EPILEPSY EDITOR'S PICK 2021

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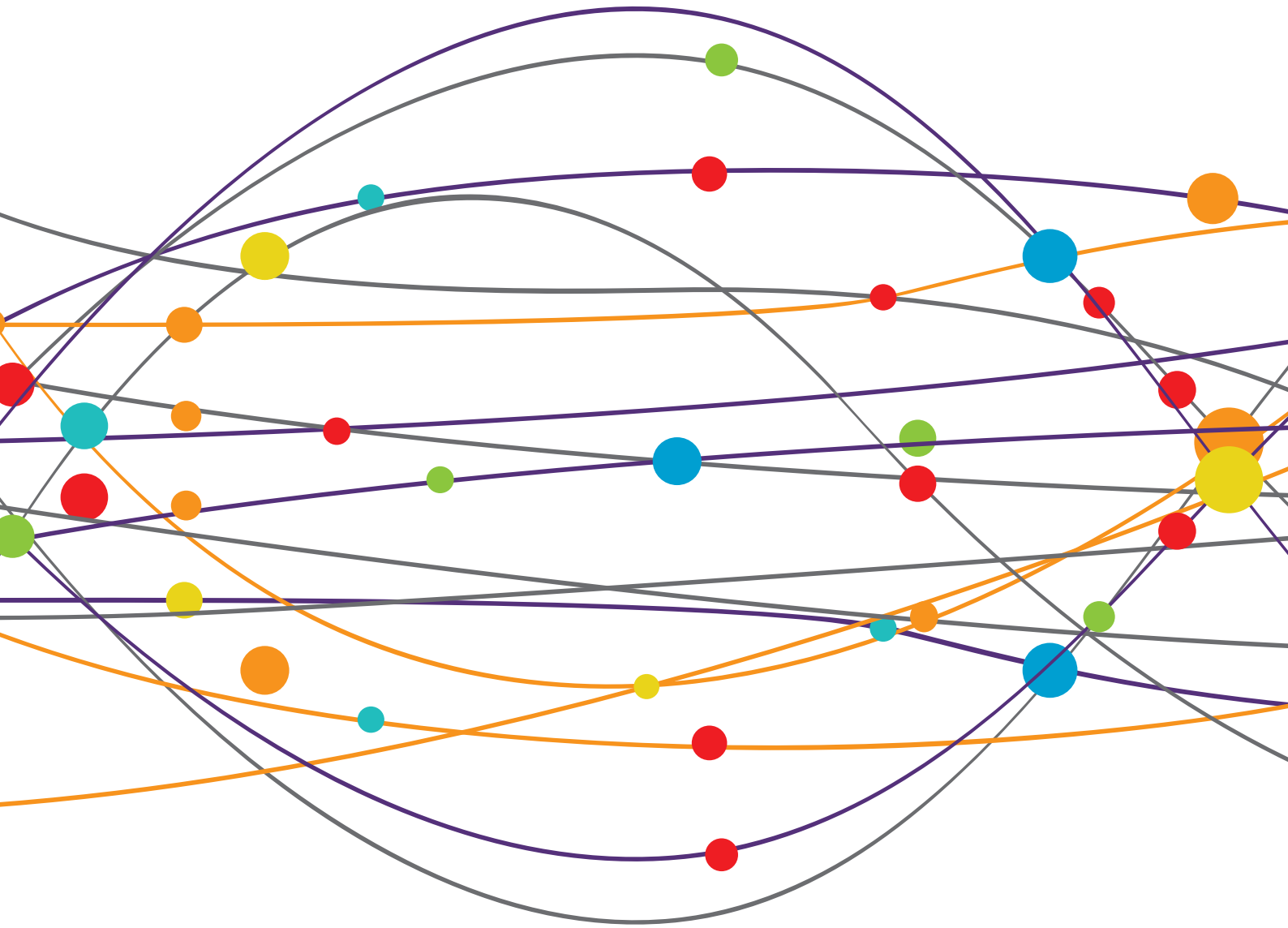

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EPILEPSY EDITOR'S PICK 2021

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Hippocampus and Insula Are Targets in Epileptic Patients With Glutamic Acid Decarboxylase Antibodies

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Background: Antibodies to glutamic acid decarboxylase (GAD ab) have been found in patients with limbic encephalitis (LE) and chronic pharmacoresistant focal epilepsy (FE). The objectives of the study were to: (1) analyze the clinical and neuroimaging course of patients with FE+GAD ab, (2) compare these characteristics with a control group, and (3) describe the most affected cerebral areas with structural and functional imaging.

Methods: Patients with FE + high titers of GAD ab and a follow-up of at least 5 years were selected. Titers of serum GAD ab exceeding 2,000 UI/ml were considered high. Evolutive clinical and radiological characteristics were studied in comparison to two different control groups: patients with bilateral or with unilateral mesial temporal sclerosis (BMTS or UMTS) of a non-autoimmune origin.

Results: A group of 13 patients and 17 controls were included (8 BMTS, 9 UMTS). The most frequent focal aware seizures (FAS) reported by patients were psychic (5/13: 33%). Somatosensorial, motor, and visual FAS (4/13:32%) (*p*: 0.045), musicogenic reflex seizures (MRS), and a previous history of cardiac syncope were reported only patients (2/13:16% each) (*p*: NS). Comparing EEG characteristics between patients and controls, a more widespread distribution of interictal epileptiform discharges (IED) was observed in FE+ GAD ab patients than in controls (*p*:0.01). Rhythmic delta activity was observed in all controls in anterior temporal lobes while in patients this was less frequent (*p*: 0.001). No IED, even in 24 h cVEEG, was seen in 6 patients (46%).First MRI was normal in 4/5 (75%) patients. During the follow-up mesial temporal lobe (MTsL) sclerosis was observed in 5/8 (62%) of patients. All patients had abnormal FDG-PET study. MTL hypometabolism was observed in 10/11 (91%) patients, being bilateral in 7/11 (63%). In controls, this was observed in 16/17 (94%), and it was bilateral in 8/17 (47%) (*p*: NS). Insular hypometabolism was observed in 5/11 (45%) patients (*P*:0.002).

Conclusions: Clinical, EEG, and FDG-PET findings in FE+GAD ab suggest a widespread disease not restricted to the temporal lobe. Progressive MTL sclerosis may be observed during follow-up. In comparison to what is found in patients with non-autoimmune MTL epilepsy, insular hypometabolism is observed only in patients with GAD ab, so it may be an important diagnostic clue.

Keywords: temporal lobe epilepsy, glutamic acid decarboxylase antibodies, autoimmune epilepsy, limbic system, insula, hippocampus

INTRODUCTION

Gamma-aminobutyric acid (GABA) is the main inhibitor neurotransmitter in the mature brain. GABAergic interneurons represent 10–20% of neurons in the cortex and they play a critical role in modulating the output of the critical excitatory pyramidal neurons. Several groups of non-pyramidal GABAergic cells have been identified. Parvalbumin (PV)-expressing, fast-spiking basket cells form the most numerous class of cortical interneurons, whose perisomatic, basket-shaped axonal boutons exert exquisite control over the spiking activity of connected neurons (1).

Antibodies against GAD, the rate-limiting enzyme for the synthesis of GABA, were initially recognized in the serum and cerebrospinal fluid (CSF) of patients with stiff person syndrome (SPS), a rare central nervous system (CNS) disorder which produces rigidity, and cramps frequently associated with other autoimmune diseases, mainly type 1 diabetes mellitus (T1DM). Moreover, GAD ab is identified in about 80% of newly diagnosed T1DM patients, although at low titers compared with those found in SPS. Since then, high levels of GAD ab have also been described in several neurological disorders (2). Among patients with epilepsy, GAD ab has been found not only in patients with limbic encephalitis (LE) but also in patients with chronic temporal lobe epilepsy (TLE) (3). A case report has suggested that GAD ab can also produce epileptogenic areas outside the temporal lobe, in particular in the inferior rolandic area (4). In patients with acute debut presenting as LE, brain MRI usually shows signal and volume changes, bilaterally in most cases, in the temporolimbic structures. Chronic cases referred for epilepsy surgery are frequently rejected because of bilateral independent seizure onset zones in both temporal lobes (5).

One study using quantitative FLAIR analysis has suggested that the amygdala is more affected than the hippocampus in patients suffering LE of different etiologies including those related to GAD ab (6). Interestingly, in patients with LE due to GAD ab, MRI with voxel based morphometry performed 2 years after the onset of epilepsy showed no hippocampal volume reduction (7). A case report has also observed no hippocampal volume loss even 7 years after epilepsy debut (8).

In general there is little information about the long-term clinical and radiological outcome of patients with epilepsy +GAD ab. In addition, information about the cerebral areas which are more involved is also scarce, and it is not known whether the condition affects temporolimbic areas only or more widespread regions.

The aims of our study were to: (1) analyze both clinical and neuroimaging (structural and functional) characteristics of patients with focal epilepsy (FE) + GAD ab at epilepsy onset and during follow-up, (2) compare these characteristics with those of a control group of clinically similar patients without antibodies, and (3) describe the most affected cerebral areas in structural and functional imaging.

MATERIAL AND METHODS

Patients and Controls

All patients with FE and high titers of GAD ab diagnosed at the Hospital Universitari de Bellvitge from May, 2006, to December, 2015, were selected. Only patients with a disease duration (from epilepsy onset) of more than 5 years were included in the study, in order to provide an adequate overview of the evolution of the disease.

In order to evaluate specific cerebral areas affected by GAD ab, FDG-PET studies performed in patients were compared with those from two different control groups: (1) a group of patients with unilateral mesial temporal lobe sclerosis (MTS) considered good surgical candidates after a congruent study with scalp video-EEG, surgically treated and seizure free after at least 1 year of follow-up but also undergoing an FDG-PET, and (2) a group of patients with bilateral MTS of known origin, provided that it was not autoimmune. The last group included all the patients controlled in Hospital de Bellvitge with bilateral MTS excluding patients with an autoimmune origin. Seizure classification was made according to the ILAE 2017 operational classification of seizure types (9).

Comorbid pathologies were divided into three categories: autoimmune, neurological, and psychiatric. Comorbid autoimmune diseases considered were the group of 12 autoimmune diseases that appear most frequently in epilepsy according to the population study of Ong et al. (10). The comorbid psychiatric diseases considered were mood and anxiety disorders, attention deficit hyperactivity disorder, and psychosis according to Kanner (11). Neurological comorbid pathologies considered were the rest of the neurological pathologies, apart from memory disturbances, that are produced by the same etiology that also produced the epilepsy.

The study was approved by the Ethical Committee of Bellvitge University Hospital. Informed consent was obtained from all patients.

MATERIAL

MRI

MRI scans were acquired using a Philips 1.5 or 3 Tesla MRI scanner (Intera, Philips Medical Systems, Amsterdam, The Netherlands) according to a standard epilepsy protocol (12). Follow-up MRI studies were done with a 3 Tesla scanner while most of the first studies had been done with a 1.5 Tesla scanner.

Fluodeoxiglucose (FDG) PET Studies

Following FDG injection and uptake under euglycemic (overnight fasting) and standardized resting conditions (eyes open, reduced ambient noise), FDG-PET scans were acquired on a Gemini TF 64 PET/CT scanner (Philips, The Netherlands; $n = 9$; 10 min 3D acquisition starting 50 min p.i. of 280 $\pm$ 62 MBq FDG). Visual readings were performed after automatic anterior-posterior commissure line realignment on transaxial, coronal, and sagittal slices spanning the entire brain.

FDG-PET/MRI Studies

After PET and MRI were carried out, images from the two techniques were normalized so they could be superimposed on each other with anatomical reliability, and were then co-recorded.

Cerebral MRI and FDG-PET scans were analyzed by a neurologist (JS or MF) independently of the neuroradiologist (SC), and by a nuclear medicine specialist (LR). The neuroradiologist and the nuclear medicine specialist were blinded to clinical characteristics and autoantibody status. The results from the two groups were compared and in case of discrepancies in a particular study, this was analyzed together and a consensus was obtained.

GAD Antibodies Determination

GAD ab was analyzed in serum and CSF (when available) with enzyme-linked immunosorbent assay (ELISA) at the Hospital de Bellvitge. All patients with focal epilepsy of unknown origin are tested for GAD ab in blood serum (20–30 patients per year). Patients with acute onset are tested for GAD also in CSF. In order to confirm the results in patients with positive GAD ab, immunohistochemistry and radioimmunoassay (RIA) were performed at Hospital Clinic of Barcelona, as described elsewhere (13). Additional immunological studies were performed, including determination in serum and CSF of onco-neuronal antibodies (Hu, Yo, Ma, Tr, amphiphysin) and antibodies against neuronal surface antigens (NSA-abs). These studies were carried out in the Neuroimmunology Unit of the Hospital Clinic of Barcelona. NSA-abs were identified by immunocyto-chemistry of rat hippocampal neuronal cultures, as described elsewhere (14).

Serum titers of GAD ab were defined as high when in excess of 2,000 IU/ml. High titers are required to consider the antibody as possibly pathogenic in the neurological symptoms (15, 16). In pharmacoresistant patients several determinations were made, in some cases before and after different immunotherapies (follow-up determinations were made at the Hospital de Bellvitge using ELISA) and results were given as exact number if titers were below 2,000 UI or >2,000 UI without specifying the exact value.

Neuropsychological Examination

A comprehensive neuropsychological examination was performed, including intelligence and memory tests. We included certain subtests (Logical Memory and Visual Reproduction) of the Wechsler Memory Scales (WMS, WMS-R, and WMS-III). Premorbid intelligence was tested with the Vocabulary subtest of the Wechsler Adult Intelligence Scales (WAIS and WAIS-III). For statistical analysis of neuropsychological variables, normalized "z" scores were computed using mean and standard deviations of the raw scores of the general population. Verbal and visual memory scores were compared with vocabulary scores individually. We consider the subjects as memory-impaired if more than one subtest "z" score was one standard deviation (SD) below the general level of intelligence (vocabulary), as proposed by Lezak (17). Memory impairment was divided into mild, moderate, or severe. Severe memory deficit was considered when subject score was below 2.5 SD and moderate between 1.5 and 2.5 SD.

Statistical Analysis

All statistical analysis was performed using SPSS for Windows (version 22.0, SPSS Inc., Chicago, IL, U.S.A.). Categorical variables were analyzed using a one-tailed chi-square analysis (with Yates correction when warranted), and continuous data were analyzed using *t*-test or Mann-Whitney U-test, ANOVA, and Kruskal-Wallis test for non-parametrical analysis. All tests were two-tailed; P-values < 0.05 were considered significant.

RESULTS

We identified 22 patients with epilepsy and GAD ab in the Epilepsy Unit database of the Hospital Universitari de Bellvitge. From this group we selected patients with high serum titers of GAD ab (>2,000 UI/ml) and a follow-up of more than 5 years. The excluded patients were 1 patient with generalized epilepsy, 2 who died during follow-up, 1 with *status epilepticus*, and 1 with pneumonia. Another patient was lost to follow-up. Finally, 13 patients with epilepsy and high titers of GAD ab were included. Among these only 2/13 (15%) patients referred an acute debut, one with limbic encephalitis that occurred during pregnancy (together with eclampsia and Hellp syndrome) and the other with an occipital *status epilepticus* in the context of celiac disease. The rest of the patients debuted with focal epilepsy. GAD ab in CSF was analyzed in 6/13 (46%) patients and was positive in all. Intrathecal synthesis was observed in 5/6 (83%). During the follow-up, several serum GAD determinations were obtained in the patients with pharmacoresistant epilepsy, and at least two determinations were obtained in all patients. The results were always above 2,000 UI/ml. No other onco-neuronal antibodies or antibodies against neuronal surface antigens (NSA-abs) were identified.

Control patients were selected from the same database. Eight patients had bilateral mesial temporal lobe sclerosis (BMTS): 2 due to meningitis, 1 to measles encephalitis, 3 to connatal anoxia, 1 encephalitis from human immunodeficiency virus, and 1 encephalitis due to parvovirus B19. Nine patients had unilateral MTS: 7 had suffered febrile seizures and 2 did not report initial

precipitating injury. As mentioned above, all UMTS were seizure-free after a temporal lobectomy. In all the control patients an immunological battery including GAD ab was performed and was negative in all cases.

Demographic and clinical characteristics of patients and controls are presented in **Table 1**.

No differences were observed between patients and controls in terms of gender and age. Patients reported earlier epilepsy onset compared to controls (*p*: 0.004), both UMTS and BMTS, and with a shorter disease duration (*p*: 0.005). After a mean disease duration of 14 years (range 6–53 years), nearly half of the patients with FE +GAD ab−6/13 (46%)—were seizure-free. Interestingly only one of the patients who was seizure-free received immunosuppressive agents, due to a liver transplant. Half of the patients with drug-resistant FE+ GAD ab had received immunotherapy (corticoids, IVIG, azathioprine, cyclophosphamide, or mycophenolate) without significant improvement in seizure frequency. All control patients with UMTS were seizure-free after surgery while none of those with BMTS were. BMTS patients suffered weekly or monthly seizures.

In comparing the seizure characteristics of FE+ GAD ab patients with those of controls (BMTS or UMTS) some differences were found: somatosensorial, motor, and visual focal aware seizures were only reported by patients with FE +GAD ab (4/13: 30%, p:0.045), suggesting a symptomatogenic zone beyond the temporal lobe. In addition, some patients reported different seizure types during the disease evolution. One man had seizures with cephalic parestesias and right focal motor clonic seizures during the first months of the disease. This seizure type completely disappeared and afterwards he started to present a déjà-vu sensation preceding a focal impaired awareness seizure. Another man reported visual focal aware seizures only at disease onset while later on this seizure type completely disappeared. Considering seizure types, focal to bilateral tonic-clonic seizures (FBTCS) as the main seizure type were only reported by patients with FE + GAD ab, and they occurred predominantly during night sleep. On the other hand, focal impaired awareness seizures (FIAS) were the predominant seizure type in controls (BMTS and UMTS) (*p*: 0.025). Two patients with FE +GAD ab reported musicogenic reflex seizures in addition to non-provoked FIAS and FBTCS. None of the controls mentioned any kind of reflex seizures (*p*: NS).

Apart from seizures, a previous history of syncope was reported in 2/13 (16%) of the patients with FE +GAD ab. None of them had a history of cardiac disorders. One of the patients, a man aged 83, was diagnosed at 70 with carotid sinus hypersensitivity and a pacemaker was implanted. He suffered no more syncopes but 2 years later he started suffering FIAS. Now on antiepileptic drugs (lamotrigine 300 mgr/day), his seizures have been completely controlled although he suffers from progressive verbal memory loss.

Comparing EEG characteristics of patients and controls, a more widespread distribution on interictal epileptiform discharges (IED) was observed in FE+ GAD ab patients than in controls, predominantly in the frontopolar and frontocentral regions, but also in the occipital and parietal regions (*p*:0.01). Rhythmic delta activity was observed in all controls in anterior temporal lobes (affecting one or both) while in patients this was less frequent (*p*: 0.001) and appeared in anterior temporal lobe areas and in frontal lobe areas (in two patients only in frontal lobe areas). Six patients (46%) had no IED even in prolonged VEEG study. Ictal EEG was obtained in all controls and in three FE+GAD ab patients. In 2/3 patients it was suggestive of unilateral temporal anterior seizures while in 1/3 bilateral independent anterior temporal lobe seizures were recorded.

Memory impairment was observed in 8/13 (61%) of the patients with FE+ GAD ab; it was moderate in three patients. Interestingly, in three of the 8 patients seizures were completely controlled. Memory decline over the last 8 years was documented in 4/8 (50%) FE+GAD ab patients. All 8/8 (100%) control patients with BMTS had moderate or severe memory impairment, both verbal and visual, with other neuropsychological deficits mostly affecting frontal lobe structures. Control patients with UMTS had mild or moderate verbal or visual memory impairment, with mild or moderate verbal memory impairment in 3/9 (33%), mild or moderate visual memory impairment in 4/9 (44%), and mild or moderate bilateral memory impairment in 2/9 (22%).

Comparing comorbidities, patients with FE+GAD ab tended to suffer other autoimmune diseases (T1DM being the most frequent) (*p*: 0.001) while patients with BMTS or UMTS tended to suffer more psychiatric comorbidities although this last difference was not statistically significant.

MRI Characteristics (see Table 2, Figures 1, 2)

In 5 (38%) FE+GAD ab patients the first available MRI was performed within the first year from the epilepsy debut. In one patient (1/5; 20%) the initial MRI, done in the first 3 months, showed bilateral amygdalar and hippocampal hyperintensities without atrophy. Greater hyperintensities were found in amygdalar tissue compared with hippocampus (patient previously reported) (3). In the rest (4/5; 80%) the first MRI was normal.

A follow-up MRI was performed in all patients. In 8 (61%) FE+GAD ab patients several (>3) MRI studies were done. The patient with bilateral amygdalar-hippocampal hyperintensities but no atrophy, when scanned 11 years later, had a normal MRI without hippocampal atrophy. Subtle unilateral hippocampal sclerosis (predominantly affecting the hippocampal tail) was found in 4 (80%) patients with an initially normal MRI. Three of them had a disease duration of between 5 and 10 years and the other a disease duration >30 years.

In a patient whose first available MRI was done 11 years after disease onset, left hippocampal sclerosis was observed. Thirteen years later the patient suffered an acute episode of limbic encephalitis (probably a relapse) affecting the right hippocampus and evolving to a bilateral MTS within a year.

In only three controls was an initial MRI available (carried out within the first year from epilepsy debut). In two, both suffering encephalitis, bilateral hippocampal/amygdalar hyperintensities were found, while in the other initial MRI was normal. In all, after <3 months bilateral hippocampal atrophy was observed.

TABLE 1 | Patients and controls.

	PATIENTS	CONTROLS		*p*
	FE+ GAD ab 13	BMTS 8	UMTS 9	
	YEARS (RANGE)	**YEARS (RANGE)**	**YEARS (RANGE)**	
Age	51 (24–83)	52(39–66)	46(29–68)	NS
Age at epilepsy onset	36(11–72)	16.(2–45)	14(1–34)	0.004
Disease duration	14 (5–53)	35(18–55)	32 (8–49)	0.005
	NUMBER (%)	**NUMBER (%)**	**NUMBER (%)**	
Gender: Male	6 (46)	4 (57)	5 (55)	NS
FOCAL AWARENESS				
No awareness	5 (33)	6 (75)	0 (0)	0.045
Psychic	5 (33)	1 (14)	3 (33)	
Epigastric	1 (8)	0 (0)	4 (44)	
Taste/olfactory	0 (0)	1 (14)	0 (0)	
Somatosensorial	1 (8)	0 (0)	0 (0)	
Visual	2 (16)	0 (0)	0 (0)	
Autonomic	0 (0)	0 (0)	1 (11)	
Motor	1 (8)	0 (0)	0 (0)	
Musicogenic reflex seizures	2 (15)	0 (0)	0 (0)	NS
Seizure type (main)				0.025
FIAS	8 (61)	8 (100)	9 (100)	
FBTCS	5 (41)	0 (0)	0 (0)	
Prior history of syncopes	2 (16)	0 (0)	0 (0)	NS
Acute onset or acute relapses*	3 (23)	5 (62)	0 (0)	NS
Seizure-free (>1 year)	6 (46)	0 (0)	0 (0)**	0.003
Memory impairment	6 (46)	8 (100)	9 (100)	NS
Bilateral	1 (10)	8 (100)	2 (22)	
Dominant	4 (30)	0 (0)	3(33)	
Non-dominant	0 (0)	0 (0)	4 (44)	
Autoimmune comorbidities***	11 (85)	0 (0)	0 (0)	0.001
Psychiatric comorbidities	0 (0)	4 (50)	1 (11)	NS
Interictal psychosis	0 (0)	2 (24)	0 (0)	
Anxiety disorders	0 (0)	1 (12)	1 (1)	
Mood disorders	0 (0)	1 (12)	0 (0)	
Other neurological comorbidities	1 (8)	2 (12)	0 (0)	NS
Mental retardation	0 (0)	1 (12)	0 (0)	
Nistagmus	1 (8)	0 (0)	0 (0)	
Mitochondrial SFN	0 (0)	0 (0)	0 (0)	
Tumor ADK colorectal/prostatic or hepatic	3(25)	1 (12)	0 (0)	NS
EEG (SLOW ACTIVITY)				
TIRDA	4 (30)	8 (100)	9 (100)	0.001
FIRDA	3 (23)	0 (0)	0 (0)	NS
EEG, IED				
Unilateral	3 (23)	3 (37)	9 (100)	NS
Temporal (>80%)	1 (8)	5 (62)	9 (100)	NS
Bilateral temporal	7 (53)	4 (50)	0 (0)	NS
Extratemporal	5 (33)	1 (12)	0 (0)	0.01
Absence of IED	6 (46)	0 (0)	0 (0)	0.003

*Electroclinical and demographic characteristics. FE+GAD ab: focal epilepsy with glutamic acid decarboxylase antibodies, BMTS: bilateral mesial temporal lobe sclerosis, UMTS: unilateral mesial temporal lobe sclerosis. FIAS: focal impaired awareness seizure, FBTCS: focal to bilateral tonic-clonic seizure. LE: limbic encephalitis. Autoinmune diseases reported: DM1, Hypothyroidism, psoriasis, celiac disease and myasthenia. ADK: adenocarcinoma. SFN: small fiber neuropathy. EEG: electroencephalogram. TIRDA: temporal rhythmic delta activity. FIRDA: frontal rhythmic delta activity, IED: interictal epileptiform discharges. *Considered acute onset: Status epilepticus, limbic encephalitis or viral or bacterial encephalitis or meningitis. **Prior to epilepsy surgery. ***One or more autoimmune diseases per patient.*

TABLE 2 | Clinical and neuroimaging characteristics of FE+ GAD ab patients at debut and during follow-up.

Patient	Age at epilepsy onset	Acute onset	Follow up (years)	Seizure semiology (focal awareness)	Initial MRI	Follow-up MRI 1	Follow-up MRI 2	Follow-up MRI 3	FDG-PET	Follow-up FDG-PET
1	26	Yes	10	Déjà vu, fear	Hippocampal and amygdalar hyperintensities and volume increase	(3 years) Normal	(7 years) Normal	(11 years) Normal	(8 years) MRTL hypermetabolism MLTL hypometabolism	(9 years) MRTL and MLTL hypometabolism
2	38	No	8	Initial: right motor Follow-up: déjà vu, MRS	Normal		(6 years) Normal		(6 years) Left insular and MRTL hypometabolism	(8 years) Bilateral insular and MTL hypometabolism
3	41	No	13	Déjà vu	Normal	(10 year) Normal	(10 year) Normal	(13 year) L. hippocampal atrophy	(5 years) MLTL hypometabolism	
4	72	No	13	No awareness FBTCS		(1 year) Normal	Pacemaker was implanted		(10 years) Bilateral MTL and insular hypometabolism	
5	30	Yes	11	Initial: visual Follow-up: déjà vu, epigastric		(4 years) Normal	(7 years) Normal	(10 years) R. hippocampal atrophy	(7 years) Bilateral MTL and left insular hypometabolism	
6	11	No	56	Paresthesia in right arm		(46 years) Normal			(52 years) Left Rolandic and insular hypometabolism	
7	50	Yes	18	No awareness FBTCS		(11 years) Left hippocampal sclerosis	(13 years) Left hippocampal sclerosis Right hippocampal aedema, hyperintensity and contrast enhancement	(15 years) Bilateral hippocampal sclerosis	(14 years) Bilateral MTL hypermetabolism Bilateral insular hypometabolism	(15 years) Bilateral MTL hypometabolism Bilateral insular hypometabolism
8	35	No	8	No awareness FBTCS		(3 years) Normal			(3 years) Bilateral MTL hypometabolism	
9	11	No	15	Visual		(8 years) Normal	(12 years) Normal	(15 years) Normal	(12 years) Bilateral MTL hypometabolism	
10	41	No	20	No awareness FBTCS		(8 years) Normal				
11	62	No	7	No awareness FBTCS	Normal					
12	39	No	31	Epigastric MRS		(11 years) Normal	(21 years) Normal	(31 years) R. hippocampal atrophy	(27 years) RMTL hypometabolism	
13	24	No	7	Déjà vu	Normal	(2 years) L. hippocampal sclerosis	(5 years) L. hippocampal sclerosis		(5 years) LMTL hypometabolism	

MRTL, mesial right temporal lobe; MLTL, mesial left temporal lobe; MRS, musicogenic reflex seizures; FBTCS, focal to bilateral tonic-clonic seizure; Hippocampal sclerosis, atrophy and hyperintensity in the hippocampus.

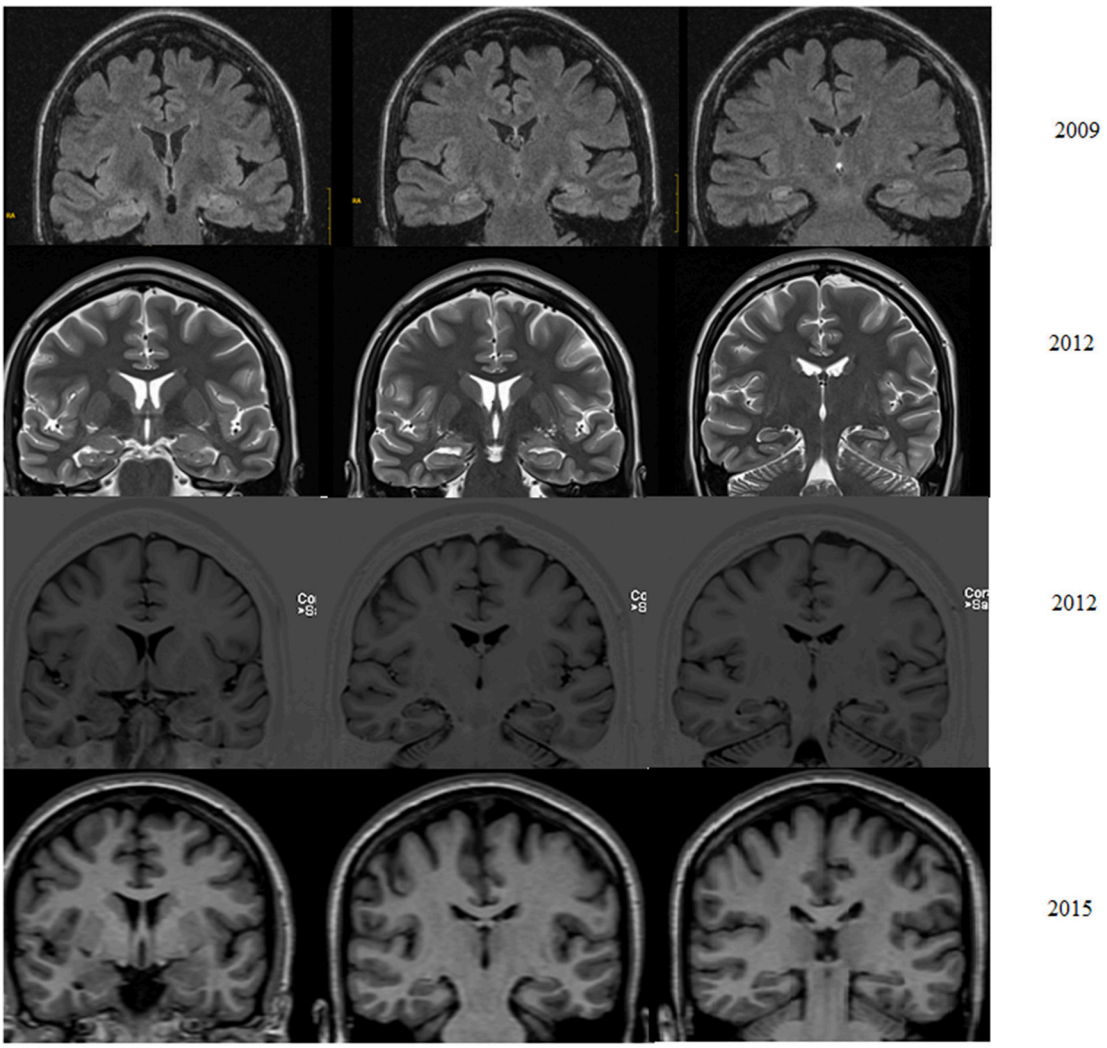

FIGURE 1 | Patient with pharmacoresistant temporal lobe epilepsy from age 25. Five years later suffers weekly focal impaired awareness seizures but no memory decline. Left hippocampal tail atrophy is found on 2012 MRI and confirmed in 2015 (Coronal T1).

