Original Article

Clinical EEG and Neuroscience
1–10

DOI: 10.1177/1550059420973095
journals.sagepub.com/home/eeg
SAGE

Quantitative EEG Analysis in Angelman Syndrome: Candidate Method for Assessing Therapeutics

Luis A. Martinez[1,2,3,*], Heather A. Born[1,2,3,*], Sarah Harris[1,2,3], Angelique Regnier-Golanov[1,2,3], Joseph C. Grieco[4], Edwin J. Weeber[4], and Anne E. Anderson[1,2,3,5]

Abstract

The goal of these studies was to use quantitative (q)EEG techniques on data from children with Angelman syndrome (AS) using spectral power analysis, and to evaluate this as a potential biomarker and quantitative method to evaluate therapeutics. Although characteristic patterns are evident in visual inspection, using qEEG techniques has the potential to provide quantitative evidence of treatment efficacy. We first assessed spectral power from baseline EEG recordings collected from children with AS compared to age-matched neurotypical controls, which corroborated the previously reported finding of increased total power driven by elevated delta power in children with AS. We then retrospectively analyzed data collected during a clinical trial evaluating the safety and tolerability of minocycline (3 mg/kg/d) to compare pretreatment recordings from children with AS (4-12 years of age) to EEG activity at the end of treatment and following washout for EEG spectral power and epileptiform events. At baseline and during minocycline treatment, the AS subjects demonstrated increased delta power; however, following washout from minocycline treatment the AS subjects had significantly reduced EEG spectral power and epileptiform activity. Our findings support the use of qEEG analysis in evaluating AS and suggest that this technique may be useful to evaluate therapeutic efficacy in AS. Normalizing EEG power in AS therefore may become an important metric in screening therapeutics to gauge overall efficacy. As therapeutics transition from preclinical to clinical studies, it is vital to establish outcome measures that can quantitatively evaluate putative treatments for AS and neurological disorders with distinctive EEG patterns.

Keywords

Angelman syndrome, quantitative EEG, epileptiform activity, EEG spectral power, minocycline

Received May 5, 2020; revised September 22, 2020; accepted October 14, 2020.

Introduction

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by epilepsy, speech delay, ataxia, intellectual disability, and autistic features.[1-3] AS is primarily caused by deletion or mutations of the maternal copy of the ubiquitin protein ligase E3A gene (*UBE3A*).[1,4,5] Genomic imprinting of *UBE3A* leads to silencing of the paternal allele in neurons and thus loss of UBE3A expression when the maternal allele is mutated or deleted. Additional investigations are necessary to understand how neuronal loss of UBE3A in AS contributes to the onset and severity of epilepsy. Seizures and interictal epileptiform activity may pose a significant risk to neurological development.[6-8] Seizures are often severe and may be pharmacoresistant,[9-11] therefore the search for improved antiepileptic drugs for AS continues to be of high importance.

EEGs have become part of the diagnostic workup for AS due to the early appearance of seizures. In addition, characteristic EEG patterns frequently emerge, including interictal epileptiform discharges and sustained regional high-amplitude rhythmic delta activity, which may be visually identified in EEG studies by visual inspection.[12,13] This qualitative interpretation of EEG events during standard clinical recordings does not translate into an objective outcome measure. Quantitative EEG

[1]Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA
[2]The Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA
[3]The Gordon and Mary Cain Pediatric Neurology Research Foundation Laboratories, Texas Children's Hospital, Houston, TX, USA
[4]Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, FL, USA
[5]Departments of Neuroscience and Neurology, Baylor College of Medicine, Houston, TX, USA

*Both the authors contributed equally as first authors.

Corresponding Author:
Anne E. Anderson, Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, 1250 Moursund Street, Suite 1225, Houston, TX 77030, USA.
Email: annea@bcm.edu

analysis (qEEG) has shown that levels of delta rhythmicity are elevated and serve as a reliable biomarker for AS.[14] Thus, quantitative EEG analysis may also be used as an outcome measure in clinical trials by tracking these changes.

qEEG measures such as spectral power analysis use a nonbiased signal processing method of decomposing raw EEG traces into discrete frequencies.[15] Power spectra provide an indirect measure of brain state or cognitive function and potentially reveal less overt, ongoing EEG abnormalities that may be overlooked during qualitative visual inspection that is commonly used in clinical EEG interpretations. qEEG is increasingly being applied in human studies, such as assessing cognitive function,[16-20] and as a predictor of drug responsiveness.[21] EEG spectral power has also been considered as a marker of function and disease severity in Rett syndrome.[22] In preclinical studies, qEEG analysis has been used with animal models of AS[14,23] revealing increased total and delta EEG spectral power, and similar results have been found when evaluating EEG recordings from humans with AS.[14,24] We retrospectively analyzed spectral power from EEG recordings collected from young AS patients during a previous prospective clinical trial evaluating safety and tolerability of minocycline (MC) that found improvements in speech, motor, and cognitive function.[25] In this prior study, visual inspection with qualitative evaluation of the EEG was performed, however, the effect of MC on abnormal EEG activity in AS was not quantified using qEEG techniques. MC is a tetracycline antibiotic with reported antiseizure and neuroprotective properties.[26-29] During the previous study, the Bayley Scales of Infant Development (BSID III) test was administered and EEG activity was recorded at 3 time points: baseline prior to MC treatment (AS-T1), the end of the 8-week treatment (AS-T2), and 8 weeks after discontinuing treatment (AS-T3). For all time points, in the prior study EEGs were qualitatively scored by assessing patterns commonly observed in AS and other abnormal characteristics. Using this qualitative system, Grieco et al[25] found moderate decreases in abnormal EEG activity at AS-T2 and AS-T3 compared with AS-T1; however, these changes were not significant. Given the improvements in BSID III test scores following treatment, we were interested in determining whether latent parallel changes in background EEG activity could be identified at this same time point using a quantitative approach.

In our study, we first compared EEG power spectra in a separate cohort from the drug study using EEG recordings from AS and age-matched neurotypical children. We then performed retrospective analysis of the separate cohort of AS patients in the MC study to test the hypothesis that the abnormally increased total and spectral power in AS were globally reduced in the EEG after MC treatment, with particular attention to the delta frequency band. This was an open label study without control subjects. Epileptiform events were analyzed prior to and following MC treatment across all brain regions to determine whether event frequency was reduced with treatment. This retrospective study supports the potential utility of qEEG analysis for assessing abnormal EEG activity as a biomarker and outcome measure in clinical trials for AS.

Methods

Study Design

qEEG analysis was performed on recordings previously acquired during EEG evaluation (neurotypical comparison with AS) or a clinical open label non-placebo controlled trial assessing safety and tolerability of MC treatment in children with AS that previously had been scored by visual assessment for the presence of abnormal EEG characteristics including abnormal rhythmic delta and theta as well as epileptiform events, with increased point values assigned to more severe abnormalities.[25] Deidentified EEG data were analyzed retrospectively in accordance with Institutional Reveiw Board (IRB) guidelines at Baylor College of Medicine/Texas Children's Hospital and University of South Florida. For the MC study, written informed consent was obtained from the parents of each participant.

