Variations in EEG discharges predict ADHD severity within individual Smith-Lemli-Opitz patients

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ABSTRACT

Objective: We sought to examine the prevalence of EEG abnormalities in Smith-Lemli-Opitz syndrome (SLOS) as well as the relationship between interictal epileptiform discharges (IEDs) and within-subject variations in attentional symptom severity.

Methods: In the context of a clinical trial for SLOS, we performed cross-sectional and repeated-measure observational studies of the relationship between EEG findings and cognitive/behavioral factors on 23 children (aged 4–17 years). EEGs were reviewed for clinical abnormalities, including IEDs, by readers blinded to participants' behavioral symptoms. Between-group differences in baseline characteristics of participants with and without IEDs were analyzed. Within-subject analyses examined the association between the presence of IEDs and changes in attention-deficit/hyperactivity disorder (ADHD) symptoms.

Results: Of 85 EEGs, 43 (51%) were abnormal, predominantly because of IEDs. Only one subject had documented clinical seizures. IEDs clustered in 13 subjects (57%), whereas 9 subjects (39%) had EEGs consistently free of IEDs. While there were no significant group differences in sex, age, intellectual disability, language level, or baseline ADHD symptoms, autistic symptoms tended to be more prevalent in the "IED" group (according to Autism Diagnostic Observation Schedule–2 criteria). Within individuals, the presence of IEDs on a particular EEG predicted, on average, a 27% increase in ADHD symptom severity.

Conclusions: Epileptiform discharges are common in SLOS, despite a relatively low prevalence of epilepsy. Fluctuations in the presence of epileptiform discharges within individual children with a developmental disability syndrome may be associated with fluctuations in ADHD symptomatology, even in the absence of clinical seizures. ***Neurology*® 2014;83:151–159**

GLOSSARY

ADHD = attention-deficit/hyperactivity disorder; **ADI-R** = Autism Diagnostic Interview, revised; **ADOS** = Autism Diagnostic Observation Schedule; **AED** = antiepileptic drug; **ASD** = autism spectrum disorder; **CI** = confidence interval; **CPRS-R:L** = Conners Parent Rating Scale–Revised Long form; ***DSM-IV-TR*** = *Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision*; **IED** = interictal epileptiform discharge; **PPV** = positive predictive value; **SI** = Symptom Inventory; **SLOS** = Smith-Lemli-Opitz syndrome.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive syndrome caused by an inborn error of cholesterol biosynthesis, which may result in intellectual disability; attention-deficit/hyperactivity disorder (ADHD); autistic features; and brain, facial, and limb malformations. While seizures are uncommon,[1,2] EEG abnormalities have not been examined systematically.

As part of a clinical trial to assess the efficacy of simvastatin, an HMG CoA-reductase inhibitor, serial clinical EEGs, and neuropsychological testing were performed, thus providing a unique opportunity to examine the characteristics of EEG in SLOS, as well as the relationship between interictal epileptiform discharges (IEDs) and behavioral symptoms in a neurodevelopmental

Supplemental data at Neurology.org

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disorder. To date, direct evidence of an etiologic role of IEDs in cognitive/behavioral alterations is lacking, except in very specific instances.

The study of the relationship between IEDs and cognitive/behavioral performance in SLOS proceeded in 3 stages: (1) an examination of the pattern of occurrence of IEDs among individuals, (2) an examination of whether subjects with IEDs on any EEG differed in baseline cognitive or behavioral characteristics from subjects without IEDs, and (3) an examination of the relationship, within-subjects, of fluctuations in the occurrence of IEDs and in attentional symptom severity. To our knowledge, this is the first study examining the association between variability in both behavior and EEG findings within individuals with a developmental disability.

METHODS

Participants and study design. Participants included 23 individuals with SLOS aged between 4.0 and 17.5 years (table 1), enrolled in a double-blinded, placebo-controlled, crossover trial of the safety and efficacy of simvastatin. Diagnosis of SLOS was based on an elevated 7-dehydrocholesterol level and identification of 2 mutant alleles of the 7-dehydrocholesterol reductase gene. Exclusion criteria included age younger than 4 years or older than 18 years, weight <10 kg, SLOS severity score[1] >30, behavioral impairments that would interfere with blood collection, and contraindications to simvastatin. All participants were receiving treatment with cholesterol supplementation before study enrollment. The sample size was based on considerations for the clinical trial.

The study was conducted between 2004 and 2009 at the inpatient NIH Clinical Center in Bethesda, MD, and at the outpatient Psychiatry Clinic at Kennedy Krieger Institute in Baltimore, MD. Study arms included two 12-month treatment blocks: (1) cholesterol plus simvastatin, and (2) cholesterol plus placebo, separated by a 2-month washout period; study visits were at 5 time points: 0, 6, 12, 20, and 26 months (see table e-1 on the *Neurology*® Web site at Neurology.org). Of the 23 participants, 18 completed the trial; reasons for incompletion included noncompliance with study procedures (n = 3), illness unrelated to study enrollment (n = 1), and concern for statin-induced myopathy (n = 1). The full results of the clinical trial will be reported elsewhere (C.A.W., personal communication).

Standard protocol approvals, registrations, and patient consents. The study was approved by the institutional review boards of the NIH NICHD and the Office of Human Subjects Research at Johns Hopkins Medicine. Informed consent was obtained from all participants and/or their legal guardians before study enrollment. The clinical trial is registered at www.clinicaltrials.gov (NCT00064792).