FDG- PET Characteristics (see Tables 2, 3 and Figure 3)

Initial FDG-PET was available in 2 patients because both experienced an acute disease onset or had a relapse. In both cases unilateral temporal lobe hypermetabolism was observed. In one patient the hypermetabolism persisted for 6 months despite immunosuppression therapy (corticoids, IVIG, and monthly cyclophosphamide).

A follow up FDG-PET was done in 11/13 (84.6%) patients more than 5 years after epilepsy debut. In 2 patients more than one FDG-PET studies was performed.

All patients had an abnormal FDG-PET study. Mesial temporal lobe hypometabolism was observed in 10/11 (91%) patients; it was bilateral in 8 (72%) and unilateral in 3 (27%). Moreover, insular hypometabolism was observed in 5/11 (45%) patients; it was bilateral in 3 (27%). In all patients except one, insular hypometabolism was observed together with mesial temporal lobe hypometabolism. Isolated unilateral insular hypometabolism was observed in one patient—a man with somatosensorial focal awareness and normal MRI.

Comparing FDG-PET Findings to MRI Findings in Patients (see Table 3)

At the end of follow-up 5 patients had a normal MRI (without hippocampal atrophy) but none of them had a normal FDG-PET. Among 5 FE+GAD ab patients who went on to show unilateral hippocampal atrophy, 3 (60%) had ipsilateral hippocampal hypometabolism and 2 (40%) bilateral hippocampal hypometabolism, with associated insular hypometabolism in one. Interestingly, 2 FE+GAD ab patients (27%) with a normal MRI showed bilateral hippocampal hypometabolism, which was associated with insular hypometabolism in one. The patients with bilateral hippocampal sclerosis also had bilateral hippocampal hypometabolism and bilateral insular

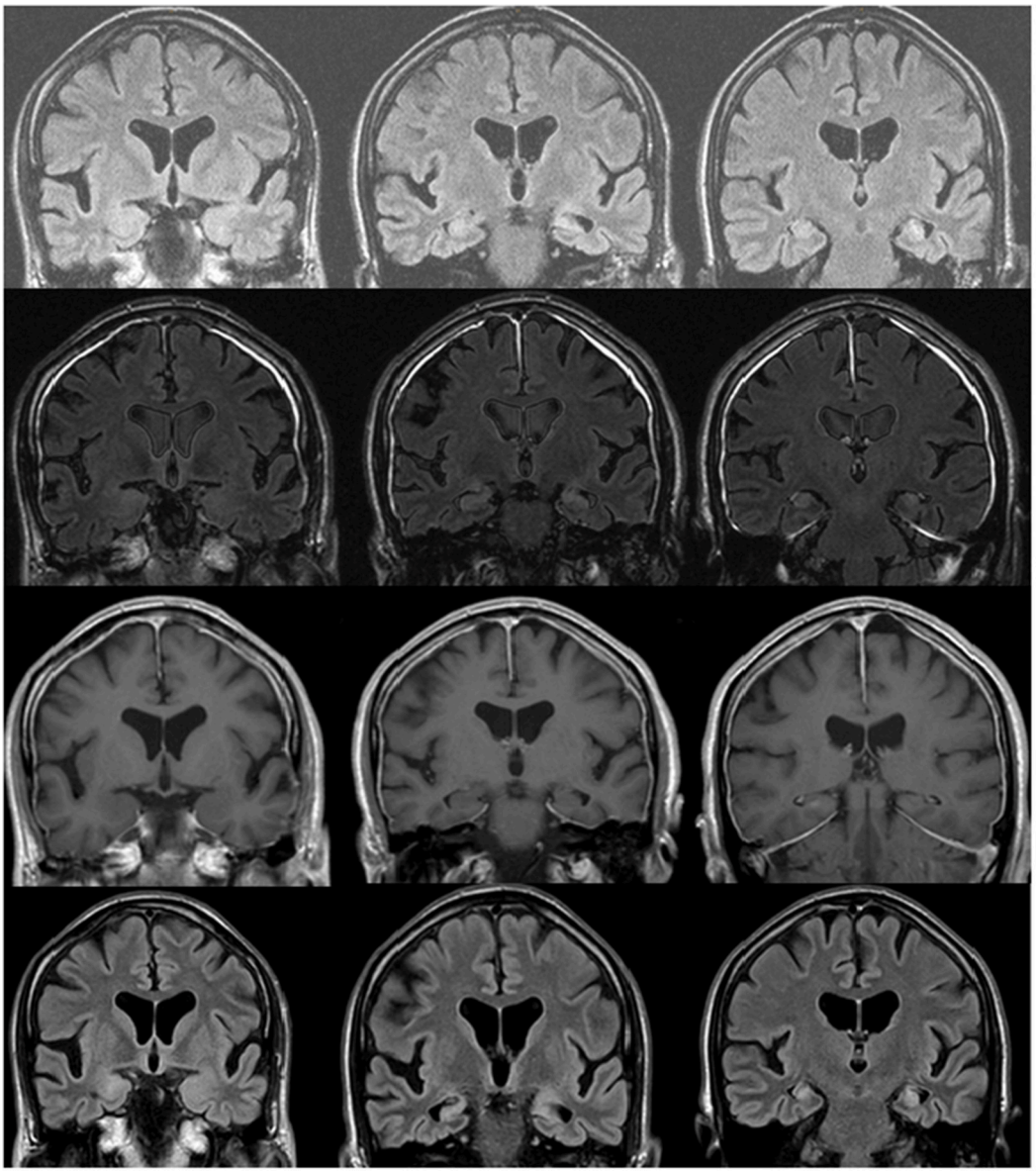

FIGURE 2 | Patient with epilepsy debut at 56. Nowadays seizure-free but with progressive memory decline. **(A)** First available MRI shows left hippocampal atrophy and hyperintensity (coronal flair). **(B)** Three years later bilateral hippocampal hyperintensities are observed without right hippocampal atrophy (coronal flair). **(C)** Three years later nodular right hippocampal contrast enhancement is observed (coronal T1 after injection of gadolinium). **(D)** After 6 months bilateral hippocampal sclerosis (atrophy+ hyperintensity) is observed (coronal flair).

hypometabolism. Overall, only 3 patients (27%) had strictly unilateral mesial temporal involvement (considering FDG-PET and MRI findings).

Comparing FDG- PET Findings of FE + GAD ab Patients and Controls (see Figure 3)

FDG-PET was performed in 7/8 (88%) BMTS controls and in 9/9 (100%) UMTS controls. Bilateral mesial temporal lobe hypometabolism was observed in all BMTS controls, and unilateral or bilateral mesial temporal lobe hypometabolism in all UMTS patients, whereas insular hypometabolism was not observed in any (p: 0.002).

DISCUSSION

In our study both clinical and paraclinical findings suggest that FE +GAD ab involves limbic areas but also the insular region, and in one patient this lobe was the only one involved.

Clinical symptoms suggestive of insular epilepsy include occurrence in full consciousness of a symptomatic sequence

TABLE 3 | Correlation between MRI findings and FDG-PET findings in patients with focal epilepsy + GAD ab.

FDG-PET \ MRI	Normal	Unilateral HIP atrophy	Bilateral HIP atrophy
Unilateral hypometabolism		3 (27%)	
Unilateral insular hypometabolism	1 (9%)		
Bilateral HIP hypometabolism	2 (18%)	1 (9%)	
Bilateral HIP + unilateral insular hypometabolism		1 (9%)	
Bilateral HIP+bilateral insular hypometabolism	2 (18%)		1 (9%)

HIP, Hippocampal, MRI, magnetic resonance imaging, FDG-PET, fluoxideoxiglucose positron emission tomography.

associating laryngeal discomfort with thoracic oppression or dyspnoea, unpleasant paraesthesia—a warm sensation focused on the perioral region or extending to a large somatic territory, and dysarthria, or dysphonic, speech defects (18). One of the patients reported seizures as an unpleasant paresthesia starting in his left arm and rapidly affecting face and tongue, with the left half of his body always in full awareness. A very specific form of sensitive focal aware seizures (pilomotor seizures) have been reported in patients with limbic encephalitis. These are probably also suggestive of insular involvement (19). Two recent case series have also suggested that insular semiology (especially other autonomic semiology) is a part of pilomotor seizures, while perisylvian semiology is highly suggestive of autoimmune epilepsy related to GAD ab or to other antibodies (20–22). Bradyarrhythmia has recently been described as a distinctive prodrome of voltage-gated potassium channel complex/leucine-rich glioma inactivated 1 antibody encephalitis (VGKC/LGI1–ab) leading to pacemaker implantation in 3 cases (23), just as in one of our patients.

The insular region is considered to be one of the cortical autonomic areas (24). GABAergic neurons have been found in several brain regions involved in the control of cardiorespiratory function (25) including the nucleus tractus solitarius (NTS) (26) and the ventrolateral medulla (27). GABA is known to play a vital role in several pacemaker networks. The most extensively studied pacemaker network is in the suprachiasmatic nucleus of the hypothalamus, an area that is responsible for controlling food intake and sleep as well as regulating body temperature and heart rate (28). We hypothesize that a loss of GABAergic neurons due to GAD ab, especially in certain autonomic brain areas (hypothalamus, insula), could affect the modulation of heart rate.

Functional neuroimaging studies also support the hypothesis of an insular involvement in patients with FE +GAD ab. In most of our patients FDG-PET showed hypometabolism in MTLE structures, but also insular hypometabolism in nearly 40% of patients. No insular hypometabolism was found in controls. Another case report showed abnormal extratemporal glucose metabolism (in this case hypermetabolism) in a patient with FE+ GAD ab (29). FDG-PET scans have been demonstrated to reliably lateralize seizure focus in MTLE, with decreased glucose uptake in the epileptogenic temporal lobe (30). It is generally believed that the region of hypometabolism is larger than the epileptogenic zone, and some studies (31) have suggested that insular hypometabolism, most frequently ipsilateral to the hippocampal sclerosis, is due to seizure propagation and does not influence seizure outcome after surgery. Other studies (32–34) have found a relationship between extratemporal hypometabolism in MTLE patients (mainly insula and frontal lobe) with poorer surgical outcome. Case studies are nowadays suggesting that pharmacoresistant FE+ GAD ab has a poor surgical outcome (35, 36). Bitemporal involvement and extratemporal involvement (insular) could be an explanation for this poor surgical outcome. In addition, insular involvement could be an important diagnostic clue in patients with MTLE epilepsy of unknown origin, by raising suspicion of an autoimmune origin due to GAD ab.

In our group of patients with FE + GAD ab, two reported suffering musicogenic reflex seizures (MRS) [Patients previously published (37)]. MRS may be a distinctive seizure type in patients with epilepsy and GAD ab.

Little information is available about long-term follow-up of patients with FE + GAD ab. In a recent study no hippocampal sclerosis (HS) was observed during the follow-up in 12 patients; however, only one of them was followed up for more than 5 years (35). In the study of Wagner et al. (6) no hippocampal volume loss was observed after two years. In another recent study which included 19 patients with FE+ GAD ab, hippocampal abnormalities were found in <30% of patients (38). In our study, where all patients were followed up for over 5 years and in the case of 2 patients more than 30 years, slight unilateral hippocampal atrophy was observed in nearly half of them and bilateral hippocampal atrophy in one. The most important finding in the study of Fredriksen et al. (38) was that patients had a disproportionate parenchymal atrophy with age, again suggesting a widespread disease not limited to the temporal lobe.

Interestingly, an anatomopathological study including 2 patients who underwent temporal lobectomy for medically refractory epilepsy + GAD ab (9 and 10 years follow-up) observed an ILAE type 3 HS or CA4 predominant HS, without lymphocytes or plasma cell infiltration (39). ILAE type 3 HS predominantly affects CA4 and the dentate gyrus. ILAE type 3 HS is the least common form of HS; patients with this form of HS typically develop epilepsy at a later age, often in the absence of an initial precipitating injury or identifiable etiology (40).

Three of our patients reported progressive memory decline even when their epilepsy was well-controlled. Tagaki et al. (41) conducted a case control study comparing cognitive performance of late-onset type one diabetic (LADA) patients with GAD ab and matched type 2 diabetes mellitus patients. They observed that verbal and visual test scores were lower in LADA+GAD ab patients and hypothesized that GAD-positive diabetic patients had an increased risk of cognitive decline compared to patients with type 2 diabetes of comparable diabetic severity.

In addition, insular atrophy and an abnormal insula functional network are also related to memory loss found in early

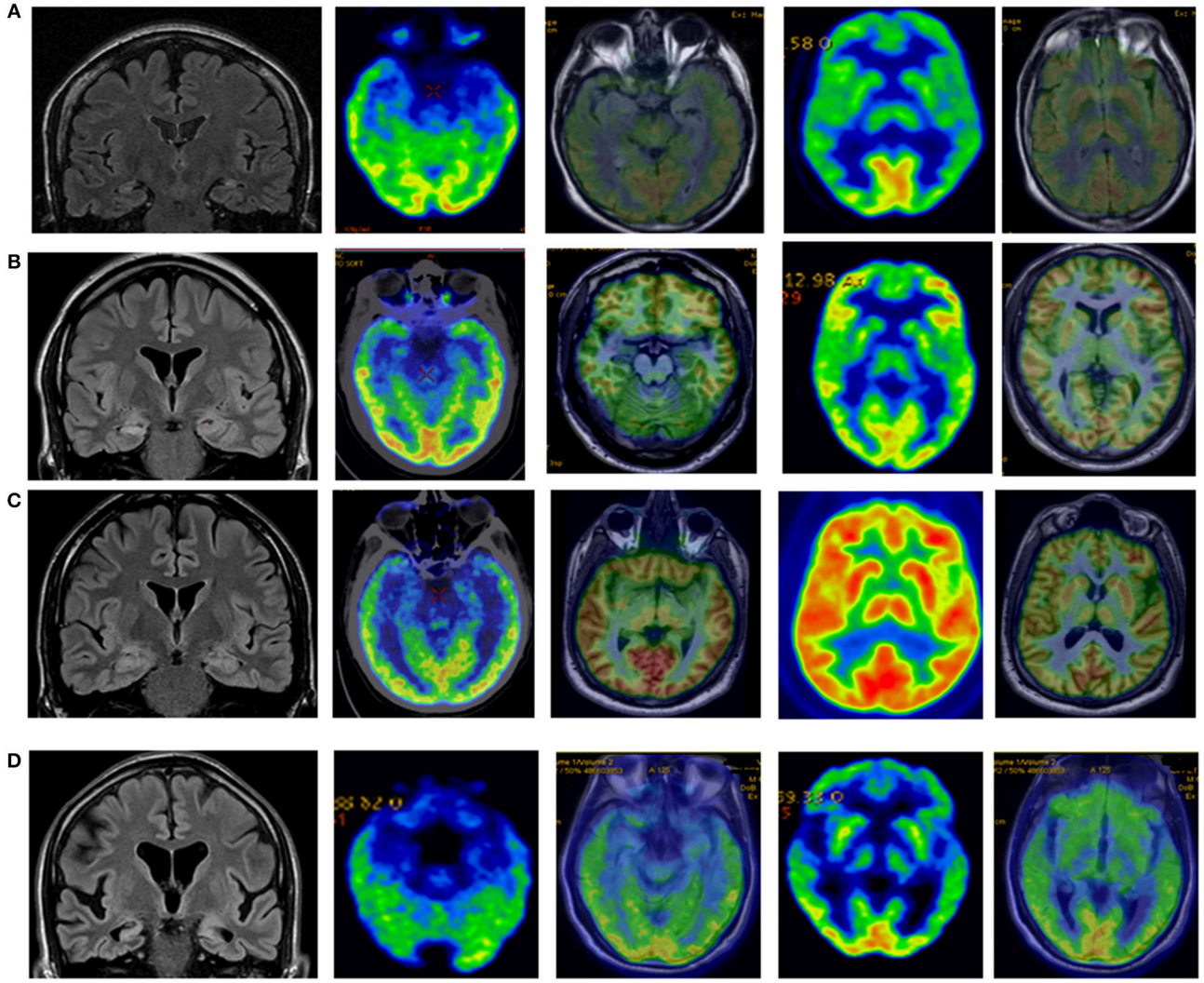

FIGURE 3 | (A) Control patient who suffered measles encephalitis at 2. Epilepsy started at 8. Currently (more than 20 years later) suffers weekly seizures and severe memory deficits. MRI shows bilateral hippocampal atrophy and FDG PET bilateral medial temporal lobe hypometabolism with normal insular metabolism. **(B)** Control patient who suffered febrile seizures. Epilepsy started at 9. He suffered weekly FIAS preceded by non-specific sensation. MRI right hippocampal hyperintensity and FDG-PET right mesial hypometabolism with normal insular metabolism. **(C)** Patient with T1DM from age 29 and temporal lobe epilepsy from age 30. MRI remains normal after 5 years but FDG-PET shows bilateral medial temporal lobe hypometabolism and left insula hypometabolism. **(D)** Patient with epilepsy from age 56. Seizure-free but progressive and severe memory decline. MRI shows bilateral hippocampal sclerosis and insular atrophy, and FDG-PET shows bitemporal medial hypometabolism and bilateral insular hypometabolism.

stages of Alzheimer disease and can be a marker of progression to Alzheimer disease in patients with mild amnestic cognitive impairment (42). Both progressive insular and hippocampal damage might account for the memory decline observed in our patients.

CONCLUSIONS

Epilepsy with GAD ab affects the limbic system unilaterally and, more frequently, bilaterally. Clinical, EEG, and FDG-PET findings suggest a widespread disease not restricted to the temporal lobe.

Progressive memory decline together with progressive hippocampal damage may be observed even in patients with well-controlled epilepsy.

Insular hypometabolism is only observed in epilepsy patients with GAD ab and not in controls with unilateral or bilateral MTS, so it may be an important diagnostic clue.

STUDY LIMITATIONS

Our study has some limitations, notably the low number of patients included and the selection bias in forming the two control groups. We included all patients with BMTS followed

up in our center with sufficient information, and also those with the "purest" UMTS. However, the most important limitations are probably the lack of initial MRI studies in some patients and most of the controls, and the lack of follow-up studies in patients with well-controlled epilepsy. Moreover, but no less important, the use of different MRI scanners (the first studies were done with a 1.5T scanner and the following studies with a 3T scanner) could have led to an underestimation of mild hippocampal atrophies in the first studies.

Larger studies with longer follow-up are needed to confirm our initial findings.

AUTHOR CONTRIBUTIONS

MF and MC conceived and designed the study. MF, SJ, CC, JM, and JB analyzed the data. SC, JS-P, LR-B, and MF analyzed MRI, FDG-PET, and MRI/PET images. MF and FM analyzed GAD determinations. MF and MC wrote the paper.

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ORIGINAL RESEARCH
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Relationship Between Seizure Frequency and Functional Abnormalities in Limbic Network of Medial Temporal Lobe Epilepsy

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Background: We compared resting-state functional connectivity (RSFC) among limbic and temporal lobe regions between patients with medial temporal lobe epilepsy (mTLE) and healthy control subjects to identify imaging evidence of functional networks related to seizure frequency, age of seizure onset, and duration of epilepsy.

Methods: Twelve patients with drug-resistant, unilateral medial temporal lobe epilepsy and 12 healthy control subjects matched for age, sex, and handedness participated in the imaging experiments. We used network-based statistics to compare functional connectivity graphs in patients with mTLE and healthy controls to investigate the relationship between functional connectivity abnormalities and seizure frequency.

Results: Among mTLE patients, we found functional network abnormalities throughout the limbic system, but primarily in the hemisphere ipsilateral to the seizure focus. The RSFCs between ipsilateral hypothalamus and ventral anterior cingulate cortex and between ipsilateral subiculum and contralateral posterior cingulate cortex were highly correlated with seizure frequency.

Discussion: These findings suggest that in mTLE, changes in limbic networks ipsilateral to the epileptic focus are common. The pathological changes in connectivity between cingulate cortex, hypothalamus and subiculum ipsilateral to the seizure focus were correlated with increased seizure frequency.

Keywords: medial temporal lobe epilepsy, partial seizure, functional magnetic resonance imaging, network-based statistics, limbic system

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INTRODUCTION

The limbic system of the brain is a complex network of structurally and functionally linked anatomic regions (1). Among its components are areas in anteromedial temporal lobe including the amygdala, hippocampal complex, and entorhinal cortex, as well as the cingulate cortex. These areas are interconnected with each other via the thalamus (particularly the anterior thalamic and medial dorsal nuclear groups as well as the midline thalamic nuclear group), and project to the hypothalamus and midbrain, and anteromedial temporal lobe. The limbic system is an important

component of the systems subserving emotion, behavior, and memory. In patients with medial temporal lobe epilepsy (mTLE), perturbations of the limbic system often result in in debilitating comorbidities and functional impairments in addition to the direct consequences of seizures.

The role of the limbic system in epilepsy is well-described, with mTLE frequently involving limbic structures and the pathological hallmark of hippocampal sclerosis (2–4). The limbic system's physiologic ability to produce and propagate synchronized activity during normal cognition makes the limbic system an ideal environment for the propagation of pathological synchronization during a seizure (5). Given the role of the limbic system in long-term recall and in emotion, it has been postulated that the high prevalence of memory, mood, and affective symptoms among patients with mTLE correlates with a disruption of normal limbic function (5–7).

Networks throughout the brain are altered in epilepsy. Network changes have been associated with cognitive decline, seizure onset zone locations, and surgical outcomes (8, 9). For example, increased thalamic "hubness" (the importance of a node in a network) prior to anterior temporal lobectomy surgery is associated with increased risk of seizure recurrence after surgery (10). Effective connectivity inferred by dynamical causal modeling has suggested that strengthened connectivity between the hippocampus and parahippocampal gyrus is associated with poor seizure control in TLE (11). Despite promising associations between limbic circuitry and clinical features, the contribution of limbic connectivity to actual seizure burden remains unclear.

To identify resting-state functional connectivity (RS FC) features associated with seizure frequency, we assessed limbic and temporal RS FC in mTLE patients and healthy controls. We used network-based statistics to identify functional connectivity abnormalities in the limbic system, and to find a relationship between those abnormalities and seizure frequency. By identifying the networks underlying clinical presentations of epilepsy, we may better target neuromodulatory approaches to the circuit components either potentiating or regulating epileptic networks.

MATERIALS AND METHODS

Participants and Clinical Scores

Consecutive mTLE patients who underwent comprehensive epilepsy evaluations including EEG monitoring at the Mayo Clinic Epilepsy Center were identified from an epilepsy research database. Twelve patients (five females) with unilateral mTLE (**Table 1**) and 12 healthy control subjects (**Table 2**), matched to the mTLE subjects by age, gender, and handedness, were studied. The control subjects were free of neurological and psychological disease. All participants provided written, informed consent in accordance with research protocols approved by the institutional review board of Mayo Clinic. We collected the numbers of seizures per month during the 3 months preceding the MRI scan as a quantitative score of disease burden. Structural MRI data were reviewed for all subjects to assess for neuroradiological abnormalities. A board certified psychiatrist (PEC) retrospectively reviewed all clinical records to ascertain if subjects had a co-occurring psychiatric disorder. In mTLE group, there were 8 subjects with neuroradiological abnormalities and 9 subjects with psychiatric disorders.

Image Acquisition

Anatomical and functional MRI images were acquired in all subjects on a Siemens 3T Magnetom Skyra system using a 32-channel array head coil and tetrahedron-shaped foam pads to minimize head movement. High-resolution structural whole-brain images were acquired using a T1-weighted sequence with $0.5 \times 0.5 \times 1.2$ mm^3 resolution, TR = 2.3 s, TI = 0.9 s, TE = 1.96 ms, and FA = 9°. Subjects were instructed to keep their eyes open during which resting-state (RS) functional magnetic resonance imaging (FMRI) data were acquired by using a gradient echo-planar sequence sensitive to blood oxygenation level-dependent contrast with $3.28 \times 3.28 \times 3.3$ mm^3 resolution, 50 slices, TR = 2.9 s, TE = 30 ms, FA = 90°, and total acquisition time of 464 s.

Preprocessing of Imaging Data

Preprocessing of all imaging data was conducted using the Analysis of Functional NeuroImages (AFNI) software package (http://afni.nimh.nih.gov). The RS FMRI data were preprocessed and denoised by the standard protocol of the AFNI package (12–14). Using the robust non-linear warping function of the AFNI package, all T1 anatomy data were registered to the MNI152-T1-2009c atlas of the Montreal Neurological Institute and linearly resampled in the 1 mm isocubic grid space. The EPI data were aligned to the T1 images, and then warped to the template brain space along with their T1 images. The registration results for the image data of all subjects were visually inspected for subcortical and cortical brain structures[1]. Note that the imaging data of three mTLE patients with seizure foci in the right hemisphere were left-right flipped before the preprocessing, to match the ipsi- and contra-lateral concept of the analysis. The terms "ipsilateral" and "contralateral" hemispheres stand, respectively for the hemisphere of seizure onset and the hemisphere opposite the side of seizure onset.

Functional Connectivity Analysis

For the functional network analysis (16, 17), masks for regions-of-interest (ROIs) in the limbic system and temporal lobe structures were defined by multiple atlases (18–21), with reference to existing studies of animal seizure models and human patients (22, 23). The entire list of ROIs and the atlas details are presented in **Figure 1A** and **Table 3**. The RS FMRI time series were separately averaged in each ROI mask, and then a Pearson correlation matrix between those was calculated as the network data of each individual subject (by a permutation test for 10,000 iterations with random network extents). A total of 253 RSFCs between ROI pairs were calculated for each subject. The group difference graph between healthy control and mTLE groups were also determined by a two-sample t-test with a threshold level at the family-wise-error-corrected $p < 0.01$ (16).

[1] Aside from the visual inspection to assess thalamic atrophy, recent studies using quantitative MRI analyses have reported global as well as segmental thalamic atrophy in TLE [see (15) for review]."

TABLE 1 | Demographic information for participants with medial temporal lobe epilepsy (mTLE).

Subject index ($N = 12$)	Video-EEG diagnosis; seizure onset zone	Handedness	Age range (year)	Epilepsy duration (year)	Seizure frequency (number of seizures per months)	Neuroradiological abnormality in the structural MRI assessment	Psychiatric symptom	Psychotropic medication
01	Right mTLE	Right	60–69	50	0.1	None	Depression, Anxiety	Citalopram
02	Left mTLE	Right	30–39	16	8	None	Remote history of alcohol and cannabis abuse (8–10 years before presentation)	None
03	Left mTLE	Right	40–49	42	8	Stable non-specific foci of increased T2/FLAIR signal in the subcortical left frontal lobe white matter	None	None
04	Right mTLE	Right	20–29	28	30	Right mesial temporal sclerosis	Anxiety	Citalopram, sertraline
05	Left mTLE	Right	10–19	16	4	None	Insomnia	Clonazepam
06	Left mTLE	Right	30–39	32	24	Left hippocampal atrophy	Depression, ADHD	Mixed amphetamine (Adderall XR)
07	Left mTLE	Right	20–29	12	3	Mild leukoaraiosis	Anxiety	None
08	Left mTLE	Left	20–29	7	2	T2 hyperintensities suggestive of migraine	Depression, nicotine-use disorder	Citalopram
09	Left mTLE	Right	20–29	1	20	None	Nicotine-use disorder (smokeless tobacco)	None
10	Right mTLE	Right	50–59	2	3	Right temporal encephalocele	None	None
11	Left mTLE	Left	20–29	22	1	Left hippocampal atrophy	None	None
12	Left mTLE	Right	30–39	6	16	Nonspecific T2 hyperintensities	Anxiety	Alprazolam

Twelve healthy control subjects were matched for age, sex, and handedness ($N = 12$, mean age difference = 0.00; $p > 0.99$). The indirectly identifiable patient data (gender, exact age, exact seizure onset age) were removed according to editorial guidance.

TABLE 2 | Demographic information for healthy control subjects (five females).

Subject index ($N = 12$)	Handedness	Age range (year)	Neuroradiological abnormality in the structural MRI assessment	Psychiatric symptom	Psychotropic medication
01	Right	50–59	None	None	None
02	Right	30–39	None	None	None
03	Right	40–49	None	None	None
04	Right	20–29	None	None	None
05	Right	10–19	Possible pineal cyst	None	None
06	Right	30–39	None	None	None
07	Right	20–29	None	None	None
08	Right	20–29	None	None	None
09	Right	20–29	None	None	None
10	Right	50–59	None	None	None
11	Left	20–29	White matter hyperintensities	None	None
12	Left	20–29	None	None	None

Correlation Analysis With Clinical Scores

We selected ROI pairs with significant group difference in their RSFC, and then performed a linear regression analysis to find correlations between functional connectivity of those selected ROI pairs and the seizure frequency for mTLE patients. R^2 values were obtained by the linear regression function of MATLAB (version 2016a, The MathWorks, Inc., Natick, MA.).

RESULTS

Functional Connectivity Difference Between mTLE and Healthy Control Groups

A total of 253 connections between ROI pairs were considered for group comparisons, and significant connectivity at the threshold level of $p_{FWE-corrected} < 0.001$ was observed in 17 and 13% of the total ROI pairs in the healthy control and mTLE group, respectively (**Figures 1A,B**). The mTLE patients showed decreased functional connectivity in the 17 ROI pairs of the limbic system, relative to the healthy control group, by a two-sample *t*-test with the default threshold level of the NBS tool ($p_{FWE-corrected} < 0.01$). Specifically, the mTLE groups showed diminished intra-limbic connectivity between ipsilateral hippocampus, subiculum, hypothalamus, mediodorsal thalamus, ventromedial prefrontal cortex (BA14), bilateral parahippocampal gyri, ventral anterior, dorsal and ventral posterior cingulate cortices, and contralateral dorsal anterior cingulate cortex (**Figures 1C**). The functional connectivity between limbic structures and temporal lobe regions showed less change compared to intra-limbic connectivity changes.

Correlations Between RSFC and Clinical Scores

Two out of 17 limbic ROI pairs, had a significant correlation between their RSFCs and seizure frequency at the threshold level of $p < 0.05$: ipsilateral ventral anterior cingulate cortex and hypothalamus ($R^2 = 0.391$, $p = 0.030$) and ipsilateral subiculum and contralateral ventral posterior cingulate cortex ($R^2 = 0.362$, $p = 0.039$) (**Figure 1C**). The results of group comparisons and their correlation with seizure frequency are summarized in the **Table 4** and **Figure 1D**.

DISCUSSION

In this study, we compared RS FC in patients with mTLE and healthy controls. Using network-based statistics, we identified connectivity changes associated with mTLE and with seizure frequency in mTLE. RS FC within the limbic system decreased in mTLE, more so than limbic—temporal connectivity. These network abnormalities primarily affected the hemisphere ipsilateral to the seizure focus. Seizure frequency correlated with RS FC between ipsilateral hypothalamus and ventral anterior cingulate cortex and between ipsilateral subiculum and contralateral posterior cingulate cortex.

Our findings are consistent with the observed decrease in functional connectivity between limbic areas in mTLE patients, which is particularly pronounced in the hemisphere ipsilateral to the seizure focus (22, 23). It is possible that the decrease in functional connectivity reflects underlying limbic pathology in the mTLE group through hypothalamus-to-mediodorsal-thalamus and hippocampus/subiculum-to-cingulate-cortex pathways (24, 25). These changes would be consistent with the affective, memory, and cognitive/behavioral symptoms experienced by mTLE patients (2).

It is also possible that the decrease in limbic RS FC is compensatory. It may be speculated that decreasing functional connectivity ipsilateral to the seizure focus helps limit the participation of the abnormal temporal lobe in limbic circuitry, thereby preserving some measure of normal limbic function. The findings reported here for FMRI connectivity are consistent with studies using intracranial electrocorticography, which show that brain regions generating seizures are functionally isolated from surrounding brain regions (26, 27).