Data Sources

Neurotypical Comparison With AS. EEG recordings lasting 30 to 60 minutes were collected at Texas Children's Hospital in Houston, TX from 6 neurotypical patients (3 males, 3 females; 5-15 years of age; average, 8.3 years; median, 8 yeas) who underwent EEG evaluation for nonseizure-related reasons and 4 patients with AS (2 male, 2 females; 6-11 years of age; average, 8.8 years; median, 9.1 years) (Figure 1). The neurotypical and AS patients were then retrospectively analyzed in parallel and neither group was part of the MC study described below.

MC Treatment. EEG records were collected at the University of South Florida during the open-label clinical study: *Minocycline in the Treatment of Angelman Syndrome* (ClinicalTrials.gov identifier: NCT01531582).[25] This study consisted of 25 children with AS (confirmed through molecular testing) at 4 to 12 years of age (average age 8.3 years; median age 8 years) treated with MC taken orally once daily for 8 weeks (3 mg/kg/d). EEG recordings lasting 30 to 60 minutes were collected at 3 time points: at baseline prior to MC treatment (AS-T1), at the end of the 8-week MC treatment regimen (AS-T2), and after a washout period of 8 weeks (AS-T3). EEGs from 21 subjects (12 male, 9 female) who completed the MC treatment were analyzed for our retrospective study with 4 subjects omitted due to excessive noise or incomplete EEG records. In the initial recruitment of participants for the study, inclusion criteria included molecular confirmation of AS and at least moderate severity of symptoms as indicated by a Clinical Global Impression–Severity score of 4 or greater. Exclusion criteria included severe or uncontrolled seizures, or medical complications: cardiovascular, respiratory, liver, kidney, or hematologic disease, or any history of systemic lupus erythematosus.[25] As is typical in AS, prescribed antiepileptic drugs varied between patients with AS; however, each was maintained on the same medication throughout the entirety of the study. Patients with AS were nonverbal, but their caregivers provided a meal regimen, therefore EEGs were conducted between meals to minimize anxiety related to hunger. EEG

sessions occurred between the hours of 8 AM and 11 AM in the presence of a parent or caregiver.

Data Acquisition and Preprocessing

All EEGs used for the present study were collected using the standard 10/20 electrode placement system (Figure 1A). EEGs were acquired using the Xltek (MC) or Nicolet (neurotypical [NT] vs AS) systems at sampling rates of 200 Hz (MC) or 500 Hz (NT vs AS), respectively. EEG recordings were made from bilateral electrodes placed in the frontal (Fp1, Fp2, F3, and F4), central (C3 and C4), temporal (T7 and T8), parietal (P3 and P4), and occipital (O1 and O2) regions of the brain according to the international 10-20 system. Prior to analysis using quantitative EEG techniques, EEG activity was referenced to the respective ipsilateral ear reference electrode (A1 or A2), band-pass filtered from 0.5 to 70 Hz, and visually inspected to identify and remove artifacts.

Spectral Analysis

Spectral power analysis was performed to quantitatively compare baseline EEG activity between the following two separate cohorts of subjects: (1) non-MC study AS and age-matched neurotypical subjects (NT vs AS) and (2) the AS subjects across the MC study treatment time points (AS-T1, AS-T2, and AS-T3). The processed EEG activity was analyzed using LabChart V8 software (AD Instruments).[23,30] From each recording, three 2-minute artifact-free epochs were selected at random during wakefulness and eyes open. EEG activity during sleep was not used for analysis, as insufficient sleep epochs were captured during study recordings. For each epoch, activity was converted to component frequencies using fast Fourier transform (FFT) analysis with an FFT size of 512 (NT vs AS comparison) or 256 (AS MC study subjects) using the Welch method with 50% window overlap.[15,31,32] For each electrode, the 3 epochs from each recording were averaged. The average values from individual electrodes were then averaged for each regional group of electrodes as listed above (frontal, central, temporal, parietal, and occipital).[14] For each region, total power was summed from 0.5 to 50 Hz and subdivided into frequency bands: delta (0.5-3 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-29 Hz), and gamma (30-50 Hz).

Epileptiform Activity Analysis

The above EEG recordings used for spectral analysis were also scanned for automated epileptiform event detection using LabChart V8 software.[30] Epileptiform events were identified in electrodes from each brain recording region (central, C3/C4; parietal, P3/P4; occipital, O1/O2; temporal, T7/T8; frontal, Fp1/Fp2; F3/F4) using the spike histogram function with criteria for waveforms of negative polarity $\geq$200 μV and within 60 ms in duration from the middle of the peak, and the frequency was reported as epileptiform events per minute. Epileptiform activity identified in our study included isolated spikes, spike-wave discharges, sharp-wave complexes, or polyspike bursts with amplitudes of 2.5$\times$ background activity.

Statistical Analysis

Statistical analyses were carried out with GraphPad Prism software 7. Data were tested for normality using the D'Agostino-Pearson's or Shapiro-Wilk test. Total EEG power and summed frequency bands from each of the averaged brain regions (frontal, temporal, etc) were compared between the neurotypical and AS groups using an unpaired Student's *t* test or a nonparametric Mann-Whitney test for groups that deviated from a normal distribution. We compared EEG spectral power between groups (neurotypical vs AS) using a 2-way analysis of variance (ANOVA) with phenotype and frequency as factors; we used Sidak's tests to make post hoc comparisons across 1-Hz frequency bins. For the MC study, total EEG power across the 3 time points were compared using the non-parametric Friedman test for repeated measures with Dunn's post hoc tests. Power spectra was analyzed by repeated measures 2-way ANOVA with Tukey's post hoc comparisons across 1 Hz frequency bins. The effect size for comparisons was assessed using Cohen's *d* corrected for sample size (AS-T1 compared with AS-T3). Data are presented as mean $\pm$ standard error of the mean (SEM); *$P < .05$, **$P < .01$, ***$P < .001$.

Results

Globally Increased EEG Spectral Power Is Present in AS Compared to Neurotypical Control Subjects

Previous reports have shown excess EEG delta power in AS relative to age-matched neurotypical subjects.[14,24] We corroborated this finding using spectral power analysis to quantify EEG activity recorded from an AS group from our clinical population in comparison to activity from a group of age- and recording site-matched neurotypical controls. Side by side comparison of representative EEG activity between a neurotypical subject and an AS subject (Figure 1B) demonstrate stark differences in signal amplitudes and prolonged runs of delta activity commonly encountered in AS across different brain regions. Runs of high amplitude delta activity in the 2- to 3-Hz range are a prominent feature in AS EEGs. However, even in the absence of obvious high-amplitude 2- to 3-Hz activity, the delta rhythm often predominated the background EEG during these short recordings (Supplemental Figure 1). Subsequent qEEG analysis revealed significantly higher total EEG power in the AS group compared with the neurotypical group in all regions ($P < .05$; Figure 1C).

