz) bands, was separated into 10-Hz-wide narrow frequency bands (i.e., 40 – 50 Hz, 50 – 60 Hz,…190 – 200 Hz). As spectral analysis was not incorporated in this program, the exact frequency of each FO could not be measured. Therefore, in each narrow frequency band, the central frequency was defined as the center frequency of the corresponding band, which represented the approximate frequency of the detected FOs from that band (e.g., 105 Hz in the 100 – 110 Hz band). The threshold of root-mean-square (RMS) power to detect FOs was set as

a ratio of 3 against the RMS power level of a background moving window (width 30 s); as this ratio was slightly higher than the original value of 2.5, the detection of FOs was rather conservative in the present study. We hoped that this would enable us to identify definite FOs from our pediatric EEG data which, like most pediatric EEG data, tended to have a rather noisy background.

Second, the automatically detected candidate FOs were marked on temporally expanded (2 s per page) and filtered EEG traces using an inhouse written program (overlaid traces with low-cut frequency [LCF] filters at 0.5, 40, and 80 Hz in green, blue, and red, respectively) (e.g., see **Figure 1**). FOs consisting of at least four consecutive oscillations were visually confirmed through the consensus of two reviewers. Oscillations that appeared to be contaminated noise or muscle activity were excluded. Signals common to almost all channels were assumed to be contaminated noise or muscle activity from the reference Aav.

Third, we evaluated the number of occurrences, frequency (central frequency of the corresponding narrow frequency band of FO detection), and duration (duration of power increase in the corresponding narrow frequency band) of FOs in each EEG dataset for further analysis. We analyzed FOs associated with visually identified epileptic discharges and those not associated with visually identified epileptic discharges

separately because FOs with and without associated spikes may have different meanings regarding epileptogenicity. FOs on different scalp electrodes were identified separately. Analysis was performed on a half-year basis during infancy and on a yearly basis in later childhood, and we investigated the changes in FO parameters that occurred with age in each patient group.

2.3. Statistical analysis

We statistically compared the number of occurrences, frequency, and duration of FOs associated with spikes between patients with and without a history of WS (Groups A and B, respectively) during the toddler period, when EEG data were available for both groups, using the Mann Whitney U test. The parameters of FOs unassociated with spikes were similarly compared. Two aspects of the FOs (i.e., association vs. lack of association with spikes; gamma vs. ripple oscillations) in each individual were compared using the Wilcoxon signed-rank test. We also statistically analyzed age-dependent changes in the number of FOs in Group A patients, that is, the differences in number of FOs between two adjacent periods which could include the pre-WS period from birth to 3 months of age, the WS period with hypsarrhythmia prior to adrenocorticotropic hormone (ACTH) (tetracosactide acetate, Cortrosyn-Z®) therapy

from 4 months to 11 months of age, or the post-WS period from one year of age on, using the Wilcoxon signed-rank test, in patients who had EEG recordings from each pair of adjacent periods. In patients with multiple EEG records in a given period, we selected the sleep EEG record with no or minimal artifacts recorded at the youngest age during the corresponding period for the present study. We also performed a similar analysis of age-dependent FO count differences between the toddler period from 2 to 5 years of age and the childhood period from 6 to 10 years of age involving all groups. Relationships were considered statistically significant if $p < 0.05$. We used JMP Japanese version 14.2.0 (SAS Institute Japan, Tokyo) for the statistical analysis.

3. Results

3.1. Clinical characteristics

Of the 25 patients (16 boys, 9 girls) with TSC who met the inclusion criteria for the present study, 23 children (15 boys, 8 girls) who had both EEG data and detailed clinical information from infancy and/or young childhood < 10 years of age were eligible and selected. The remaining two patients were excluded because their EEG data had been recorded at hospitals other than OUH. Demographic data and clinical courses of the patients are provided in **Supplementary Table S1** and **Figure 2**, respectively.