It is unclear why connectivity pairs in the cingulate cortex and hypothalamus and in the cingulate cortex and subiculum emerged as associated with seizure frequency. Previous network

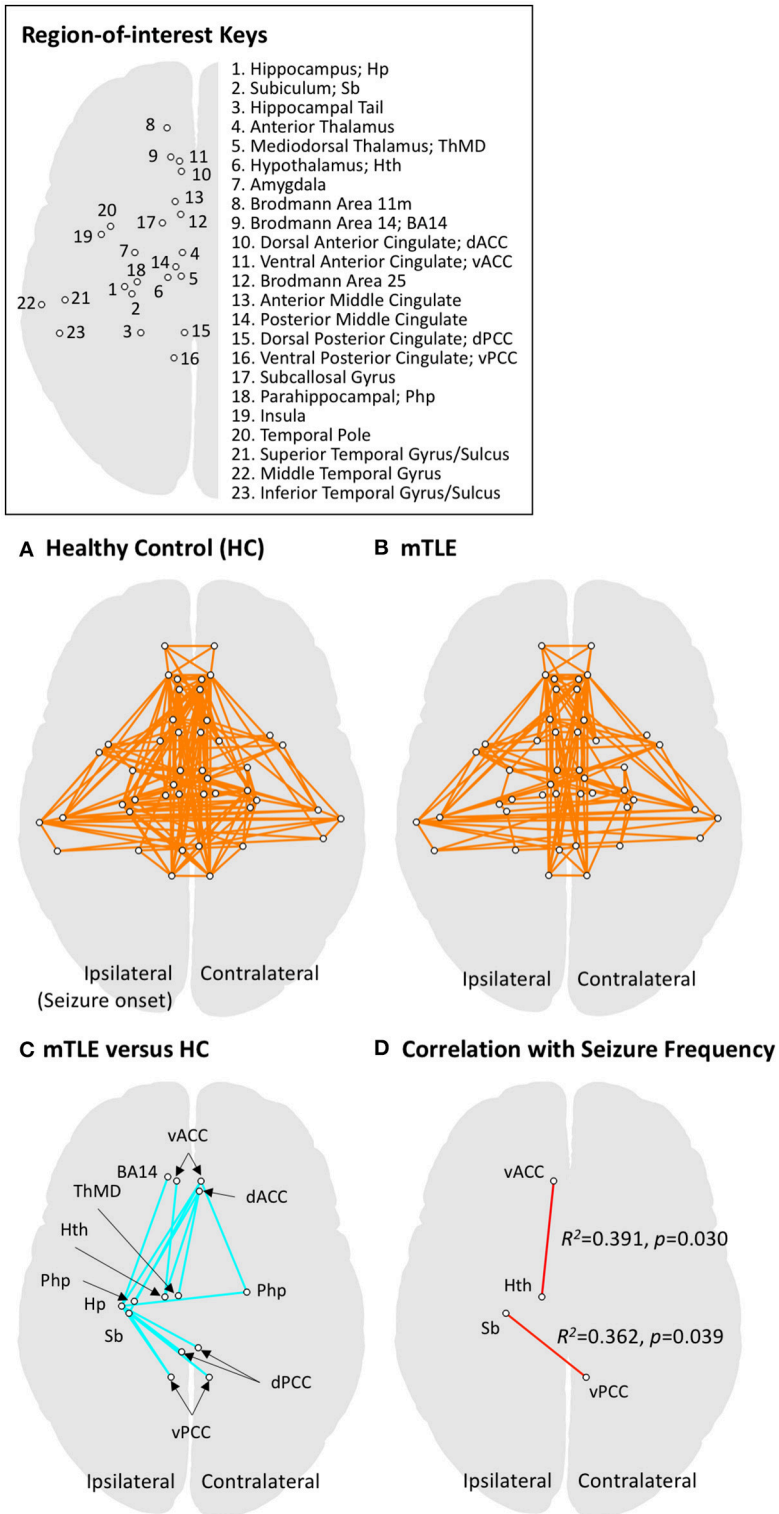

FIGURE 1 | Functional connectivity results for regions of interest (ROIs) in the limbic system and temporal lobe. ROIs in limbic and temporal lobe areas were investigated using network-level analysis (also see **Table 3** for details on defining ROIs). **(A)** The graphical representation of functional networks in limbic and temporal lobe regions for the healthy controls (HC), derived by one-sample *t*-test to the zero mean with a threshold level at $p_{FWER-corrected} < 0.001$. Circles connected by a line represent a pair of ROI nodes and their functional connection. Anatomical labels for the nodes (circles) are described in the upper right panel. **(B)** The graphical representation of functional networks in drug-resistant medial temporal lobe epilepsy (mTLE). The same statistical approach to panel A was applied. The mTLE

(Continued)

FIGURE 1 | patients showed decreased limbic functional connectivity relative to the HC group. The functional connectivity among limbic networks and temporal lobe regions, however, showed less change compared to intra-limbic connectivity changes. **(C)** Group difference between functional networks in HC and mTLE groups. A two-sample *t*-test was performed with a threshold level at $p_{FWER-corrected} < 0.01$. The intra-limbic connectivity between ipsilateral hippocampus, subiculum, hypothalamus, mediodorsal thalamus, ventromedial prefrontal cortex (BA14), bilateral parahippocampal gyri, ventral anterior, dorsal and ventral posterior cingulate cortices, and contralateral dorsal anterior cingulate cortex are decreased in the mTLE group. **(D)** The functional networks significantly correlated with seizure frequency in mTLE patients at a threshold level of $p < 0.05$. Functional connectivity between ipsilateral ventral anterior cingulate cortex and hypothalamus, and that between ipsilateral subiculum and contralateral ventral posterior cingulate cortex showed significant correlation with seizure frequency. The ROI pairs (circles) in limbic system, showing significant difference in functional connectivity between mTLE and HC groups are also summarized in the **Table 4**.

TABLE 3 | Definition of regions-of-interest (ROIs) in limbic structures and seizure-relevant areas for the network-level analysis.

ROI index	Region	Base atlas	Remarks
HIPPOCAMPAL FORMATION			
1	Hippocampus (Hp)	FS-Hp	CA1, CA2, CA3, and CA4 were merged into one mask.
2	Subiculum (Sb)	FS-Hp	Subiculum, parasubiculum, and subsubiculum were merged.
3	Hippocampal tail	FS-Hp	
DIENCEPHALON			
4	Anterior nuclei of thalamus	Morel-Th	Anterior dorsal, medial, and ventral nuclei were merged.
5	Mediodorsal nucleus of thalamus (ThMD)	Morel-Th	Magnocellular and parvocellular mediodorsal nuclei were merged.
6	Hypothalamus (Hth)	FS-aseg	Hypothalamus is included in the inferior diencephalon mask.
SUBCORTEX			
7	Amygdala	FS-aseg	
VENTROMEDIAL PREFRONTAL CORTEX (VmPFC)			
8	Brodmann area 11m	MNI-VmPFC	
9	Brodmann area 14 (BA14)	MNI-VmPFC	BA14c, BA14m, BA14r, and BA14rr were merged.
CINGULATE CORTEX			
10	Dorsal anterior (dACC)	MNI-VmPFC	Brodmann area 24
11	Ventral anterior (vACC)	MNI-VmPFC	Brodmann area 32
12	Subgenual anterior (BA25)	MNI-VmPFC	Brodmann area 25
13	Anterior middle	FS-a2009s	
14	Posterior middle	FS-a2009s	
15	Dorsal posterior (dPCC)	FS-a2009s	
16	Ventral posterior (vPCC)	FS-a2009s	
17	Subcallosal gyrus	FS-a2009s	
OTHER CORTICAL AREAS			
18	Parahippocampal gyrus (Php)	FS-a2009s	
19	Insula	FS-a2009s	
20	Temporal pole	FS-a2009s	
21	Superior temporal gyrus/sulcus	FS-a2009s	
22	Middle temporal gyrus	FS-a2009s	
23	Inferior temporal gyrus/sulcus	FS-a2009s	

FS-HP, -aseg, -a2009s are FreeSurfer templates for hippocampal subfields, automatic segmentation, and cortical parcellation, respectively. Morel-Th is the reconstructed template image of Morel histological thalamic atlas, and MNI-VmPFC is the atlas for ventromedial prefrontal cortex of the Montreal Neurological Institute.

studies have focused on the clinical outcome of seizure freedom and not seizure frequency. One study of seizure propagation networks showed that seizure freedom associated with decreased functional connectivity in bilateral midline structures including the precuneus and midcingulate (28). This finding, together with our observation that select cingulate connectivity features are associated with seizure frequency, may implicate midline connectivity as an overall indicator of limbic network health. Importantly, without larger studies pooling subjects with similar disease courses, this proposal is purely speculative.

With the limbic system acting as a centerpoint for a range of cognitive and seizure-related processes, broader network changes may help explain the role of specific limbic regions in epilepsy (29). A pilot study of functional connectivity in deep brain stimulation (DBS) for epilepsy has suggested that patients who respond to DBS at the anterior nucleus of the thalamus have increased thalamic connectivity to the DMN (30). Intrinsic DMN connectivity increases prior to inter-ictal epileptiform discharges in TLE before returning to its baseline, decreased connectivity after the discharge (31). Further study of how paired connectivity features interact with other resting-state functional networks is warranted.

The primary limitation of this study was the small sample size. We did not conduct structured psychiatric interviews for this study and only retrospectively collected psychiatric histories from the medical record. Future efforts building on the present work will include prospective and comprehensive structured psychiatric assessments. A deeper understanding of the cause and nature of limbic connectivity in patients with mTLE may prove important in treating mTLE and the comorbidities associated

TABLE 4 | The ROI pairs (circles) in limbic system, showing significant difference in functional connectivity between mTLE and HC groups.

Node pair		*t*-value	*p*-value (two-tailed)	Correlation with seizure frequency
Hippocampus (ipsilateral)	- vACC (contralateral)	−5.60	<0.0001	Not significant
	- vPCC (ipsilateral)	−5.46	<0.0001	Not significant
	- vPCC (contralateral)	−5.37	<0.0001	Not significant
	- dPCC (ipsilateral)	−6.41	<0.0001	Not significant
	- dPCC (contralateral)	−4.11	0.0005	Not significant
	- Ventromedial prefrontal cortex (BA14, ipsilateral)	−3.96	0.0007	Not significant
	- Parahippocampal gyrus (contralateral)	−3.30	0.0033	Not significant
Subiculum (ipsilateral)	- vACC (contralateral)	−3.64	0.0014	Not significant
	- vPCC (ipsilateral)	−3.65	0.0014	Not significant
	- vPCC (contralateral)	−3.77	0.0011	$R^2 = 0.391$, $p = 0.030$
	- dPCC (ipsilateral)	−4.31	0.0003	Not significant
Hypothalamus (ipsilateral)	- vACC (ipsilateral)	−3.72	0.0012	$R^2 = 0.362$, $p = 0.039$
	- dACC (contralateral)	−3.67	0.0013	Not significant
Mediodorsal Thalamus (ipsilateral)	- dACC (contralateral)	−3.94	0.0007	Not significant
Parahippocampal gyrus (ipsilateral)	- vACC (contralateral)	−3.11	0.0051	Not significant
	- dACC (contralateral)	−4.82	<0.0001	Not significant
Parahippocampal gyrus (contralateral)	- vACC (contralateral)	−3.36	0.0028	Not significant

with limbic network dysfunction. Also, we flipped the EPI data for left-handed subjects, but there are reports showing that mTLE patients have different behaviors regarding to the anatomical and functional features on the laterality (32–34). To observe more details on the laterality of mTLE connectivity, the further investigation with larger cohorts is required.

In conclusion, we identified functional connectivity changes associated with mTLE and cingulate and limbic network connectivity abnormalities that correlated with increased seizure frequency.

ETHICS STATEMENT

All participants provided written, informed consent in accordance with research protocols approved by the institutional review board of Mayo Clinic.

AUTHOR CONTRIBUTIONS

HJ, KW, IB, and GW: design and conceptualization of the study. HJ, DK-J, PC, EB, and GW: analysis and interpretation of the data. HJ, IB, and DK-J: drafting the manuscript for intellectual content. PC, DJ, EB, GW, and IB: revising the manuscript for intellectual content. KW and DJ: major role in the acquisition of data.

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A Comparison of Parenteral Phenobarbital vs. Parenteral Phenytoin as Second-Line Management for Pediatric Convulsive Status Epilepticus in a Resource-Limited Setting

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Introduction: Pediatric convulsive status epilepticus (CSE) which is refractory to first-line benzodiazepines is a significant clinical challenge, especially within resource-limited countries. Parenteral phenobarbital is widely used in Africa as second-line agent for pediatric CSE, however evidence to support its use is limited.

Purpose: This study aimed to compare the use of parenteral phenobarbital against parenteral phenytoin as a second-line agent in the management of pediatric CSE.

Methodology: An open-labeled single-center randomized parallel clinical trial was undertaken which included all children (between ages of 1 month and 15 years) who presented with CSE. Children were allocated to receive either parenteral phenobarbital or parenteral phenytoin if they did not respond to first-line benzodiazepines. An intention-to-treat analysis was performed with the investigators blinded to the treatment arms. The primary outcome measure was the success of terminating CSE. Secondary outcomes included the need for admission to the pediatric intensive care unit (PICU) and breakthrough seizures during the admission. In addition, local epidemiological data was collected on the burden of pediatric CSE.

Results: Between 2015 and 2018, 193 episodes of CSE from 111 children were enrolled in the study of which 144 met the study requirements. Forty-two percent had a prior history of epilepsy mostly from structural brain pathology (53%). The most common presentation was generalized CSE (65%) caused by acute injuries or infections of the central nervous system (59%), with 19% of children having febrile status epilepticus. Thirty-five percent of children required second-line management. More patients who received parenteral phenobarbital were at a significantly reduced risk of failing second-line treatment compared to those who received parenteral phenytoin ($RR = 0.3$, $p = 0.0003$). Phenobarbital also terminated refractory CSE faster ($p < 0.0001$). Furthermore, patients

who received parenteral phenobarbital were less likely to need admission to the PICU. There was no difference between the two groups in the number of breakthrough seizures that occurred during admission.

Conclusion: Overall this study supports anecdotal evidence that phenobarbital is a safe and effective second-line treatment for the management of pediatric CSE. These results advocate for parenteral phenobarbital to remain available to health care providers managing pediatric CSE in resource-limited settings.

Attachments: CONSORT 2010 checklist

Trial registration: NCT03650270

Full trial protocol available:

https://clinicaltrials.gov/ct2/show/NCT03650270?recrs=e&type=Intr&cond=Status+Epilepticus&age=0&rank=1

Keywords: Africa, convulsive status epilepticus, management, pediatrics, phenobarbital

KEY POINT BOX

- Parenteral phenobarbital as a second-line treatment terminates refractory pediatric convulsive status epilepticus more effectively than parenteral phenytoin
- Parenteral phenytoin for convulsive status epilepticus is associated with increased admission rates to pediatric intensive care
- Midazolam infusion for convulsive status epilepticus increases the demand on the intensive care units

INTRODUCTION

The management of status epilepticus (SE) continues to be a significant challenge in modern epileptology. This enduring and self-perpetuating seizure activity can have a plethora of semiologies with generalized convulsive SE (CSE) being the most common. Seizures that do not self-terminate within 5 min (or recur repeatedly) are less likely to do so without therapeutic intervention (1). Therefore, the practical definition of SE is any seizure that is >5 min in duration or multiple discrete seizures between which there is no extended period of recovery (2).

Children commonly present with CSE to pediatric emergency medical centers (3–5). While epidemiological data on pediatric CSE are lacking globally, this is particularly true for resource-limited settings such as in sub-Saharan Africa. CSE remains an important medical emergency as without effective management, neurological sequelae and mortality can ensue (5, 6). In addition, SE poses a significant cost to healthcare institutions due to the intensive management and monitoring that these patients require (7). Furthermore, all the current treatment guidelines for CSE are primarily based on evidence gathered from high-income, resource-equipped countries (8–10). In contrast, there remains a lack of robust data guiding the management of SE, especially in children and in those patients based in Africa.

The treatment guidelines that are available for CSE recommend the use of benzodiazepines as the first line-agents (9). These include either diazepam, lorazepam or midazolam as they have comparable antiseizure efficacy (11). However, in a subset of patients, mostly children, these agents are ineffective (12). When this occurs, these patients require second-line agents which are typically more difficult to administer and have a greater potential for severe adverse effect profile (13). There is currently poor evidence regarding which second-line agent to use in pediatrics. Historically parenteral phenytoin is used, but there is little evidence to support its use. However, there are multi-center randomized-controlled studies currently underway in high-income settings aimed at determining the optimal second-line intervention for pediatric CSE. These include: the Emergency Treatment with Levetiracetam or Phenytoin in Status Epilepticus (EcLiPSE) study based in the United Kingdom and recruiting participants from 6 months to 18 years of age (14); the Established Status Epilepticus Treatment Trial (ESETT) based in the United States which is comparing parenteral fosphenytoin, valproate and levetiracetam in patients older than 2 years (15); and the Convulsive Status Epilepticus Pediatric Trial (ConSEPT) study in New Zealand comparing parenteral formulations of phenytoin against levetiracetam in patients between 3 months and 16 years of age (16). While these studies will provide important data, their results cannot be easily extrapolated or translated to patients in Africa who face significant healthcare restraints and do not have ready access to the newer, more expensive agents like parenteral levetiracetam.

Within our area of practice, if parenteral phenobarbital and phenytoin is ineffective in terminating refractory CSE, typically the next line of intervention is a parenteral infusion of midazolam (17–20). However, administering these agents usually requires additional infrastructure (e.g., infusion pumps) and admission to an intensive care unit due to the significant risk of cardiopulmonary depression. Access to pediatric intensive care (PICU) unit is limited across Africa and therefore the use of a midazolam infusion is viewed with caution.

Phenobarbital is widely used across Africa as a low cost and effective addition to the antiseizure arsenal, particularly in pediatric epilepsies (21). Currently, there exist only a few studies demonstrating how the use of parenteral phenobarbital

can be effective in terminating CSE in neonates, pediatrics and adults (22–24). Our experience at the Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, South Africa is that parenteral phenobarbital is an effective and preferred treatment for refractory pediatric CSE. Moreover, our impression is that repeated doses of parenteral phenobarbital is both more effective and safer than the traditional approach of using parenteral phenytoin followed by a midazolam infusion. However, to date we have not been able to provide evidence to support this anecdotal evidence. Furthermore, validating the use of parenteral phenobarbital is becoming increasingly important by the frequent limited access across sub-Saharan Africa and worldwide which often results in it needing to be imported via complex regulatory channels (25–27).

The aim of this study was to demonstrate the efficacy of parenteral phenobarbital (PHB) as second-line management for pediatric CSE refractory to benzodiazepines. We compared two treatment protocols, one containing PHB as the second-line agent and the other using parenteral phenytoin (PHY). We compared these agents by looking at how effective they were in terminating CSE (primary outcome), whilst also comparing differences in the need for PICU admission and breakthrough seizures (secondary outcomes). In addition, for patients that fail second-line treatment, we reviewed the use of repeated parenteral boluses of PHB vs. the use of a midazolam (MDZ) infusion. Furthermore, our study also collected local epidemiological data on the burden of pediatric CSE.

MATERIALS AND METHODS

Study Design and Inclusion Criteria

This was an open-label single-center randomized parallel clinical trial which was conducted at the RCWMCH and ran between March 2015 and March 2018. The study was stopped at this time as access to PHB became limited. All children from 1 month to 15 years of age who presented with CSE needing therapeutic intervention were entered into this study by the attending medical staff. Study data were collected using REDCap hosted by the University of Cape Town's eResearch Center and the study was approved by the UCT Human Research Ethics Committee (UCT HREC 297/2005). At recruitment, initially verbal consent was obtained from the child's parent or legal guardian for the intervention as it was part of routine medical care at RCWMCH. After the CSE had been terminated, full written consent was obtained from the parents or legal guardian in order to use the child's clinical data for research purposes. In addition, if medical records were incomplete or missing, these children were also excluded. Children who were on chronic treatment with phenobarbital or phenytoin and or had received intravenous phenobarbital or phenytoin prior to admission were excluded from the study.

Definitions and Data Collection

CSE was defined as any convulsive seizure that lasted longer than 5 min ("continuous") or multiple discrete seizures between which there was no extended period of recovery between events ("intermittent") (2). The time of CSE onset was defined as the time provided by the caregiver who accompanied the child. The time to admission and to treatment were recorded by the attending medical emergency unit staff. If children were admitted multiple times, each admission was captured independently. Upon first entry into the study, demographics and past medical history were captured for each child. Thereafter, data pertaining to each admission was captured separately, including initial seizure presentation, referral, treatment, investigations, and length of admission. In addition, the etiology of CSE was recorded and then classified according to the framework proposed by Trinka et al. (2). This included: "Acute," CSE caused by acute systemic illness or CNS injury (e.g., metabolic or electrolyte abnormalities, infection, stroke); "Remote," CSE followed previous CNS injury (e.g., post-stroke, post-infective, post-traumatic); "Progressive," CSE was the result of extending CNS disease (e.g., brain tumor); "Electroclinical," CSE presenting as part of a defined electroclinical syndrome (e.g., Dravet syndrome); and "Unknown," cause for CSE not found during admission. However, as it was not possible to perform EEG on all patients, the EEG axis was excluded. Febrile CSE was defined as CSE provoked by hyperthermia (>38.4 degrees Celsius) in the absence of prior afebrile seizures and evidence of acute central nervous system disease (28). Adverse events were defined as an acute decompensation in the child's state that followed the use of antiseizure medication, typically in the form of respiratory depression and or hypotension. All children presented in status are managed in an acute care setting where they are monitored for adverse events as part of routine emergency care. If these became evident, appropriate resuscitation and respiratory support measures were taken (e.g., inotropic support, assisted ventilation) and, when necessary, the child was referred to the PICU for further monitoring and management.

Admission Procedure

Children with CSE presenting to the RCWMCH were admitted to the medical emergency unit where they received standard monitoring and airway protection. This included continuous monitoring of heart rate, respiration, blood pressure and peripheral temperature as well as point-of-care tests such as urinalysis, glucose and blood gas analysis (including oxygen saturation). In addition, routine blood samples were sent to the laboratory including inflammatory markers and electrolytes. Additional blood investigations were performed as required. Children received urgent computed tomography (CT) imaging for focal seizures without a known cause or in the presence of focal neurological deficit, signs of raised intracranial pressure and/or prolonged depressed level of consciousness following seizure termination (20). Magnetic resonance imaging (MRI) was not performed in the acute setting. Where indicated, lumbar puncture was performed once the patient was stabilized and raised intracranial pressure excluded.

Treatment Protocols

Upon entry into the study, children were randomly allocated (at a ratio of 1:1) to one of two treatment protocols [**Figure 1** (PHB) and **Figure 2** (PHY)]. These protocols are based on the Emergency Triage Assessment and Treatment

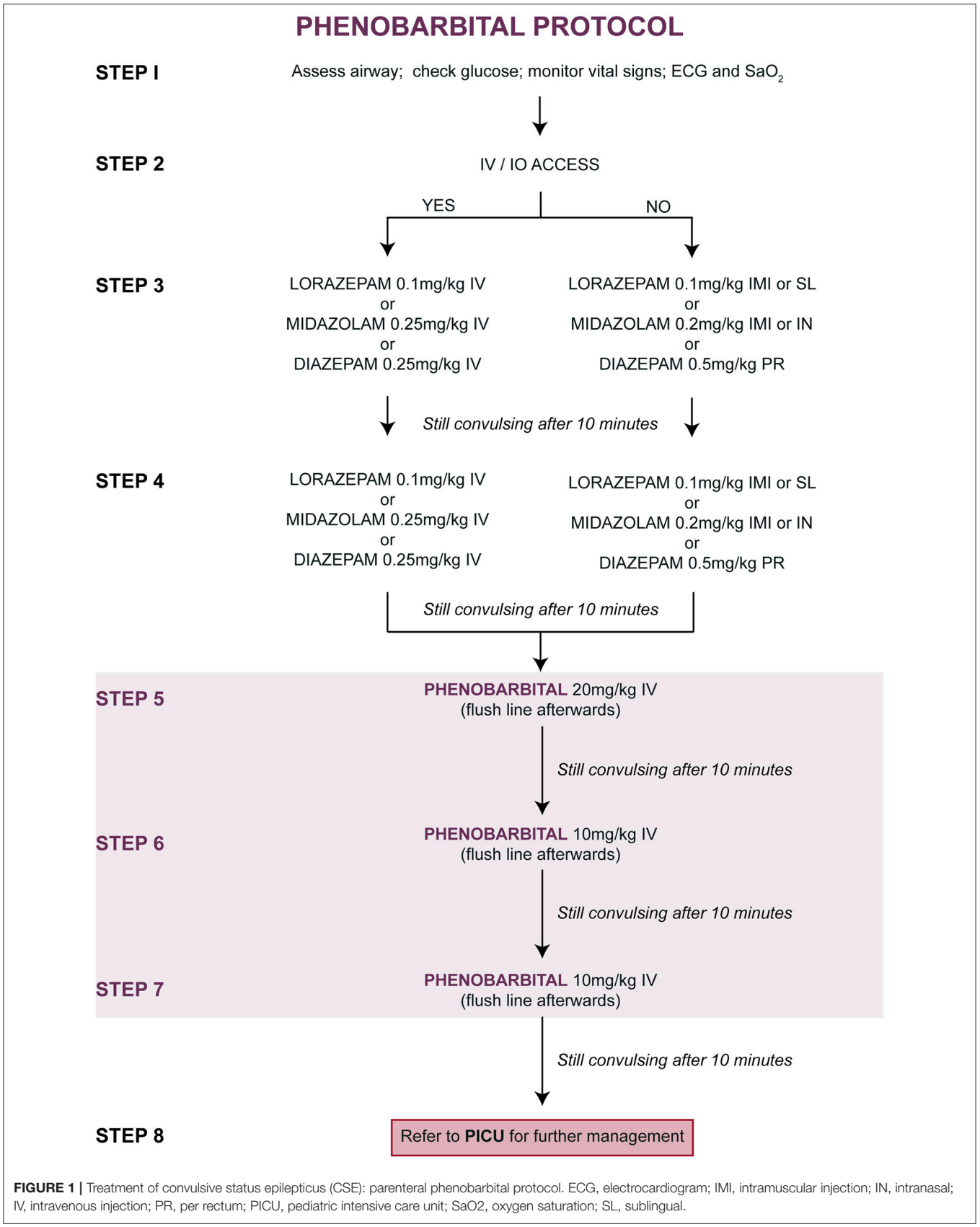

FIGURE 1 | Treatment of convulsive status epilepticus (CSE): parenteral phenobarbital protocol. ECG, electrocardiogram; IMI, intramuscular injection; IN, intranasal; IV, intravenous injection; PR, per rectum; PICU, pediatric intensive care unit; SaO2, oxygen saturation; SL, sublingual.

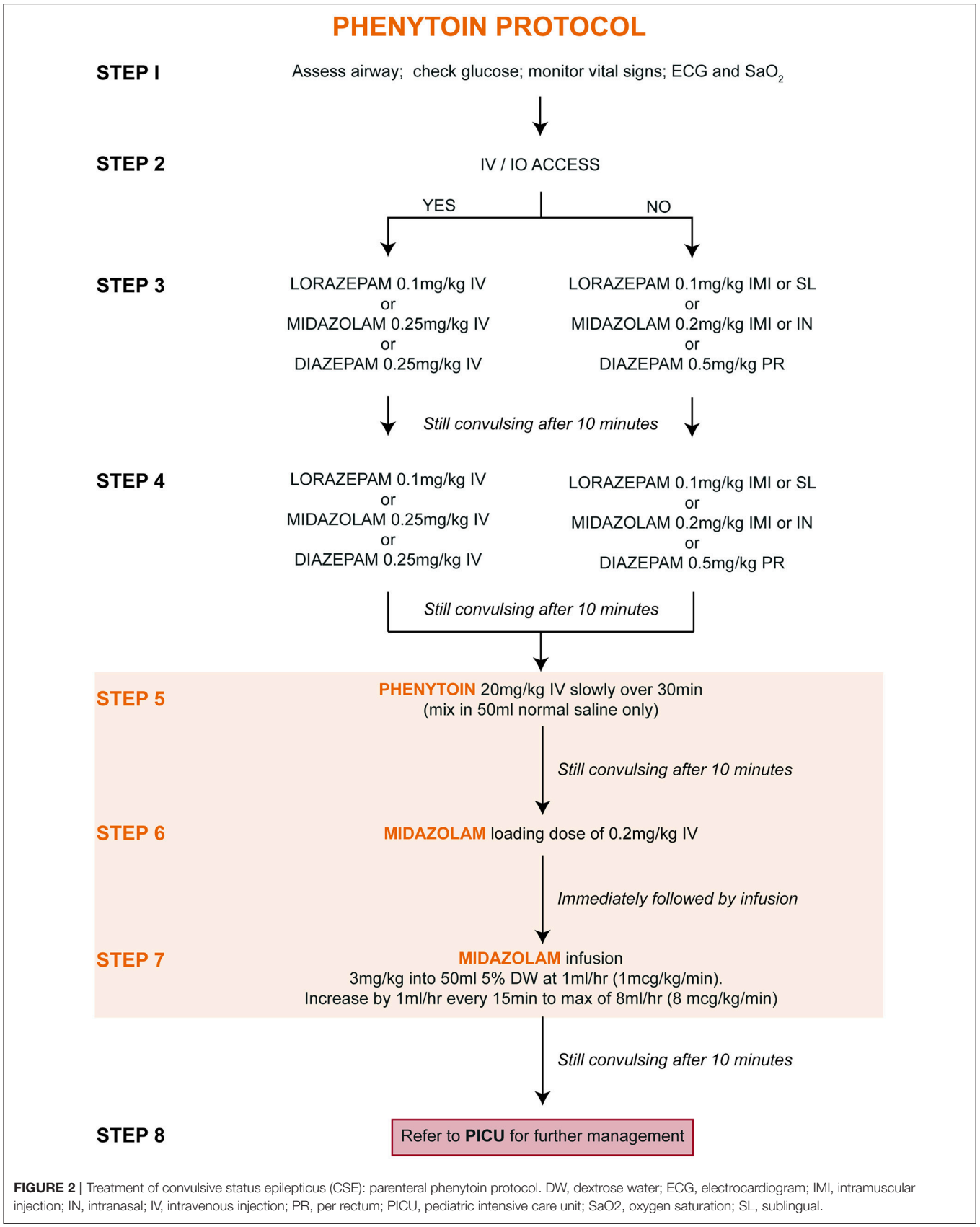

FIGURE 2 | Treatment of convulsive status epilepticus (CSE): parenteral phenytoin protocol. DW, dextrose water; ECG, electrocardiogram; IMI, intramuscular injection; IN, intranasal; IV, intravenous injection; PR, per rectum; PICU, pediatric intensive care unit; SaO2, oxygen saturation; SL, sublingual.

(ETAT) guidelines and used in the sub-Saharan African setting for the management of SE (20). Pre-hospital intravenous administration of benzodiazepines by emergency services were included, however all other routes of administration were not counted due to the lack of consistency in their administration. Children who did not respond to either the PHB or the PHY treatment protocols were referred to the PICU. Other reasons for admission to the PICU included respiratory depression following administration of the second-line agent, need for inotropic support, etiology-related concerns requiring intensive monitoring (e.g., severe electrolyte imbalances) and or prolonged state of a depressed level of consciousness.

Outcome Measures

We focused on short-term outcomes when comparing the response to the administration of the different second-line treatments given to patients with refractory CSE (i.e., who did not respond to first-line benzodiazepines). We performed an intention to treat (ITT) analysis that includes all patients that were randomized and allocated a treatment (29). All randomized patients were included in the analysis regardless of whether they received the allocated treatment. This approach was used to minimize the effect of protocol deviations as well as patients not progressing to second-line therapy on the random assignment of protocols. We do acknowledge that this approach only allows for a conservative measure of the effect of the treatments, but more accurately accounts for the inconsistencies that are inherent in clinical practice. The primary outcome was the success of the second-line agent in terminating refractory CSE. Specifically, we compared how many episodes of refractory CSE were terminated after a single dose of parenteral PHB or PHY. Secondary outcomes included the need for PICU admission and seizure recurrence (termed "breakthrough seizures") within the first 24 h following termination of CSE. Furthermore, we calculated the number needed to treat (NNT) to show how many patients with refractory CSE would need to have followed the parenteral PHB protocols over the parenteral PHY protocol to prevent admission to the PICU (calculation performed using ClinCalc (30)).