EEG recording and interpretation. Routine clinical overnight EEGs (duration 5–7 hours) were performed at each study visit, using a NeuroScan system (Compumedics NeuroScan, Charlotte, NC) with typical clinical EEG recording parameters and a standard international 10-20 montage; in 19 recordings, a double-distance montage was used when the child's adverse behavior prevented the use of a full montage. Eighty-five EEGs were successfully recorded (1–5 EEGs per participant) (table e-1); 30 EEG results were not available because of subject withdrawal from the study (n = 15), technical difficulties (n = 7), and subject noncompliance (n = 8). EEG analysis consisted of standard clinical interpretation by a board-certified clinical neurophysiologist (J.B.E. or W.H.T.), with coding of focal or diffuse slowing, slowing of the posterior basic rhythm, and the presence of IEDs (sharp waves and spikes). Interrater reliability, using 6 EEGs, was assessed for J.B.E. with W.H.T. ($\kappa = 1$), and J.M.S. with J.B.E. ($\kappa = 1$). To assess the frequency of IEDs, spike count was calculated on all EEGs with IEDs by reviewing 100-second epochs during the awake state and N2 sleep.

Neuropsychological and behavioral measures. To categorize the severity of intellectual disability of each participant (table e-2), baseline adaptive and intellectual functioning was assessed using the Vineland Adaptive Behavior Scales Survey Form, second edition,[3] and the Stanford-Binet Intelligence Scales, fifth edition.[4] Because approximately 39% of the participants were "nonverbal," the nonverbal IQ score was used in lieu of the full-scale IQ. Nonverbal mental age equivalency, using Stanford-Binet Intelligence Scales, fifth edition, nonverbal IQ, or the visual reception subscale of the Mullen Scales of Early Learning[5] was obtained for the purposes of valid interpretation of the Autism Diagnostic Observation Schedule (ADOS)[6] and the Autism Diagnostic Interview, revised (ADI-R).[7]

Overall classification of baseline autism spectrum disorder (ASD) (including both autism and pervasive development disorder not otherwise specified) group status of "ASD" or "none" was determined by an experienced psychiatrist (E.T.) for 17 of 23 participants, based on the combined results of 3 measures: (1) ADOS (ADOS-2 diagnostic algorithm), (2) ADI-R, and (3) *DSM-IV-TR*[8] criteria. The ADOS-2 Comparison Score and the total score of the "Social Affect" and "Restricted and Repetitive Behavior" subscales were also examined (table 1). ADI-R and ADOS data were excluded for 6 of 23 participants with nonverbal mental ages less than 24 months (table e-2).

Symptoms of inattention and hyperactivity were assessed using both "trait" and "state" measures; trait symptoms, or overall pattern of behaviors, were examined using selected baseline visit subscale *t* scores (table e-2) from the Conners Parent Rating Scale–Revised Long form (CPRS-R:L).[9] The CPRS-R:L is a widely used standardized assessment of ADHD and other problem behaviors in children and adolescents. Items demonstrate significant discriminant validity ($p < 0.001$) for *DSM-IV* ADHD symptom criteria. State behaviors, or those behaviors observed over the past 6 months (since the last study visit), were examined using the age-appropriate Symptom Inventory (SI).[10–12] The SIs are parent rating scales that screen for symptoms of psychiatric disorders observed in children and adolescents. Symptom severity scores for the ADHD categories (i.e., inattentive type, hyperactive/impulsive type, combined type) were obtained for each study visit. The Child Symptom Inventory–4 has significant test-retest reliability ($p < 0.0001$) and high correlations with other frequently used parent rating scales for ADHD.[12] Five participants (22%) were receiving medication for ADHD at various visits during the study (viz., clonidine, guanfacine, dexmethylphenidate, amphetamine salts). All neuropsychological and behavioral measures were administered at the same visits as the EEGs.

Data analysis. Data analysis was performed in 3 stages. In the first stage, binomial intraclass correlation[13–15] was used to determine whether EEGs with IEDs were clustered nonrandomly in

Table 1 Demographic and behavioral data

	Total SLOS group (n = 23)			IED group (n = 13)			No-IED group (n = 9)			Between-group analyses	
Demographic/baseline behavioral variable	No.	%	Mean ± SD	No.	%	Mean ± SD	No.	%	Mean ± SD	2-Tailed *p* value	Odds ratio
Sex			NA			NA			NA	0.08[F,t]	6.67
Male	14	60.87		10	76.92		3	33.33			
Female	9	39.13		3	23.08		6	66.67			
Age, mo	23	NA	96.87 ± 42.86	13	NA	84.77 ± 37.21	9	NA	107.44 ± 46.15	0.23[S]; 0.21[W]	NA
Nonverbal mental age	21[a]	NA	38.81 ± 20.56	11[a]	NA	34.55 ± 20.38	9	NA	41.00 ± 20.36	0.49[S]; 0.41[W]	NA
VABS-II adaptive behavior composite	23	NA	48.35 ± 17.69	13	NA	48.00 ± 17.26	9	NA	49.22 ± 20.28	0.88[S]; 0.85[W]	NA
ID classification			NA			NA			NA	0.83[F]	NA
None	2	8.70		1	7.69		1	11.11			
Mild	9	39.13		6	46.15		3	33.3			
Moderate/severe	12	52.17		6	46.15		5	55.56			
Language level			NA			NA			NA	0.20[F]	4.08
Verbal	14	60.87		6	46.15		7	77.78			
Nonverbal	9	39.13		7	53.85		2	22.22			
ADI-R results[b]			NA			NA			NA	0.63[F]	2.0
None	10	58.82		5	55.56		5	71.43			
ASD	7	41.18		4	44.44		2	28.57			
ADOS-2 Comparison Score	16	NA	5.69 ± 2.24	9	NA	6.33 ± 1.58	6	NA	4.33 ± 2.66	0.14[S]; 0.15[W]	NA
ADOS-2 Social Affect + Restricted Repetitive Behavior total	17	NA	12.88 ± 5.22	9	NA	15.11 ± 3.82	7	NA	10.00 ± 6.00	0.07[S]; 0.07[W,t]	NA
ADOS-2 results[b]			NA			NA			NA	0.06[F,t]	Infinity
None	3	17.65		0	0.00		3	42.86			
ASD	14	82.35		9	100.00		4	57.14			
DSM-IV-TR results			NA			NA			NA	0.38[F]	2.67
None	7	30.43		3	23.08		4	44.44			
ASD	16	69.57		10	76.92		5	55.56			
Overall ASD results[b]			NA		33.33	NA			NA	0.62[F]	2.67
None	7	41.18		3	3		4	57.14			
ASD	10	58.82		6	66.67		3	42.86			
CPRS-R:L *DSM-4* ADHD inattentive (*t* score)	21	NA	58.43 ± 9.49	13	NA	59.15 ± 8.92	7	NA	58.57 ± 11.03	0.9[S]; 0.76[W]	NA