The regional averaged spectral power values were broken down into component frequencies and summed frequency bands. In line with the known characteristics of AS EEG patterns,[14,24] significantly increased delta power was found in the frontal, temporal, parietal, and occipital regions analyzed for AS compared with the neurotypical group ($P < .05$; Figure

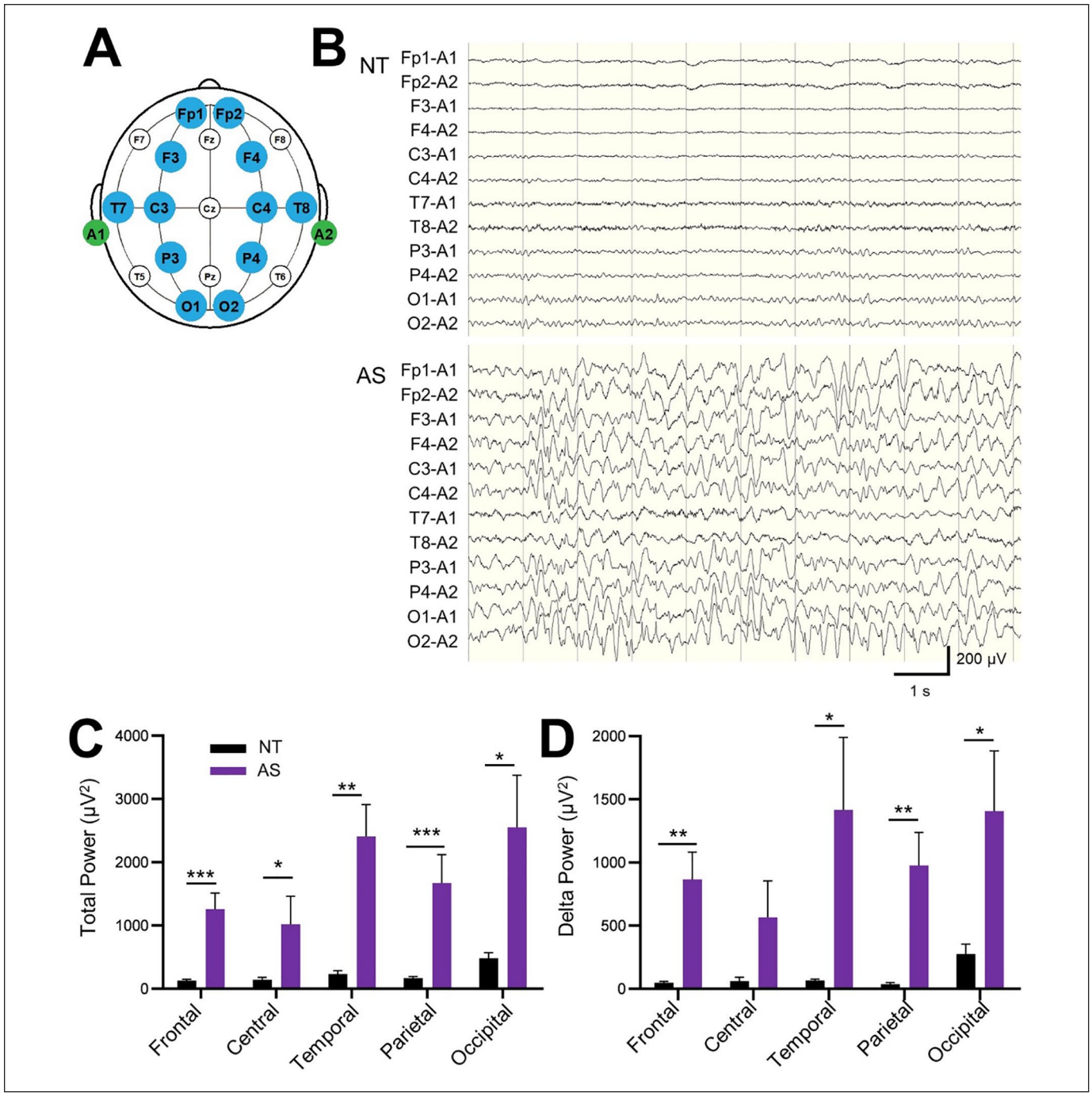

Figure 1. Qualitative and quantitative differences in baseline Angelman syndrome (AS) compared with neurotypical (NT) subjects. (A) Montage showing standard placement of scalp EEG electrodes that were used for qEEG analysis (filled blue) and reference electrodes (filled green). Each channel was referenced to its ipsilateral ear reference (eg, T7-A1, T8-A2). (B) Representative EEG traces from age-matched NT and AS subjects showing prolonged runs of high-amplitude 2 to 3 Hz activity in AS. (C) Total EEG power is increased in the AS group compared with age-matched NT group. (D) All regions except central show significantly increased EEG power in the summed delta frequencies in AS.

1D, Supplemental Figure 2). When comparing neurotypical with AS across individual frequencies, the AS group showed significant increases in all regions ($P < .001$) and post hoc differences in delta and theta frequencies (frontal, $P < .001$ for 1-4 Hz; central, $P < .001$ for 1-3 Hz, $P < .05$ for 5-6 Hz; temporal, $P < .001$ for 1-3 Hz; parietal, $P < .001$ for 1-6 Hz; occipital, $P < .001$ for 1-6 Hz; Figure 1D, Supplemental Figure 2).