Sample Size

There is limited evidence quantifying the effectiveness of parenteral PHB and PHY for terminating pediatric CSE. The evidence available suggests second-line treatment with a single dose of parenteral PHB is effective in terminating pediatric CSE in 77% of cases (24). In contrast, Rai et al. (31) show that second-line treatment with PHY is effective in 97% of cases. We therefore based our estimated incidence of the primary outcome, termination of CSE, as 77% for the PHB group and 97% for the PHY group. We used ClinCalc (30) to calculate sample size with an independent dichotomous endpoint (two-sided test) using a Type I error probability (α) set at 0.05 and a Type II error probability (β) set at 0.2 (power 80%). The calculated sample size to see a difference in efficacy between the second-line agents was 86, with 43 episodes required in each of the two treatment groups. However, as the supply of parenteral PHB became limited, the study was stopped before the desired sample size was achieved. A *post-hoc* power calculation was performed to measure the statistical power with the acquired sample size. As response to a particular second-line agent was our primary outcome, after study completion we performed a *post-hoc* power analysis to recalculate the actual statistical power against what we had expected (using the ClinCalc (30)). As the actual incidence of CSE termination was 86% for patients who received PHB compared to 45% for those who received PHY, the *post-hoc* power was 94% which is higher than the 80% we had originally calculated whilst designing this study.

Randomization, Blinding and Concealment

Randomization of protocols was performed using a Research Randomizer (32) using a simple randomization technique (33). Study protocols were prepared in sealed, opaque envelopes by RJB and the numbers allocated by a separate party who was not otherwise involved in the study. Envelopes were placed in a secure box in the medical emergency unit. On admission into the study, the attending doctor managing an eligible patient took an envelope containing the protocol that would then be assigned to that patient. Due to differences in administration, the doctors implementing the protocols could not be blinded. However, after completion of patient recruitment and data collection, all patient identifiers were removed. In addition, those patients who had received second-line therapy were kept unknown until after the data analysis was performed.

Follow-Up

Patients were followed-up throughout their admission. Formal measurements of long-term outcomes (including neurodevelopmental assessment) are intended for a future follow-up study.

Data Analysis

Data were analyzed using custom scripts written on MATLAB (Statistics Toolbox, Release 2018a The MathWorks, Inc., Natick, Massachusetts, United States). We used a pair-wise deletion to deal with missing data that was not present in the patient's records. For continuous data, normality was established using the Shapiro-Wilk test and thereafter parametric (i.e., *paired* or *unpaired student's t-tests*) or nonparametric tests (i.e., *Mann-Whitney U-test*) were performed. Data that were not normally distributed were reported as median with the interquartile range (*IQR*). Categorical data were summarized in contingency tables with differences between groups identified using the *Fisher-exact* or *chi-squared* (X^2) tests. Contingency tables were used to calculate associations between exposures (i.e., treatments received) and outcomes (i.e., success in terminating CSE, need for admission to PICU, etc.). Associations are reported as relative risk (RR) with its associated 95% confidence interval. Significance was defined as $p < 0.05$.

RESULTS

Over a three-year period, a total of 193 episodes of CSE were entered into the study with 40 of these being re-admissions (**Figure 3**). Forty-nine episodes needed to be excluded as they either did not meet the definition of CSE at admission or their

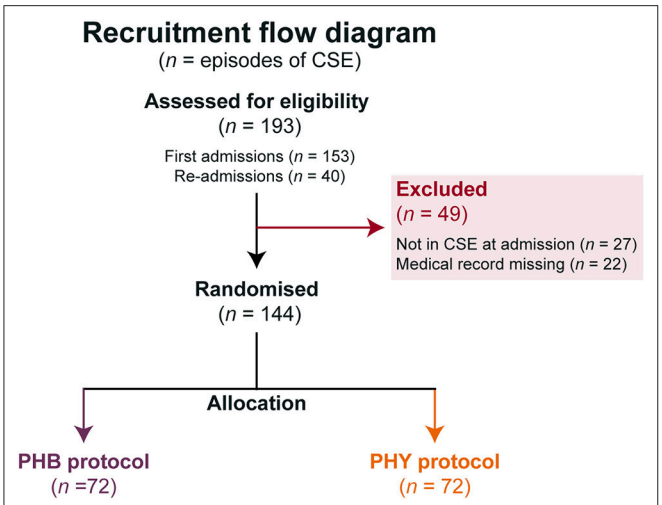

FIGURE 3 | Flow chart of protocol allocation. Number of episodes of pediatric convulsive status epilepticus (*n*) studied and allocation to the different second-line treatment protocols. All patients allocated a pathway were included in the intention-to-treat analysis. BZP, benzodiazepines (including diazepam, lorazepam and midazolam); CSE, convulsive status epilepticus; PHB, phenobarbital; PHY, phenytoin.

TABLE 1 | Demographics of patient cohort recruited into study.

	Full cohort (*n* = 111)	PHB protocol (*n* = 52)	PHY protocol (*n* = 59)	*p*
GENDER				
Female	58 (52.3%)	26 (50.0%)	32 (54.2%)	0.71
Male	53 (47.7%)	26 (50.0%)	27 (45.8%)	
PAST MEDICAL HISTORY				
HIV-infected	3 (2.7%)	2 (3.8%)	1 (1.7%)	0.60
Cerebral palsy	20 (18.0%)	10 (19.2%)	10 (16.9%)	0.75
Previous TBM	3 (2.7%)	1 (1.9%)	1 (1.7%)	>0.99
Previous TBI	2 (1.8%)	1 (1.9%)	2 (3.4)	>0.99
NEONATAL HISTORY				
Pre-term (<37 weeks)	18 (16.2%)	7 (13.5%)	8 (13.5%)	0.76
Documented HIE	10 (9.0%)	4 (7.7%)	6 (10.2%)	0.74
SEIZURE HISTORY				
Previous admission for seizures	51 (45.9%)	28 (53.8%)	23 (39.0%)	0.13
Confirmed epilepsy diagnosis	47 (42.3%)	26 (50.0%)	21 (35.6%)	0.18
EPILEPSY ETIOLOGY				
Genetic	12 (25.5%)	7 (26.9%)	5 (23.8%)	0.54
Infectious	1 (2.1%)	1 (3.8%)	–	0.47
Structural	25 (53.2%)	12 (46.2%)	13 (61.9%)	>0.99
Unknown	9 (19.2%)	6 (23.1%)	3 (14.3%)	0.3

HIE, hypoxic ischemic encephalopathy; HIV, human immune-deficiency virus; TBI, traumatic brain injury; TBM, tuberculous meningitis.

TABLE 2 | Overview of presentation of CSE episodes included in this study.

	Total episodes (*n* = 144)	PHB protocol (*n* = 72)	PHY protocol (*n* = 72)	*p*
AXIS I: SEMIOLOGY				
Focal onset evolving into bilateral SE	28 (19.4%)	13 (18.1%)	15 (20.8%)	0.83
Generalized	93 (64.6%)	46 (63.9%)	47 (65.3%)	>0.99
Unknown focal or generalized	23 (16.0%)	13 (18.1%)	10 (13.9%)	0.65
TYPE Of CSE				
Continuous	73 (50.7%)	40 (55.6%)	38 (52.8%)	0.87
Intermittent	71 (49.3%)	32 (44.4%)	34 (47.2%)	
AXIS II: ETIOLOGY				
Acute	86 (59.7%)	45 (62.5%	41 (56.9%)	0.93
Electroclinical	20 (13.9%)	10 (13.9%)	10 (13.9%)	>0.99
Remote	24 (16.7%)	13 (18.1%)	11 (15.3%)	0.82
Unknown	14 (9.7%)	4 (5.6%)	10 (13.9%)	0.16
Febrile status epilepticus	27 (18.8%)	14 (19.4%)	13 (18.1%)	>0.99
AXIS IV: AGE				
Age at admission- median months (*IQR*)	28.1 (15.5–66.0)	25.7 (13.1–65.6)	22.2 (14.8–46.3)	0.48
Infancy (1 month−1 year)	25 (17.4%)	9 (12.5%)	16 (22.2%)	0.19
Childhood (>1 year−12 years)	119 (82.6%)	63 (87.5%)	56 (77.8%)	

SE, status epilepticus; CSE, convulsive status epilepticus.

medical records could not be found to complete data collection. This left a total of 144 episodes from 111 patients. There was a 50:50 split between the children that were allocated to either the PHB or PHY protocol (72 episodes in each group). All episodes of the CSE in these two groups were included in the intention-to-treat analysis. The demographic and past medical history for both the total patient cohort ($n = 111$) and each treatment arm is shown in **Table 1**. Of the full cohort, 46% of children had previously been admitted for seizures and 42% had a preceding diagnosis of epilepsy. In those children with epilepsy, structural (53%) and genetic (26%) causes were the most common.

In terms of overall presentation of pediatric CSE (**Table 2**), the most common seizure semiology was generalized CSE (65%) due to an acute etiology (60%). At the time of admission, the median age for the full cohort was 28.1 months (*IQR* 15.5–66.01). There was a near equal prevalence of continuous (51%) vs. intermittent episodes (49%) of CSE. In addition, 19% of admissions met the diagnostic criteria for FSE. Between the patients randomized to the PHB and PHY treatment groups, there were no differences in patient demographics nor in the presentation of CSE.

Looking at management, overall 48% required second-line intervention with a further 13% requiring third-line intervention (**Table 3** and **Figure 4A**). The median time to first-line treatment was 50 min (*IQR* 33.8–70.5). Overall, 20% of children presenting in CSE required admission to the PICU mostly due to concerns of respiratory depression (66%). Breakthrough seizures occurred in 13% of patients.

With regards to comparing the two second-line treatment protocols (**Table 3**), of the 72 episodes allocated in each group, only 36 episodes (50%) progressed to second-line intervention in the PHB group and 33 (46%) in the PHY group. Of the patients who required second-line treatment, we found after

TABLE 3 | Overall management of CSE and differences in outcomes between PHB and PHY groups.

	Total episodes ($n = 144$)	PHB protocol ($n = 72$)	PHY protocol ($n = 72$)	*p*
RESPONSE TO TREATMENT (RESPONDED/TOTAL)				
First-line: benzodiazepines	75/144 (52.1%)	36/72 (50.0%)	39/72 (54.2%)	0.74
Second-line: PHB or PHY	50/69 (72.5%)	31/36 (86.1%)	15/33 (45.5%)	0.0003
Third-line: repeated PHB or MDZ	18/19 (94.7%)	4/5(66.7%)	18/18 (100%)	0.002
Fourth-line: PICU	1 (100%)	1/1 (100%)	-	>0.99
TREATMENT TIMES (MINUTES)				
Onset to admission	40.0 (25.0–65.0)	35.0 (25.0–60.0)	40.0 (22.8–65.0)	0.99
Admission to treatment	5.0 (5.0–10.0)	5.0 (5.0–10.0)	5.0 (5.0–10.0)	0.61
Onset to first-line treatment	50.0 (33.8–70.5)	50.0 (34.0–72.0)	50.0 (32.0–70.0)	0.83
Total CSE duration	73.0 (48.0–109.0)	64.0 (45.0–103.5)	83.0 (53.0–115.0)	0.04
First-line treatment to arrest	16.5 (5.0–45.0)	5.0 (3.0–12.0)	9.0 (3.0–14.0)	0.29
Second-line treatment to arrest	28.0 (13.3–41.5)	10 (10.0–21.8)	28.0 (24.5–33.0)	<0.0001
Third-line treatment to arrest	13.0 (8.0–25.0)	13.0 (13.0–28.0)	12.0 (6.5–22.0)	0.39
PICU				
Required admission	29 (20.1%)	9 (12.5%)	20 (27.8%)	0.04
REASONS FOR PICU ADMISSION				
Inotropic support needed	2 (6.9%)	–	2 (10.0%)	>0.99
Respiratory depression	19 (65.5%)	5 (55.6%)	14 (70.0%)	0.68
Prolonged LOC	7 (24.1%)	3 (33.3%)	4 (20.0%)	0.64
Seizure control	1 (3.5%)	1 (11.1%)	–	0.31
Breakthrough seizures	18 (12.5%)	8 (11.1%)	10 (13.9%)	0.8

LOC, prolonged loss of consciousness; PICU, pediatric intensive care unit.

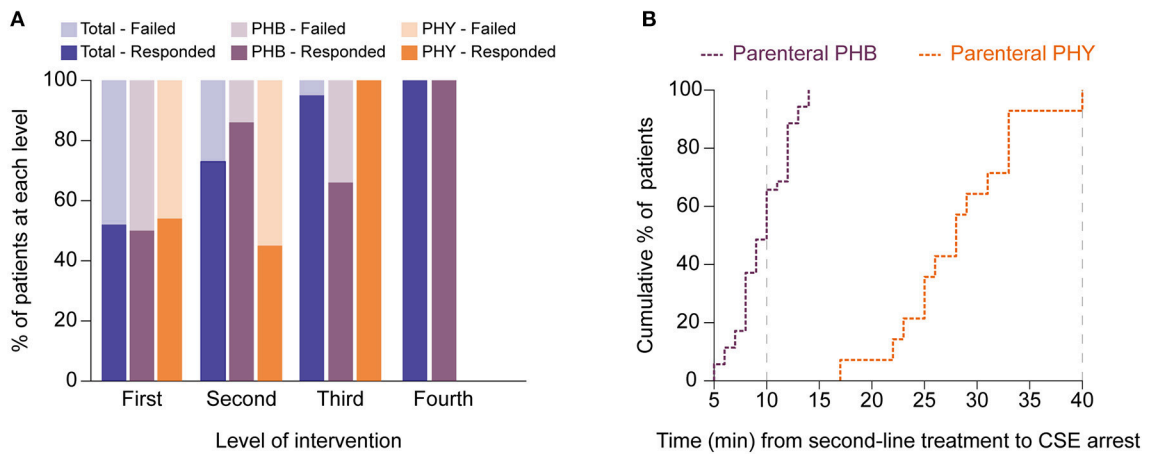

FIGURE 4 | Second-line treatment with parenteral phenobarbital terminates pediatric convulsive status epilepticus more successfully and faster than parenteral phenytoin. **(A)** Bar graph showing differences in responses to different levels of intervention between the total cohort (blue) as well as for the phenobarbital (PHB, purple) and phenytoin (PHY, orange) treatment groups. Dark shading indicates percentage of patients that responded to level whilst lighter shading shows those patients who failed that level. **(B)** Cumulative percentage plot showing the proportion of patients who responded (i.e., CSE terminated) to either parenteral phenobarbital (purple) vs. parenteral phenytoin (orange) at specific time points (in minutes) after the second-line agent was introduced. Gray dashed lines indicate when the protocol recommends progression to a third-line agent. Y axis represents the cumulative percentage of patients whose CSE had terminated whilst X axis represents time in minutes.

a single dose of parenteral PHB, 86% of children responded with the CSE termination. In contrast, CSE was terminated in only 46% of children who received a single parenteral dose of PHY. Furthermore, we noted that in the patients who required third-line treatment, 67% of patients who responded to repeated parenteral boluses of PHB with one patient requiring fourth-line intervention given in the PICU. In contrast, 100% of patients given the MDZ infusion responded.

To measure the efficacy of a particular treatment, we used contingency tables to compare the number of children who had their refractory CSE successfully terminated using second-line treatment with either PHB or PHY. Using this approach we were then able to calculate the relative risk (*RR*) of failing second-line treatment (i.e., CSE not terminating within 10 min after the full dose of the agent had been given). We found that patients who received parenteral PHB as second-line treatment had a significantly lower risk of needing third-line intervention compared to those who received PHY (14 vs. 54%, $RR = 0.3$, *95% CI* 0.1–0.7, $p = 0.0003$). Furthermore, in the patients who responded to second-line treatment, we found that PHB terminated CSE significantly faster compared to PHY (*median* 10 min, *IQR* 10.0–21.8 vs. *median* 28.0 min, *IQR* 24.5–33.0, $p < 0.0001$; **Figure 4B**). Of those patients who did respond to third-line treatment, there was no difference in time to CSE arrest between those that received repeated PHB boluses or the MDZ infusion (*median* 13.0 min, *IQR* 13.0–28.0 vs. *median* 12.0, *IQR* 6.5–22.0, $p = 0.4$).

We also noticed that overall fewer patients allocated to the PHB protocol required admission to the PICU compared to those allocated to the PHY protocol (13 vs. 28%, $RR = 0.57$ *95% CI* 0.32–0.99). We calculated a NNT of 6.5, indicating that 6.5 patients with refractory CSE would have to have followed the parental PHB protocol in order to reduce a single admission to the PICU that would have otherwise occurred if they had followed the parenteral PHY protocol. We then assessed the risk of needing admission to PICU at each level of intervention. Notably we found that in patients who required second-line intervention, there was no significant difference between the PHB and PHY groups (30 vs. 7%, $RR = 1.45$, *95% CI* 1.1–2.0, $p = 1.4$). However, in patients who required third-line treatment, those that received the repeated boluses of parenteral PHB were at a significantly lower risk of requiring PICU admission compared to those that received the MDZ infusion (25 vs. 100%, $RR = 0.05$, *95% CI* 0.01–0.4, $p = 0.003$). Lastly, we saw no difference in the proportion of patients who had breakthrough seizures within the first 24 h after CSE was terminated between the two groups (11 vs. 14%, $p = 0.8$).

DISCUSSION

In conducting this study, we have been able to describe the local burden of CSE while also providing new data on the efficacy of parenteral PHB as second-line management. A large proportion of our cohort with CSE had previously presented with seizures or had an established diagnosis of epilepsy prior to their entry into the study (**Table 1**). In contrast, Sadarangani et al. (5) reported only 23% of their Kenyan children presenting in confirmed CSE had a prior history of seizures. The majority of our children with epilepsy had an underlying structural cause. Surprisingly, the proportion of children with a documented past medical history of human immunodeficiency virus (HIV), tuberculous meningitis (TBM), traumatic brain injury (TBI) or hypoxic ischemic encephalopathy (HIE) was relatively small despite the high prevalence of these conditions in the South African context (34, 35). In the emergency setting the past medical history may not have been adequately recorded, leading to possible underestimation of these conditions.

The majority of our cohort presented with generalized CSE (**Table 2**). While this proportion is similar to that reported by Chin et al. (4) in their UK-based study, we suspect that generalized FSE was overestimated in our cohort due to the high prevalence of structural epilepsies. There may in fact be a much higher burden of seizures with unrecognized focal onset evolving into bilateral SE. The most common cause for pediatric CSE was an acute CNS injury or infection with 19% of the cases being FSE. These figures are again similar to what has been previously reported in both Kenya and the United Kingdom (4, 5).

The management of pediatric CSE in our cohort contrasts similar studies conducted in resource-equipped settings (**Table 3**). Firstly, the time from onset to admission was higher in our cohort (median 40 min) although we expected there to be a greater delay in our setting given the known barriers to accessing care. Secondly, the time from seizure onset to administration of first-line BZPs was significantly longer in our cohort (median of 50 min compared to 28 min reported in the Chin et al. (13) cohort). Our study was set in an urban environment and we would therefore expect the time from onset to admission to be even longer for children in rural settings, as suggested by previous work done in Kenya (5). Thirdly, the total seizure duration reported in our study was longer (73.5 min compared to 65 min reported in the Chin et al. cohort). Chin et al. (13) have previously identified a lack of prehospital treatment, delayed admission time, more than two benzodiazepines and intermittent CSE as risk factors for CSE lasting longer than 60 min. In addition, we reported a higher proportion of patients who did not respond to first-line benzodiazepines (48 vs. 35% reported in the Chin et al. cohort). The proportion of patients requiring PICU admission (20%) was similar to the Chin et al. cohort (20%). This was surprising, as we expected that the longer delay in treatment would result in a greater need for PICU intervention. However, this may be explained by differences in accessibility to PICU, as in our setting the access to PICU is significantly more limited compared to a more resource-equipped hospital.

Our intention-to-treat comparative analysis has shown that parenteral PHB is a more effective and efficient second-line agent for refractory CSE compared to parenteral PHY. This is evident in PHB terminating CSE faster and more successfully by decreasing the need for higher intervention and admission to PICU. However, we do acknowledge that this difference may have a modest effect clinically as the calculated NNT was 6.5 indicating that for every 6.5 patients treated with the PHB protocol, one patient is prevented from being admitted to the PICU if they otherwise followed the PHY protocol. A major contributing factor to why PHY is less effective than PHB is likely due to the longer time needed to administer it (36). The reason why patients in the PHY group required admission to the PICU was due to increased need for third-line with a midazolam infusion, which is known to cause significant respiratory depression. The need for admission to the PICU is of particular importance due to the lack of PICUs, and PICU beds, available in resource-limited

healthcare settings. Furthermore, for the children who required third-line intervention, we found no significant difference in efficacy between using repeated parenteral boluses of PHB vs. using an MDZ infusion.

Previous work by Malamiri et al. (24) from Iran compared parenteral sodium valproate against intravenous phenobarbital as second-line management of pediatric refractory CSE. Their results show that parenteral sodium valproate appears more effective in decreasing recurrence of seizures within 24 h as well as decreasing adverse effects (namely respiratory depression) compared to parenteral phenobarbital. However, parenteral sodium valproate is not commonly used within Africa for the management of pediatric CSE with the common alternative to parenteral phenobarbital being parenteral phenytoin. Apart from our study, there is currently no evidence comparing parenteral phenobarbital vs. phenytoin for second-line management of pediatric CSE. The only direct comparison can be found in the study by Treiman et al. (22) who showed that first-line management with parenteral phenobarbital appeared more effective in terminating CSE in adults. Therefore, for those practicing within sub-Saharan Africa there remains a demand to compare the commonly used parenteral phenobarbital against parenteral phenytoin which remains as the recommended second-line agent on pediatric CSE management guidelines.

Our findings conflict with those of Sreenath et al. (37) who claim that parenteral lorazepam and the combination of diazepam and phenytoin is 100% effective in terminating CSE. Moreover, Rai et al. (31) also suggested that phenytoin is 97% effective in terminating pediatric status epilepticus. To explain this difference in PHY efficacy, it is important to consider differences in the underlying cause of CSE. Specifically, FSE is thought to be associated with sodium-channel mutations that would impact the efficacy of PHY. Notably, PHY was only effective in terminating FSE in 14% of pediatric cases (38). Comparing our study with Sreenath et al. (37), we noted that 27% of the children in our study who received PHY had FSE compared to 7.9% in theirs.

In terms of the generalizability of this study, we believe it provides useful clinical data for other resource-limited healthcare settings. Our data shows that parenteral phenobarbital is an effective antiseizure medication in the management of pediatric CSE, thereby validating previous reports (26, 27). However, the findings of this study may not be globally relevant as in resource-rich settings there is ready access to newer antiseizure medications (namely intravenous levetiracetam). This study is also not comparable to the larger double-blinded randomized controlled studies currently underway (EcLiPSE, ESETT and ConSEPT). Nevertheless, we have been able to demonstrate that within a resource-limited setting, parenteral PHB remains the most effective treatment. While in our setting at RCWMCH we are able to give PHY followed by a midazolam infusion with appropriate monitoring and access to PICU, in the majority of centers across Africa this is not viable. In contrast, PHB is an effective agent for the management of CSE while decreasing demands on healthcare resources. PHB is often quoted to cause hypotension and respiratory depression, but there is little data to support this. Previous work by Crawford et al. (39) reported that even very high doses of parenteral PHB is safe, with few adverse effects. Respiratory depression and hypotension following treatment for CSE were found to be related to confounding factors including excessive levels of benzodiazepines and/or the underlying etiology.

While we attempted to uphold scientific rigor throughout the design and implementation of this study, it is not without limitations. Most notably, the reliability of the reporting of both time of seizure onset and seizure semiology by the child's caregivers should be viewed with caution. During the recruitment of children into this study, our sample size was affected by a large number of exclusions. This was in part due to incorrect diagnosis of CSE, highlighting a need to train local practitioners in the identification of CSE according to the latest guidelines. In addition, 22 children were excluded due to missing paper-based medical records. This reflects a concerning inefficiency within our healthcare system which negatively impacts research efforts and, more importantly, the care of patients. Furthermore, as EEG was not routinely performed in the acute setting, we were not able to exclude non-convulsive SE after CSE was terminated (40). However, standard operating procedure of the neurology service ensures that any child with persistent reduced level of consciousness post seizure termination, or abnormal movements post seizure termination, undergoes EEG. This study was also vulnerable to bias as the practitioners administering the protocols were not blinded. While the analysis was blinded, it was not possible to blind the implementation due to differences in administration of the agents.

CONCLUSION

This study has characterized the burden of pediatric CSE within our local setting, providing important epidemiological insight. We hope these findings will be useful for those managing pediatric CSE within resource-limited settings while also advocating for parenteral PHB to be retained.

ETHICS STATEMENT

This study was approved by the University of Cape Town Human Research Ethics Committee (reference number: 297/2005). Signed consent forms were obtained from the parents or legal guardian of all children recruited into this study.

AUTHOR CONTRIBUTIONS

RB was responsible for study design, data collection, data analysis, and writing of the manuscript. SA was involved in the study design, data collection and writing of the manuscript. AS-C and AN assisted in study design and data collection. HB was involved in study design, data collection, data analysis, and supervising the writing of the manuscript. JW was the principle investigator and supervised the study design, data collection, data analysis, and writing of the manuscript.

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REVIEW
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Deep Brain Stimulation and Drug-Resistant Epilepsy: A Review of the Literature

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Introduction: Deep brain stimulation is a safe and effective neurointerventional technique for the treatment of movement disorders. Electrical stimulation of subcortical structures may exert a control on seizure generators initiating epileptic activities. The aim of this review is to present the targets of the deep brain stimulation for the treatment of drug-resistant epilepsy.

Methods: We performed a structured review of the literature from 1980 to 2018 using Medline and PubMed. Articles assessing the impact of deep brain stimulation on seizure frequency in patients with DRE were selected. Meta-analyses, randomized controlled trials, and observational studies were included.

Results: To date, deep brain stimulation of various neural targets has been investigated in animal experiments and humans. This article presents the use of stimulation of the anterior and centromedian nucleus of the thalamus, hippocampus, basal ganglia, cerebellum and hypothalamus. Anterior thalamic stimulation has demonstrated efficacy and there is evidence to recommend it as the target of choice.

Conclusion: Deep brain stimulation for seizures may be an option in patients with drug-resistant epilepsy. Anterior thalamic nucleus stimulation could be recommended over other targets.

Keywords: anterior thalamic nucleus, electrical stimulation, neuromodulation, neurostimulation, refractory epilepsy, target

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INTRODUCTION

Deep brain stimulation (DBS) is a neurointerventional technique that involves implanting electrodes and a pacemaker-like device to deliver pulses of electricity to specific areas of the brain. Although the mechanism of action remains to be fully elucidated, it is suggested that DBS acts via focal modulation of specific functional circuits within the brain (1, 2). The fact that the same DBS parameters and targets can benefit different neurological disorders suggests that DBS does not act against the pathophysiology of any specific disorder, but rather modulates existing and active pathologic brain circuits and it is well-tolerated (3).

The success of DBS for the treatment of Parkinson's disease (PD), in conjunction with the benefits of being adjustable, reversible, and exhibiting a good safety profile, has prompted investigation into the potential utility of neuromodulation via DBS for other diseases (4). Tens of thousands of patients suffering from different forms of neurological disorders have been treated with DBS worldwide (5), including tremor, dystonia, obsessive–compulsive disorder, depression, Tourette's syndrome, headache, chronic pain, eating disorders, and epilepsy (6). Anti-epileptic drugs (AEDs) can control seizures in most patients with epilepsy. However, at least 30% of adults with epilepsy do not achieve seizure control with AEDs (7) and surgery to remove or disconnect the epileptogenic zone is not always an appropriate option (8). These patients may be candidates for neurostimulation.

The number of potential neural targets in drug-resistant epilepsy (DRE) has increased over the years. The majority of available literature suggests targeting the anterior thalamic nucleus (ATN). In the following sections, we review the clinical outcomes for the most commonly chosen targets for the treatment of epilepsy. We included the ATN, centromedian thalamic nucleus (CMTN), hippocampus, basal ganglia (caudate nucleus, subthalamic nucleus), posterior hypothalamus and cerebellum.

MATERIALS AND METHODS

We performed a literature search of the Medline®, Embase®, Index Medicus®, Scopus, and Cochrane databases from January 1980 to October 2018 that incorporated Medical Subject Headings and text words for literature related to "deep brain stimulation" and "drug-resistant epilepsy." We also searched bibliographies of pertinent reviews: original articles reference lists, book chapters and relevant conference proceedings to find additional documents. We included original retrospective and prospective studies assessing the impact of DBS on seizure frequency in patients with DRE regardless of language or country of origin. Children were classified as subjects younger than 18 years. Systematic reviews, meta-analyses, randomized controlled trials, and observational studies were included. We also included studies on experimental, animal or molecular models in search of an integrative review (basic and clinical science). The following outcomes were assessed: seizure reduction; seizure freedom; and time of follow-up. Discrepancies were solved by consensus. Categorical data were expressed as percentages and quantitative data as mean, standard deviation, and range. All statistical analyses were performed with SPSS statistical software package (SPSS for Mac, v.21, SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Of the 429 abstracts identified by the search, 145 were reviewed as full-text articles. Seventy-two articles fulfilled eligibility criteria and described outcomes in 826 patients. The majority of patients were diagnosed with generalized or secondary generalized seizures (75%), while 7.2% included exclusively patients with focal seizures. Age ranged between 5 and 66 years, with a median of 30 years. All patients included in the studies had DRE.

Historical Perspective

The beginnings of DBS date back to the late 19th century. Several authors identified the functional anatomy of the brain using animal models that went against the established beliefs and dogmas of the time (9). Horsley and Clarke (10) were pioneers in the development of stereotactic frameworks for experimental use in animals. Subsequently Spiegel et al. (11) developed the use of X-ray pneumoencephalography in 1947 allowing to visualize the living brain more accurately. As well, this enabled the creation of stereotactic atlases to guide surgeries. In 1950s we saw the introduction of neuro-ablative techniques for the treatment of Parkinsonian tremor, with the study of Albe Fessard et al. (12). They were the first to report the use of high frequency electrical stimulation (∼100–200 Hz) targeting the intermediate ventral thalamic nucleus with clinical improvement in tremor severity (12). The emergence of levodopa as a highly effective pharmacological treatment for PD in the 1960s limited the development of DBS, although several authors such as Hosobuchi et al. (13) and other research groups continued their studies in other pathologies, such as chronic pain and disorders with impaired level of consciousness with encouraging results.

As further evidence accumulated through the 1980s in regards to adverse effects of levodopa, such as dyskinesias, and patients who were resistant to treatment, a better understanding of the basal ganglia (14) emerged. Furthermore, the subthalamic nucleus (STN) was identified initially as a central locus of PD (15) and therefore represented an important potential surgical target (16). This was eventually introduced into clinical practice by Pollak et al. (17). Shortly thereafter, they identified the globus pallidus interna (GPi) as another target in 1994 (18).

From these advances, the use of DBS expanded to other pathologies such as epilepsy. The first studies investigating the anti-epileptic effects of DBS in epilepsy were published in the 1970s and 1980s (19–21). Since then, numerous studies have been published evaluating the effectiveness and safety of DBS in epilepsy; however, most studies have limitations as generally report small number of patients with variable results. To date, only one large randomized control clinical trial has been published (22), generating modest evidence of benefit.

Pathophysiology

The basic rationale for DBS as an effective anti-epileptic therapy is similar to what has been postulated for movement disorders: potential cellular inhibition or excitation (neuro-modulation) within the target structure (23). The stimulation will then either help to disrupt seizure propagation or raises the overall seizure threshold. If appropriately coordinated, low frequency stimulation (LFS) has been shown to restore normal neuronal electrical activity, while high frequency stimulation (HFS) is typically more effective at disrupting the propagation of synchronous neuronal activity (24). In hippocampal rat slice models, HFS enhances the inhibitory tone of the network and prevents the synchronization and propagation of epileptiform burst discharges (25).