Continued

Table 1 Continued

Demographic/baseline behavioral variable	Total SLOS group (n = 23)			IED group (n = 13)			No-IED group (n = 9)			Between-group analyses	
	No.	%	Mean ± SD	No.	%	Mean ± SD	No.	%	Mean ± SD	2-Tailed *p* value	Odds ratio
CPRS-R:L *DSM-4* ADHD hyperactive (t score)	21	NA	61.29 ± 12.33	13	NA	63.39 ± 12.49	7	NA	57.71 ± 13.02	0.35[s]; 0.35[w]	NA
CPRS-R:L *DSM-4* ADHD combined (t score)	21	NA	60.57 ± 10.82	13	NA	62.00 ± 10.90	7	NA	59.00 ± 11.66	0.57[s]; 0.54[w]	NA
CPRS-R:L cognitive problems/inattention (t score)	21	NA	62.05 ± 9.89	13	NA	63.31 ± 10.29	7	NA	61.57 ± 9.07	0.71[s]; 0.70[w]	NA
CPRS-R:L hyperactivity (t score)	21	NA	64.29 ± 13.16	13	NA	66.46 ± 13.09	7	NA	60.57 ± 14.40	0.37[s]; 0.35[w]	NA
CPRS-R:L ADHD index (t score)	22	NA	62.55 ± 10.71	13	NA	63.38 ± 9.19	8	NA	62.38 ± 13.60	0.84[s]; 0.65[w]	NA
CPRS-R:L global index restless impulsive (t score)	21	NA	62.95 ± 12.04	13	NA	63.62 ± 11.42	7	NA	62.43 ± 14.74	0.84[s]; 0.76[w]	NA
CPRS-R:L global index total (t score)	21	NA	61.71 ± 11.97	13	NA	61.62 ± 12.01	7	NA	62.43 ± 13.65	0.89[s]; 0.88[w]	NA

Abbreviations: ADHD = attention-deficit/hyperactivity disorder; ADI-R = Autism Diagnostic Interview, revised; ADOS-2 = Autism Diagnostic Observation Schedule, second edition; ASD = autism spectrum disorder; CPRS-R:L = Conners Parent Rating Scale–Revised Long form; *DSM-IV-TR* = *Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision*; ID = intellectual disability; IED = interictal epileptiform discharge; NA = not applicable; SLOS = Smith-Lemli-Opitz syndrome; VABS-II = Vineland Adaptive Behavior Scales Survey Form, second edition.

[a] Specific nonverbal mental age could not be calculated for 2 participants because their low performance on Stanford-Binet Intelligence Scales, fifth edition, resulted in age equivalency of "<24 months" and the Mullen Scales of Early Learning was not administered.

[b] ADOS and ADI-R results are unavailable for 6 participants because of nonverbal mental age <24 months.

[t] Indicates a trend (i.e., $p < 0.1$).

[F] The *p* values were calculated using Fisher exact test for count data.

[s] The *p* values were calculated using Student *t* test.

[w] The *p* values were calculated using Wilcoxon Mann-Whitney *U* exact test.

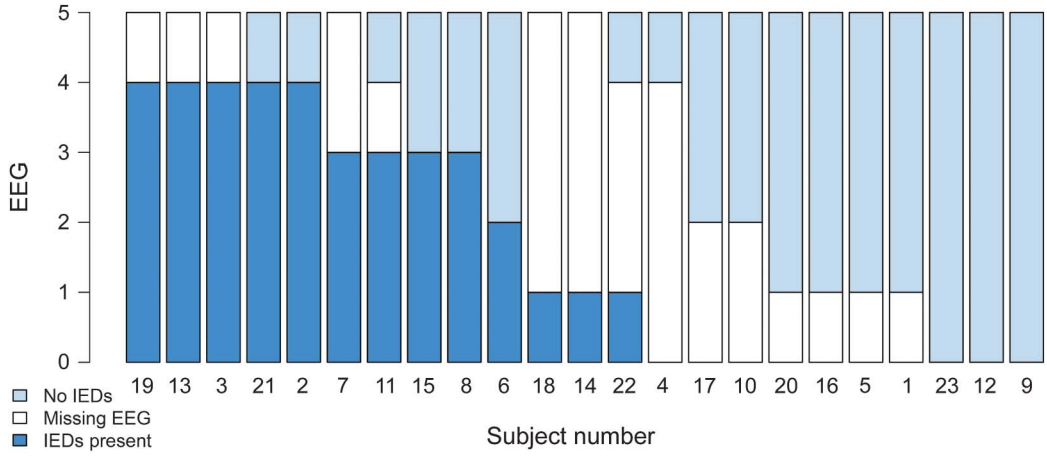
individuals with SLOS (figure 1). For the second stage, between-group analysis was conducted to assess "trait" trends in the "IED group" vs the "no-IED" group. For the purpose of study group placement, the IED group was defined as presence of IEDs on any EEG (n = 13; 56.5%), or no-IED if at least 3 EEGs were successfully completed, and no IEDs were identified on any EEG (n = 9; 39.1%). The rationale for this grouping scheme is based on previous studies demonstrating that, in individuals with epilepsy, a single EEG is typically specific but not sensitive for IEDs, while the recording of 3 or more EEGs improves sensitivity to >80%.[16,17] Only one participant (S21) in our study had a documented history of epilepsy and antiepileptic drug (AED) treatment; oxcarbazepine (not a known spike suppressor) was initiated between the 6- and 12-month visits. Other notable histories included a questionable history of a single seizure in the remote past (S12), and teacher-reported "staring spells" (S15); neither child was diagnosed with epilepsy.