Among the selected 23 patients, median age at the first visit to OUH was 12 months (range, 0 – 116 months), median age at the first EEG recording was 15 months (range, 1 – 117 months), and median duration of follow-up was 62 months. All patients had epilepsy with a median age of seizure onset of 8 months (range, 0 – 36 months). Ten patients had a family history of TSC, 13 had cardiac rhabdomyomas, 21 had dermatologic findings, and all patients had central nervous system lesions. The clinical diagnostic criteria of TSC were fulfilled in all but pt. #7, who fulfilled the genetic diagnostic criteria due to a mutation of the *TSC1* gene (exon 7 c.526dupT [Y176fsX217]) which had been detected at Tottori University during testing for clinical purposes with written informed consent (**Supplementary Table S1**).

Patients were categorized as follows as indicated in **Table 1**. Eleven patients (pts. #1 – 11) who were diagnosed with WS during infancy were classified into Group A: their median age at first visit was 4 months (range, 0 – 116 months), that at first EEG recording was 6 months (range, 2 – 117 months), that at seizure onset was 5 months (range, 0 – 11 months), and that at the onset of WS was 7 months (range, 2 – 11 months). Within Group A, Subgroup A-1 (pts. #1 – 5) included five patients who had been followed up with EEG examinations from before the onset of WS: four had focal seizures preceding the onset of epileptic spasms. Focal spike-waves antecedent to

hypsarrhythmia were observed in all five of these patients. Subgroup A-2 included three other patients in Group A (pts. #6 – 8) who were diagnosed with WS at their first visit because epileptic spasms and hypsarrhythmia were already present: only one patient had focal seizures prior to the onset of epileptic spasms. Subgroup A-3 included the remaining three patients in Group A (pts. #9 – 11) who had been diagnosed with WS at other hospitals prior to their first visit to OUH: none of them had focal seizures or known focal spike-waves preceding the onset of epileptic spasms. All 11 patients with WS in Group A received ACTH therapy for a total of four weeks; although spasms were at least temporarily suppressed by this treatment, seizures relapsed shortly in all patients. Vigabatrin (VGB) was used in three of the seven children who had WS at the time of its formal approval, and one of them (pt. #3) is seizure-free to date. In contrast, two other patients (pts. #5 and 7) who had intracerebral tumor-like lesions underwent surgery; although both had transient seizure freedom after the operation, both experienced a later relapse of seizures and one of them (pt. #5) continues to have seizures.

The remaining 12 patients (pts. #12 – 23) who did not develop WS were categorized as Group B. Their median age at first visit was 31.5 months (range, 0 – 103 months), that at first EEG recording was 31.5 months (range, 1 – 103 months), and that at seizure onset was 13 months (range, 2 – 36 months). Of these twelve patients, nine

who had focal seizures and/or generalized tonic-clonic seizures (GTCS) and only focal spike-waves in EEG were diagnosed with focal epilepsy (Subgroup B-1 (pts. #12 – 20)). One patient (pt. #21) who had no EEG record before young childhood was categorized as Subgroup B-2. The remaining two patients (pts. #22 and 23) who had epileptic spasms from 3 years of age and coexisting generalized and multifocal epileptic discharges in EEG were assumed to have generalized epilepsy (Subgroup B-3). Of the 12 children in Group B, eight were seizure-free at the last follow-up as a result of medical treatment using various antiepileptic drugs.

3.2. Detected FOs and their age-dependent changes

We analyzed a total of 107 EEG records in all diagnostic groups and identified a total of 10,274 FOs in the interictal EEG data (6,521 and 3,753 in Groups A and B, respectively). Of these, 10,036 (97.7%) were associated with spikes (6,367 and 3,669 in Groups A and B, respectively), and the remaining 238 (2.3%) were not associated with spikes (154 and 84 in Groups A and B, respectively). FO counts in each group/subgroup and those associated/unassociated with spikes are indicated in **Table 2**. The number of FOs associated with spikes per patient (median, 145 [range, 0 – 2,691]) was significantly greater than the number unassociated with spikes (median, 5 [range, 0 –

57]) (p=0.0001). A comparison of Groups A and B regarding the number of FOs associated and unassociated with spikes during the toddler period did not reveal any significant difference between the groups (median, 16 [range, 0 – 83] versus 1.5 [0 – 97] in FOs associated with spikes [p=0.3367]; median, 1 [range, 0 – 17] versus 1 [0 – 9] in FOs unassociated with spikes [p=0.3599]).