There are three different theories behind the selection of targets: the stimulus can be: (1) directly applied to the suspected seizure onset zone; (2) applied in deep subcortical structures to interrupt epileptic networks or; (3) applied over deeply located fiber bundles, which are connected to different structures within the brain (1, 2). The direct stimulation of the epileptogenic zone alters the tissue excitability and neuronal synchronization and can have an inhibitory effect, without causing functional deficits (1). Direct targets include the hippocampus, amygdala, hypothalamus or specific cortical zones. The indirect stimulation of deep structures may suppress neuronal circuits that favor seizure emergence. These targets include the cerebellum, basal ganglia and thalamus (2). Stimulation of fiber bundles in structures such as fornix or corpus callosum alter the threshold of seizure induction by modulation of neuronal circuits which are located in different but connected brain structures (26) (see **Figure 1**).

Networks

Papez circuit links hippocampal output via the fornix and mammillary nuclei to the ATN. Projections from the ATN then travel through the cingulum bundle to the parahippocampal cortex and complete the circuit by returning to the hippocampus. Animal studies have supported the role of this circuit as being important in seizure occurrence (27, 28). Alterations in Papez' circuit have been observed in multiple forms of epilepsy. The cerebellum, STN, and CMNT all have projections to the circuit of Papez and have therefore been considered as potentially therapeutic DBS targets for seizure reduction (29).

Another important network is the cortico-thalamo-cortical loop, which has been associated with absence epilepsy (30) and motor seizures (31) in animal models. Lesional studies in non-human primates with focal epilepsy showed almost complete suppression of seizures with thalamotomy (32). More recent studies using optogenetic techniques have shown that both thalamic and cortical neurons can trigger seizures, contrary to previous hypotheses (33–35). It has been proposed that the thalamocortical pathway acts as a "choke point" in the disruption of seizure propagation (30).

Basic Mechanisms

The mechanism of action underlying DBS remains poorly understood. Its action on neuronal circuits is likely multifactorial and complex. The initial DBS study suggested that its effect was inhibitory (36). This inhibition could be explained by either the blockade of depolarization and inactivation of voltage-gated currents, or, alternatively, by activation of GABAergic afferents in the stimulated nucleus (37). It is not completely clear if the therapeutic effects of DBS occur due to the stimulation of neurons, glial cells, passage fibers or afferent inputs to target neurons (38). Some studies have identified that activation thresholds were lowest in myelinated axons, and sequentially increased in unmyelinated axons, dendrites, and cell bodies (39, 40). In addition to orthodromic activation of efferent axons, multiple studies conducted in animal models and humans with PD have shown that DBS excites afferent axons in an antidromic manner (41, 42).

Other studies have shown that DBS stimulates neurons and astrocytes, producing a release of glutamate, D-serine and ATP (43). The activation of astrocytes leads to neuronal modulation through brain flow and neurovascular factors (44, 45). In addition, a "microlesion effect" has been proposed. It is demonstrated by clinical improvement in patients prior to turning the device ON, which favors the astrocytes activation hypothesis (46). Electrotaxis, the mechanism by which progenitor cells migrate through the electric current produced by DBS (47), seems to be based on inducing synthesis of growth factors and gene expression, which enhance neuroplasticity and neurogenesis (48, 49).

Neurostimulation is classified according to the method of stimulation (50). An open-loop neurostimulation system applies chronic, intermittent or continuous stimulation to inhibit epileptiform activity without reference to the patient's clinical symptoms or ongoing electroencephalogram (EEG) activity. Using an implanted seizure detection device, closed-loop stimulation provides more efficient treatment by adjusting the stimulation settings in response to EEG changes, before application of electrical stimuli (2). A burst of stimulation is applied, with the intention of terminating the detected bioelectrical change (51). Recent evidence supports the concept that closed-loop stimulation (feedback-controlled) can be more effective than open-loop therapy (52). The responsive neurostimulator system (RNS, NeuroPace, Inc., Mountain-view, CA, USA) is a prime example of a closed-loop system and has been approved by the FDA for use in epilepsy. This system applies electrical stimulation to a previously defined seizure-onset zone, triggered by detection of electrophysiological signatures of a seizure in real-time (53). RNS has demonstrated efficacy in the reduction of epileptic seizures in long-term studies, increases in quality of life scores and acceptable safety (51, 54).

TECHNIQUE

The DBS procedure consists of three steps: (1) preoperative planning, (2) surgical implantation, and (3) postoperative assessment. Prior to surgery, stereotactic coordinates for the target region are obtained by merging magnetic resonance imaging (MRI) of the patient's brain with a brain atlas (55). DBS for DRE is carried out with the patient under local or general anesthesia. The surgical procedure typically takes up to seven hours to complete and involves a multidisciplinary team of surgeons, epileptologists, and technical device staff. First, the exact placement and trajectory path for the electrode lead is determined. Next, burr holes are carefully drilled at the planned entry points for the electrodes. Region-specific neuronal activity, as a functional landmark, is used to verify the target structure during surgical procedure. One or more permanent microelectrodes are inserted into the brain using imaging guidance. Intraoperative fluoroscopy and postoperative MRI or computed tomography (CT) scans are acquired to confirm electrode placement. Finally, lead extenders are tunneled subcutaneously down the neck to below the clavicle, in which the pulse generator is implanted.

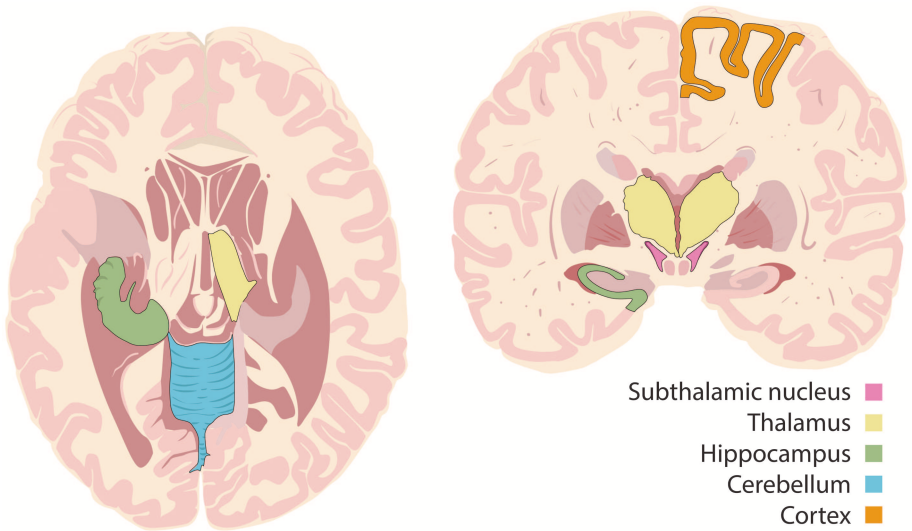

FIGURE 1 | Targets of deep brain stimulation.

Old frame-based systems utilize a fixed frame that surrounds the patient's head entirely. New frameless systems are essentially skull-mounted aiming systems. The patient's head is registered to a scan containing the planned trajectory using intraoperative imaging, and a neuro-navigation system is then used to align the surgical trajectory with the plan. Compared to their frame-based counterparts, the new frameless systems provide benefits, such as increased patient comfort and shorter operating times. A recent American meta-analysis found a clinically insignificant loss of accuracy with frameless methods, they can therefore represent a reasonable alternative to frame-based methods (56).

DBS Parameters

Stimulation parameters in epilepsy have been chosen empirically in the last 40 years, based on investigator experience in other pathologies, (primarily movement disorders), and center preferences. In some centers, epilepsy specialists even use electrodes that have been developed specifically for the treatment of PD (57). It is difficult to draw conclusions from existing studies suggesting parameters and predictors of efficacy, as they have been done with a small number of patients.

The evidence is scarce and often contradictory. The SANTE study found no favorable parameters for frequency, voltage, current, or pulse width after a long term follow up (22). There is no clear difference between cycling and continuous stimulation (58), or unilateral and bilateral stimulation (57). To date, usual stimulation parameters are: frequency $\geq$100 Hz and voltage at 1–10 V for stimulation of the ANT; frequency $\geq$130 Hz and voltage at 1–5 V for hippocampal and STN stimulation; HFS at voltage 1–10 V for stimulation of the CMNT; and low (10 Hz) or high (200 Hz) stimulation for the cerebellum (59). However, as there are currently no clinical randomized control trials comparing the different stimulation paradigms, the optimal parameters for epilepsy remain unknown.

Most DBS systems use a continuous, high frequency (100–250 Hz) pulse train (55). After surgery, several postoperative outpatient sessions are conducted over the course of 3–6 months, by a clinician who optimizes stimulation parameters based on patient feedback and seizure control (60). Often the clinician is a neurologist or an epilepsy nurse, who determines the optimal parameters including amplitude, frequency and pulse width.

TARGETS OF STIMULATION

Thalamus

Anterior Thalamic Nucleus (ATN)

The ATN is the most widely used target for DBS in treatment of DRE (22). It has been preferred because of its size, its distance from vascular structures (24), and its extensive connections. Several studies have indicated that the anterior thalamic region is crucial to the maintenance and propagation of seizures (61). This is explained by its connections to the limbic system through the fornix and mammillothalamic tract with widespread and extended projections to the cingulate, entorhinal cortex, hippocampus, orbitofrontal cortex, and caudate, all of which have been implicated in the pathogenesis of focal epilepsy (62).

Small open-label, uncontrolled, pilot studies have shown clinical benefits. A 49% reduction of seizure frequency was reported in four patients after 44 months of follow-up (58). Similarly, a mean reduction of 75% was observed in four patients with mesial temporal lobe epilepsy (TLE) after treatment with DBS-ATN (63). Lee and colleagues investigated 15 patients with DRE, who underwent placement of bilateral DBS electrodes in the ATN. They showed a significant decrease (70%) in seizure frequency and it was concluded that the short-term outcome of ATN-DBS directly reflects the long-term outcome (64). Additionally, Kerrigan et al. (65) reported four out of five patients with significant reductions in the frequency and

severity of seizures after a 36 month follow-up, without any complications. Hodaie et al. (61) reported a mean reduction of 54% in seizure frequency during a 15 month follow-up, also without adverse effects. Finally, Andrade et al. (66) described five of six patients (83%) with at least 50% improvement in seizure frequency over a mean follow-up period of 5 years. Sleep disruption and neuropsychiatric symptoms have been reported as a voltage-dependent adverse effect of DBS in ATN in patients with epilepsy (67).

These studies led to a randomized clinical trial in 2010 called *Stimulation of the Anterior Nucleus of the Thalamus for Epilepsy* (SANTE) (22). This study was a multicenter, double blind, clinical randomized control trial investigating the use of bilateral DBS of the ATN for the treatment of localization-related epilepsy. One hundred and ten patients with focal or secondarily generalized seizures, intractable to drug treatment, were divided into two groups. Half of the participants received stimulation, and half of the patients received no stimulation over a 3-month blinded phase. Subsequently, all patients received un-blinded stimulation. This trial reported significant improvement in seizure frequency, especially in focal seizures with altered awareness and severe seizures after 25 months of follow-up. Of the 110 patients initially enrolled in the study, 81 (74%) completed the follow-up period. Among these patients, the median decrease in seizure frequency was 56%, ranging widely from a 26% increase in frequency to complete seizure freedom (six patients). Median seizure frequency reduction continued to improve over the 3 years of the trial, with a 41%, 56%, and 68% median seizure frequency reduction at 1 year, 2 years, and 3 years of DBS, respectively, with 29% greater reduction in seizure attacks in the stimulated group compared to the control group, observed during the last month of treatment (22).

No surgery-related symptomatic hemorrhages or deaths were reported, although two participants had transient, stimulation-induced seizures. Other adverse events included paraesthesias at the implant site in 18%, local pain in 11%, and infection in 9% of cases. Depression and memory impairment were more frequent in the stimulated group compared with the controls. Patients with temporal lobe seizures showed a greater benefit during the blinded phase compared with those with extratemporal or multifocal seizure onsets (62). Some studies have suggested that bitemporal mesial epilepsy may be the most responsive to ATN stimulation (22). Direct targeting in the ATN using high-resolution MRI is likely superior to indirect targeting due to extensive individual variation in the location of the ATN and may therefore improve the efficacy of DBS (68). Furthermore, performance of ATN-DBS parameters with simultaneous EEG recording during the ATN-DBS has been suggested to improve the therapeutic efficacy by monitoring of EEG desynchronization (69). As demonstrated in previous studies, DBS had a better effect over time.

Centromedian Nucleus of Thalamus (CMNT)

Dense cluster of axons project from CMNT, a midline thalamic structure, to the dorsolateral part of putamen. The CMNT also projects to the cerebral cortex, principally to the motor and premotor cortices (70). Anatomical patterns of CMNT connections support its role in the pathophysiology of generalized seizures. Animal studies have demonstrated the CMNTs role in the initiation of seizures (71, 72) as well as in improvement of level of postictal consciousness after stimulation of the CMNT (73).

Stimulation of the CMNT in humans for treatment of DRE was first performed by Velasco and colleagues (74). CMNT stimulation appears to be more suitable for the control of absence and generalized seizures, especially in patients with primary or secondary Lennox Gastaut syndrome (LGS) with up to 80% of patients showing a good response. It does not appear to be effective for the treatment of focal seizures with altered awareness (74). Targeting the parvocellular division of the CMNT bilaterally, Velasco et al. (75) observed a reduction in seizure frequency in 13 patients with LGS. However, this was an open-label uncontrolled case series. In the only controlled pilot study of CMNT stimulation, preformed in seven patients, Fisher and colleagues found a 50% reduction in seizure frequency in three patients, treated with 24 h/day continuous stimulator trains (76).

Eleven patients with generalized or frontal lobe DRE epilepsy were recruited at King's College Hospital (London, United Kingdom) and at the University Hospital La Princesa (Madrid, Spain) (77). They underwent bilateral DBS targeting the CMNT. Among the eleven patients, seven (64%) demonstrated improvement. Among the five patients with frontal lobe epilepsy, only one patient (20%) had significant improvement (more than 50% of reduction in seizure frequency) during the blind period; and two (40%) during the long-term extension phase. However, all six patients (100%) with generalized epilepsy had significant improvement in seizure frequency during the blind period; and in the long-term extension phase, five of the six (83%) patients showed more than 50% improvement in the frequency of seizures. Among patients with generalized DRE epilepsy, DBS implantation and stimulation of the CMNT appeared to be effective and safe. One patient (9%) had the device removed 6 months after implantation due to infection and one patient (9%) reported a transient agraphia in the first 4 days following implantation. Improvement in seizure attacks was observed during months 3 to 50 with the DBS device turned OFF (77). Additional large and well-controlled studies for CMNT stimulation are needed to identify the efficacy, mechanism and the target population (**Table 1**).

Hippocampus

TLE is the most frequent focal epilepsy syndrome in humans and is frequently associated with hippocampal sclerosis and DRE. Temporal lobe resection is the optimal therapy for patients with refractory mesial TLE (97). Unfortunately, this is contraindicated in a considerable number of patients, including those whose seizures originate in both temporal lobes, those at risk for a postoperative decline in memory, cognitive function and language, and those who have had a previous temporal lobectomy but continue to have seizures originating from the contralateral temporal lobe (98).

TABLE 1 | Clinical studies of Bi-ATN and CMT-DBS for the treatment of Epilepsy.

Author/year	Mean age (years)	Design	n	Seizure type (s)	Follow up (months)	Average seizure reduction (range)
BI-ATN						
Upton et al. (21)	24	Open label	6	CPS	>36	4/6 had "significant clinical control"
Sussman et al. (78) (Abstract)	NR	Open label	5	CPS, SGTC	12–24	60% showed "improvement"
Hodaie et al. (61) Andrade et al. (66)	30	Single blind	5 (+1)	GTC, DA, CPS, AA, SGTC, PM	4–7 years	55% (24–89%)
Kerrigan et al. (65)	36	Open label	5	SPS, CPS, SGTC	20.4 (6–36)	48% (−57–98%) of "serious seizures"
Lee et al. (79) (& Bi-STN)	22	Open label	3	TS, DA, HM, AM, SGTC	6 (2–10)	75.4% (50–90.6%); 3/3 RR
Lim et al. (58)	27	Open label	4	G, P, STGC	24	49% (35–76%); 1/4 RR
Osorio et al. (63)	31	Single blind	4	(Bi- MTLE) SGTC, CPS, SPS, DA	36	75.6% (53–92%); 4/4 RR
Andrade et al. (80)	29, 45	Open label	2	(DRA) SGTC, MYO, CS, GTC	120	98% of SGTC in one; 66% total in other
Fisher et al. (22) (SANTE)	36	Clinical trial (Double blind, randomized, parallel group)	54 55*	CPS, SGTC	24	26% above controls after 2 months; 56% median reduction after 2 years
Lee et al. (64)	31	Open label	15	CPS, GTC, SPS	39 (24–67)	70.5% (0–100%); 13/15 RR
Oh et al. (81)	33	Open label	9	CPS, SGTC	34.6 (22–60)	57.9% (35.6–90.4%); 7/9 RR
Van Gompel et al. (82) (& Bi-HC)	26, 32	Open label	2	SPS, CPS, SGTC	3	80% in one; 53% in other; 2/2 RR
Piacentino et al. (83)	38	Open label	6	(LGS), CPS, SGTC	>36	3/5 RR**
Voges et al. (67)	37	Case-Cohort study, Open label	9	CPS, SGTC	28	7/9 RR
Lehtimaki et al. (84)	35	Open label	15	NR	25.2 (9–52)	10/15 RR
Salanova et al. (85) (SANTE)	NR	Clinical trial	83	SANTE study	61	69%
Krishna et al. (86)	32	Open label	16	SPS, CPS, SGTC, GTC, MYO, DA	52	65% (−500–99%) at 3 y; 11/16 RR
Franco et al. (87)	51, 48	Open label	2	(SBH) CPS, SGTC	18, 12	61% in one, 75% in other; 2/2 RR
Valentin et al. (88)	15	Open label	1	SPS	12	>60%
Nora et al. (89)	30	Open label	1	GTC	40	87%
Piacentino et al. (90)	48	Open label	1	CPS, GTC	60	100%***
Jarvenpaa et al. (91)	38	Open label	16	NR	24	12/16 RR
CMNT						
Velasco et al. (92)	18	Open label	5	GTC, CPS, MYO, DA	6–37	80–100% GTC; 60–80% CPS
Fisher et al. (76)	28	Clinical trial (Double blind, cross over)	6	GTC	9	30%
Velasco et al. (74)	19	Clinical trial (Open label)	13	(LGS) AA, GTC, CPS, SGTC	41.2 (12–4)	81.6% (53.1–100%) LG; 57.3% (13–98.6%) SGTC
Chkhenkeli et al. (93) (& HCN, CDN)	21–40 range	Single blind	5 of 54	SPS, GTC, CPS, SGTC, TS, PM	≤18	4/5 "worthwhile improvement";1/5 no improvement¥
Velasco et al. (75)	13	Open label	13	(LGS) AA, GTC	46 (23–132)	80% (30–100%)
Andrade et al. (66)	NR	Open label	2	GTC, SPS, CPS, SGTC	≤7 years	0/2 RR
Cukiert et al. (94)	29	Open label	4	DA, AA, MYO, TS, TC, TA	18 (12–24)	80% (65–98%)
Valentin et al. (95)	27	Open label	1	RSE	6	100%

(Continued)

TABLE 1 | Continued

Author/year	Mean age (years)	Design	n	Seizure type (s)	Follow up (months)	Average seizure reduction (range)
Valentin et al. (77)	37	Single blind	11	G or FLE	24 (12–66)	82% (40–100%) G; 49% (0–92%) FLE; 6/6 RR in G; 1/5 RR in FLE
Son et al. (96)	29	Open label	14	(LGS) SPS, CPS, GTC, G, DA, MYO, AA	18.2	68% (25–100%); 11/14 RR
Valentin et al. (88)	10, 8	Open label	2	GTC, TS, TA, DA, MYO	48, 18	>60% in one; no significant reduction in other

Seizure types: *G, Generalized; P, Partial; CS, Clonic; SPS, Simple partial seizure; CPS, Complex partial seizure; DA, Drop attack: atonic; DIA, Dialeptic; GTC, Generalized tonic-clonic; AA, Atypical absence; SGTC, Secondarily generalized; PM, Partial motor; TS, Tonic; TC, Tonic-clonic; TA, Typical Absence; HM, Hypermotor; AM, Automotor; MYO, Myoclonic; RSE, Refractory status epilepticus; DRA, Dravet Syndrome; SBH, Subcortical band heterotopia; LGS, Lennox-Gastaut Syndrome.*

Targets: *ATN, Anterior thalamic nucleus; CMNT, Centromedian nucleus of thalamus; HCN, Head of caudate nucleus; CDN, Cerebellar dentate nucleus.*

Seizure onset: *Bi, Bilateral; Uni, Unilateral; TLE, Temporal lobe epilepsy; MTLE, medial temporal lobe epilepsy; FLE, Frontal lobe epilepsy.*

Outcomes: *RR: Responders rate (Seizure reduction ≥50%); Serious Seizures: Potentially injurious seizures (SGTC or CPS associated with falls).*

Other: *NR, Not report.*

**SANTE trial control patients (22).*

***One died, not related to DBS (83).*

****The patient had long-term significant reduction in seizure frequency even with an absent electric stimulation (90).*

¥ Engel Classification (93).

Twenty years ago, hippocampal LFS [2-Hz pulses −500 μA base to peak, 1 ms duration biphasic square-wave pulses] was used to trigger seizures in experimental models (99). Recently, studies done in rat models of TLE have demonstrated that LFS applied at a frequency of 1 Hz, significantly reduced the excitability of the neuronal tissue, resulting in decreased seizure frequency (100). Remarkably, hippocampal HFS can protect hippocampal neurons against kainate neurotoxicity in macaques, likely via the inhibition of apoptosis (101).

Continuous electrical stimulation, especially with high frequencies (130 Hz), has been shown to completely inhibit picrotoxin- and high-K+-induced epileptiform activity in animal *in vivo* models of epilepsy (102, 103). Several clinical investigations support the anti-seizure effect of electrical stimulation of the hippocampus. Direct stimulation over the suspicious epileptogenic zone within the hippocampus is applied in this procedure. Two studies describe a decrease in interictal epileptiform discharges (93, 104). Téllez-Zenteno and his group reported a 15% seizure frequency reduction in four patients after unilateral DBS in the hippocampus (98). Boon et al. (105) studied the effect of DBS in medial temporal lobe in 10 patients with DRE. After mean follow-up of 31 months, one patient was seizure free, one demonstrated a more than 90% reduction in seizure frequency; five of 10 patients had ∼50% seizure-frequency reduction; two had a reduction of 30–49%; and one remaining patient was a non-responder, with no change in seizure frequency. No serios clinical adverse effects (except an asymptomatic intracranial hemorrhage in one patient) or alterations in clinical neurological testing have been reported. In another study, McLachlan et al. (106) studied the effect of continuous bilateral electrical stimulation of the hippocampus in two patients with seizures originating from bilateral mesial temporal lobes. Seizure frequency decreased by 33% in both patients during stimulation and remained 25% lower for the 3 months after stimulation was turned off. No consistent changes were seen in objective or subjective measures of memory. No other adverse effects were reported. More surprisingly, Vonck et al. (107) described three patients with neuromodulation of the amygdala-hippocampal junction who exhibited a 50–90% decrease in seizures. The larger prospective, controlled, double-blind study evaluating the effects of Hippocampus-DBS in 16 TLE patients has shown that HFS (130 Hz) is effective in reducing seizure frequency in patients with refractory TLE. Fifty-percent of this cohort became seizure-free (108).

To evaluate anatomical and functional changes in amygdala-hippocampal function after DBS in TLE patients, several studies have been conducted. Velasco et al. (109) found that by extending the period of follow-up from 18 months to seven years, patients could be divided into two groups: patients with normal and abnormal MRI studies. Ninety five percent of patients with non-lesional epilepsy exhibited more than 95% of improvement, while only 50–70% of patients with hippocampal sclerosis identified on MRI showed improvement with DBS. None of these patients exhibited neuropsychological deterioration. Micro-lesions have been reported in a few epileptic patients, following the diagnostic implantation of depth electrodes in the temporal lobe (110, 111). Altogether, DBS appears to be a safe and valuable option for patients who suffer from drug-resistant TLE in whom resective surgery is contraindicated. Hippocampal DBS has also been found to be a relatively safe procedure. No irreversible cognitive or psychiatric deficits were encountered (112). However, even with the potential benefits, hippocampal stimulations cannot be considered as a first line therapy, in lieu of a resective procedure (**Table 2**).

Basal Ganglia

Caudate Nucleus (CN)

The CN may represent a deep target for the treatment of epilepsy. Low frequency electrical stimulation has been found to be

TABLE 2 | Clinical studies of HC-DBS for the treatment of Epilepsy.

Author/year	Mean age (years)	Type of study	n	Seizure type (s)	Follow up (months)	Average seizure reduction (range)
HC						
Velasco et al. (113, 114)	24	Open label	10	(TLE) CPS, SGTC	2 weeks	100% after 6 days
Vonck, 2002 (115)	33	Open label	3	(MTLE) CPS, GTC	5 (3–6)	77% (50–94%)
Vonck et al. (116)	NR	Open label	7	(TLE) NR	14 (5.5–21)	43% (0–100%)
Tellez-Zenteno et al. (98)	32	Clinical trial (Double blind, cross over)	4	(MTLE) CPS, SGTC	6 blind	26% (ON) vs. −49% (OFF)
Boon et al. (105)	NR	Open label	10	(MTLE) CPS, SPS, SGTC	31 (15–52)	50% (<30–100%)
Velasco et al. (109, 117)	29	Clinical trial	9	(MTLE) CPS, SGTC	18 (1 blind)	83% (50–100%); 9/9 RR
McLachlan et al. (106)	45, 54	Clinical trial (Double blind, cross over)	2	NR	3	33% (ON) vs. 4% (OFF)
Boex et al. (110) Bondallaz et al. (118)	34	Open label	8	(MTLE) CPS, SGTC	44	67% (0–100%); 6/8 RR
Tyrand, 2012 (119)	32	Open label	12	(TLE) NR	0	58.1%*
Morrell et al. (53) (RNS trial) Heck et al. (54) (RNS trial)	34.9 (18–66)	Clinical trial	95 of 191	SPS, CPS, SGTC	3 blind 48	38% (ON) vs. 17% (OFF) 53%, 55% RR
Vonck et al. (107)	NR	Open label	11	(MTLE) CPS, SPS, SGTC	96 (67–120)	70% (0–100%); 8/11 RR;
Cukiert et al. (57)	37	Single blind	9	(TLE) CPS, SPS, SGTC	30.1	61% (−50–100%); 7/9 RR
Jin et al. (120)	NR	Open label	3	CPS, SGTC	35	93% (91–95%)
Lim et al. (121)	35	Open label	5	CPS, SGTC	38	45% (22–72%); 3/5 RR
Cukiert et al. (108)	38.4	Clinical trial (Double blind, randomized)	16	SPS, CPS	8 (6 blind)	3/14 RR in CPS, 7/16 RR in SPS

Seizure types: *P, Partial; CS, Clonic; SPS, Simple partial seizure; CPS, Complex partial seizure; DA, Drop attack: atonic; DIA, Dialeptic; GTC, Generalized tonic-clonic; AA, Atypical absence; SGTC, Secondarily generalized; PM, Partial motor; TS, Tonic; HM, Hypermotor; MYO, Myoclonic; PME, Progressive myoclonic epilepsy.*
Targets: *ATN, Anterior thalamic nucleus; VIM, Ventral intermediate nucleus of thalamus; STN, Subthalamic nucleus; HC, Hippocampus.*
Seizure onset: *Bi, Bilateral; Uni, Unilateral; TLE, Temporal lobe epilepsy; MTLE, medial temporal lobe epilepsy; FLE, Frontal lobe epilepsy.*
Outcomes: *RR: Responders rate (Seizure reduction ≥50%).*
Other: *NR, Not report.*
**Outcome: Reduction of interictal activity with biphasic stimulation in Hippocampal sclerosis (119).*

efficacious when targeting the caudate (112). Interestingly, high frequency (30–100 Hz) stimulation of the head of the caudate nucleus (HCN) caused enhancement of epileptiform spikes from the ipsilateral hippocampus and amygdala while, on the contrary, LFS of the caudate reliably produced inhibitory effects bilaterally. The caudate loop is a functional entity comprised of the HCN, thalamus and neocortex. Activation of HCN is associated with hyperpolarization of cortical neurons, suggesting that suppression of seizure activity may be a result of stimulation-induced inhibition of the cortex (122).

Trials studying the effect of caudate stimulation on patients with epilepsy have not been systematically conducted and have yielded only marginal results. Sramka and Chkhenkeli (123) published a study of 74 patients showing reduced interictal epileptiform activity with both caudate and dentate nucleus stimulation. Chkhenkeli et al. (93) stimulated the ventral HCN at low frequency (4–8 Hz) in 38 patients, producing seizure freedom in 21 patients, while improving 35 patients overall (92%). This group was the first to describe benefit with striatal stimulation in cases of drug-resistant TLE. Their study was based on a pathophysiological hypothesis related to the balance of output between pro-convulsant and anti-convulsant structures, however, their work suggests that LFS of the CN is anti-epileptic (124). These results highlight the ability of the basal ganglia to modulate cortical epileptogenicity. Controlled clinical studies are necessary to determine efficacy and safety of this anatomical location. Currently, the CN is not the most frequently targeted structure in the treatment of DRE.

Subthalamic Nucleus (STN)

STN DBS has been explored as an option to treat motor seizures through the disruption of pathological cortical synchronization. Inhibition of the STN may potentially release the inhibitory effect of the substantia nigra pars reticulata on the dorsal

midbrain anticonvulsant zone, thus raising the seizure threshold. This mechanistic rationale has arisen from observations in animal models (125, 126). The compact and distinct anatomical structure of the STN makes it a superior surgical target for electrode stimulation, which has been previously demonstrated in DBS-STN for treatment of PD.

Benabid et al. (127) reported a series of three patients with DRE who were implanted with STN-DBS. All patients were reported to exhibit important reductions in seizure frequency with stimulation; the first two patients exhibited reductions of 83 and 50%. Afterwards, the group reported a case describing a 5-year-old girl with DRE caused by focal centroparietal dysplasia, followed for 30 months. She had a 81% improvement in the number of seizures. This was most substantially reflected in reduction in cluster seizures (89%) and diurnal seizures (88%) (128).

Small studies have been conducted in severely impaired patients with severe DRE. Chabardes et al. (129) demonstrated a mean of 60% seizure frequency reduction in 80% of patients (4/5). They included patients aged from 5 to 38 years (17.6 $\pm$ 12.7). The stimulation was well-tolerated and appeared to demonstrate efficacy, however there were some complications related to the procedure in 40% (2/5) of patients; namely, a device infection in one patient, and a post-implantation subdural hematoma in a second patient, requiring re-operation.

Forty percent (2/5) of patients who underwent STN-DBS at the Cleveland Clinic Foundation reported a seizure rate reduction of 60% at 10 months of follow-up and 80% at 16 months (130). STN/substantia nigra DBS resulted in 30–100% reduction in seizure frequency of five patients with drug-resistant myoclonic epilepsy (131). STN-DBS may be a favorable target in certain epilepsy syndromes, but controlled, blinded trials are required to demonstrate efficacy and safety.

Cerebellum

The cerebellar nuclei have been some of the oldest targets in DBS, with initial uncontrolled trials in the 1970s (20) demonstrating potential efficacy in the treatment of epilepsy. Nuclear activation of inhibitory Purkinje cells, likely results in the suppression of excitatory cerebellar output to the thalamus and therefore, decreased excitatory thalamo-cortical projections, resulting in overall decreased cortical excitability (132). Disrupting thalamo-cortical activity has proven to be a useful approach to stop generalized spike-and-wave discharges in mice. It has been demonstrated that cerebellar nuclei are modulators of pathological oscillations during absence seizures (133).

Seventy six percent of epileptic patients (87/115) treated with cerebellar nuclei modulation demonstrated benefit with a reduction in seizure frequency. Overall, 27% reported seizure freedom and 49% reported reduction in seizure frequency and severity. Those patients with generalized tonic-clonic seizures benefited the most (134).