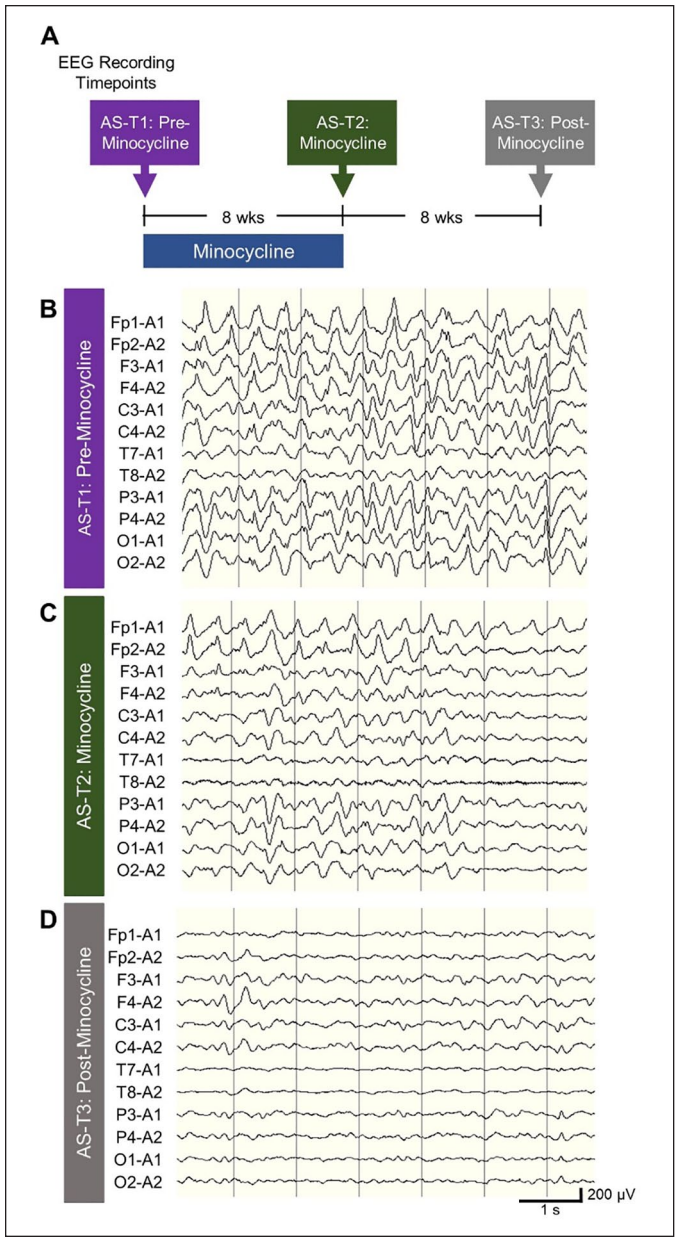

Figure 2. Representative EEG traces from an Angelman syndrome (AS) subject over the course of the minocycline (MC) study. (A) Diagram outlining the MC treatment study in participants with AS. (B) EEG activity at baseline prior to the start of MC treatment (AS-T1), (C) at the end of 8 weeks of MC treatment, and (D) at 8 weeks after the end of MC treatment. Following the same subject across the 3 time points demonstrates a progressive decrease in signal amplitude and 2- to 3-Hz activity.

Effect of MC Treatment on EEG Spectral Power in AS

We analyzed spectral power in EEGs that were collected at 3 time points during the clinical trial (Figure 2). No significant differences in total EEG power were found between AS-T1 and AS-T2 ($P > .05$, Figure 3A). However, nearly all brain regions displayed significant reductions in total EEG power when comparing AS-T1 and AS-T3 (frontal, $P = .0036$, Cohen's $d = 0.54$; central, $P = .041$, Cohen's $d = 0.60$; temporal, $P = .010$, Cohen's $d = 0.65$; parietal, $P = .0061$, Cohen's $d = 0.54$; Figure 3A). Total power at AS-T3 was also reduced compared to AS-T2 in the temporal ($P = .026$; Cohen's $d = 0.66$) and parietal ($P = .041$; Cohen's $d = 0.50$) regions (Figure 3A).

Next, we examined whether delta frequencies, which show excess power at baseline in AS were reduced with MC treatment. Frontal, central, temporal, and parietal regions exhibited significant reductions in AS-T3 summed delta band power compared to AS-T1 (frontal, $P = .0021$, Cohen's $d = 0.49$; central, $P = .026$, Cohen's $d = 0.63$; temporal, $P = .026$, Cohen's $d = 0.76$; parietal, $P = .0036$, Cohen's $d = 0.66$; Figure 3 and Table 1). Comparisons at 1-Hz frequency bins between baseline, MC treatment, and washout following MC treatment showed significant decreases at AS-T3 in all regions due to treatment ($P < .001$) and post hoc differences in delta frequencies (Supplemental Figure 3). To further investigate changes in EEG power spectra following treatment, we evaluated whether reductions in power were present in frequencies analyzed outside the delta range. The frontal and parietal regions exhibited reductions in summed theta power at AS-T3 compared to AS-T1 (frontal, $P = .026$, Cohen's $d = 0.40$; parietal, $P = .016$, Cohen's $d = 0.22$; Figure 3B and E). Additional reductions were found in alpha (frontal, $P > .05$, Cohen's $d = 0.61$; central, $P = .010$, Cohen's $d = 0.57$; temporal, $P > .05$, Cohen's $d = 0.63$; parietal, $P = .0061$, Cohen's $d = 0.54$; Figure 3B-E), beta (frontal, $P > .05$, Cohen's $d = 0.63$; central, $P = .0061$, Cohen's $d = 0.61$; temporal, $P = .026$, Cohen's $d = 0.34$; parietal, $P = .0012$, Cohen's $d = 0.64$; occipital, $P = .0012$, Cohen's $d = 0.61$; Figure 3B-F), and gamma (parietal, $P > .05$, Cohen's $d = 0.68$; occipital, $P = .026$, Cohen's $d = 0.76$; Figure 3E and F) frequency band power when comparing AS-T3 with AS-T1.

Effect of MC Treatment on Epileptiform Events Associated With AS

Epileptiform events in the form of isolated spikes, spike-wave discharges, and bursts of spikes with an increased amplitude compared to background EEG activity were collectively quantified at all three time points within each region (Figure 4A). Frontal (Fp2) and occipital (O1) areas exhibited significant reductions in epileptiform events at AS-T3, while similar effect sizes were seen across all brain regions (Fp1–AS-T1: 4.95 ± 2.62 compared to AS-T3: 1.71 ± 0.92, Cohen's $d = 0.36$; Fp2–AS-T1: 4.25 ± 2.53 compared to AS-T3: 1.64 ± 0.90, $P = .025$, Cohen's $d = 0.37$; C3–AS-T1: 16.70 ± 7.24 compared to AS-T3: 8.00 ± 2.87, Cohen's $d = 0.33$; C4–AS-T1: 16.40 ± 6.20 compared to AS-T3: 6.17 ± 2.39, Cohen's $d = 0.46$; T7–AS-T1: 19.21 ± 6.32 compared to AS-T3: 6.84 ± 2.10, Cohen's $d = 0.56$; T8–AS-T1: 15.65 ± 5.10 compared to AS-T3: 6.59 ± 2.07, Cohen's $d = 0.49$; P3–AS-T1: 21.28 ± 6.32 compared to AS-T3: 6.84 ± 2.10, Cohen's $d = 0.43$; P4–AS-T1: 21.15 ± 6.35 compared to AS-T3: 9.85 ± 3.30, Cohen's $d = 0.47$; O1–AS-T1: 26.05 ± 6.82 compared to AS-T3: 15.42 ± 4.43, $P = .045$, Cohen's $d = 0.39$; O2–AS-T1: 28.48 ± 6.55 compared to AS-T3: 14.07 ± 3.82, Cohen's $d = 0.57$; Figure 4B and C).