The number of gamma oscillations per patient (median, 118 [range, 0 – 1,100]) and that of ripples (median, 69 [range, 0 – 1,605]) were not significantly different (p=0.6475). Furthermore, a histogram of FO frequency distribution shows a single peak at 75 Hz, which is close to the border dividing gamma from ripple bands in the present 10-Hz-wise frequency band segmentation (**Supplementary Figure S1)**.

Age-dependent changes in FO counts are depicted in **Figure 3.** In all five patients who had EEG recordings before WS (Subgroup A-1), the number of FOs associated with spikes was zero during the pre-WS period and increased during the WS period (median, 258 [range, 95 – 608]), although this increase did not reach statistical significance (p=0.0625) (**Figure 4A**). FOs unassociated with spikes in these patients did not differ between the two periods (median, 0 [range, 0 – 1] versus 3 [range, 0 – 19]) (p=0.1250) (**Figure 4D**).

In the eight patients who had WS in infancy and EEG recordings during and after

WS (Subgroups A-1 and A-2), the number of FOs associated with spikes was significantly greater during the WS period before ACTH therapy (median, 242 [range, 95 – 723]) than during the post-WS period (median, 0 [range, 0 – 124]) (p=0.0078) (**Figure 4B**). The number of FOs unassociated with spikes was not significantly different between the two periods (median, 3 [range, 0 – 19] versus 0 [range, 0 – 14]) (p=0.6875) (**Figure 4E**).

In the ten patients from among all groups who had EEG recordings during both the toddler and childhood periods, the number of FOs associated with spikes was not significantly different between the two periods (median, 3 [range, 0 – 97] and 0 [range, 0 – 20], respectively) (p=0.0625) (**Figure 4C**). FOs unassociated with spikes in these patients likewise did not differ between the two periods (median, 1 [range, 0 – 17] and 0 [range, 0 – 19], respectively) (p=0.5625) (**Figure 4F**).

The number of FOs did not show consistent age-dependent changes, although there were some patients whose FOs increased along with worsening EEG and seizures; one (pt. #4) received a second round of ACTH therapy during the toddler period while four others (pts. #9, 11, 21 and 23) received a second round during childhood **(Figures 2, 3**).

We found a total of 4,404 gamma oscillations (2,821 and 1,583 in Groups A and B, respectively). Of these, 4,302 (97.7%) were associated with spikes (2,753 and 1,549 in

Groups A and B, respectively), and the remaining 102 (2.3%) were not associated with spikes (68 and 34 in Groups A and B, respectively). Age-related changes in gamma oscillations were similar to the general age-related changes in FOs, as depicted in **Supplementary Figure S2**.

There were a total of 5,870 ripple oscillations (3,700 and 2,170 in Groups A and B, respectively). Of these, 5,734 (97.7%) were associated with spikes (3,614 and 2,120 in Groups A and B, respectively), and the remaining 136 (2.3%) were not associated with spikes (86 and 50 in Groups A and B, respectively). Age-related changes in ripple oscillations were also similar to the general changes in FOs, as depicted in **Supplementary Figure S3**.

3.3. Age-related changes in frequency of FOs

The observed record-wise median frequency of FOs associated with spikes ranged from 45.0 to 185.0 Hz (10,036 FOs; median 85.0 Hz) in all patients considered together, from 55.0 to 185.0 Hz (6,367 FOs; median 85.0 Hz) in Group A, and from 45.0 to 165.0 Hz (3,669 FOs; median 75.0 Hz) in Group B. The record-wise median frequency of FOs that were not associated with spikes ranged from 45.0 to 195.0 Hz (238 FOs; median 85.0 Hz) in all patients, from 45.0 to 195.0 Hz (154 FOs; median 85.0 Hz) in Group A,