Velasco et al. (135) conducted a double blind, randomized control pilot study with five DRE patients with motor seizures. They implanted stimulating electrodes on the supero-medial cerebellar cortex and evaluated the efficacy and safety. After 6 months of stimulation, all patients reported a seizure rate reduction, on average 41% (14–75%) compared with the control group. At the end of 24 months, the three (60%) patients who completed follow up reported a further seizure reduction of 24% (11–38%) (135). In regards to the safety profile of the intervention, 60% of patients required another procedure owing to electrode migration, and 20% (one patient) suffered a severe local infection that finally resulted in long-term antibiotic therapy and removal of the entire stimulation system (136). After these preliminary studies, cerebellar stimulations have not been used in recent studies.

Hypothalamus

Due to the presence of epileptiform activity during depth electrode recordings of the mammillary bodies, the posterior hypothalamus has been suggested as a DBS target (137). The mammillo-thalamic tract stimulation has been used to treat gelastic seizures secondary to hypothalamic hamartomas, and has shown an improvement in seizure frequency and severity (138). A report of two patients by Franzini et al. (139) showed a reduction up to 80% in seizure frequency from baseline after 9 months of follow-up. This target may be unfavorable due to consequences of potential hemorrhage in this region during electrode implantation, as well as possible alterations in sleep-wake cycle (140). A recent study targeting the posteromedial hypothalamus reported nine patients with DRE, associated with intractable aggressive behavior, achieved a significant decrease in the frequency of epileptic seizures after up to 5 years of follow-up, achieving an average seizure reduction of 89.6% (141). Current experience with hypothalamic stimulation is too limited to draw firm conclusions. Other targets such as the caudal zona incerta, nucleus accumbens and fornix have been also explored (see **Table 3**).

ADVERSE EFFECTS

There are well-known side effects and potential complications associated with DBS, which have been mainly elucidated by the literature regarding DBS in the treatment of movement disorders (153). The overall complication rate for DBS surgeries in patients with PD was 7%, which included mechanical complications (3%), hemorrhage or infarction (1%), lead removal (1%), hematoma (0.4%), and infection (0.4%) (154). Comparatively limited information is available regarding the specific complications of DBS in patients with epilepsy. The most common stimulation-related side effects in the SANTE studies were mainly those expected from implanted electrodes, including stimulation-related paresthesias (22.7%), implant site pain (20.9%), implant site infection (12.7%), and subclinical bleeding around electrodes (22). Other known complications include: wound infection; lead or extension fracture; erosion; lead tract fibrosis; electrode migration; external interference with other devices; equipment infection; pain; transient worsening or new seizures; and dizziness (59); skin complications [such as abrasions, ulcerations and aseptic necrosis (155)]; hardware discomfort; ineffective product (85, 156); and peri-electrode edema (157) that may produce disorientation, gait instability, headache, seizure or acute confusion (155).

TABLE 3 | Clinical studies of STN, Cerebellum (CB), HNC, CZI, pHT, NA, and Fornix -DBS for the treatment of Epilepsy.

Author/year	Mean age (years)	Type of study	n	Seizure type (s)	Follow up (months)	Average seizure reduction (range)
STN						
Benabid et al. (128)	5	Open label	1	SPS	30	80.7%
Chabardes et al. (129)	18	Open label	5	TS, CS, GTC, HM	18 (8–30)	51.4% (0–80.7%)
Shon et al. (142)	23, 22	Open label	2	(FLE) TS	18, 6	86.7% in one; 88.6% in other
Handforth et al. (143)	45, 46	Open label	2	P	26–32	50% and 33%
Lee et al. (79) (& ATN)	20	Open label	3	DIA, SGTC, TS	18, 30, (1 loss)	49.1% (20–71.4%)
Vesper et al. (144)	39	Open label	1	(PME), GTC, MYO	12	50% MYO, 100% GTC
Wille et al. (131) (& VIM)	32	Open label	5	(PME), GTC, MYO	24 (12–42)	30–100%
Capecci et al. (145)	35, 30	Open label	2	PM, GTC, DA, CPS, AA	48, 18	65% in one and 0% in other
CB						
Cooper et al. (20)	29	Open label	15	CPS, SGTC, GTC, MYO, TA	27	10/15 "improved"
Van Buren et al. (19)	27	Double blind, cross over	5	CPS, SGTC, GTC, MYO	15–21 range	No significant reduction
Levy et al. (146)	29	Open label	6	GTC	7–20 range	2/6 RR
Bidznski et al. (147)	NR	Open label	14	NR	10–16 days	13/14 "improved"; 1/14 no change
Wright et al. (148)	30	Clinical trial (Double blind, cross over)	12	GTC, DA, A, MYO, CPS	6 blind	No statistically significant; 11/12 patients felt it helped
Davis et al. (149)	NR	Open label	27	Spastic seizures	17 years	23/27 improved; 4/27 no improvement
Chkhenkeli et al. (93) (& HCN, CDN)	21–40 range	Single blind	11 of 54	GTC, CPS, SGTC, TS, PM	≤18	5/11 seizure free; 5/11 "worthwhile improvement"; 1/11 no improvement*
Velasco et al. (135)	26	Clinical trial (Double blind, cross over)	5	GTC, TS, DA, MYO, AA	24 (3 blind)	67% (ON) vs. 7% (OFF); 76% (62–89%) GTC; 57% (24–90%) TS
HCN						
Chkhenkeli et al. (124)	NR	Open label	57	NR	NR	Unclear
Chkhenkeli et al. (93) (& CDN)	NR	Open label	38 of 54	GTC, CPS, SGTC, TS, PM	≤18	21/38 Seizure free; 14/38 "worthwhile improvement"; 3/38 no improvement*
CZI						
Franzini et al. (139) (& pHT)	26	Open label	2	(RS)SPS, SE	6, 48	85% in one, and remission of SE in other; 2/2 RR
Anderson et al. (150)	20	Open label	3	(NSPM)GTC, MYO, TA	4.3 years (3–6)	3/3 "improved"
pHT						
Franzini et al. (139)	20, 36	Open label	2	DA, MYO, CPS	9, 60	75% in one and 80% in other; 2/2 RR
Benedetti et al. (141)	21	Open label	5	SPS, CPS, GTC, AA, DA,	5 years	89.6% (25–100%); 5/5 RR
NA						
Schmitt et al. (151)	42	Open label	5	SPS, CPS, GTC	6	37.5% median; no significant changes in mean frequencies; 2/5 RR of DS
Kwoski et al. (152)	37	Clinical trial (double blind, cross over)	4	SPS, CPS, SGTC	15 (6 blind)	17.2% (ON) vs. −1,6 (OFF) of DS at 28 days; 3/4 RR of DS
FORNIX						
Koubeissi et al. (26)	41	Open label	7	(MTLE), SPS, CPS	1–9 days	Seizure odd reduced by 92% for day 1–2

Seizure types: *SPS, Simple partial seizure; CPS, Complex partial seizure; TA, Typical Absence; GTC, Generalized tonic-clonic; SGTC, Secondarily generalized; SE, Status epilepticus; DA, Drop attack: atonic; AA, Atypical absence; MYO, Myoclonic; PM, Partial motor; TS, Tonic; RS, Rassmusen syndrome; NSPM, North Sea Progressive Myoclonic Epilepsy.*
Targets: *CB, Cerebellum; HCN, Head of caudate nucleus; CZI, Caudal zona incerta; pHT, Posterior hypothalamus; NA, Nucleus accumbens; CDN, Cerebellar dentate nucleus.*
Seizure onset: *Bi, Bilateral; Uni, Unilateral; MTLE, medial temporal lobe epilepsy.*
Outcomes: *RR: Responders rate (Seizure reduction ≥50%); DS: Disabling seizures (CPS+ GTC).*
Other: *NR, Not report.*
** Engel Classification (93).*

Psychiatric Side Effects

Depression and Suicide

A large variability in the use of diagnostic scales measuring depression is noted in the existing literature regarding DBS. It is therefore difficult to directly compare the results of the studies. Anterior thalamic DBS was initially reported to be associated with higher rates of memory deficits and depression (158); however, 5-year (85) and 7-year follow-up (156) of the SANTE study population found no significant deterioration in cognition or depression scores. Randomized multicenter and observational studies have shown improvement in depression and anxiety scores after DBS in patients with PD (159, 160). However, higher prevalence of depression after STN-DBS has also been reported (158, 161). PD patients treated with STN-DBS were also found to have a higher suicide rate, in a paper published a decade ago. Suicidal behavior was frequently associated with postoperative depression and altered impulse regulation (162, 163). A recent randomized controlled trial did not find a direct association between suicide and DBS (164). This may be indicative of improved patient selection criteria in recent years. This is further complicated by the fact that suicide after DBS has occurred not only with different anatomic targets (particularly with thalamic and GPi stimulation) but also with other diseases (dystonia and essential tremor) (165). Although the evidence is contradictory and scarce in epilepsy, all patients should be carefully screened for suicide risk as part of the presurgical workup for DBS surgery. Additionally, patients should be monitored closely for depression and suicidality post-operatively.

Apathy

Apathy has been frequently reported as a possible adverse effect of STN-DBS, however the existing literature is controversial. Some authors have found significant worsening of apathy scores 3–6 months after surgery (166). They hypothesized a direct influence of STN-DBS on the limbic system by diffusion of stimulus to the medial limbic compartment of STN. Recently, a metanalysis assessing apathy following bilateral DBS of the STN in PD concluded that the reduction of dopaminergic medication after surgery may be the cause of worsening apathy in this patient population (167). Other authors have failed to find significant differences in apathy prevalence or severity between surgical and non-surgical patients (168). On the contrary, some publications have stated positive psychiatric side effects of DBS (169). Results of STN stimulation in patients with PD in a Polish study confirmed the positive effects of stimulation on drive and ability to feel pleasure. The authors demonstrated improvement of mood, sleep and apathy following the first month after initiation of stimulation, which was seen independently of improvements in motor symptoms (170).

Cognitive Deterioration

Cognitive decline reported after DBS mainly affects frontal subcortical cognitive functions, such as verbal fluency, processing speed, attention, learning, and working memory (171). Worse cognitive outcomes after surgery remained unchanged, regardless of DBS settings or "on" and "off" motor states, suggesting the cause might be related to lead trajectory or location (172). Some psychiatric side effects have been also described, such as psychosis (for example, delusions of marital infidelity resembling Othello syndrome), and impulse control disorders, such as binge eating, hypersexuality, hypomania, and secondary increase in body weight (155).

Other Side Effects

Other less mentioned and more infrequently encountered, but nonetheless important complications include post-surgical headache, instability and gait disorders with falls (170, 173), as well as speech disorders (dysarthria, intelligibility, pitch variation in speech, worsening hypophonia, stuttering, and speech articulation problems) (155, 174). When the device is less frequently used, the complications are higher, for instance, bilateral cerebellar stimulation has been associated with re-operation in 60% and serious complications in 20% of the patients (136).

EPILEPSY COMORBIDITIES

People with epilepsy in the general population have a two to five-fold risk of somatic comorbid conditions compared to people without epilepsy. Thus, there is a clear need for an integrated approach in patients with epilepsy, especially in those with DRE who are good candidates for electrical stimulation. The process of localizing DBS targets is undergoing continuous evolution. The clinical effects of DBS are likely due to the activation of complex, widespread neuronal networks, directly and indirectly influenced by the stimulation of a single isolated target. The delivery of such stimulation may aid in the discovery of strategically combined targets for electrical stimulation to treat additional neurological, psychiatric, cognitive and somatic disorders. Computational modeling (experimental and clinical), engineering designs, and neuroimaging techniques play a critical role in this process (175).

Depression

Depression is the most common comorbidity of epilepsy, affecting between 10% to 60% of patients with seizures (176). Different targets have been used in patients with refractory major depressive disorder, such as subcallosal cingulate gyrus (Brodmann area 25), ventral capsule/ventral striatum, medial forebrain bundle, and the nucleus accumbens. The overall effect sizes have shown a significant reduction in Hamilton depression rating scale scores after DBS stimulation in these four regions (177). Selected patients with refractory depression and DRE could benefit from stimulation of these targets.

Obesity

There is some evidence that obesity may be more common in people with epilepsy than in the general population (178). The pathophysiology of obesity is complex, involving both altered patterns of eating and satiety, as well as compulsive behavior surrounding food intake. Proposed stimulation targets to treat obesity therefore include the hypothalamus and nucleus accumbens (179). To date, experience with DBS for the treatment of obesity is limited. Although surgery has been proven to be safe, no definitive conclusions can be made as to whether it is

effective. There is an opportunity to study this further in patients with epilepsy and obesity, especially in those with gelastic seizures secondary to hypothalamic hamartomas. Further work is needed to address target selection, the kinetics of DBS, and the ideal stimulation parameters in this population.

Psychogenic Non-epileptic Seizures

Psychogenic non-epileptic seizures (PNES) are one of the most common differential diagnoses of epilepsy. PNES are involuntary episodes of any combination of altered movement, sensation, or awareness that bears resemblance to epileptic seizures, but are not accompanied by epileptiform electrical discharges (180). Unfortunately, PNES are an exclusion criterion for DBS candidates in most of the epilepsy centers. There are few reports of the experience with psychogenic movements disorders and DBS. This literature suggests that DBS does not produce side effects in patients with psychogenic disorders. One patient with psychogenic parkinson-like disorder underwent HFS of the STN for approximately 5 years without side effects, prior to the device being turned off (181). Similarly, authors from Bethesda described two cases of psychogenic dystonia who underwent DBS in the GPi, after initially being thought to have organic dystonia (182). DBS did not lead to any benefits or side effects for these patients. These reports further highlight the safety of DBS in neuronal tissue.

LIMITATIONS

Many of the available publications are non-randomized, unblinded, uncontrolled small studies. Therefore, the evidence is susceptible to major biases:

a) Regression to mean: patients are typically implanted when there is high seizure burden. In all chronic diseases, phases of high activity and lower activity are observed, thus seizure burden may have returned to baseline without any therapy.
b) Placebo effect: even in some controlled studies, there is no "sham-surgery" arm that would allow for estimation of the effect of an invasive procedure on the subjective seizure assessment.
c) High expectations of both investigator and patients, resulting highly suggestible results.

As well, in most studies, concomitant drug changes were allowed, but were usually not reported; therefore, the effect of stimulation cannot be accurately measured. Finally, stimulation paradigms were frequently chosen not on a pathophysiological basis, but rather on convenience and/or chance: e.g., the 50 Hz and pulse in STN stimulus is based on previously chosen paradigms for PD treatment. There are usually four or five different stimulation variables that are freely combined, resulting in hundreds of different combinations. Further investigations are necessary to solve these limitations in this promissory area.

FUTURE DIRECTIONS

The scope of DBS is increasing rapidly in parallel with the understanding of brain circuitry dysfunction in many pathologies (183). Investigators are only just beginning to realize the full potential of this growing field (184). The future of DBS depends on technological advances in the area: the focus should be the improvement of clinical knowledge as well as improved practicality (smaller size devices, increased battery life, greater tolerance and safety profiles, improved software). Further understanding of the mechanisms underlying cerebral circuits in epilepsy and its comorbidities is required, in order to further define specific criteria and predictors in the selection of patients who could benefit from DBS. This will be only possible investing resources in basic research, essential as it forms the foundation upon which translational research and clinical trials are built on. The poor results of DBS treatment in some patients as reported in the literature, may be mitigated in the future with improvement in patient selection, better target identification, and the development of more effective stimulation paradigms, such as closed-loop stimulation (184).

The identification of optimal targets for specific subgroups of patients, including patients with comorbidities, especially those with psychiatric disorders, is crucial for further development (185). Basic science studies have been fundamental in advancing the understanding of the complexity of alterations in neuronal networks and continue to provide valuable information for researchers and clinicians, allowing for further clinical developments, particularly in the identification of these practical targets. Modern methodologies such as EEG-fMRI studies are allowing for the delineation of epileptogenic neuronal networks, which is increasingly accepted over the classic concept of the epileptogenic zone (186). Analysis of epileptic neuronal networks will help guide treatments in a more precise way, hopefully resulting in more efficacious treatments.

Advances in surgical procedures may include the implementation of new electrodes to treat a single symptom synergistically or multiple symptoms at once, which would additionally allow for the establishment of different stimulation parameters for each electrode contact.

Finally, optogenetics rests on the use of genetically-encoded, light sensitive proteins, such as opsins, to modulate neuronal activity, intracellular signaling pathways, or gene expression with spatial, directional, temporal, and cell-type specificity (187). Epilepsy is one the disorders that has been widely explored in this regard. The ability to inhibit or activate different neurons with high specificity and resolution has turned it into an exciting research tool and a possible therapeutic intervention targeting neurons with abnormal activity (188). Studies in several animal models have shown a reduction in seizure activity both electrographically and clinically (189–191). Even so, there are still several limitations that must be overcome prior to its application in humans, particularly the ∼1000-fold difference in brain volume between the rodent models and the much larger human neuronal circuits that makes optogenetic control difficult.

CONCLUSION

DBS is one of the most remarkable interventions in the history of functional neurosurgery. Whether it will significantly improve

the outcomes of DRE patients remains to be seen. DBS tends to have better results in patients with generalized epilepsy, although it has been used with success in some patients with focal-onset seizures. The best DBS targets for each epileptic syndrome, as well as the optimal combination of stimulation variables for each target remains speculative, however ATN stimulation has been performed in the highest number of patients and with the most rigorous study protocol allowing it to be recommended over the other targets. Results in hippocampal and frontal stimulation are suboptimal and should be reserved only for patients in whom resective procedures are contraindicated. Surgery should be recommended before potential hippocampal or frontal stimulation is considered. Targets, such as the CN and cerebellar nuclei, need more exploration. There is a need for large, well-designed randomized control trials to validate and optimize the efficacy and safety of DBS.

AUTHOR CONTRIBUTIONS

NZ and FS conceived and designed the analysis. NZ and LL initiated the collaborative project, collected the data, performed the analysis and wrote the paper. JO-H and LL monitored data collection, cleaned the data, and drafted the paper. AC and JT-Z revised the paper.

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REVIEW
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Advances in the Potential Biomarkers of Epilepsy

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Epilepsy is a group of chronic neurological disorders characterized by recurrent, spontaneous, and unpredictable seizures. It is one of the most common neurological disorders, affecting tens of millions of people worldwide. Comprehensive studies on epilepsy in recent decades have revealed the complexity of epileptogenesis, in which immunological processes, epigenetic modifications, and structural changes in neuronal tissues have been identified as playing a crucial role. This review discusses the recent advances in the biomarkers of epilepsy. We evaluate the possible molecular background underlying the clinical changes observed in recent studies, focusing on therapeutic investigations, and the evidence of their safety and efficacy in the human population. This article reviews the pathophysiology of epilepsy, including recent reports on the effects of oxidative stress and hypoxia, and focuses on specific biomarkers and their clinical implications, along with further perspectives in epilepsy research.

Keywords: biomarkers, epilepsy, epileptogenesis, inflammation, neurological disorders, blood-brain barrier breakdown

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INTRODUCTION

Epilepsy, a condition affecting the central nervous system (CNS), is characterized by the occurrence of repeated seizures along with a chronic complex of somatic, vegetative, and psychiatric symptoms. Epilepsy can be defined as when the patient experiences at least one of the following: (a) two or more unprovoked (or reflex) seizures more than 24 h apart, (b) one unprovoked (or reflex) seizure and, over the next 10 years, a recurrence risk of at least the general recurrence risk (60%) after two unprovoked seizures or (c) a diagnosis of an epilepsy syndrome. Patients with epilepsy are prone to generate epileptic seizures and consequential social, psychological, cognitive, and neurobiological disabilities (1). It is estimated that 1–2% of the world's population is affected by epilepsy (2, 3). It may occur in all age groups and is connected with a burden of socioeconomical, behavioral, psychiatric, and other medical issues for both the patient and their close ones (1, 4).

Epileptogenesis describes the process of structural modifications leading to seizure activity in a normal brain (5). Throughout recent years, many hypotheses have been proposed to explain the underlying etiopathogenesis of epilepsy, including neurodegeneration (6, 7), disturbance of brain-blood barrier (BBB) (8), amygdala dysregulation, alterations of the glutamatergic system (9), oxidative stress (10), hypoxia (11), and the epigenetic modification of DNA (12). Moreover, the majority of studies on inflammation and epilepsy indicate the important role of inflammatory markers in epileptogenesis through the dysregulation of cytokine balance in the CNS or through the complement pathway. These hypotheses may not exclude one another and may in fact be concurrently presented leading to the culmination of epilepsy. As ∼40% of cases of epilepsy have an unknown etiology, further investigations into the potential causes are essential in order for physicians to provide an optimal treatment for patients (13).

WHO defines biomarkers as "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction" (14). The role of finding novel markers in post-epileptic brain damage is a possible grasping point for the prevention of complications and for the development of targeted methods of treatment in the future. The need of an investigation into new biomarkers is also augmented by the relatively low specificity of EEG, which remains the main diagnostic tool in epilepsy (15). Biomarkers may play a role in individualized epileptic treatment, based on the patients' biomarker profile. As there are many types of epileptic conditions, each condition would have a certain panel of biomarkers. Biomarkers would also play a role in monitoring anti-epileptic treatment and may have a potential value in determining patients who would benefit more from surgical therapy.

NEUROINFLAMMATION AND OXIDATIVE STRESS

Neuroinflammation is considered to be a primary factor in epileptogenesis. Reactive oxygen species (ROS) has been indicated to play a crucial role as mediators in the process of neuronal injury (16–18). Currently, there are two suggested pathways of ROS production, the non-enzymatic and the enzymatic pathway. The non-enzymatic pathway is indicated to be triggered by the ionization process, UV radiation and toxic influence of chemicals and drugs. The enzymatic pathway, on the other hand, is a result of intracellular damage by enzyme-mediated processes including respiratory chain, xanthine oxidoreductase (XOR), peroxisomal oxidases, enzymes of the cytochrome P450 family, cyclooxygenases (COX), lipoxygenases and NADPH oxidases (NOX). ROS is considered to be a waste product of these enzyme-mediated reactions (19). Recent studies suggest that ROS may play a crucial pro-epileptic role including pro-inflammatory cytokine production and microglial activation during epilepsy. McElroy et al. have additionally proposed the role of ROS in modulating the course of neuroinfection (20).

The increased production of ROS leads to microglial activation, ultimately resulting in the release of pro-inflammatory cytokines (20). Cytokines play an essential role in these processes not only because they are responsible for the aggravation of immune response, but also because they regulate the pro- and anticonvulsive neuronal hyperexcitability (21, 22). In light of McElroy et al.'s investigations, this concept was supported by the results of decreased microglial activation through redox-sensitive m-Tor pathway following the administration of anti-oxidative factors (20).

Interestingly, other studies have demonstrated that the main cytokine activator, cyclooxygenase-2 (COX-2), was triggered via ROS through transforming growth factor-B-activated kinase 1 (TAK1) pathway (23, 24). These investigations brought together ROS, COX-2 TAK1 pathway in the neuroinflammatory process. To support this concept, it was verified that the temporal lobe epilepsy (TLE) is associated with microglia activation, which in turn leads to the production of ROS and other cytotoxic factors (25–28). The activation of microglia through oxidative stress promotes the apoptosis of pericytes through ROS elevation (29).

The Role of HMGB1 in Oxidative Stress

High mobility group box-1 (HMGB1) has recently emerged as a potential biomarker of epilepsy (30). It takes part in the immune response via activating macrophages and endothelial cells, leading to the release of tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1), interleukin-6 (IL-6) by connecting to the receptor for advanced glycation end products (RAGE) and to TL4 (Toll-like receptor 4). This specific connection triggers NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation and thus the elevation of pro-inflammatory proteins levels (31). Furthermore, via stimulating TLR4 and neutrophils, HMGB1 is the factor that leads to oxidative stress (32, 33). The HMGB1-mediated HMGB1-TLR2/4-NF-κB pathway has been shown to take part in epileptogenesis via microglial activation. HMGB1 has been additionally indicated as a potential therapeutic agent in epilepsy and as a non-invasive biomarker, which could identify patients with high risk of epilepsy (34). The level of HMGB1 has been shown to increase within 3–4 h after a drug-resistant epilepsy (DRE) seizure, proving HMGB1 to be a promising marker (35). Zhu et al. also reported the elevation of HMGB1 within 24 h after an episode of seizure in children, in comparison to the control group. The authors suggested that HMGB1 can be a prognostic factor of the frequency of seizures in the course of epilepsy (36).

Hypoxia and Epilepsy

Hypoxia resulting from ischemic events can lead to the energetic disturbances of homeostasis. The following dysregulation of ATP-dependent ion-pumps drives the imbalance of sodium, calcium, and potassium ions concentration, leading to the release of excitatory amino-acids such as glutamate (37, 38). As a consequence, an uncontrolled electric stimulation is provoked, resulting in cellular brain injury (39–41). Hypoxia inducible factor (HIF-1), a heterodimer protein consisting of two subunits a and b, is involved in ischemic processes (6). The level of HIF-1a depends on the partial concentration of oxygen (42). In normoxemia, HIF-1a is rapidly brought down by the protein von Hippel Lindau (pVHL)-mediated process of ubiquitin-proteasome pathway. Hypoxia, on the other hand, blocks the degradation of HIF-1a, resulting in its accumulation within the cell (43). Factors responsible for the stabilization of HIF-1a may include insulin, insulin-like growth factor, platelet-derived growth factor (PDGF), epithelial growth factor, and interleukin-1B (41). Moreover, HIF-1a is an important regulator of gene expression in the peripheral tissues and the CNS which is currently in a hypoxic state. The effect of HIF-1a is the promotion of physiological processes including angiogenesis, glycolysis and glucose transporter 1 (GLUT1) membrane recruitment (44). The final effect of glycolysis is the accumulation of pyruvate in neuronal cells which is then converted into butyric acid via butyric dehydrogenase. This accumulation of by-products

may lead to a decreased pH within the inner environment of neuron, leading to its dysfunction and altered metabolic state. The ketogenic diet which is based on lowered glucose intake may omit this pathway regulated by HIF-1a and alternatively promote beta-oxidation, converting substrates to acyl-CoA (45). Taking this into consideration, the efficacy of a ketogenic diet for patients with DRE can be beneficial. In addition, it has been shown that a ketogenic diet also improves the outcomes of patients with GLUT-1 deficiency syndrome (46). Studies on epilepsy-induced rat models and post-mortem human histopathologic brain samples supported a significant correlation between HIF-1a elevation and epilepsy occurrence (42–44, 47) Numerous analyses have supported the positive correlation between HIF-1 and the elevation of COX-2 production. It has been demonstrated that HIF-1a binds to hypoxia responsive element on the COX-2 promotor located in DNA, resulting in the up-regulation of COX-2 and PGE-2 (prostaglandin E2) (48). This could potentially explain the mechanism of febrile seizures in pediatric patients, seizures resulting from perinatal ischemia, and seizures occurring after strokes and transient ischemic attacks (TIA). Investigations and further understanding of the basis of hypoxia along with oxidative stress as the underlying cause of epilepsy could lead to the discovery of new potential epilepsy biomarkers. For the first time, we suggest that both pathways of hypoxia and oxidative stress may contribute to brain damage and epileptogenesis through COX-2 activation (**Figure 1**).

MicroRNA AS THE NOVEL DIAGNOSTIC TOOLS FOR EPILEPSY

MicroRNAs (miRNAs) are a group of single-stranded, endogenous, non-coding molecules. It is estimated that 1–5% of both animal and human genes are involved in the coding miRNA (49). To date, over 500 genes which take part in miRNA coding have been discovered and the number is still rising. miRNAs take part in both physiological and pathological processes through its regulation of homeostasis. Research has revealed the involvement of miRNAs in cellular processes including cellular division, cellular cycle control, cell differentiation, apoptosis, angiogenesis, and oncogenesis (50).

Moreover, miRNA is involved in the immunological system through its regulation of the immune responses during infection (51–54). For this reason, miRNAs have been suggested to be involved in epileptogenesis (55). Three hypotheses explaining the origin of miRNA in biofluids were proposed. The first hypothesis suggests the passive entry of miRNA into the systemic circulation as a result of mechanical cellular damage, which may take place during neuroinflammation. The second hypothesis presumes that miRNAs enter the circulation via the actively-secreted microvesicles (MV), which could also be involved in intracellular communication. The third hypothesis proposes that miRNAs may be actively secreted as a response to a large variety of stimuli. This mechanism is preceded by the formation of the complex of miRNA-Argonaut proteins (Ago) and HDL (56, 57).

Due to the feasibility of comparing histopathological nervous tissue samples from both human and animals, the biological processes involving miRNAs have been extensively studied. It has been suggested that epigenetic modifications implicated in the development of DRE through the modulation of gene expression are involved in the absorption of anti-epileptic drugs (58).

Due to miRNA profiling in patients with epilepsy, the significance of miRNA in the regulation of protein levels in epileptogenesis has been identified. Elevated levels of miRNA-23a, miRNA-34a, miRNA-132, miRNA-146a in epilepsy, in particular, were frequently detected. Additionally, elevated levels of miRNA-21, miRNA-29a, miRNA-132, identified as regulated by p53, were noticed subsequent to episodes of seizures (59). The significance in the plasma levels of miRNA-134 within the course of antiepileptic drugs usage has been reported, in which miRNA-134 could potentially serve as a peripheral biomarker reflecting the acute epileptic episode during the course of the treatment (60). Similarly, miRNA-4521, as reported by Wang et al. (61), could serve as a potential biomarker in refractory epilepsy. It has also been stated that the levels of miRNA-301a-3p collected from the blood were different in patients with DRE compared to epileptic patients who were responsive to therapy. Thus, it was suggested that miRNA-301a-3p could be a marker for an early diagnosis of DRE (59). In another study, the silencing expression of miRNA-132 was shown to lead to a decreased number of seizures. It was suggested that the silencing of miRNA-132 has an impact on the MFs-CA3 pathway, which may provide beneficial outcomes for patients with epilepsy (62).

Table 1 presents the recent reports on miRNA detected in biofluids from patients with epilepsy.

The Role of miRNA in the Brain Blood Barrier Damage

Elevated levels of miRNA-132 were observed in animal models of CA3 status epilepticus (SE). Microinjection of antagomir against miRNA-132 in animal models have additionally been found to produce an anti-inflammatory effect (62). As the concept of inflammation and BBB damage has been proposed, the link between miRNA-132 and epileptogenesis was indicated (69). Elevated levels of miRNA-34a after SE has additionally been supported. Following the microinjection of antagomir against mir-34a, an inhibition of caspase-3 was reported, suggesting a possible association with increased neuronal survival and decreased level of nerve tissue apoptosis. In turn, studies on animal models and people with TLE have demonstrated the regulatory role of miRNA-146 during epilepsy (70, 71).