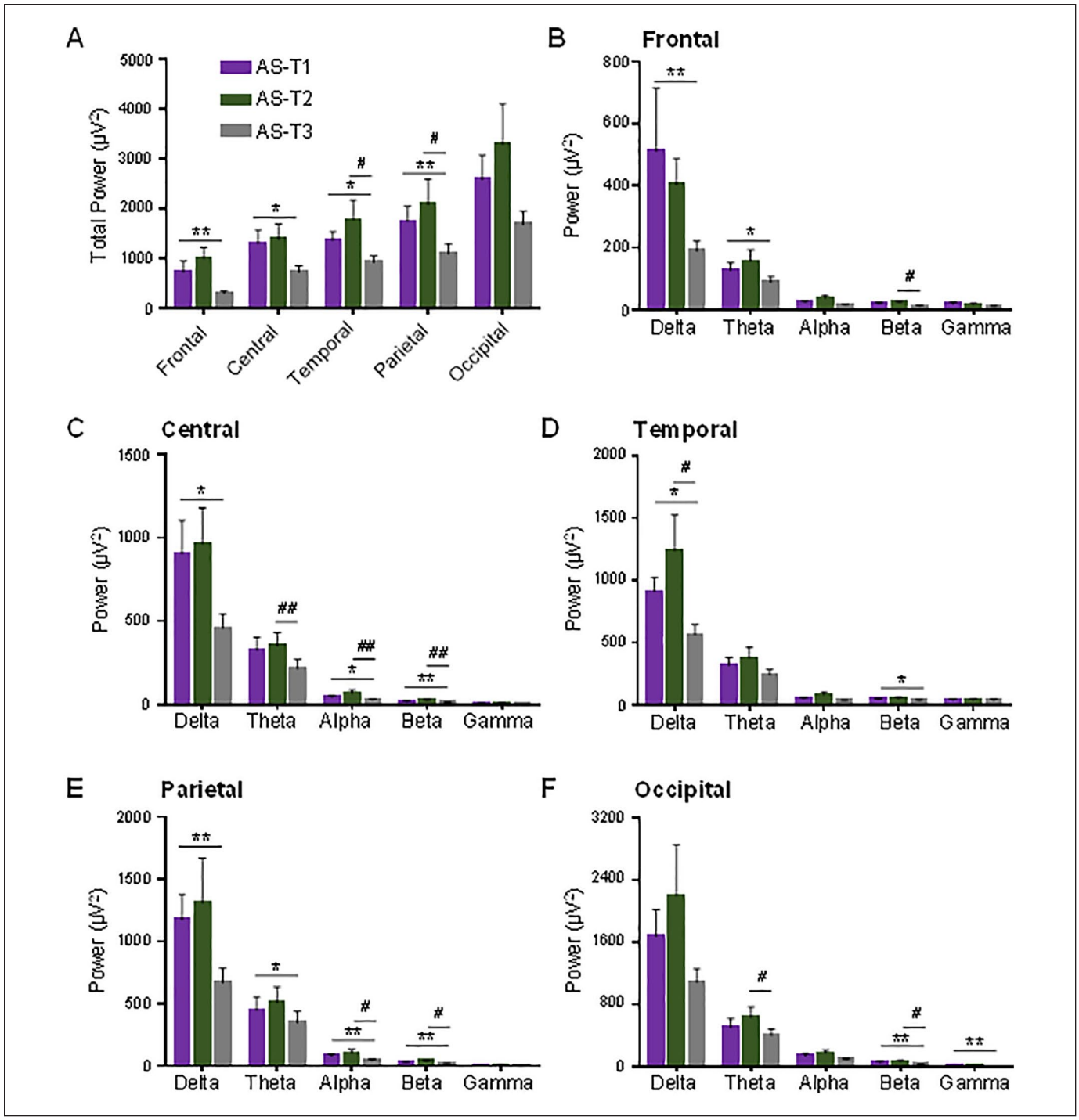

Figure 3. Comparison of total and spectral power in Angelman syndrome (AS) throughout the minocycline (MC) treatment study. (A) Total EEG power at the end of the 8-week MC treatment (AS-T2) is not significantly different compared to baseline activity (AS-T1). However, total power is reduced in most brain regions evaluated after the 8-week washout period (AS-T3). (B) Frontal, (C) central, (D) temporal, and (E) parietal regions exhibit significant reductions in EEG power in the delta frequency band, while (F) no significant difference was found in the occipital region. $^{*}P < .05$, $^{**}P < .01$ AS-T1 versus AS-T3; $^{\#}P < .05$, $^{\#\#}P < .01$ AS-T2 versus AS-T3.

Discussion

Early reports in AS described abnormal EEG patterns with high-amplitude delta activity.[13,33-37] As an outcome measure in the clinical trial examined here, the original study reported the EEG findings based on qualitative scores obtained by visual inspection for electrographic features of AS.[25] In our study, we applied qEEG analysis, removing the qualitative aspect of EEG review. Comparisons between pre- and post treatment power spectra showed significantly reduced abnormal background

Table 1. Regional Averages of EEG Total and Spectral Frequency Power in Subjects With Angelman Syndrome (AS) at the Different Treatment Time Points.[a]

	Region	Total (μV^2)	Delta (μV^2)	Theta (μV^2)	Alpha (μV^2)	Beta (μV^2)	Gamma (μV^2)
AS-T1	Frontal	698.99 ± 215.60	511.43 ± 201.81	126.65 ± 24.15	23.33 ± 3.74	19.12 ± 3.69	18.46 ± 6.23
	Central	1309.35 ± 269.84	905.21 ± 201.12	326.84 ± 80.27	48.13 ± 8.98	21.19 ± 3.65	7.98 ± 2.68
	Temporal	1369.39 ± 172.98	910.65 ± 114.14	318.47 ± 62.81	53.10 ± 8.28	46.08 ± 6.97	41.08 ± 8.37
	Parietal	1742.19 ± 310.29	1175.31 ± 204.72	449.23 ± 105.92	79.51 ± 14.96	31.32 ± 4.88	6.82 ± 1.58
	Occipital	2388.57 ± 493.86	1674.35 ± 343.45	506.61 ± 116.39	139.70 ± 33.15	57.87 ± 13.06	10.05 ± 2.51
AS-T2	Frontal	632.22 ± 129.06	402.75 ± 84.15	155.20 ± 37.14	36.66 ± 9.90	22.17 ± 4.09	15.43 ± 5.02
	Central	1420.45 ± 311.39	965.5 ± 215.39	352.18 ± 80.85	69.20 ± 20.17	25.08 ± 5.24	8.48 ± 3.96
	Temporal	1791.97 ± 392.03	1239.35 ± 287.10	376.58 ± 88.05	81.97 ± 23.72	53.38 ± 10.87	40.70 ± 11.49
	Parietal	1970.74 ± 505.68	1310.19 ± 359.63	509.41 ± 128.10	104.55 ± 29.74	38.44 ± 8.94	8.14 ± 3.75
	Occipital	3082.15 ± 819.81	2196.90 ± 658.02	634.53 ± 133.64	174.47 ± 42.49	68.78 ± 18.82	7.46 ± 1.74
AS-T3	Frontal	311.07 ± 44.24**	188.22 ± 31.53**	88.32 ± 17.58*	14.79 ± 2.15	10.72 ± 1.77#	9.01 ± 2.59
	Central	721.65 ± 134.61*	458.52 ± 87.82*	216.89 ± 55.26##	29.45 ± 4.83*,##	12.98 ± 1.98**,##	3.79 ± 1.22
	Temporal	908.38 ± 133.49*,#	564.42 ± 81.57*,#	241.59 ± 45.25	33.82 ± 4.63	33.70 ± 8.90*	34.85 ± 13.82
	Parietal	1089.14 ± 204.14**,#	669.13 ± 118.38**	346.51 ± 94.02*	50.60 ± 7.28**,#	19.79 ± 2.73**,#	3.10 ± 0.56
	Occipital	1615.57 ± 248.65	1086.34 ± 173.20	401.86 ± 82.87#	92.31 ± 11.51	31.32 ± 3.36**,#	3.74 ± 0.48**

[a]Summary of the averaged values for each brain region for the AS group at baseline prior to (AS-T1), after (AS-T2), and following the washout period (AS-T3) with minocycline (MC) treatment for the delta (0.5-3 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-29 Hz), and gamma (30-50 Hz) frequency bands. Values are mean ± SEM. Differences are indicated for comparisons between the AS groups.
*$P < .05$, **$P < .01$ (AS-T1 compared to AS-T3); #$P < .05$, ##$P < .01$ (AS-T2 compared to AS-T3).