A generalized linear mixed-effects model[18,19] found no significant within-subject treatment effect (simvastatin vs placebo) on the presence of IEDs; thus, we did not stratify subsequent analyses based on treatment. Between-group analyses (IED vs no-IED) of demographics (age and sex) and baseline neuropsychological/behavioral symptoms (intellectual and language level, autistic behaviors, and inattention/hyperactivity) were performed using both the 2-tailed Student *t* test and Wilcoxon Mann-Whitney *U* exact test to satisfy assumptions regarding sample size and data distribution. Fisher exact test was used for categorical variables (table 1).

Finally, within-subject analyses of the occurrence of IEDs and concurrent ADHD state symptoms were examined via a linear mixed-effects model. The group classification (IED/no-IED) from the prior analysis was not relevant to this step. SI ADHD severity scores (attention, hyperactivity/impulsivity, and combined type) were used as the response variables; the presence of IEDs was a fixed effect, and subject identity factor was a random effect. Each visit was treated as an independent observation, because there was no a priori assumption that ADHD severity would increase or decrease over time. Each subject served as his or her own control, and the results therefore reflect average changes in ADHD scores at visits in which the EEG revealed IEDs, vs visits for the same subject in which the EEG revealed no IEDs. Although estimated changes in mean ADHD scores, associated with presence vs absence of IEDs, are based only on those individuals who had both EEGs with and without IEDs (n = 7), the variance estimates of ADHD scores account for and benefit from ADHD scores from all participants. Subsequent analyses examined the effect of IED as a rate (rather than a binary variable), separately for waking and N2 sleep. To assess the potential impact of other factors on ADHD severity, post hoc hierarchical models added one additional fixed-effect covariate: ADHD medication usage, age at enrollment, sex, and time (visit number). In each model, the presence of IEDs was added hierarchically as the final predictor. Observations with missing data were excluded from all models. The random effect model imposes a marginal correlation structure. No residual correlation unaccounted for by the random effects was assumed.

RESULTS Demographic and behavioral data are summarized in table e-2 and table 1. EEG results are summarized in table e-1. The occasional use of double-distance montages (n = 19) did not appear to have a negative impact on EEG sensitivity, as 12 (63%) had identified abnormalities, 11 of which were epileptiform in nature. The most common

Figure 1 Plot showing clustering of EEG results (presence/absence of IEDs) by subject



EEG distribution within each subject. IED = interictal epileptiform discharge.

IED topology was central-temporal (i.e., "rolandic"). IEDs were quite frequent when present: spike counts ranged from 0 to 123 spikes/100 seconds during N2 sleep (mean ± SD = 52 ± 34), excluding one EEG in which no N2 sleep was achieved; and 0–106 spikes/100 seconds during waking (mean ± SD = 21 ± 25), excluding one EEG that captured no waking.

For the first stage of analysis, binomial intraclass correlation ($\rho = 0.64$) suggested moderately strong clustering of epileptiform EEG abnormalities within individual subjects (figure 1), thus revealing that EEGs with IEDs were not randomly distributed among all subjects.

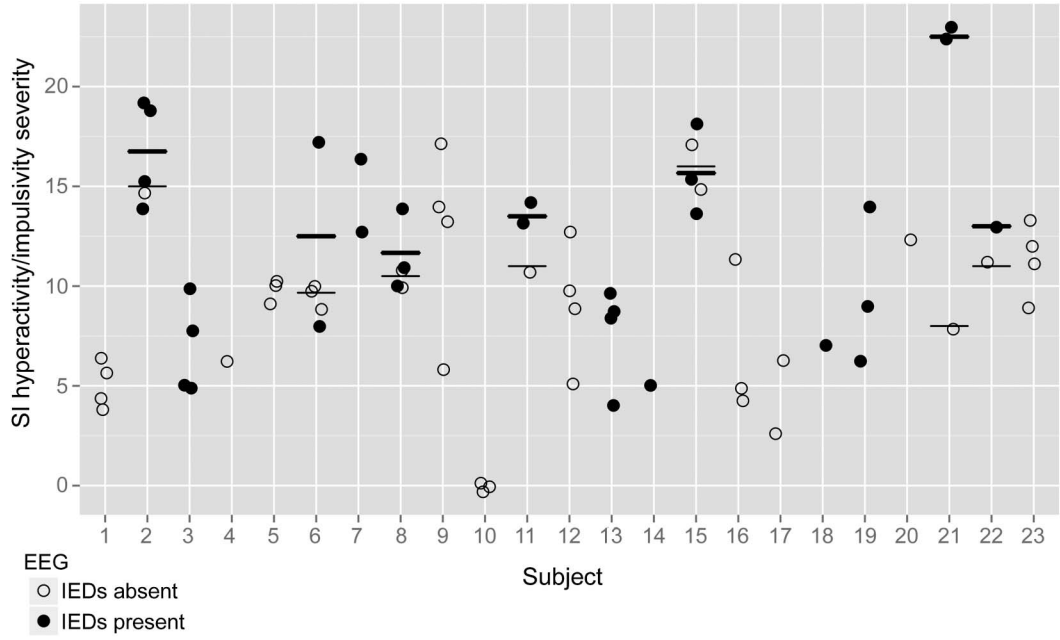
For the second stage of analysis, we predicted that the IED group would show elevated ADHD and autistic symptoms compared with the no-IED group. As seen in table 1, there were no significant between-group differences in age, ADHD trait symptoms, severity of intellectual disability, verbal ability, ADI-R scores, or *DSM-IV* ASD diagnosis; however, the IED group demonstrated trend level increases in male predominance and in baseline autistic "state" behaviors, with all 9 individuals (100%) in the IED group receiving ADOS-2 classifications of "autism" or "ASD." Further analyses of the ADOS-2 results revealed similar trend level increases in the total score of the Social Affect and Restricted and Repetitive Behavior subscales, but no group differences in the ADOS-2 Comparison Score (table 1) were seen.