Oxidative Stress and miRNA

Increasing number of studies indicate a role of oxidative stress as the underlying cause of many diseases. Cellular redox signals are mediated by miRNA, an important regulator of homeostasis. On the basis of epigenetic modification, miRNA regulates ROS at the stage of post-transcriptional degradation of NOX4 and Nrf2rna, which is the down-regulatory mechanism of ROS production, resulting in decreased synthesis of ROS (72, 73). For example, miRNA-129-5p negatively regulates HMGB1 during epilepsy. The TLR4/NF-kB signaling pathway is activated by elevated levels of HMGB1. It has been shown that miRNA-129-5p plays a role in the inhibition of the development

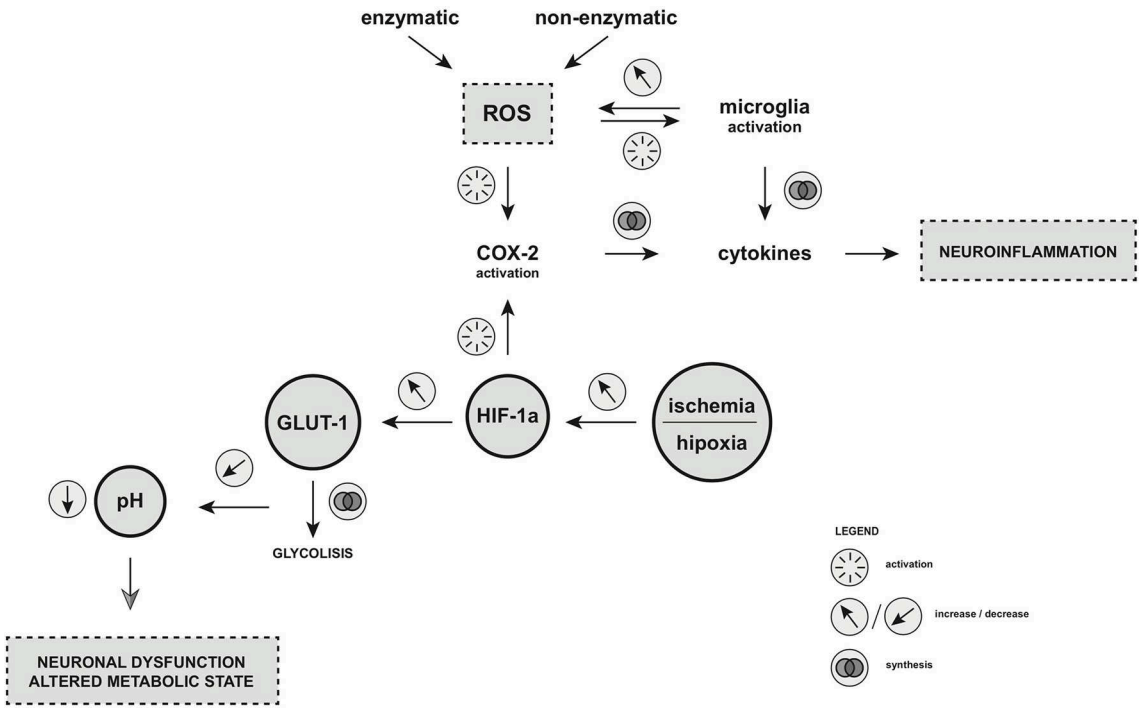

FIGURE 1 | Oxidative stress and hypoxia as the key players of epileptogenesis. Enzymatic and non-enzymatic pathway of ROS production as well as increased level of HIF-1α under hypoxic/ischemic condition leads to COX-2 activation. As the result, microglia is activated and cytokines production is augmented, which leads to neuroinflammation. HIF-1α is also the factor that regulates the glucose metabolism in the central nervous system (CNS) through GLUT-1 synthesis. Dysregulation of HIF-1a production may result in an accumulation of pyruvate in neuronal cells which is then converted into butyric acid via butyric dehydrogenase. This accumulation of by-products may lead to a decreased pH within the inner environment of neuron, leading to its dysfunction and altered metabolic state. ROS, reactive oxygen species; COX-2, cyclooxygenase-2; HIF-1α, hypoxia inducible factor-1 alpha; GLUT-1, glucose transporter-1. Illustration by Paulina Szuba.

of autoimmune encephalomyelitis-related epilepsy rat model by targeting HMGB1. Dysregulation of miRNAs' physiology involved in maintaining ROS homeostasis may possibly lead to oxidative damage and disease progression (74).

Hypoxia and miRNA

Under conditions of hypoxia, the expression of HIF1A mRNA is elevated and HIF-1a protein stabilization is increased. HIF1, the intracellular messenger of hypoxia, is transferred to the nucleus and regulates the expression of target genes. HIF1 binds to the HRE sequence to the cluster mir-200a-mir429 on chromosome 1, leading to an increased expression of miRNA-429. Subsequently, miRNA-429 in the cytosol binds to a sequence located in the 3'UTR of the HIF-1a miRNA, leading to the decreased activity of HIF-1 (75). The upregulation of miRNA-429 in human hippocampal tissues from TLE and hippocampal sclerosis-convergence have been further supported, indicating the high utility of this miRNA (76).

Circulating miRNA as Biomarker: Prospects and Limitations

For decades, circulating miRNAs have been a research material of interest. In contrast to the miRNA samples obtained from invasive surgical procedures, biofluids, particularly blood-derived plasma and serum, is easily accessible. A full blood or serum test is minimally invasive compared to procedures such as a lumbar puncture. miRNA studies in biofluids have become increasingly accessible and applicable due to the development of new molecular investigative methods. The possibility of using miRNA derived from blood as a sensitive marker, both as a prognostic and predictive factor of many diseases, is invaluable for modern researches (77–79).

On the other hand, many reports point to the uncertain efficacy of circulating miRNA. The origin of miRNA in the bloodstream remains unclear. In addition, reports point to the equivocal specificity of miRNA, which can be modified under the influence of various extrinsic factors such as tobacco, pregnancy, diet, or alterations to the circadian cycle (80–82).

THE ROLE OF THE COMPLEMENT SYSTEM IN EPILEPSY

The complement system is composed of more than 30 proteins which interact in a strictly organized manner to destroy pathogenic agents and to protect normal tissues from the deposition of immune complexes (83). There are three pathways leading to complement activation: classic, alternative and lectin (84). Each pathway leads to the activation of fragment C3, which is cleaved to form opsonin C3b and C3a, promoting the

TABLE 1 | Reports on miRNA detected in biofluids from patients with epilepsy.

miRNA	Regulation	Species	Material	Comments	References
hsa*-miR-30a-5p	Up-regulation	Human	Biofluid	Expression was analyzed by microarray and RT-qPCR. MiR-30a was overexpressed in the serum of epilepsy patients during seizures onset. The expression of miR-30a was positively associated with seizure frequency.	(63)
mir-143-3p; mir-145-5p; mir-365a-3p; mir-532-5p	Up-regulation	Human	Biofluid	Up-regulated in serum in patients with mTLE. MiRNA measured 30 min after seizures	(64)
miR-106b; miR-146a; miR-301a	Up-regulation	Human	Biofluid	Up-regulated levels in serum derived from patients with epilepsy in comparison to healthy control group	(65)
miRNA-129-2-3p	Up-regulation	Human	Biofluid	Upregulated miR-129-2-3p confirmed by qRT-PCR expression in plasma samples of refractory TLE group	(66)
hsa-miR-342-5p; hsa-miR-4446-3p; hsa-miR-30b-5p	Down-regulation	Human	Biofluid	Downregulated in DRE group compared to drug-responsive group and control group	(67)
hsa-miR-134-5p	Down-Regulation	Human	Biofluid	Downregulated in plasma samples from MTLE patients when compared with healthy controls	(68)
hsa-miR-194-5p; hsa-miR-15a-5p; hsa-miR-144-5p; hsa-miR-181c-5p; hsa-miR-889-3p	Down-regulation	Human	Biofluid	Downregulated in serum in patients with epilepsy	(67)
hsa-let-7d-5p; hsa-miR-106b-5p; hsa-miR-146a-5p; hsa-miR-130a-3p	Up-regulation	Human	Biofluid	Upregulated in serum samples from TLE patients	(59)

hsa, homo sapiens.

activation of the lytic pathway, acting as anaphylotoxin and causing damage to cell membranes and pathogens. C5a formed through this process attracts macrophages and neutrophils, and also activates mast cells (85).

The complement system plays a critical role in the innate immune system and is one of the main mechanisms of the effector adaptive humoral response (86). It mediates the reaction against infectious agents through a coordinated sequence of the enzymatic cascade, leading to the elimination of foreign cells by pathogen recognition, opsonization, and lysis (87). Although it is essential in maintaining immune balance, inappropriate activation of the complement cascade can lead to tissue damage and contribute to the development and progression of various pathologies (88).

Increased Concentrations of Ingredients, Biomarkers

Studies from human and animal models have indicated that the regulation of the complement cascade contributes to the development of epilepsy (89). The concentration of serum C3 in untreated patients with epilepsy were shown to be significantly higher than in that of healthy controls (90). Recent studies have reported an elevated concentration of the classical pathway components in patients with epilepsy compared to healthy controls and in untreated epileptic patients compared to those who are undergoing treatment (91–94).

Investigation into the plasma concentrations of a panel of complement analytes in epileptic subjects presented a highly predictive model comprising of 6 complement analytes (C3, C4, properdin, FH, C1Inh, and Clu) which distinguish between epilepsy cases and controls (89). This may useful for the development of prognostic markers and effective epilepsy therapies.

The Classical Pathway

In the classical complement pathway, the proteolytic cleavage of the C3 fragment into C3a and C3b requires the linkage of the C1q to cell surfaces, C1s and C1s proteases (95).

Soluble C3a promotes the recruitment of microglia and inflammation, whereas C3b can be subsequently split into C3bα, C3bβ, and iC3b, all of which can act as opsonins. Recent studies have shown new non-canonical roles for phagocytic C1q-C3 signals in improving synaptic connectivity (96, 97). For instance, the C1q and C3b analytes are associated with the removal of synapses during the development of the visual system and the elimination of unwanted structures of synaptic hippocampus in models of neurodegenerative disorders (98–101). This indicates the role of the classical complement pathway in the epileptogenic remodeling of synaptic circuits associated with status epilepticus and TLE (92, 102–104).

Furthermore, it has been shown that the C1q-C3 signaling can modify the expression of the pro-inflammatory tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL1β) (105, 106). In turn, the upregulation of TNF-α levels in microglia has been observed in the condition of the SE-induced activation of C5 (107). It has been further indicated that the SE-provoked increases in C1q signaling and the generation of C3a and C3b-mediated activation of C5a/b may contribute to the initiation and/or preservation of neuroinflammation in epilepsy (108). Further investigation would be required, however, to deepen the understanding of complement cascade in this matter.

The C1q analyte has been additionally proven to prevent further necrosis and inflammation by promoting phagocytosis

of cellular debris and apoptotic cells (109), which could be considered a neuroprotective mechanism subsequent to SE (110, 111). C1q has also been shown to reduce the lipopolysaccharide-induced microglial release of IL-6 and TNF-α, and thus may play a role in helping to reduce the pro-inflammatory responses induced by SE (105, 112). Regardless of pathway activation, the final stage of the complement cascade leads to the formation of a membrane-attacking complex (MAC). MAC joins the cell membranes, creating a porous functional channel, which leads to the flow of ions and ultimately to the osmotic lysis of the attacked cell (113). The infusion of single proteins of the membrane attack the complex pathway (C5b6, C7, C8, and C9) to the hippocampus of awake, freely moving rats has been shown to induce cytotoxicity and behavioral and electrographic convulsions (83).

Therapeutic Potential

The therapeutic implications in modulating the complement cascade has been previously demonstrated (114). The anti-C5 antibody directed toward the final complement pathway is of high therapeutic significance. Treatment with eculizumab blocks the cleavage of C5 and prevents the formation of MAC while leaving the rest of the complement system intact (115). Most importantly, eculizumab appears to be well-tolerated in all approved clinical settings (116). Eculizumab and other designed inhibitors of the complement cascade are likely to achieve clinical utility that goes far beyond paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, including autoimmune disease, transplantation, neurodegenerative, and other CNS diseases, including epilepsy (117, 118).

ROLE OF CYTOKINES IN EPILEPTOGENESIS

Recent clinical and experimental findings have supported the premise of inflammation as a major pathological basis in epileptogenesis. Inflammation can be studied through the measurements of inflammatory cytokines, which are soluble mediators of cell communication that are critical in immune regulation. Inflammatory cytokines' potentiation of free radical species and alterations in glutamatergic neurotransmission, ultimately result in neuronal excitoxicity, and consequential structural alterations (such as BBB disruption) within the brain which have been consistently observed in epileptic individuals (119, 120).

Within the CNS, cytokines are produced as a response to various inflammatory stimuli. In recent years, studies have shown that epileptic seizures can induce the production of cytokines, which in turn contributes to further inflammation and structural changes, thereby establishing an ongoing cycle of events contributing to the development and progression of epilepsy (121). Both pro- and anti-convulsive effects have been reported for cytokines, suggesting the diverse nature of cytokine networks and the complex relationship between the immune system and epilepsy. Here, we review the different mechanism of cytokine involvement in the development of epilepsy.

Free Radical Generation

Pro-inflammatory cytokines are indicated to inhibit neurogenesis through the direct induction of neuronal death via reactive oxygen species (ROS) generation and excitotoxic mechanisms. Due to its high intrinsic metabolic rate and low levels of protective antioxidants, the brain is highly susceptible to free radical neuronal damage. The generation of ROS from a preceding inflammatory event may result in progressive oxidative damage, cellular destruction and neuroprogression (122). Pro-inflammatory cytokines including IL-1B, TNF-α, and IFN-y have been shown to potentiate the effects of these free radicals (123). Consequentially, mechanisms of neuroprogression, including neurodegeneration and reduced neurogenesis, play a part in the underlying pathophysiology of the epileptic brain.

Alterations in Glutamatergic Neurotransmission

Alterations in glutamatergic neurotransmission could trigger neuronal excitotoxicity, impaired neuroprotection, and the necessary conditions for the development of epilepsy (124, 125).

IL-1B has been indicated to alter the glutamate transporter expression leading to a decreased reuptake of glutamate. Resulting excess synaptic glutamate lead to subsequent N-Methyl-D-aspartic acid (NMDA)-mediated excitotoxicity and cellular damage (126). Particularly within the neurons of the hippocampus, the binding of IL-1B to the IL-1 receptor induces the phosphorylation of the NMDA receptor and the potentiation of its activity. This results in an increased neuronal calcium influx and subsequent cell death (127).

Blood-Brain Barrier Compromise

Neuroinflammation induces structural changes to the brain parenchyma, one of which is the leakage of the BBB and thereby the changes in its functional properties (21). These alterations lead to cellular damage and neuronal hyperexcitability, leading to the reduced threshold for seizure induction. BBB disruption can be triggered by direct insult to the endothelium or via systemic factors, including activation of circulating leukocyte and release of molecular mediators that increase vascular permeability (128). Studies have shown BBB failure after exogenous administration of pro-inflammatory cytokines including IL-1, IL-6, TNF-a, and IFN-y, suggesting a link between the systemic immune system and neuronal dysfunction (129).

COX-1 and COX-2

COX-2 is indicated to play an important role in the post-seizure inflammation and hyperexcitability of the brain, possibly contributing to secondary damage in the brain and the increased likelihood of repetitive seizures. One pathway is through their synthesis of PGE2, which has excitatory effects. Activation of a single PGE2 receptor (EP2) has been shown to exacerbate the rapid upregulation of IL-6 and IL-1B in activated microglia and reduce the production of TNF-a, IL-10. EP2 thus regulates innate

immunity in the CNS by alternating the balance between pro- and anti-inflammatory cytokines (130).

Particularly in DRE, the cellular expression of COX-1 and COX-2 and relationship to the efflux transporter expression is particularly important for elucidating the underling effects of inflammation. The "transporter hypothesis" of DRE suggests that the overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein (p-gp) and BCRP at BBB may prevent anti-epileptic drugs from reaching their targets. P-gp, an ATP-dependent efflux pump, has the function of pumping foreign substances out of the cell. P-gp up-regulation was in part caused by elevated COX-2 activity and pharmacologic inhibition of COX-2 has been shown to allow greater uptake of P-gp substrate phenytoin (131, 132).

The contribution of COX-2 inhibition in neuroprotection and its potential role in adjunct therapeutic strategy remains inconclusive, as there has yet to be a selective COX-2 inhibitor which has shown a favorable therapeutic outcome. Although short-term exposure might be useful, the accompanying risk of cardiovascular adverse effects makes it unlikely that chronic COX-2 inhibition can be used in the long-term treatment of epilepsy (130).

Pro-Inflammatory Cytokines in Epilepsy

The seizure-induced activation of the cytokine network may suggest the interplay of the nervous-immune-endocrine systems in the pathological process of epileptic seizures. IL-1B and IL-8 are pro-inflammatory cytokines that activate additional cytokine cascades and increase seizure susceptibility and organ damage, whereas IL-1 receptor antagonist and IL-10 act as anti-inflammatory cytokines that have protective and anticonvulsant effects (22). It remains unclear whether increased cytokine levels in plasma and CSF of epilepsy patients relate to a cerebral inflammatory process alone, or arise as a result of postictal peripheral muscular recovery or circulating immune cells. Several studies related cytokines to changes in neuronal excitability and suggested a potential role for targeted therapy (21, 133).

IL-1B

While IL-1 cytokines are constitutively expressed at very low levels in the human CNS, they are often elevated in the brain under certain pathological states such as during an active seizure, hypoxic injury, and during the process of an infection (22). Recent clinical studies have reported changes in levels of IL-1B in the blood, CSF, and brain tissue (22). A significant difference was found where the level of in IL-B in CSF was increased in patients with generalized tonic-clonic seizures compared to the control group. The increased levels also show a significant positive correlation with the duration and frequency of seizures (134). A decrease in IL-1ra/IL-1B ratio was reported after a seizure, that leads to increased influence of the pro-inflammatory IL-1B and may implicate a pro-inflammatory state in the brain (135).

Other studies found no significant differences in the IL-1B concentration in blood and CSF after generalized tonic-clonic seizures (22). Studies in patients with focal epilepsy, mesial TLE, and febrile seizures similarly showed that postictal plasma concentrations of IL-1B did not significantly differ from baseline levels (21, 127, 136, 137). However, an increased level of IL-1B was found in post-mortem samples of patients with TLE when compared to autopsy controls (127).

Therapies for auto-inflammation including IL-1 blockade have been indicated in the treatment of refractory epilepsy (138). Febrile infection-related epilepsy syndrome, a rare but devastating encephalopathy occurring after a febrile illness, showed an improvement with anakinra while in refractory status epilepticus. This suggests that this treatment may be a useful adjunctive medication for certain cases of refractory epilepsy syndromes (139).

IL-6

IL-6 is a primary pro-inflammatory cytokine involved in the acute phase of the immune response. Seizures cause changes in levels of IL-6 both in CSF and in the peripheral blood. The magnitude of these changes is related to the severity of seizures. IL-6 levels are strongly increased after recurrent GTCS, whereas after single tonic-clonic or prolonged partial seizures IL-6 levels are increased to a lesser extent (140, 141). IL-6 levels have been reported to be significantly higher in the daily generalized motor seizures than in either intermittent seizures or in control subjects (142). This indicates the positive correlation between the magnitude of IL-6 activation and severity of cerebral epileptic activity. A meta-analysis of serum IL-6 levels in TLE patients revealed marginal but significant IL-6 elevation when compared to controls (143). While IL-6 seems to be consistently increased in epilepsy patients, it is not possible to explain whether it is a cause or consequence of the disease. A case study indicated that after blocking IL-6R with the monoclonal antibody tocilizumab, stable remission of epileptic symptoms could be achieved. This suggests the possible therapeutic implications and efficacy of tocilizumab in the treatment of synaptic diseases which needs to be further confirmed by controlled studies (144).

IL-8

IL-8, a pro-inflammatory cytokine, plays a role in the promotion of neuronal growth after injury and in the stimulation of nerve growth factor production, constituting both damaging and reparative functions involved in the pathogenesis traumatic brain injury. IL-8 is found to be significantly increased in the serum of patients with partial onset seizures (145). It is reported to be associated with seizure severity (measured by seizure frequency, VA score, or NHS3) in TLE, extra-temporal lobe epilepsy, and idiopathic generalized epilepsy (136). In neonatal seizures, IL-8 levels significantly increased within 24 h and remained increased after 48 and 72 h (22).

TNF-a

Although TNF-a is a prominent pro-inflammatory marker, there are limited reports on the significance of TNF-a in epileptic patients. No significant differences were found in the serum of patients with daily or intermittent generalized motor seizures (142). A study reported decreased frequency of CD8+ T-lymphocytes expressing TNF-a in mTLE patients, in which lower frequency could be explained by the migration of pro-inflammatory CD8 T-cells to brain areas affected

by repetitive seizures, thus reducing their frequency in the peripheral blood (133). A study reported that in DRE resulting from Rasmussen's encephalitis, some patients showed seizure improvement following adalimumab administration, an anti-TNF-a therapy. In this study, patients had over a 50% decrease in seizure frequency and shown an improvement in their functional deficit. Further studies are necessary to confirm the results of the efficacy of adalimumab and its further therapeutic implications in epilepsy (146).

Anti-inflammatory Cytokines in Epilepsy

IL-1Ra

IL-1Ra, the antagonist of IL-1 receptor type 1, limits IL-1B-mediated actions through the inhibition of IL-1B's biological activities and its receptor binding. IL-1Ra is induced in response to seizures, and is indicated to exert neuroprotective and anticonvulsant effects. Increased levels of IL-1Ra is observed after episodes of seizures. Its elevation after generalized seizures is higher than its increase after complex partial seizures, indicating its reflection on the seizure severity (21). In a study on neonatal seizures, IL-1Ra was continuously inactivated with significantly lower concentration in seizure group within 72 h of seizure attack. It is hypothesized that this lack of consistent IL-1Ra induction in response to epileptogenic environment may be characteristic of neonatal seizures, making the neonatal period more vulnerable to seizures (22).

IL-10

IL-10 plays an important regulatory, anti-inflammatory role, counteracting various pro-inflammatory processes during infection as well as in autoimmune disorders. The anti-inflammatory effects of IL-10 is mediated through the deactivation of macrophages, which in turn decreases the production of pro-inflammatory cytokine production by T-cells.

Although increased levels of pro-inflammatory cytokines were primarily found in patients with epilepsy, significant elevations of CSF IL-10 were also observed in epileptic patients (22). It has been hypothesized that the increase of IL-10 in CSF of epilepsy patients can be due to counteracting mechanisms to the pro-inflammatory stimuli. As an example, in neonatal seizures, IL-10 levels were elevated in plasma 48–72 h after seizure onset. This may indicate the enhanced protective role of IL-10 which has an anticonvulsive effect in neonatal seizure patients (22).

Other Cytokines

EPO

Different cell types within the nervous system, including neurons, glial cells, endothelial cells, produces EPO, and expresses EPO-R (147). Several studies have demonstrated that EPO could enhance phagocytosis in polymorphonuclear cells and reduce the activation of macrophages, thus modulating the inflammatory process. EPO could play a protective role in neuronal survival after an epileptic seizure. A significant difference in EPO levels in the CSF has been observed between seizure groups and control subjects. Changes in the levels of EPO after generalized tonic-clonic seizures has been reported to positively correlate to the duration and frequency of seizures (148).

Hs-CRP

High-sensitivity CRP (Hs-CRP) is a useful biomarker to detect chronic, subtle inflammation, which is not detected by conventional CRP values. It is significantly higher in the daily generalized motor seizures than in either intermittent seizures or control (142).

CCL2

Chemokines, expressed in microglia, astrocytes, and endothelial cells, plays a role in the guidance of inflammatory mediators toward the source of inflammation and in the activation of leukocytes (149). CCL2 is one of the primary elevated inflammatory chemokines observed in patients with pharmacoresistent epilepsy. It is of particular interest after results from animal studies reveal its upregulated expression in addition to the enhancement of seizure frequency as a result of induced systemic inflammation. Inversely, exogenous administration of anti-CCL2 antibodies suppress LPS-mediated seizure enhancement in chronically epileptic animals. Although there are limited results from human studies, these observations may point to the significance of CCL2 in the molecular pathways that link peripheral inflammation with neuronal hyperexcitability (150).

In **Table 2** we present the summary and characteristics of the above-mentioned factors.

Figure 2 summarizes the discussed pathways which could potentially lead to the development of epilepsy.

PROTEINS AND AMINO ACIDS ROLE IN EPILEPSY

Aspartate, Glycine, Glutamate, and NMDA Receptor

The role of aspartate and its N-methyl-D-aspartate receptor (NMDA-R) has been widely discussed in the previous decade. A clear connection exists between this amino acid and epileptogenesis, though the exact mechanism is still debatable. NMDA-Rs are ionic glutamate receptors. Several characteristic attributes of NMDA-Rs include co-agonist activation, extracellular Mg^{2+}-induced voltage-dependent blockade, elevated permeability to Ca^{2+}, and slow gating and deactivation kinetics (151).

Aspartate and glutamate can both activate NDMA-R, with glutamate being the one with a stronger stimulation potential. Glycine is a required co-agonist. However, it can be exchanged for stronger binding D-serine (152). Ronne-Engström et al. presented the changes of amino acids level in extracellular fluid (ECF) taken by microdialysis from the epileptogenic brain regions of patients suffering from epilepsy. There was the greatest increase in the aspartate level, and the levels of glutamate, serine, and glycine also increased significantly (153). This indicated the possible role of NDMA-R in epileptogenesis.

There are different mutations concerning NMDA-R subunits. GluN1 subunit is encoded by GRIN1 gene at chromosome 9q34.3, with twelve mutations previously described (154, 155). GluN2A is encoded by GRIN2A gene at chromosome 16p13.2,

TABLE 2 | Cytokines and their main role in epileptogenesis.

	IL-1B	IL-6	IL-8	TNF-a
Action	• Pro-inflammatory cytokine • Elevated under certain pathological states (active seizure, hypoxic injury, infection)	• Pro-inflammatory cytokine • Involved in the acute phase of the immune response	• Pro-inflammatory cytokine promote neuronal growth after injury • Stimulates the production of nerve growth factor	• Pro-inflammatory cytokine
Generalized tonic-clonic seizures	• No changes in plasma levels, no significant differences • CSF IL-1B levels show an increase after seizure with a significant positive correlation with the duration and frequency of seizures	• Levels strongly increased after single and recurrent GTCS • IL-6 significantly higher in the daily generalized motor seizures than in either intermittent seizures or control	• Associated with seizure severity	• No significant differences
Partial seizures	• No changes in plasma levels	• Levels increased in prolonged partial seizures but to a lesser extent than in GTCS	• Elevated levels in serum	
Neonatal seizures	–	–	• Significantly increased within 24 h; remained increase after 48–72 h	
Mesial temporal lobe epilepsy	• Increased level in brain tissue	• Increase frequency of CD4+ T-lymphocytes expressing IL-6 • IL-6 increased in serum	• Associated with seizure severity	• Decrease frequency of CD8+ T-lymphocytes expressing TNF-a in mTLE patients
Febrile seizures	• No significant differences in CSF and serum	• No significant serum elevation		
References	(22, 127, 134–139)	(140–144)	(22, 136, 145)	(133, 142, 146)

	IL-1Ra	IL-10	EPO	CRP	CCL2
Action	• Anti-inflammatory cytokine • Limits IL-1B-mediated pro-inflammatory actions through the inhibition IL-1B's biological activities and receptor binding • Neuroprotective and anticonvulsant effects	• Anti-inflammatory cytokine • Suppression of pro-inflammatory cytokine production	• Enhance phagocytosis in polymorphonuclear cells • Reduce the activation of macrophages • Possible protective role in neuronal survival after an epileptic seizure	• Biomarker to detect chronic, subtle inflammation	• Guide inflammatory mediators toward the source of inflammation • Activation of leukocytes
Generalized tonic-clonic seizures	• Levels increased after seizure, higher in generalized seizure than after complex partial seizures	• Increased level in CSF	• CSF EPO levels show an increase after seizure with a significant positive correlation with the duration and frequency of seizures	• High-sensitivity CRP (Hs-CRP), IL-6 significantly higher in the daily generalized motor seizures than in either intermittent seizures or control	• Elevated in patients with pharmacoresistent epilepsy
Partial seizures	• Levels increased after seizure	• Increased level in CSF			
Neonatal seizure (hypoxic-ischemic encephalopathy-induced seizure)	• Levels increased within 24 h; rapid decreased after 48–72 h	• Increased within 24 h; remained increase after 48–72 h			
References	(21, 22)	(22)	(147, 148)	(142)	(149, 150)

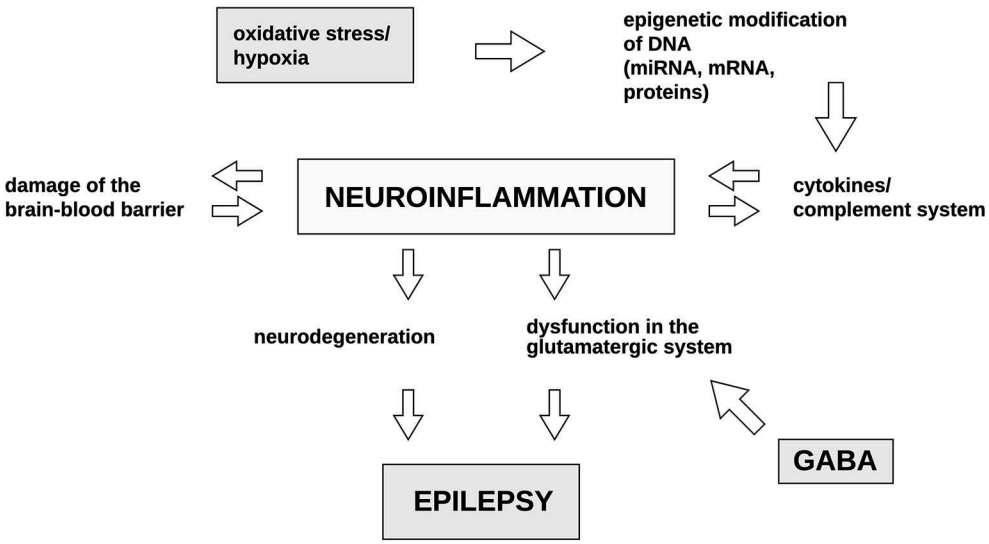

FIGURE 2 | Potential mechanisms occurring during epileptogenesis and their correlations with one another. Both oxidative stress and hypoxia have been previously reported to induce epigenetic modifications of DNA. This may result in the activation of cytokines and the complement system. Consequently, the resulting neuroinflammatory processes may in turn induce the production of cytokines and the elements of the complement system in a feedback loop. Neuroinflammation is the primary factor which leads to BBB destruction, neurodegeneration and the dysfunction in the glutamatergic system resulting in the dysregulation of GABA synthesis. Culmination of the above-mentioned mechanisms result in epilepsy. GABA, gamma-Aminobutyric acid. Illustration by Paulina Szuba.

which is a genome hotspot, and very susceptible to mutation, eighty-two of which were previously described (155, 156). These mutations are also suspected to be strongly connected with epileptogenesis. GRIN2B, encoding the CluN2B subunit is located on chromosome 12p13.1, with thirteen mutations connected with epileptogenesis (155, 157). One epileptogenic mutation is also known in the GRIND2D gene on chromosome 19q13.3, encoding the GluN2D subunit (155, 158).

Different mutations present with different disease phenotypes. GRIN2A mutation is commonly associated with childhood epilepsy syndromes within epilepsy-aphasia spectrum, such as benign epilepsy with centrotemporal spikes (BECTS), Landau-Kleffner syndrome (LKS), and epileptic encephalopathy with continuous-spike-and-waves-during-slow-wave-sleep (CSWSS) (159, 160), whereas GRIN1, GRIN2B and GRIN2D mutations present with developmental delay and more severe phenotypes of epilepsy (161, 162). This is connected with the embryonic expression of subunits encoded by these genes and the fact that GluN1 is a subunit required for proper functioning of NDMA-Rs throughout the brain (163). However, GRIN2A can also lead to more severe phenotypes, such as early-onset epileptic encephalopathy. Among relatives, the genetic penetrance may vary and thus not every member will be affected by epilepsy (155).

Studies have indicated the inflammatory background of NMDA-R-associated epilepsy. IL-1β and HMGB1 use interleukin 1 receptor type I (IL-1R1) and Toll-like receptor 4 (TLR4), respectively, to activate Src kinases-dependent NR2B phosphorylation and to enhance NMDA-mediated Ca^{2+} influx (164, 165). Furthermore, HMGB1 uses physical, non-receptor interaction with presynaptic NMDA receptors, resulting in the release of Ca^{2+}-dependent glutamate. This mediates inflammatory cell loss and epileptogenesis by the excitability of CA1 neurons via reduced NMDA-induced outward current (166). NMDA-dependent long-term synaptic depression in the hippocampus is also connected with the activation of JAK/STAT pathway by IL-6, which has a significantly higher occurrence of epileptogenic neuronal damage (167). Additionally, the exposure to lipopolysaccharides in early childhood correlates with a further poor developmental outcome due to chronic changes to NDMA-R and its units' expression in hippocampus and cortex (168, 169).

Martell et al. showed the correlation between the activation of NMDA receptors and voltage-dependent intrinsic oscillations in intracellular whole-cell patch clamp recordings of neocortical pyramidal neurons, with a simultaneous instability of the neuronal system, presented by the whole-cell I-V curve and the lower frequencies in resonance. This suggests the role of NMDA-R in both producing low-frequency oscillations and in promoting cell responsiveness to lower frequencies, which could make these neurons more prone to epileptogenesis (170).