EEG power across all brain areas, with significant decreases in the delta frequency band. The pattern of changes in delta power at AS-T3, with no significant difference between AS-T1 and AS-T2, parallels the scores in auditory comprehension, total language ability, fine motor ability, and communication that were significantly improved at AS-T3 (reported in the initial clinical study).[25] The underlying mechanism of action of MC is not completely understood. Neuroinflammation has been linked to epilepsy, and seizures can provoke the release of inflammatory factors, thus potentially establishing an ongoing vicious cycle.[38] MC has been suggested to exhibit neuroprotective properties in the context of epilepsy due to its anti-inflammatory activity[26,39] and may also protect synapses by inhibiting matrix metalloproteinaise-9 (MMP-9). In patients with fragile X syndrome (FXS), MC attenuated EEG amplitude in the temporal cortex.[40] In mouse models of FXS, MC reduced audiogenic seizure severity, which has been hypothesized to result from inhibition of MMPs[41] or promotion of dendritic spine maturation.[42,43] Furthermore, Grieco et al[25] showed that a mouse model of AS treated with MC exhibited rescue of deficits in hippocampal long-term potentiation, a synaptic mechanism reported to underlie learning and memory. It is unclear if EEG changes would have progressed similarly if treatment had been continued beyond 8 weeks or if reduced EEG power was a delayed effect resulting from MC withdrawal. Follow-up assessment of EEG power and behavior in AS subjects beyond the washout period potentially would be beneficial to determine if the observed improvements are maintained in the absence of MC treatment. Preclinical studies have demonstrated sustained effects after discontinued MC treatment. For example, MC may induce a sustained suppression in cytokine gene expression in the brain after irradiation exposure or improved neurological function following stroke after 30 days MC withdrawal.[44,45] Similarly, the delayed effects observed here at AS-T3 may result from ongoing changes in gene expression beyond AS-T2. Given that excess delta power has been previously identified in EEG studies with AS subjects and linked to the loss of functional *UBE3A*,[14,24] the reduction in abnormal delta power found at AS-T3 in parallel with improved BSID III scores supports the use of qEEG analysis as a quantifiable and translational biomarker for evaluating potential therapeutics in future studies. BSID III scores were not available for these analyses to examine a statistical correlation with the changes in EEG delta power.

Spike frequency, a form of interictal epileptiform activity that reflects neuronal network dysfunction with epileptogenic potential[46,47] was also reduced in association with washout following MC treatment. We were able to quantify epileptiform events across multiple brain regions, and found significant differences in some, but not all brain regions analyzed (Figure 4), while in the initial clinical trial, Grieco et al[25] found improvements in EEG scores, but these changes did not reach statistical significance. Our approach differed by analyzing brain regions individually with qEEG techniques compared to the more global visual scoring used in the original reports for this clinical study. Although not performed in the original MC trial, any future studies evaluating MC treatment in AS should have a larger sample size, placebo, and neurotypical control groups.

There are inherent confounds in the original study that could not be controlled for in these subsequent analyses. Treatment of AS seizures often requires multiple antiepileptic drugs, and medications taken concurrently with MC treatment were not considered in the initial study design, although over the course of the study no changes in antiepileptic drug regimens were made, so it is unlikely they contributed to the reported EEG changes. Grieco et al[25] also noted that 21 of the participants with AS were of maternal deletion subtype, which is associated with more severe epilepsy in AS; however, phenotypic