and from 45.0 to 195.0 Hz (84 FOs; median 85.0 Hz) in Group B. A comparison of Groups A and B regarding the frequency of FOs associated and unassociated with spikes during the toddler period revealed no significant difference between the two groups (median, 85.0 Hz [range, 75.0 – 185.0] vs. 75.0 Hz [55.0 – 105.0] in FOs associated with spikes [p=0.2240]; median, 75.0 Hz [range, 70.0 – 195.0] vs. 95.0 Hz [60.0 – 165.0] in FOs unassociated with spikes [p=0.5157]). As **Figure 5** depicting age-related changes in FO frequency shows, no tendency toward frequency deviation was found.

3.4. Age-related changes in duration of FOs

The observed record-wise median duration of FOs associated with spikes ranged from 35.0 to 104.0 ms (10,036 FOs; median 59.0 ms) in all patients, from 35.0 to 104.0 ms (6,367 FOs; median 57.5 ms) in Group A, and from 46.0 to 99.0 ms (3,669 FOs; median 60.0 ms) in Group B. The record-wise median duration of FOs that were not associated with spikes ranged from 29.0 to 116.0 ms (238 FOs; median 51.0 ms) in all patients, from 29.0 to 116.0 ms (154 FOs; median 48.0 ms) in Group A, and from 40.0 to 80.0 ms (84 FOs; median 55.5 ms) in Group B. A comparison of Groups A and B regarding the duration of FOs associated and unassociated with spikes during the toddler period revealed no significant difference between the two groups (median, 60.0 ms [range, 37.5

– 104.0] vs. 62.0 ms [48.0 – 73.0] in FOs associated with spikes [*p*=1.0]; median, 50.0 ms [range, 32.5 – 98.5] vs. 47.0 ms [40.0 – 80.0] in FOs unassociated with spikes [*p*=0.6166]). There were no apparent age-related changes in FO duration, as shown in **Figure 6**.

4. Discussion

A general relationship has been suggested between FOs in scalp EEGs and epilepsy severity [12,13], and FOs are particularly abundant in EEGs with hypsarrhythmia in WS and the CSWS pattern [14,16,17]. This is the first study to directly investigate the long-term longitudinal age-related changes in FOs in various types of epilepsies in children with the single identical etiology of TSC. We endeavored to clarify the complex pathophysiology of epilepsies associated with TSC from the perspective of scalp EEG FOs by using a semi-automatic detection tool in order to increase the objectivity of the analysis.

In the present study, although the number of FOs per patient during the toddler period did not significantly differ between the groups with and without history of WS (Groups A and B, respectively), the counts of FOs associated with spikes were much greater during the WS period than during the post-WS period. Such a marked increase

in FOs during hypsarrhythmia must be a unique phenomenon and may be related to functional disruption of the physiological brain networks in epileptic encephalopathy. Although the increase in spike-associated FOs from zero during the pre-WS period to abundance during the WS period did not reach statistical significance, probably because of our small number of participants, we are confident that a repetition of this analysis in a larger patient population would indicate significance. The increase in FO counts observed in association with worsening seizures and generalized/multifocal spike-waves in childhood in Group B-3 may also be related to the functional disturbance of the brain. There were some minor fluctuations in the FO parameters during long-term follow-up, which may be linked to still unknown factors involved in epilepsy and/or TSC. Longitudinal changes in epileptic FO are not yet fully understood, particularly in the contexts of the many other types of etiologies and forms of epilepsy. Improving our knowledge of typical patterns of age-related change in FOs may help elucidate pathophysiological mechanisms, especially those related to the age-dependent severity of epileptic encephalopathy.