For the third stage of analyses, as predicted, the linear mixed-effects model revealed that the presence of single-visit IEDs within individuals predicted higher ADHD symptom severity (i.e., same visit SI scores) occurring over the previous 6 months, compared with visits without IEDs: combined type symptoms ($p = 0.004$, $\chi^2_1 = 8.3$; mean increase = 27%, 5.3 points; 95% confidence interval [CI] = 1.8–8.9), hyperactive/impulsive symptoms ($p = 0.0046$, $\chi^2_1 = 8.0$; mean increase = 34%, 2.9 points; 95% CI = 0.93–4.9), and a trend level increase in inattentive symptoms ($p = 0.068$, $\chi^2_1 = 3.3$; mean increase = 21%, 2.3 points; 95% CI = −0.13–4.8). Figure 2 illustrates the hyperactive-impulsive results. When the binary variable of IED presence/absence was converted to a continuous variable of spike counts, spike counts were significant predictors of all ADHD severity scores ($p < 0.001$), although with smaller effect sizes than seen in the binary model. Controlling for ADHD medication use, age, sex, or visit did not change the results substantively.

DISCUSSION Results of this study suggest that in children with relatively mild SLOS, (1) IEDs are common, but epilepsy is not; (2) EEG findings are variable from session to session, but cluster within particular individuals over serial testing; (3) there was no evidence that propensity of IEDs was significantly associated with age, sex, cognitive or language level, or overall diagnostic classification of ASD; and (4) within-subjects, the presence of IEDs at a particular visit was associated with parent reports of increased ADHD symptom severity, relative to severity reported at another visit during which no IEDs were demonstrated.

While in most clinical situations IEDs predict the presence of epilepsy with high specificity and moderate sensitivity,[20] there are particular clinical scenarios in which they are less closely associated with epilepsy. For example, rolandic spikes, the most common variety of IEDs in this cohort, are seen in specific genetic intellectual disability syndromes (Rett syndrome[21] and fragile X syndrome[22]), but can also be associated with the pediatric syndrome of benign epilepsy with

Figure 2 Plot illustrating the within-subject regression model examining the effect of presence vs absence of IEDs on hyperactivity/impulsivity severity



Within-subject regression of ADHD symptoms vs presence of IEDs. SI ADHD severity score (specifically the hyperactive/impulsive type) is on the y-axis, and the x-axis indexes subject number. The magnitude of the effect of interest, i.e., within-subjects change in ADHD severity as related to presence or absence of IEDs, is estimated from ADHD severity score differences in participants who had at least one EEG with IEDs and at least one EEG without IEDs. Variance estimations, however, benefit from information from all participants. For individuals who had EEGs with and without IEDs, horizontal lines indicate mean ADHD severity scores for visits with IEDs (heavy lines) and without IEDs (thin lines). In 6 of the 7 subjects who had EEGs both with and without IEDs, the mean ADHD hyperactive/impulsive severity score was higher at visits with IEDs than at visits without IEDs. ADHD = attention-deficit/hyperactivity disorder; IED = interictal epileptiform discharge; SI = Symptom Inventory.

centrotemporal spikes, or occur as a normal developmental variant.[23,24] Whereas certain classes of IEDs have a high positive predictive value (PPV) for epilepsy (e.g., temporal lobe IEDs: 91%), rolandic spikes generally have a lower PPV (estimated at 38%).[25] While 57% of our SLOS cohort had at least one EEG with IEDs (PPV = 8%), the vast majority (22/23; 96%) did not have diagnosed epilepsy. This observation strongly suggests the use of caution when interpreting IEDs in patients with SLOS, so as to not overinterpret IEDs' ability to predict epilepsy in this population.

While IEDs were not strongly correlated with epilepsy in our cohort, they did appear to have clinical relevance, demonstrated by their association with, on average, a 34% increase in hyperactive/impulsive symptom severity. Although this observation of a within-subject, serial association appears to be novel, prior results from different populations have suggested that IEDs may disrupt cognitive and behavioral function. One cross-sectional study of referrals to a neurology clinic for ADHD demonstrated a substantial rate of IEDs (20%),[26] although the prevalence estimation may have been confounded by referral bias. Research using a different time scale (second-by-second) has also demonstrated brief impairments in attention associated with individual IEDs.[27] Within-subject variability of the presence/frequency of sharp waves in other populations has been previously noted,[28] although not previously tied to cognitive variability. Few interventional data are available to establish a causal relationship between IEDs and cognitive impairment, although a pilot treatment study in benign epilepsy with centrotemporal spikes demonstrated that therapy with levetiracetam suppressed rolandic spikes and improved auditory processing ability.[29] If such a causal association were firmly established in SLOS and other developmental disorders, it may guide future clinical trials of AEDs as "spike suppressors" for the purpose of normalizing EEGs and thus improving cognition and behavior, independently of therapy to ameliorate seizures.