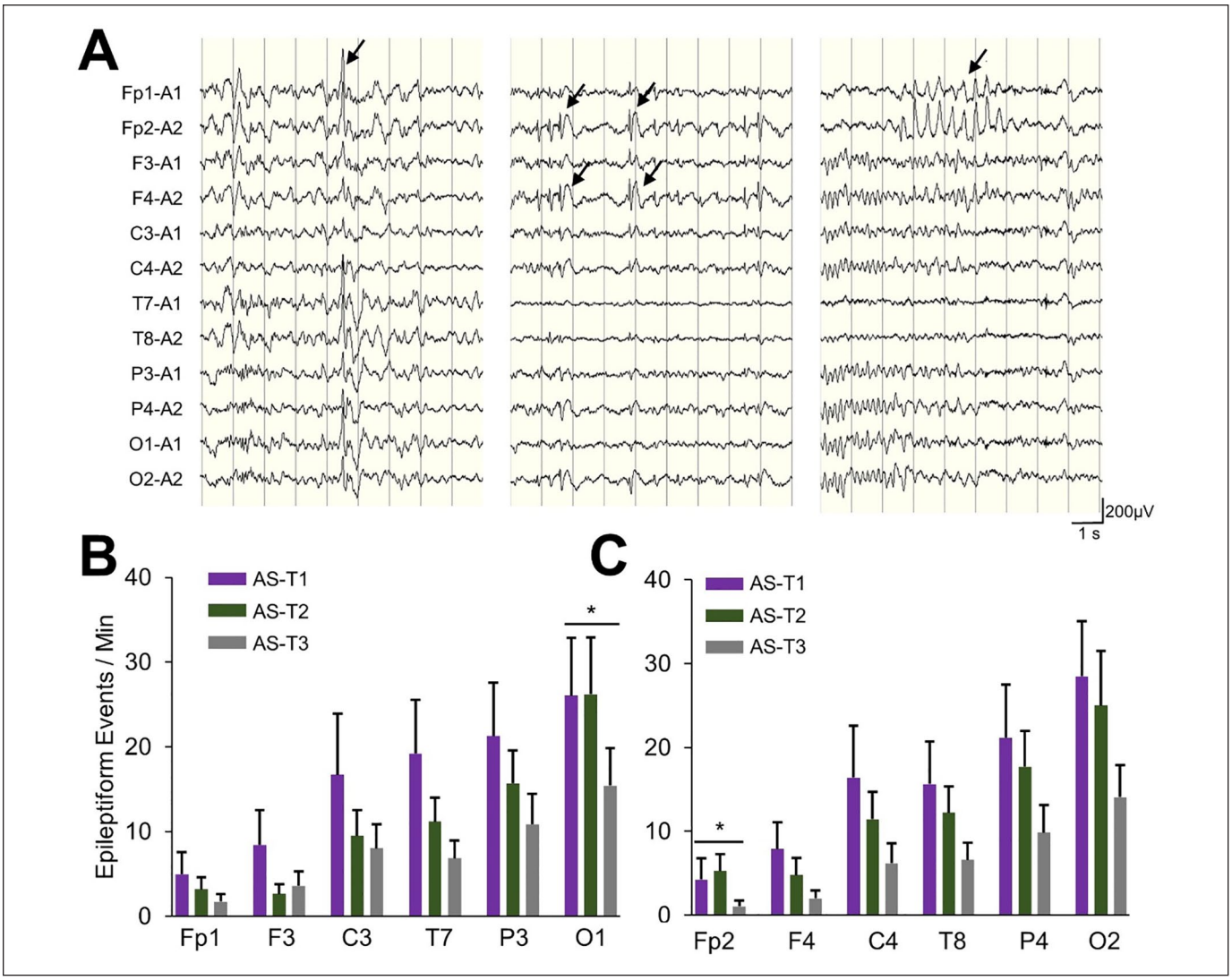

Figure 4. Effect of minocycline (MC) treatment on epileptiform events. (A) Representative EEG traces from AS subjects showing examples of epileptiform events (arrows; isolated spikes, spike-wave discharges, spike bursts). Epileptiform events were quantified in (B) left hemisphere regions and (C) right hemisphere regions. The frequency of epileptiform events was significantly reduced in O1 and Fp2 regions at AS-T3, while the decreases in the O2, Fp1, central, temporal, and parietal regions were not statistically significant. *$P < .05$.

differences occur within this group depending on the type of breakpoint.[48,49] While this retrospective study was unable to correlate genotype with specific EEG phenotypic expression or the extent of EEG changes following drug treatment, recent work highlighted a more severe EEG phenotype in those with deletion versus nondeletion type AS.[24] Another limitation of the MC study is the lack of an AS placebo group or parallel neurotypical groups. Such a comparison could increase the validity of these results. However, the study we have performed was a retrospective analysis of the EEG recordings already obtained during the previous clinical study, which was a prospective open-label trial evaluating the safety and tolerability of MC and thus was designed without neurotypical or placebo controls.

It is uncertain what correlation elevated EEG power may have with other AS-related phenotypes; however, targeting the normalization of EEG total and spectral power in AS may be a viable strategy for improving neuronal network function and an important metric for screening treatment strategies to gauge efficacy. A similar qEEG approach can also be applied in preclinical studies to screen novel therapeutics with the potential for translation, since animal models of AS exhibit similar EEG abnormalities as those seen in AS humans, including high delta power and epileptiform events[14,23] and increased susceptibility to seizures.[23,50-52] To promote the transition of novel therapeutic compounds from animal studies to clinical trials, it is critical to establish quantifiable and consistent biomarkers and outcome measures that can better evaluate treatment efficacy. Furthermore, although studies are consistently revealing increased delta power in AS EEGs, to reduce intra-/intersubject variability it will be necessary to establish qEEG analysis standards that can be applied across different acquisition

environments and which can be easily implemented during clinical trials. Overall, our findings support and extend previous work that has identified increased total, delta, and theta power as characteristic of AS EEG activity and highlight the potential use of qEEG analysis as a diagnostic biomarker or outcome measure in clinical trials.

Author Contributions

LAM contributed to conception and design; contributed to analysis and interpretation; drafted manuscript; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. HAB contributed to conception and design; contributed to analysis and interpretation; drafted manuscript; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. SH contributed to analysis; critically revised manuscript; and gave final approval. AR contributed to analysis; critically revised manuscript; and gave final approval. JCG contributed to design; contributed to acquisition; critically revised manuscript; and gave final approval. EJW contributed to design; contributed to acquisition; critically revised manuscript; and gave final approval. AEA contributed to conception and design; contributed to interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Luis A. Martinez https://orcid.org/0000-0002-0771-9726
Heather A. Born https://orcid.org/0000-0001-9934-648X
Anne E. Anderson https://orcid.org/0000-0001-7639-2504

Supplemental Material

Supplemental material for this article is available online.

References

1. Clayton-Smith J, Laan L. Angelman syndrome: a review of the clinical and genetic aspects. *J Med Genet*. 2003;40:87-95.
2. Williams CA, Driscoll DJ, Dagli AI. Clinical and genetic aspects of Angelman syndrome. *Genet Med*. 2010;12:385-395.
3. Thibert RL, Larson AM, Hsieh DT, Raby AR, Thiele EA. Neurologic manifestations of Angelman syndrome. *Pediatr Neurol*. 2013;48:271-279.
4. Chamberlain SJ, Lalande M. Angelman syndrome, a genomic imprinting disorder of the brain. *J Neurosci*. 2010;30:9958-9963.
5. Tomaić V, Banks L. Angelman syndrome-associated ubiquitin ligase UBE3A/E6AP mutants interfere with the proteolytic activity of the proteasome. *Cell Death Dis*. 2015;6:e1625.
6. Ibrahim GM, Cassel D, Morgan BR, et al. Resilience of developing brain networks to interictal epileptiform discharges is associated with cognitive outcome. *Brain*. 2014;137(pt 10):2690-2702.
7. Holmes GL. Effect of seizures on the developing brain and cognition. *Semin Pediatr Neurol*. 2016;23:120-126.
8. Ung H, Cazares C, Nanivadekar A, et al. Interictal epileptiform activity outside the seizure onset zone impacts cognition. *Brain*. 2017;140:2157-2168.
9. Kuenzle C, Steinlin M, Wohlrab G, Boltshauser E, Schmitt B. Adverse effects of vigabatrin in Angelman syndrome. *Epilepsia*. 1998;39:1213-1215.
10. Østergaard, John R, Balslev T. Efficacy of different antiepileptic drugs in children with Angelman syndrome. *Dev Med Child Neurol*. 2001;43:718-719.
11. Park SH, Yoon JR, Kim HD, Lee JS, Lee YM, Kang HC. Epilepsy in Korean patients with Angelman syndrome. *Korean J Pediatr*. 2012;55:171-176.
12. Boyd SG, Harden A, Patton MA. The EEG in early diagnosis of the Angelman (happy puppet) syndrome. *Eur J Pediatr*. 1988;147:508-513.
13. Vendrame M, Loddenkemper T, Zarowski M, et al. Analysis of EEG patterns and genotypes in patients with Angelman syndrome. *Epilepsy Behav*. 2012;23:261-265.
14. Sidorov MS, Deck GM, Dolatshahi M, et al. Delta rhythmicity is a reliable EEG biomarker in Angelman syndrome: a parallel mouse and human analysis. *J Neurodev Disord*. 2017;9:17.
15. Welch P. The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms. *IEEE Trans Audio Electroacoust*. 1967;15:70-73.
16. Fonseca LC, Tedrus GMAS, Chiodi MG, Cerqueira JN, Tonelotto JMF. Quantitative EEG in children with learning disabilities: analysis of band power. *Arq Neuropsiquiatr*. 2006;64(2B):376-381.
17. Franko E, Wehner T, Joly O, et al. Quantitative EEG parameters correlate with the progression of human prion diseases. *J Neurol Neurosurg Psychiatry*. 2016;87:1061-1067.
18. Leuchter MK, Donzis EJ, Cepeda C, et al. Quantitative electroencephalographic biomarkers in preclinical and human studies of Huntington's disease: are they fit-for-purpose for treatment development? *Front Neurol*. 2017;8:91.
19. de Medeiros Kanda PA, Anghinah R, Smidth MT, Silva JM. The clinical use of quantitative EEG in cognitive disorders. *Dement Neuropsychol*. 2009;3:195-203.
20. Bjørk MH, Sand T, Bråthen G, et al. Quantitative EEG findings in patients with acute, brief depression combined with other fluctuating psychiatric symptoms: a controlled study from an acute psychiatric department. *BMC Psychiatry*. 2008;8:89.
21. Chiarenza GA, Chabot R, Isenhart R, et al. The quantified EEG characteristics of responders and non-responders to long-term treatment with atomoxetine in children with attention deficit hyperactivity disorders. *Int J Psychophysiol*. 2016;104:44-52.
22. Roche KJ, LeBlanc JJ, Levin AR, et al. Electroencephalographic spectral power as a marker of cortical function and disease severity in girls with Rett syndrome. *J Neurodevelop Disord*. 2019;11:15. doi:10.1186/s11689-019-9275-z
23. Born HA, Dao AT, Levine AT, et al. Strain-dependence of the Angelman Syndrome phenotypes in Ube3a maternal deficiency mice. *Sci Rep*. 2017;7:8451.
24. Frohlich J, Miller MT, Bird LM, et al. Electrophysiological phenotype in Angelman syndrome differs between genotypes. *Biol Psychiatry*. 2019;85:752-759.