Regarding the frequency characteristics of scalp FOs in pediatric epilepsies, gamma oscillations predominate in WS during infancy [12] while ripple oscillations are mainly observed in benign childhood epilepsy with centrotemporal spikes (BECTS) and CSWS

in childhood [13,14,23]. Ohuchi et al. [14] reported that frequency at around 140 Hz was the main frequency of FOs in childhood epilepsy without TSC. In contrast, in TSC patients with epilepsy in the present study, FOs centered in frequency at around 75 Hz at the border between gamma and ripple bands, and no consistent age-dependent frequency changes were identified from infancy through childhood, suggesting that shifts in the dominant frequency of FOs are not regulated by a simple age-dependent developmental process. Because the frequency distribution of FOs suggested that FOs can be grouped into a single spectrum in TSC, we analyzed FOs mainly comprising gamma and ripple oscillations as a single group. Further analysis of age-dependent changes in the counts, frequency, and duration of FOs conducted on the two bands separately did not reveal any band-specific characteristics. Based on these findings, we present most of our FO data collectively in the present study. The characteristics of FOs may differ depending on epilepsy type and underlying etiology, and the present findings may be applicable only to the pathological condition of TSC. We need more studies on FOs involving many more patients with different epilepsy types and etiologies, especially TSC and others.

In the present study, the number of FOs unassociated with spikes was significantly smaller than that of FOs associated with spikes, and there were no apparent age-related

changes in this trend even when hypsarrhythmia was involved. Ohuchi et al. [14] have indicated that ripples unassociated with spikes may be largely irrelevant to epileptogenicity. Mooji et al. [24] have reported the presence of ripples in spike-free EEGs recorded from children with and without epilepsy, and suggested that these spike-free EEG ripples may be physiological. Similarly, in the present study limited to TSC patients, FOs unassociated with spikes were considered to have little relationship with epileptogenicity. Therefore, it may be justifiable to focus on FOs associated with spikes in order to exclude possibly non-epileptic FOs. The separation between epileptic and non-epileptic FOs is indicated to be realized by the usage of a modulation index (MI), a summary measure quantifying the strength of coupling between high-frequency activity and 3 to 4 Hz slow-waves (see Motoi et al. [25]), and we need to pursue studies in this direction.

We are aware of several limitations in the present study. First, this is a single-center retrospective study involving a small number of patients, and EEG data could not be recorded regularly in some patients, particularly before the onset of WS. FO counts could not be investigated as factors related to prediction of the onset of WS, treatment response, or epilepsy and intellectual prognosis. We hope to solve such problems by increasing the number of participants in future studies. Epilepsy severity involves

multiple aspects, including persistence versus freedom of seizures. Developmental disorders are another important consideration, and may depend on brain lesions or epilepsy or their combination. Various anti-epileptic treatments, such as VGB, may have different effects on FOs. These issues were not within the scope of the present study, but the relation of FOs to some of these phenomena would be interesting research topics. A multivariate analysis that involves many more patients and uses these factors as fixed-effect predictor variables would reveal the detailed relationship between FOs and particular factors, such as active seizures in a given EEG study, history of epileptic spasms, and age. Second, our methods of visual correction of FOs might have biased their detection. Due to our use of a semi-automatic detection tool, the rate of FOs in the present study cannot be directly compared to those in other reports relying on visual review [13]. Third, because the EEG sampling rate was 500 Hz, it was difficult to analyze fast ripples (250 – 500 Hz) in scalp EEGs, which are known to have a grave clinical meaning as biomarkers of impending epileptogenesis [26].

In conclusion, we objectively confirmed that epileptic scalp FOs are associated with severity of epileptic encephalopathy, particularly in the context of WS, during long-term evolutional clinical courses of epilepsy associated with TSC.

Acknowledgments

We thank Dr. Nicolás von Ellenrieder at the Montreal Neurological Institute, McGill University, for kindly providing us with the Matlab program for semi-automatic HFO/FO detection that was used in the present report. We also thank Professor Eiji Nanba at the Division of Functional Genomics, Research Centre for Bioscience and Technology, Tottori University, for providing us with the clinical genetic test result regarding patient #7.

K. Kobayashi was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT KAKENHI Grant Number 15H05874 [Non-linear Neuro-oscillology]) and by Health and Labour Research Grants from the Ministry of Health, Labour and Welfare, Japan (H24-nanchitou-ippan-029, H26-nanchitou-ippan-051, H29-nanchitou-ippan-010, and 19GC1013). T. Akiyama was supported by Grants-in-Aid from the Japan Society for the Promotion of Science (JSPS KAKENHI Grant Number JP15K09622).