The relationship between IEDs and cognition in patients with ASD, but without diagnosed clinical epilepsy, is an area of current investigation. Specifically, while children with nonsyndromic ASD have an epilepsy prevalence of 20% to 35%, the prevalence of IEDs is as high as 60%.[30] Discussions are ongoing regarding potential therapeutic uses of specific AEDs to improve behavior through IED suppression, independent of seizure treatment. While our data do not address whether IED suppression would improve cognition, nor whether

the IEDs themselves are causal (vs correlative) in behavioral performance variations, we present the first test-retest data demonstrating an association between the presence of IEDs and alterations in neuropsychological function across 6-month intervals.

Although particular individuals were more likely than others to demonstrate IEDs, this propensity was not associated with demographic or behavioral variables. Baseline data of autistic symptoms revealed no significant between-group (IED vs no-IED) differences in ASD classification according to ADI-R or *DSM-IV-TR* criteria, although trend-level associations were seen between presence of IED on the one hand, and ADOS-2 classification of ASD and total ADOS-2 score on the other. The clinical significance of this association is unclear, because our limited sample of individuals who had both IED on EEG and valid ADOS data did not allow for further analyses of ADOS findings; however, a more in-depth analysis of autistic features of our SLOS cohort is currently underway and will be reported elsewhere.

There are several limitations to this study. First, while this was a relatively large sample for a rare disease, our sample size is nevertheless fairly small in absolute terms. Other significant differences may emerge with larger samples in both between-group and within-subjects analyses. Lack of compliance resulted in incomplete data for several participants. In particular, incomplete sets of EEG data may have resulted in a type II error associated with misclassification of subjects as no-IED when the missing EEGs would have shown IEDs; however, this possibility is mollified somewhat by the fact that 3 EEGs have been shown to be >80% sensitive for sharp waves in individuals who have them.[16,17] Results of behavioral measures could potentially be influenced by unreliable parent informants, although it is not expected that such bias would preferentially affect a single group. Clinician and tester bias was reduced through blinding, as IED group status was not determined until after testing was complete. Rating scales based on retrospective reviews of behavior resulted in less direct correlations between current ADHD symptoms and presence of same-day IEDs. Stronger inferences could be made from repeated measurements of same-day EEG and behavioral performance (e.g., a continuous performance task). Finally, replication of these studies in larger populations with other etiologies for developmental delay will be necessary to establish the generalizability of our finding; interventional clinical trials will be necessary to firmly establish a causal association between IEDs and fluctuations in cognitive abilities. Serial studies should examine specific behaviors that may be influenced by the presence of IEDs on EEG in SLOS (and other neurodevelopmental disorders).

Comment: EEGs not likely helpful for behavioral assessments in Smith-Lemli-Opitz

Neurologists' interpretations that a routine EEG shows interictal epileptiform discharges (IEDs) have high variability and low sensitivity in cohorts of idiopathic epilepsies.[1] However, given the wide availability of EEG and the heterogeneous nature and observational criteria for behavioral diagnoses, identifying brain-based biomarkers such as IEDs could be helpful.

To this end, Schreiber et al.[2] present a lucid secondary analysis of data from an as-yet unpublished, 26-month clinical trial, conducted from 2004 to 2009, of the HMG CoA-reductase inhibitor simvastatin for 23 children aged 4 to 18 years with Smith-Lemli-Opitz syndrome. EEGs were performed at 6-month intervals, so participants had up to 5 EEGs (median 4). Thirteen of 22 individuals analyzed had at least one EEG with IEDs, of whom one had epilepsy (specificity 43%; positive predictive value 8%).

Did the categorical factor "presence of IEDs on any EEG" indicate worse behavioral problems? Univariate comparison between the group with any IEDs (n = 13, 77% male, mean age 7 years) and the group with no IEDs (n = 9, 33% male, mean age 9 years) across 20 factors and behavioral scores suggested worse autism (uncorrected $p = 0.07$) but not attention-deficit/hyperactivity disorder (ADHD) scores in the any-IED group. However, the baseline group differences and decisions about multiple comparisons support need for replication.

Within individuals, did visits with IEDs show worse behavior than visits with no IEDs? In the 7 individuals with some IED-positive and some IED-negative visits, mean ADHD symptom inventory scores averaged 27% higher on the IED-positive visits (linear mixed-effects model $p = 0.004$). However, 85 EEGs were done as part of this study. Given the clustering of EEG results within subjects, the number needed to test in clinical practice would be quite high.

This is an important early study, but further work is needed to justify routine research and clinical applications.

1. Gilbert DL, Sethuraman G, Kotagal U, Buncher CR. Meta-analysis of EEG test performance shows wide variation among studies. Neurology 2003;60:564–570.
2. Schreiber JM, Lanham DC, Trescher WH, et al. Variations in EEG discharges predict ADHD severity within individual Smith-Lemli-Opitz patients. Neurology 2014;83:151–159.

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Study funding: No targeted funding reported.

Disclosure: D. Gilbert has received honoraria from the Tourette Syndrome Association/Centers for Disease Control and Prevention and the American Academy of Pediatrics; serves on the medical advisory board for the Tourette Syndrome Association; and has received book royalty from Elsevier. Dr. Gilbert has received research support (for Tourette Syndrome, ADHD) from the NIH (NIMH R01 MH092520, NIMH R01 MH081854), from the Cincinnati Children's Hospital Research Foundation, the University of Cincinnati, the Tourette Syndrome Association, Otsuka Pharmaceuticals (clinical trial, Tourette Syndrome), Ecopipam Pharmaceuticals (clinical trial, Tourette Syndrome), and AstraZeneca (clinical trial, Tourette Syndrome). Go to Neurology.org for full disclosures.