25. Grieco JC, Ciarlone SL, Gieron-Korthals M, et al. An open-label pilot trial of minocycline in children as a treatment for Angelman syndrome. *BMC Neurol*. 2014;14:232.
26. Plane JM, Shen Y, Pleasure DE, Deng W. Prospects for minocycline neuroprotection. *Arch Neurol*. 2010;67:1442-1448. doi:10.1001/archneurol.2010.191
27. Wang DD, Englot DJ, Garcia PA, Lawton MT, Young WL. Minocycline- and tetracycline-class antibiotics are protective against partial seizures in vivo. *Epilepsy Behav*. 2012;24:314-318.
28. Beheshti Nasr SM, Moghimi A, Mohammad-Zadeh M, Shamsizadeh A, Noorbakhsh SM. The effect of minocycline on seizures induced by amygdala kindling in rats. *Seizure*. 2013;22: 670-674.
29. Garrido-Mesa N, Zarzuelo A, Gálvez J. Minocycline: far beyond an antibiotic. *Br J Pharmacol*. 2013;169:337-352.
30. Nguyen LH, Brewster AL, Clark ME, et al. mTOR inhibition suppresses established epilepsy in a mouse model of cortical dysplasia. *Epilepsia*. 2015;56(4):636-646.
31. Frohlich J, Senturk D, Saravanapandian V, et al. A quantitative electrophysiological biomarker of duplication 15q11.2-q13.1 syndrome. *PLoS One*. 2016;11:e0167179. doi:10.1371/journal.pone.016717
32. den Bakker H, Sidorov MS, Fan Z, et al. Abnormal coherence and sleep composition in children with Angelman syndrome: a retrospective EEG study. *Mol Autism*. 2018;9:32. Published 2018 Apr 27. doi:10.1186/s13229-018-0214-8
33. Sandanam T, Beange H, Robson L, Woolnough H, Buchholz T, Smith A. Manifestations in institutionalised adults with Angelman syndrome due to deletion. *Am J Med Genet*. 1997;70(4):415-420.
34. Smith A, Wiles C, Haan E, et al. Clinical features in 27 patients with Angelman syndrome resulting from DNA deletion. *J Med Genet*. 1996;33(2):107-112. doi:10.1136/jmg.33.2.107
35. Laan LA, Renier WO, Arts WF, et al. Evolution of epilepsy and EEG findings in Angelman syndrome. *Epilepsia*. 1997;38:195-199.
36. Korff CM, Kelley KR, Nordli DR. Notched delta, phenotype, and Angelman syndrome. *J Clin Neurophysiol*. 2005;22:238-243.
37. Valente KD, Andrade JQ, Grossmann RM, et al. Angelman syndrome: difficulties in EEG pattern recognition and possible misinterpretations. *Epilepsia*. 2003;44:1051-1063.
38. Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. *Exp Neurol*. 2013;244:11-21.
39. Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol*. 2001;166:7527-7533.
40. Schneider A, Leigh MJ, Adams P, et al. Electrocortical changes associated with minocycline treatment in fragile X syndrome. *J Psychopharmacol*. 2013;27:956-963.
41. Dziembowska M, Pretto DI, Janusz A, et al. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. *Am J Med Genet A*. 2013;161A:1897-1903.
42. Bilousova T V, Dansie L, Ngo M, et al. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J Med Genet*. 2009;46: 94-102.
43. Dansie LE, Phommahaxay K, Okusanya AG, et al. Long-lasting effects of minocycline on behavior in young but not adult Fragile X mice. *Neuroscience*. 2013;246:186-198.
44. Mehrotra S, Pecaut MJ, Gridley DS. Minocycline modulates cytokine and gene expression profiles in the brain after whole-body exposure to radiation. *In Vivo*. 2014;28:21-32.
45. Xian NT, Wang Q, Koike MA, et al. Monitoring the protective effects of minocycline treatment with radiolabeled annexin v in an experimental model of focal cerebral ischemia. *J Nucl Med*. 2007;48:1822-1828.
46. Urrestarazu E, Jirsch JD, LeVan P, et al. High-frequency intracerebral EEG activity (100-500 Hz) following interictal spikes. *Epilepsia*. 2006;47:1465-1476.
47. Staley KJ, White A, Dudek FE. Interictal spikes: Harbingers or causes of epilepsy? *Neurosci Lett*. 2011;497:247-250.
48. Varela MC, Kok F, Otto PA, Koiffmann CP. Phenotypic variability in Angelman syndrome: comparison among different deletion classes and between deletion and UPD subjects. *Eur J Hum Genet*. 2004;12:987-992.
49. Valente KD, Varela MC, Koiffmann CP, et al. Angelman syndrome caused by deletion: a genotype-phenotype correlation determined by breakpoint. *Epilepsy Res*. 2013;105:234-239.
50. Jiang YH, Pan Y, Zhu L, et al. Altered ultrasonic vocalization and impaired learning and memory in Angelman syndrome mouse model with a large maternal deletion from Ube3a to Gabrb3. *PLoS One*. 2010;5:e12278.
51. Mandel-Brehm C, Salogiannis J, Dhamne SC, Rotenberg A, Greenberg ME. Seizure-like activity in a juvenile Angelman syndrome mouse model is attenuated by reducing Arc expression. *Proc Natl Acad Sci USA*. 2015;112:5129-5134.
52. Ciarlone SL, Wang X, Rogawski MA, Weeber EJ. Effects of the synthetic neurosteroid ganaxolone on seizure activity and behavioral deficits in an Angelman syndrome mouse model. *Neuropharmacology*. 2017;116:142-150.