M. Matsuhashi was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT KAKENHI Grant Number 15H05875 [Non-linear Neuro-oscillology]). The Department of Epilepsy, Movement Disorders and Physiology, Kyoto University, is involved in an Industry–Academia

Collaboration Course and supported by Eisai Co., Ltd., Nihon Kohden Corporation, Otsuka Pharmaceutical Co., Ltd. and UCB Japan Co. Ltd.

Conflict of Interest Disclosures

The authors have no other conflicts of interest to disclose.

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Figure legends

Figure 1. Representative fast oscillations (FOs) in the scalp EEG recorded from a patient with focal epilepsy and subsequent West syndrome (WS) in Subgroup A-1 (case 9 in Supplementary Table S1).

Temporally expanded and overlaid EEG traces are shown with three different low-cut frequency filters (0.5 Hz, 40 Hz, and 80 Hz in green, blue, and red, respectively) using the average EEG of A1 and A2 as a reference (Aav). FOs with at least four consecutive waves associated with spikes are indicated by pink squares (a representative FO is shown in the magnified pink box).

Figure 2. Clinical courses

The patients in Group A with WS and those in Group B without WS are indicated on the left and right hand sides of the illustration, respectively.

Figure 3. Age-related changes in the number of FOs

A and B: FOs associated with spikes in Groups A and B, respectively. C and D: FOs not associated with spikes in Groups A and B, respectively.

Data from Group A are indicated by thick lines (A-1, solid line; A-2, broken line;

A-3, dotted line) while data from Group B are indicated by thin lines (B-1, solid line; B-2, broken line; B-3, dotted line). Note that the scale is considerably different between the FOs associated and not associated with spikes.

Figure 4. Age-dependent changes in FO counts

Comparison of the number of FOs associated with spikes between the pre-WS and WS periods in Subgroup A-1 (A), between the WS and post-WS periods in combined Subgroups A-1 and A2 (B), and between the toddler and childhood periods in all patients (C). D-F: Comparison of the number of FOs not associated with spikes between the same pairs of periods.

Figure 5. Age-related changes in frequency of FOs

A and B: FOs associated with spikes in Groups A and B, respectively. C and D: FOs not associated with spikes in Groups A and B, respectively. Data from Group A are indicated by thick lines (A-1, solid line; A-2, broken line; A-3, dotted line) while data from Group B are indicated by thin lines (B-1, solid line; B-2, broken line; B-3, dotted line).

There is no apparent tendency toward change in the frequency of FOs related to age,

state of epilepsy, or association with spikes.

Figure 6. Age-related changes in duration of FOs

A and B: FOs associated with spikes in Groups A and B, respectively. C and D: FOs not associated with spikes in Groups A and B, respectively. Data from Group A are indicated by thick lines (A-1, solid line; A-2, broken line; A-3, dotted line) while data from Group B are indicated by thin lines (B-1, solid line; B-2, broken line; B-3, dotted line).

There is no apparent tendency toward change in the duration of FOs related to age, state of epilepsy, or association with spikes.

Legends of Supplementary Figures

Supplementary Figure S1. Histogram of FO count distribution across frequency regarding oscillations associated (A) and not associated (B) with spikes.

Supplementary Figure S2. Age-related changes in counts of gamma oscillations

A and B: gamma oscillations associated with spikes in Groups A and B, respectively. C and D: gamma oscillations not associated with spikes in Groups A and B, respectively. Data arrangement is similar to that in Fig. 3.

The pattern of age-related changes in gamma oscillations is largely similar to that in whole FOs.

Supplementary Figure S3. Age-related changes in counts of ripple oscillations

A and B: ripple oscillations associated with spikes in Groups A and B, respectively. C and D: ripple oscillations not associated with spikes in Groups A and B, respectively. Data arrangement is similar to that in Fig. 3.

The pattern of age-related changes in ripple oscillations is largely similar to that in whole FOs.