AUTHOR CONTRIBUTIONS

John M. Schreiber, MD, contributed to the manuscript through providing study concept and design, analysis and interpretation of study data, and drafting and revising the manuscript for content. Diane C. Lanham, MA, contributed to the manuscript through providing study concept and design (behavioral variables), acquisition of study data, analysis and interpretation of study data, and drafting and revising the manuscript for content. William H. Trescher, MD, contributed to the manuscript through providing study concept and design, acquisition of study data, analysis and interpretation of study data, and reviewing and revising the manuscript for content. Susan E. Sparks, MD, PhD, contributed to the manuscript through participating in study protocol concept and design, acquisition of study data, and study supervision and coordination. Christopher A. Wassif, MS, contributed to the manuscript through providing study concept and design, and reviewing and revising the manuscript for content. Brian S. Caffo, PhD, is a biostatistician who recommended and supervised the statistical analyses, and reviewed the manuscript for content. Forbes D. Porter, MD, PhD, is a principal investigator of the study and contributed to the manuscript through providing study concept and design, reviewing and revising the

manuscript for content, study supervision, and obtaining funding. Elaine Tierney, MD, is a principal investigator of the study and contributed to the manuscript through providing study concept and design, reviewing and revising the manuscript for content, study supervision, and obtaining funding. Andrea L. Gropman, MD, contributed to the manuscript through providing study concept and design, and reviewing and revising the manuscript for content. Joshua B. Ewen, MD, contributed to the manuscript through providing study concept and design, acquisition of study data, performance of statistical analyses, analysis and interpretation of study data, and drafting and revising the manuscript for content.

STUDY FUNDING

This research was supported by Autism Speaks, the intramural research program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and by the Kennedy Krieger/Johns Hopkins NICHD Intellectual and Developmental Disabilities Research Center core grant P30 HD024061. This publication was made possible by the Johns Hopkins Institute for Clinical and Translational Research (ICTR), which is funded in part by grant UL1 TR 000424-06 from the National Center for Advancing Translational Sciences (NCATS), a component of the NIH, and NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the Johns Hopkins ICTR, NCATS, or NIH. This project was also supported by a Bench to Bedside award to F.D.P. from the Office of Rare Diseases, NIH Clinical Center, and NICHD; and by National Institute of Neurological Disorders and Stroke/NIH grant K23 NS073626 awarded to J.B.E.

DISCLOSURE

J. Schreiber performs EEGs in his clinical practice (approximately 10% effort) and bills for this procedure. He was employed as a clinical fellow at the NIH during his involvement in this study. He received travel funding from the J. Kiffin Penry Epilepsy MiniFellow Network in 2011. D. Lanham reports no disclosures relevant to the manuscript. She received compensation for travel to an investigator's meeting and commercial research support (Seaside Pharmaceuticals), government research support (NICHD core grant P30 HD024061 and NIDA/NICHD American Recovery and Reinvestment Act), and nonprofit foundation support (Autism Speaks). She currently receives 100% institutional support from Kennedy Krieger Institute (KKI) for psychiatry research. W. Trescher performs clinical EEGs (20% effort) and bills for this service. He interpreted EEGs for this study; during Dr. Trescher's tenure at KKI, reimbursement for these tests were billed directly to the NIH Intramural Program, and during his subsequent time at Hershey Medical Center, they were billed to the NIH Intramural Program indirectly through KKI. He also received institutional support (30% effort) for research from Hershey Medical Center from 2005 to 2008. S. Sparks serves on the advisory board and received travel funding and research funding from BioMarin Pharmaceutical. She received travel and research funding from Genzyme, Santhera, and Sarepta. Additional grant funding has been received from NIH: NIH/NICHD 3U54HD053177 and NIH/NIAMS 5P50AR060836-03, and research support from the Muscular Dystrophy Association. In addition, she receives travel and honoraria from the Society of Inherited Metabolic Disease in her role as faculty for the North American Metabolic Academy. C. Wassif is supported by the intramural research program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and is a member of the RSH/SLOS Medical and Scientific Advisory Board. B. Caffo has performed paid statistical consulting for Sapphire Consulting and AgeneBio. F. Porter is supported by the intramural research program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. He has also received research support from the RSH/SLOS Foundation, Ara Parseghian Medical Research Foundation, Autism Speaks, and the National Niemann-Pick Disease Foundation. Dr. Porter is a member of the RSH/SLOS Foundation and the National Niemann-Pick Disease Foundation Medical and Scientific Advisory Boards. E. Tierney served on the RSH/SLOS Foundation scientific committee, and during the past 2 years was funded by Autism Speaks grant 2990, Autism Treatment Network AIR-P HRSA 1 UA3 MC 11054, FDA 5R01FD004247-02, NIH Bench to Bedside R01 EB003543, and received vitamin and cholesterol supplements from Solace Nutrition to be used for a research study. A. Gropman serves on the data safety monitoring board for BioMarin and received honoraria for developing educational material for Shire and Hyperion. She is a consultant for GeneDx. She served as an expert witness in the trial of Crouse v. Cabell Huntington Hospital and reviewed medical records in a vaccine injury compensation case. She received honorarium for serving as a guest editor for ASENT, special issue on mitochondrial disorders. She received research funding from the National Urea Cycle Foundation, the Propionic Acidemia Foundation, and the O'Malley Fund. Dr. Gropman holds stock options in Life Sciences Technology as part of an employee stock option plan. J. Ewen performs clinical EEGs in his clinical practice (approximately 20% effort) and bills for this service. His laboratory performed EEGs for this study for which he billed the NIH Intramural Program. He has received grant support over the period of this study and subsequently from the NIH (T32 HD07414, K12 NS001696, P01 HD24448, P50 HD052121, K23 NS073626) and Hunter's Dream for a Cure (private foundation support unrelated to this study). Go to Neurology.org for full disclosures.

Received August 21, 2013. Accepted in final form February 18, 2014.

REFERENCES

1. Kelley RI, Hennekam RC. The Smith-Lemli-Opitz syndrome. J Med Genet 2000;37:321–335.
2. Ryan AK, Bartlett K, Clayton P, et al. Smith-Lemli-Opitz syndrome: a variable clinical and biochemical phenotype. J Med Genet 1998;35:558–565.
3. Sparrow SS, Balla DA, Cicchetti DV. Vineland Adaptive Behavior Scales—Interview Edition. Circle Pines, MN: American Guidance Service, Inc.; 2000.
4. Roid G. Stanford-Binet Intelligence Scales, 5th ed. Rolling Meadows, IL: Riverside Publishing; 2003.
5. Mullen E. Mullen Scales of Early Learning Manual, AGS Edition. Circle Pines, MN: NCS Pearson, Inc.; 1995.
6. Lord C, Rutter M, DiLavore P, Risi S, Gotham K, Bishop S. Autism Diagnostic Observation Schedule Second Edition (ADOS-2) Manual (Part 1): Modules 1–4. Torrance, CA: Western Psychological Services; 2012.
7. Rutter M, Le Couteur A, Lord C. Autism Diagnostic Interview–Revised. Los Angeles: Western Psychological Services; 2003.
8. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV. Washington, DC: American Psychiatric Association; 2000.
9. Conners CK. Conners' Rating Scale–4. New York: Multi Health Systems; 1997.
10. Gadow KD, Sprafkin J. Adolescent Symptom Inventory–4: Norms Manual. Stony Brook, NY: Checkmate Plus; 1998.
11. Gadow KD, Sprafkin J. Early Childhood Inventory–4: Screening Manual. Los Angeles: Western Psychological Services; 2000.
12. Gadow KD, Sprafkin J. Child Symptom Inventory–4: Screening and Norms Manual. Los Angeles: Western Psychological Services; 2002.
13. Goldstein H, Browne H, Rasbash J. Partitioning variation in multilevel models. Understanding Stat 2002;1:223–231.
14. Lesnoff M, Lancelot R. Aod: analysis of overdispersed data [online]. Available at: http://cran.r-project.org/package=aod. Accessed March 20, 2013.
15. R Core Team. R: a language and environment for statistical computing [online]. Available at: http://www.R-project.org. Accessed July 1, 2012.
16. Marsan CA, Zivin LS. Factors related to the occurrence of typical paroxysmal abnormalities in the EEG records of epileptic patients. Epilepsia 1970;11:361–381.

17. Salinsky M, Kanter R, Dasheiff RM. Effectiveness of multiple EEGs in supporting the diagnosis of epilepsy: an operational curve. Epilepsia 1987;28:331–334.
18. Bates D, Maechler M, Bolker B. lme4: linear mixed-effects models using S4 classes. R Package Version 0.999999-0 [online]. Available at: http://CRAN.R-projects.org/package=lme4. Accessed March 20, 2013.
19. Faraway JJ. Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models. Boca Raton, FL: Chapman & Hall/CRC; 2006.
20. Goodin DS, Aminoff MJ. Does the interictal EEG have a role in the diagnosis of epilepsy? Lancet 1984; 1:837–839.
21. Cooper RA, Kerr AM, Amos PM. Rett syndrome: critical examination of clinical features, serial EEG and video-monitoring in understanding and management. Eur J Paediatr Neurol 1998;2:127–135.
22. Kluger G, Bohm I, Laub MC, Waldenmaier C. Epilepsy and fragile X gene mutations. Pediatr Neurol 1996;15: 358–360.
23. Eeg-Olofsson O, Petersen I, Sellden U. The development of the electroencephalogram in normal children from the age of 1 through 15 years: paroxysmal activity. Neuropadiatrie 1971;12:375–404.
24. Bernardina B, Beghini G. Rolandic spikes in children with and without epilepsy (20 subjects polygraphically studied during sleep). Epilepsia 1976;17:161–167.
25. Kellaway P. The incidence, significance, and natural history of spike foci in children. In: Henry C, editor. Current Clinical Neurophysiology: Update on EEG and Evoked Potentials. New York: Elsevier; 1981:151–175.
26. Silvestri R, Gagliano A, Calarese T, et al. Ictal and interictal EEG abnormalities in ADHD children recorded over night by video-polysomnography. Epilepsy Res 2007;75:130–137.
27. Aarts JH, Binnie CD, Smit AM, Wilkins AJ. Selective cognitive impairment during focal and generalized epileptiform EEG activity. Brain 1984;107(pt 1):293–308.
28. Ewen JB, Vining EP, Smith CA, et al. Cognitive and EEG fluctuation in benign childhood epilepsy with central-temporal spikes: a case series. Epilepsy Res 2011; 97:214–219.
29. Kossoff EH, Los JG, Boatman DF. A pilot study transitioning children onto levetiracetam monotherapy to improve language dysfunction associated with benign rolandic epilepsy. Epilepsy Behav 2007;11:514–517.
30. Spence SJ, Schneider MT. The role of epilepsy and epileptiform EEGs in autism spectrum disorders. Pediatr Res 2009;65:599–606.

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