In a recent study involving post-traumatic epilepsy (PTE)—kindled rat models and a small group of patients with temporal lobe refractory epilepsy, Liu et al. (171) shows a significant decrease in microfilament heavy chain level in the epileptic brain tissue, which is a reflection of axonal impairment. He also observed an elevated level of amyloid precursor protein (APP), but its contributions toward epileptogenesis remain unclear. On one hand, reduced level of APP is connected with increased susceptibility to seizure, as reported by Steinbach et al. (172). However, the elevated level of APP is also connected to hyperexcitability (173). What was observed by Liu et al. was the ability of NMDA-R antagonists to both counteract the

accumulation of APP and reverse previous accumulations, while NMDA-R activation can lead to the blockage of axonal transport which is crucial for maintaining physiological neuronal function. The hypothesis is that the process is controlled by the upregulated activity of Cdk5 and GSK-3β (neurofilament kinases), in which elevated levels were observed in brain tissues of both human and rat models. Both kinases use different pathways to slow down the axonal transport. GSK-3β downregulates kinesin-based motility (174) and increases neurofilament bundling (175), while Cdk5 uses the phosphorylation of NFH side arms (176), and the pathway via Lis1/Ndel1-dependent regulator in conditions of stress (177).

The potential clinical impact of these findings was tested on a rat model with kainic-induced SE, relating to limbic system protection by NDMA-R inhibitors. Hippocampal and limbic system damage has been considered as potential starting points for the later development of epilepsy among children with febrile seizures (178). Studies have also indicated that the lesions in the limbic system underlie the predisposition to febrile seizures (179) or are secondary to early epileptic signs (180, 181).

Therefore, the clinical implication of limbic system damage and its role in neuroprotection remains equivocal. The inhibition of NMDA-R by dizocilpine after SE showed processes of neuroprotection in most affected limbic system regions, except for the hilus of the dentate gyrus and the substantia nigra pars reticulata. Although the hilus was susceptible to damage during SE, it was not progressive in the NDMA-R inhibitor group. Thus, dizocilpine was suggested to have a potentially protective role. A reduced level of fragmented DNA and histological apoptosis markers suggests that the inhibition of NDMA-R could prevent neuronal apoptosis. The prevention of the loss of dentate granule cells is of clinical importance as the resulting hyperexcitability in damaged regions may ultimately lead to the development of epilepsy. However, the neuropathological indication of neuronal protection did not correlate with the clinical prevention of spontaneous recurrent seizures (SRS) (182). Additionally, a study on ketamine, another NDMA-R antagonist, on pilocarpine-induced SE rat model reproduced similar results to those from the kainic model concerning the development of SRS (183). On the other hand, the limitation of the dizocilpine study was the injection of only a single dose of NMDA-R inhibitor, which cannot exclude the fact that, with repetitive inhibition, the neuropathological protection could be followed by clinical improvement (182).

The development of proper treatment based on NDMA-R inhibition is still an ongoing process, with its first data reported mostly through case studies (155). In the case of a 6-year-old child with GRIN2A mutation and early-onset epileptic encephalopathy, non-responsive to conventional methods of treatment, good response to memantine (159), an FDA-approved drug used clinically for the treatment of Alzheimer's disease, was observed. In rat models, memantine was observed to significantly lower the reduction of NFH by decreasing Cdk5 and GSK-3β, showing probable mechanisms of its protective role. GLuN1 and GLuN2B inhibitor, ifenprodil, showed similar, promising results (171). A number of experiments of *in vitro* electrophysiological models included different NMDA-R inhibitors, such as ketamine, magnesium, dextromethorphan, dextrorphan, amantadine, and TCN-201 (159). TCN-201 in rat models significantly reduced the number of epileptiform events (184). The most commonly used inhibitor, memantine, occurs as a stronger, and safer NMDA-R inhibitor than amantadine (185). Ketamine has lower potency and is therefore less effective than memantine. An analysis of the potential of a selective GluN2B negative allosteric modulator, radiprodil, was proven to be more effective than other NDMA-R inhibitors in some variations of gene mutations (186).

NDMA-R inhibitors are not always equally effective when used in monotherapy. In a case study of two children with GluN2D V667I mutation, one remained refractory to the monotherapy of memantine, midazolam, pentobarbital, ketamine or magnesium, while polytherapy with ketamine and magnesium i.v. proved to be beneficial. In another case, in which the patient was found to be refractory to memantine and polytherapy of memantine, sulthiame, and lamotrigine improved his condition. This indicates the possible usage of NDMA-R antagonists in polytherapy along with conventional anticonvulsant (158).

The Role of Glutamine Synthetase

Glutamine synthetase (GS) is an enzyme characteristic for astrocytes. It has a leading role in the glutamine-glutamate-ammonia cycle. Glutamine is involved in many biological processes including the Krebs' cycle and is a precursor to the neurotransmitters gamma-aminobutyric acid (GABA) and glutamate (187, 188). It connects processes of cell metabolism, the detoxification of ammonia, glutamate, and the neurotransmitter pool role (189, 190).

Physiologically, glutamate released from synapses is converted by GS into non-toxic glutamine due to its uptake by glial cells, mostly via the excitatory amino acid transporter 2 (EAAT2), and then transported back to neurons, repeating the cycle (191, 192). GS is expressed on glial cells, where it is responsible for 80% of glutamate transport (193, 194). The cycle of glutamate and glutamine is also affected by the malfunction of the EAAT2 (195, 196), phosphate-activated glutaminase (197) and the vesicular glutamate transporter 1 (VGLUT1) (198).

Chronically elevated levels of extracellular glutamate lead to increased excitotoxicity (199–201), as observed in various neuropsychiatric disorders (202), including refractory epilepsy. The studies on GS after SE in animal models are equivocal. In kainate models, in the latent phase GS expression was higher, while it decreased in the chronic phase (203, 204). In the pilocarpine model, however, GS in the chronic phase appeared redistributed rather than downregulated (205).

Mesiotemporal sclerosis (MTS) is characterized by region-specific neuronal loss (206–208), reactive alterations in astrocytes, gliosis, and mossy fiber sprouting (209, 210). Astrogliosis, a characteristic feature for MTS, presents with an upregulation of the intermediate filament marker, glial fibrillary acidic protein (GFAP) (211–214). This pathomechanism has been observed in TLE, a type of refractory epilepsy (215).

In TLE patients and in rodent epilepsy models, regions of the hippocampus affected by cell death shows downregulated GS, leading to an increase in extracellular glutamate concentration,

ultimately resulting in neural hyperexcitability, excitotoxicity, and neurodegeneration in epilepsy (205, 216). Using magnetic resonance spectroscopy, it was observed that what underlies this downregulation is the disruption of glutamate-glutamine cycle. Not only was the glutamate level increased and the glutamine level decreased, the process of cycling was slower (217). The glutamate receptor subunits GluR1 and GluR2 in the hippocampus appeared to be upregulated. In two studies of resected tissue obtained from subjects affected by TLE, however, no changes in glutamate transporter expression were found (215, 218).

In brain microdialysis studies, it was observed that the increase in glutamate level occurred seconds before a seizure started and the peak was observed for no shorter than 15 min after the end of EEG recordings of the seizure (215, 219). Analogs of glutamate or glutamate itself can trigger seizures and its antagonists counteracts a seizure occurrence (220, 221). In addition, genetic deletion in GS or EAAT2 expression (222) or the injection of its inhibitor such as methionine sulfoximine led to spontaneous seizure in a rodent model (223–226). Currently, there are no known substance that would alleviate epileptogenesis caused by GS or EAAT2 disruption. The role of genetic engineering in treatment is yet to be described.

P2X7 Receptor (P2X7R)

One of the most discussed receptors with a well-studied role in epileptogenesis is P2X7R, a cell surface-expressed, purinergic, ionotropic receptor for ATP, which is released in the event of neurotrauma (e.g., seizure). In rodent models, P2X7R, in contrast to other receptors from P2X family, is only expressed in the postnatal period, reflecting a correlation with CNS maturation and the development of purinergic signaling, required for proper development (227–229). Abnormalities in purinergic signaling lead to abruptions in neuronal migration and axonal outgrowth, disrupting proper synaptogenesis, and the development of microglia and astroglia (230, 231).

ATP release roots through both a physiological, activity-regulated manner and through neuronal and glial damage. All of P2X receptors bind to ATP (232). P2X7R requires a high amount of this ligand, a characteristic feature for pathological conditions (233). The activation of P2X7R is connected with immunological reactions of microglia and the release of caspase-1-dependent interleukin-1b (IL-1b), regulated by inflammasomes (234). This interleukin, in turn, promotes glutamatergic signaling and upregulates the activity of cycloxygenase-2 (COX2), nitric oxide synthase (NO synthase) and TNF-a, leading to increased excitability (125, 235). The expression of P2X7R on a molecular level seems to be regulated by the Specificity protein 1 (Sp1) transcription factor in neuro 2a cells (236) and posttranscriptional silencing by microRNAs. The latter was observed in mice model with induced status epilepticus (237).

TNF-a is primarily a product of macrophages and T-cells, existing at low levels in the physiological brain. It can be rapidly upregulated in pathological conditions by glial cells, neurons and the epithelium. The main pathways of TNF-a activity include NF-kB binding leading to cell death (238) and the activation of p38 mitogen-activated protein kinase resulting in cell survival (239). Studies on the P2X7R agonist, 2′-3′-O-(benzoyl-benzoyl) ATP (BzATP), and its antagonist, oxidized ATP (OxATP), indicates a crucial role of TNF-a in the homeostatic balance between neuronal cell death and neuroprotection. The activation of P2X7R, followed by TNF-a activation, was shown to reduce glutamate-induced neuronal cell death (240, 241). The modulation of P2X7R by its agonists and antagonists in a rat pilocarpine epilepsy model indicated that the activation of P2X7R and its induction of TNF-a can lead to more evident neuronal damage within the hippocampus (242). However, in KASE model, P2X7R antagonist treatment was not associated with astroglial protection (243, 244).

The inhibition of P2X7R is also connected with the reduction of neutrophil infiltration after SE via Monocyte Chemoattractant Protein 1 (MCP-1) (245–247). Immunoreactivity is detected in microglia and further regulates the activity of Macrophage Inflammatory Protein 2 (MIP-2), leading to neuronal damage (248). In addition, P2X7R modulates glutamate and GABA release in the hippocampus (233, 249–252), lowering the intracellular potassium level and depolarizes sodium and calcium entry (253). It is possible for P2X7R to modulate the activity of neurons by PanX1, a membrane channel opened by P2X7R, which modulates neuronal cell death and neuronal activity (254).

P2X7R is upregulated in seizures within the hippocampus and the cortex in mouse models. This upregulation in HI seizures models is prolonged, leaving the brain susceptible to further epileptiform events and epilepsy development (255). This would lead to a rapid or an enhanced release of pro-inflammatory cytokines such as IL-1b, resulting in a prolonged neuroinflammatory response and further injury (256, 257) and a disruption of cognitive and hippocampal function of brain regulated by IL-1b, which seem to be affected in HI seizures in rodent model (255).

In various clinical studies, the injection of P2X7R antagonists such as A-438079, JNJ-47965567, Brilliant Blue G and JNJ-42253432 lead to reduced seizure intensity. It also limits the immunological reaction via caspase-activation and neuroinflammatory genes transcription (232). The antagonists proved helpful in different neurological abnormalities, such as Alzheimer's disease, traumatic brain injury and Parkinson's disease (258–262). They were also studied in non-neurological conditions and appeared safe and well-tolerated, although they showed no efficacy in those diseases (263–265).

A-438079 injection proved effective in kainic rat models of status epilepticus. The neuroprotective outcome of A-438079 was also observed in global hypoxia invoked in rats, a model for neonatal hypoxic-ischemic (HI) seizures. However, the effect on post-hypoxia seizure was limited due to the short-term study duration. A high dose of the antagonist did not present comparable results, indicating a short duration of action and a narrow therapeutic window (251). In another study of a KASE epilepsy model, A-438079 combined with lorazepam caused seizures cessation during status epilepticus. However, it is unclear whether the drugs had a crucial role (266). Comparable results in HI seizures mice model was also observed for JNJ-47965567,

with a similar clinical limitation for its usage (232). None of the antagonists presented full cessation of seizures, indicating that seizures can be triggered by a different neurotransmitter rather than by ATP (267, 268). JNJ-42253432 led to less severe phenotype of epileptiform activities though failed to suppress SRS (243).

Brilliant Blue G (BBG), a selective P2X7R antagonists reducing Ca^{2+} influx in neuronal cells, which in turn increases glutamate transporter 1 (GLT-1)/Glutamate aspartate Transporter (GLAST) mRNA stability, reducing glutamate release. This leads to the recovery of astrocytic GLT-1/GLAST function and consequential higher glutamate reuptake (269). This is crucial for the prevention of excitotoxicity. In rat models, BBG administration helped in PTZ-induced kindling animals to improve cognitive functions, such as learning and memory, which can be a clinical sign of reduced hippocampal injury and cell death (270). However, BBG had a non-satisfactory anticonvulsive effect in 6 Hz electroshock-induced mice model, not affecting the seizure threshold (271).

Aquaporin 4 and Its Role in Neuroexcitation

Aquaporin 4 (AQP4) is a protein from the aquaporin family of hydrophobic membrane channels, serving as a water channel in accordance to the osmotic gradient (272–275).

AQP4 is expressed by glial cells in the brain and the spinal cord, mainly within points of contact between astroglial end-feet and blood vessels and astrocyte membranes ensheathing the glutamatergic synapses (276–278). In mice models, AQP4 deficiency has been connected to prolonged seizures along with deficit extracellular K^+ clearance. This has been explained through the role of water and ion homeostasis in blocking hyperexcitability. Accordingly, the expression of AQP4 has been reduced in the perivascular membrane within the epileptically-altered sclerotic regions of the hippocampus (272). Moreover, AQP4 immediately decreases its immunoreactivity post-SE in kainic-induced epileptic mice models, which correlates with the prolonged seizures observed (279, 280). However, it is unclear whether this is due to the initial change during SE or that SRS trigger recurrent changes. The changes were observed mainly in stratum lacunosum moleculare, the molecular layer and the dentate gyrus, affecting the fine processes of astrocytes as well as its end-feet (272, 280, 281). Immunoreactivity diffused to a greater extent in neurophils, especially in the areas of dysmorphic neurons. In a compensatory manner, AQP4 mRNA levels are increased (272, 282). The exact mechanism of this reaction is unclear (280, 283). It is probable, in accordance to the mathematical modeling of the AQP4-deficiency model of water and ionic (potassium) transport in brain ECF, that post-neuroexcitation changes in rate and extent of alterations in extracellular space volume affect changed K^+ dynamics and what is more, also on astrocyte water permeability. It may also have an influence on long-range K^+ buffering and gap junction coupling (283). The other theory suggests that the cause and the result are the opposite: diffuse immunoreactivity in the piriform cortex and the hippocampus with an expression mostly observed at end-feet astrocytes, after SE results in areas lacking AQP4 in piriform cortex. The role of mislocalization of APQ4 with the reduction of channel in adluminal end-foot membranes rather than in the abluminal ones, that stable level is underlined in some papers, with the results of testing suggesting no changes in expression, but rather in the localization of AQP4 in subjects with epileptic seizures (284, 285). Both lowered expression and incorrect localization on end-foot membranes can lead an alteration in homeostasis. Additionally, AQP4 is described as a factor influencing synaptic plasticity by neurotrophin mediation, especially neurotrophin BDNF, leading to long-term potentiation, depression and location-specific object memory in mice models (286–290).

Glial fibrillary acidic protein (GFAP) is another astrocyte marker, characterized by its intermediate filaments. In kainate-induced SE, the levels of GFAP were visibly elevated in all areas of the hippocampus excluding the stratum sadiatum and stratum lacunosum moleculare. Immediately after SE, no changes in GFAP protein expression were observed but a trend toward increased protein was observed later post-SE, while GFAP mRNA anteceded the increase in GFAP levels (291, 292). It led to further sclerotization of the hippocampus, a phenomenon characteristic for further development of TLE. This is another indication of protein markers of astrocytes playing a role as a marker for the development epilepsy after an epileptiform event (272).

The theory of an inflammatory cause of epilepsy has not been reflected in the possible role of AQP4 in epileptogenesis. In mice models, AQP4 deficiency has an alleviating effect on experimental autoimmune encephalomyelitis as well as on inflammation after intracerebral lypopolysacharydes (LPS) administration (293–295). AQP4 stimulates AQP4-dependent cell to swell and promotes cytokine release. It also activates astrocyte Ca2+ signaling via TRPV4 as a reaction to an osmotic stimuli (284).

The therapeutic possibilities are currently limited. In several small studies, substances such as tetraethylammonium (TEA+), azetazolamide, carbonic anhydrase inhibitors, bumetanide, and its analog AqB013 and others may have the potential to inhibit AQP4, but the results remain inconclusive (296–302). Antiepileptic drugs, such as zonisamide, lamotrigine, phenytoin and topiramate, were observed as AQP4 inhibitors. The safety level of various substances with inhibiting capacities, including NSC168597, NSC164914, and NSC670229, is uncertain (303). A promising molecule is TGN-020, a structurally similar substance to carbonic anhydrase and antiepileptic drugs. Up to this point, its peritoneal injection was shown to reduce ischemic cerebral edema and infarct volume in a rat model of ischemic stroke, without any studies on its potential role in epilepsy treatment (303). Similar results have shown the effects of IMD-0354, an inhibitor of both kinase IKKβ and AQP4, in lowering the intracranial pressure in mice models after acute water intoxication and as a form of pro-drug (a phenol phosphate) to reduce brain edema, improving the neurological state of mice after an ischemic stroke (304). The studies conducted on different models are also prone to an assessment distortion due to potential factors affecting or mimicking AQP4's role and the need for blood-brain barrier penetration of potential inhibitors (284).

Matrix Metalloproteinase-9 and Epilepsy

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases which play a role in regulating the cell-matrix composition. They are produced by neurons and, to a lesser extent, by glial cells. They play a crucial role in prenatal (in embryogenesis and morphogenesis) and postnatal development (in remodeling of tissues). They could potentially play an important role in the pathomechanism of neurodegenerative disorders (such as Alzheimer's disease), ischemia, neurotrauma, neoplasms (305–308), inflammation (309) and epilepsy (310–314). In pathological conditions, the stimulation of MMPs is upregulated by cytokines from immune cells and glia (315–317).

MMP-9, a gelatinase, is activated extracellularly from inactive zymogen. Matrix metalloproteinases (TIMPs), especially TIMP-1, have a controlling role over MMP-9. MMP-9 can influence cerebral epithelium via the proteolysis of type IV collagen. To a lesser degree, it degrades other types of collagen (V and XI), laminin and aggrecan core protein. It is also involved in learning (318, 319) and in neuronal plasticity. It serves an important role in controlling the extracellular matrix protein composition (320, 321) through the proteolysis of molecules responsible for signaling and adhesion, growth factors (321–324) and receptors for neurotransmitters (321, 325). The changes in synapses and its structures facilitates synaptic transmission and, consequentially, the excitability of neurons (312, 318). It has also been reported to contribute to neuroinflammation and to neuronal apoptosis in epilepsy in animal models.

Synaptic transmission is also influenced via MMP-9 by NMDA and AMPA glutamate receptors, reducing its efficacy after multiple seizures, as observed in 4-aminopyridine (4-AP) induced epilepsy model (325–328).

There are various possible pathways of MMP-9 activation. During an epileptic activity, the activation of MMP-9 depends on Ca2+ entry (329). However, the prolonged synthesis and accumulation of the precursor form of MMP-9, resulting in increased functional MMP-9, is unlikely to depend on the immediate increases in neuronal Ca2+ levels. During kainine-induced seizures, Ser-proteases such as tPA/plasmin and thrombin stimulate the release of MMP-9 (330, 331). The active form of MMP-9 can be quickly transformed from the constitutive pool of zymogen (312). Further synthesis can be observed due to neuronal intermediate early genes activity (332) or to transcription activation in neurons, stimulated by pro-inflammatory cytokines such as IL-1b and TNF-a, released from glia (333–336). These cytokines may use MAPK/Erk pathway to activate transcription (321, 336). The inhibition of MMP-9 in microglial cells after LPS stimulation decreases the level of pro-inflammatory cytokines such as IL-1b and IL-6 and inhibits the transcription of iNOS (inducible nitric oxide synthase) (337), modifying the pro-inflammatory activity of MMP-9.

Several studies suggest that the CNS attempts to reduce alterations in neuronal excitability through neuroplasticity (338–340). However, no epilepsy model presents signs of neuroplasticity, such as aberrant synaptogenesis or axonal sprouting (341) or structural effects on dendritic spine density (342, 343), leaving this hypothesis unclear.

In pathological conditions in which MMPs are more stimulated to activation, MMP-9 activity leads to the disruption of BBB. The BBB leakage results in further immunological reaction and immune cells' recruitment and migration. This can contribute to a worsening of the state of the patient, causing brain edema, hemorrhage or further spreading of infarct (344–348). For instance, in rat models, the leakage led to epileptiform activity with a positive correlation between MMP-9 levels and seizure frequency (349, 350).

In rat models after kainate seizures, MMP-9 activity and MMP-9 mRNA levels were significantly increased exclusively in the hippocampal dentate gyrus, correlating with the changes in the hippocampal dendritic architecture. This could be connected to synaptic abnormalities, such as the quantity of synapses and dendrites and to dysregulated synaptic transmission (311, 312). MMP-9 knockout mice were less sensitive to pentylenetetrazol (PTZ) kindling-induced epilepsy, with a simultaneous decrease of mossy fiber synaptogenesis (313), while MMP-9 overexpression results in increased dendritic spine proliferation and the misposition of synaptogenesis in the hippocampus. There is a positive correlation between MMP-9 level and seizure duration in acute encephalopathic patients. In patients affected by viral infections, higher MMP-9/TIMP-1 ratio which was measured after prolonged febrile seizures is indicated to be connected with dysfunctional BBB (351) and an increase susceptibility to febrile seizures or encephalopathy (253). On the other hand, higher MMP-9 levels in the CSF is observed in these patients with bacterial infections of the CNS who present with neurological complications, such as secondary epilepsy (352). In patients with systemic lupus erythematosus, patients with higher levels of MMP-9 were more prone to seizure activity and other neuropsychiatric symptoms in the course of their disease (353). Whether or not MMP-9 plays a significant role in seizure-induced neuroapoptosis is a question which necessitates further research.

Excitotoxicity leading to neuronal and hippocampal apoptosis in conjunction with high MMP-9 activity was observed in kainate-induced epilepsy models (310, 354). In pilocarpine models of epilepsy, the same phenomenon was also observed. The apoptosis was connected to signals of neuronal cell survival, mediated by integrin, and interrupted by MMP-9, after pilocarpine-induced status epilepticus (355).

The homeostatic balance in MMP-9 levels can also play a protective role. Its protective homeostatic plasticity involves extracellular substrates, including integrins (321, 324, 356), cadherins (357) and b-dystroglycan (322, 323, 358), which helps to control dendritic spinal shape and induce its reversible loss by b-dystroglycan or ICAM-5, consequentially affecting the entire synapse (313, 358, 359). This effect depends on MMP-9 mRNA activity-dependent dendritic transport, enhanced in the kainate epilepsy model (311). It is vital in obtaining reduced neuronal excitability and thus, the optimal conditions for recovery (360). In mice models, lower levels of MMP-9 led to reduced seizure-evoked pruning of dendritic spines, leading to decreased neuronal loss (313).

In models with 4-aminopyridine (4-AP) induced seizures and in Wistar Glaxo Rijswijk (WAG/Rjj) rats, no cell damage

was observed. 4-AP models presented with generalized cortical seizures and WAG/Rjj rats presented with absence epilepsies, which typically generate spike and wave discharges after 4 months of age. MMP-9 and zymogen levels were increased in regions affected by seizure activity in these models (regions of the seizures' generalization within the cortex in the 4-AP model and the thalamus and cortical regions in WAG/Rjj during higher seizure activity). In WAG/Rjj, additionally, a diurnal peak was observed, which correlates with the sleep-wakefulness transition and the seizure activity. This indicates that cortical seizures promote the precursor and the active form of MMP-9. MMP-9's elevation could be an effect of elevated neuronal activity rather than that of neuronal death, as no apoptosis was observed in the WAG/Rij model of absence seizures and in the 4-AP model in the zones affected by seizure propagation (361). Additionally, higher levels of MMP-9 in WAG/Rjj rats treated with the anti-absence seizure drug ethosuximide (ETX) were reported. This is due to the suppression of the sleep-wake disturbances until ETX started to interfere with sleep pattern, which resulted in the downregulation of MMP-9 (362, 363).

The therapeutic potential of MMP-9 inhibitors remains inconclusive. In animal models, the MMP-9 inhibitor, S24994, has a protective role on the hippocampus in kainate-induced epilepsy. In kainate or picrotoxin models, it reduces dendritic spines after seizure activity (313). Monoclonal antibodies can also be beneficial and genetic engineering could provide further insights.

As in the case of AQP4, there are FDA-approved drugs with an inhibiting potential. Tetracyclines, statins, resveratrol, estrogen, and indomethacin are medications which have been observed to reduce MMP-9 levels. Tetracyclines (minocycline, doxycycline), via the prevention of BBB leakage, reduced CNS inflammation and size of infarction (364–368). Statins (atorvastatin, simvastatin, pravastatin) improved clinical outcome in acute coronary syndrome patients (369, 370). In animal models, atorvastatin and minocycline reduced seizure activity and inhibited neuroinflammation and neuronal apoptosis (371–374).

In **Table 3** we present the summery and characteristics of above-mentioned proteins.

CONCLUSIONS

In this review, we summarize the current findings on the potential biomarkers of epilepsy. For the first time, we suggest that both processes of hypoxia and oxidative stress may lead to a neuroinflammatory state, ultimately resulting in epileptogenesis. Inflammatory factors may play an essential role in epilepsy. MiRNAs, regulated by epigenetic modifications, can be detected from biofluids. The diverse pathways and numerous molecules from recent investigations provide opportunities for further research regarding the diagnosis and treatment of epilepsy. The level of cytokines can be used to predict the disease severity and be useful in monitoring treatment efficacy. Medications targeting cytokines inhibitors can improve disease prognosis.

TABLE 3 | Proteins and their role in epileptogenesis.

Type of protein	Aminoacids involved in epileptogenesis	Activation or regulation	Effects	Possible therapeutic substances	References
N-methyl-D-aspartate receptor (NMDAR)	Aspartate, glutamate, glycin, serin	↑	Producing low-frequency oscillation, promoting cell responsiveness to lower frequencies	Magnesium, dextromethorphan, dextrorphan, dizocilpine, memantine, ifenprodil, ketamine, amantadine, TCN-201, radiprodil	(152, 153, 158, 159, 171, 182, 185, 186)
Glutamine synthetase (GS)	Glutamine, glutamate	↓	Increased extracellular glutamate concentration, leading to hyperexcitability, excitotoxicity, neurodegeneration	-	(199–205) (215–218) (213–219)
P2X7 receptor (P2X7R)	Glutamate (via caspase-1-dependent interleukin-1b)	↑	Increased excitability, reduced glutamate-induced neuronal cell death	A-438079, JNJ-47965567, Brilliant Blue G, JNJ-42253432	(232, 243); (232, 258–266); (267–271)
Aquaporine 4 (AQP4)	–	↓	Hyperexcitability, prolonged seizures	Tetraethylammonium (TEA+), azetazolamide, carbonic anhydrase inhibitors, bumetanide, AqB013, antiepileptic drugs (zonisamide, lamotrigine, phenytoin, topiramate), TGN-020, IMD-0354, NSC168597, NSC164914, NSC670229	(276–290) (296–304)
Matrix metalloproteinase 9 (MMP-9)	Aspartate, glutamate, glycin, serin	↑	Disruption of blood-brain barrier, higher seizure frequency, increased susceptibility to febrile seizures	S24994, monoclonal antibodies, tetracyclines (minocycline, doxycycline), statins (atorvastatin, simvastatin, pravastatin), resveratrol, estrogen, indomethacin	(305–375)

There is no consensus in which miRNA, protein or amino acid could serve as an ideal marker for epilepsy and further neuronal damage. Its connection to epilepsy is most likely through features connected with specific epileptiform events, rather than generally to epilepsy as a uniform disease. Each type of epilepsy presents with a different seizure phenotype, distinct behavioral changes, and further complications and comorbidities, suggesting the possibility of differences in the underlying etiology on a molecular level.

There are some common limitations among many studies on the molecular etiopathogenesis and development mechanisms in epileptiform events and epilepsy. First of all, only a few studies are performed on human cell lines. Even in these cases, the sample is not obtained from biopsy, but from fresh cadavers or during surgical treatment of neoplasms or epileptic lesions. Due to this collecting method, the sample obtained may have been altered and even damaged on the molecular level, leading to disturbances in studies results. Due to genetic modifications, the rodent model is becoming increasingly accurate in its resemblance to the conditions of human CNS but it could not serve as a relevant biological model. Secondly, the processes of inducing seizures can have a great influence on the behavior of neurons, glia cells, and their proteins. Thirdly, most studies presented results from a small sample size over a limited period of time. This can also lead to biased results and disturbances in their statistical analysis.

It is important to pay attention to the increasing number of molecules with a future therapeutic potential which are under investigation due to their influence on proteins and amino acids. There is also an open field for genetic engineering to enhance the power of established particles to regulate the excitability of brain cells. Nonetheless, we should remember that small rodent groups may not develop potential adverse reaction which may on the other hand be evident in human organisms. Because of this, FDA-approved drugs with modifying potentials can be the first step to novel therapy, based on protein, and amino acids activity in CNS. Extensive data exists regarding the molecular details of epileptogenesis. Although there are no conclusive answers, we can establish a starting point for further research on the therapeutic potential and clinical implications of proteins and amino acids reactions and collaboration in brain electric homeostasis. The role of finding novel markers of brain damage after post-epileptiform events is a possible grasping point for the prevention of complications and for new, targeted methods of treatment in the future.

AUTHOR CONTRIBUTIONS

DK was responsible for the Project administration, supervision, visualization, writing–review and editing. PI and WK were responsible for supervision, writing–review and editing. ZL, NL, SS, and AL were responsible for writing–review and editing.

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Diagnostic Yield of Epilepsy Panel Testing in Patients With Seizure Onset Within the First Year of Life

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Purpose: We aimed to evaluate the diagnostic yield of epilepsy gene panel testing in epilepsy patients whose seizures began within the first year after birth. We included 112 patients with seizure onset before 12 months and no known etiology.

Methods: Deep targeted sequencing with a custom-designed capture probe was performed to ensure the detection of germline or mosaic sequence variants and copy number variations (CNVs).

Results: We identified pathogenic or likely pathogenic variants in 53 patients (47.3%, 53/112), including five with pathogenic CNVs. Two putative pathogenic mosaic variants in *SCN8A* and *KCNQ2* were also detected and validated. Those with neonatal onset (61.5%, 16/26) or early infantile onset (50.0%, 29/58) showed higher diagnostic rates than those with late infantile onset (28.5%, 8/28). The diagnostic rate was similar between patients with a specific syndrome (51.9%, 27/52) and those with no recognizable syndrome (43.3%, 26/60).

Conclusion: Epilepsy gene panel testing identified a genetic cause in nearly half of the infantile onset epilepsy patients. Since the phenotypic spectrum is expanding and characterizing it at seizure onset is difficult, this group should be prioritized for epilepsy gene panel testing.

Keywords: epilepsy, seizure, genetic test, diagnostic yield, target panel sequencing

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INTRODUCTION

With technological advances and declining costs, molecular genetic testing using next-generation sequencing technology is rapidly being incorporated into clinical practice. Although genome-wide testing methods such as whole-exome or whole-genome sequencing are the ultimate goal, selective gene panel tests also have multiple advantages in clinical application (1, 2). Epilepsy gene panel testing is one successful example that has been implemented in clinical practice.

To date, many studies have reported on the clinical utility of epilepsy gene panel testing. Although many custom-designed epilepsy gene panels produce similar lists of genes with pathogenic variants, there is substantial variability in their diagnostic rates, which range from 10

to 50% (3–15). This suggests that the diagnostic yield in these studies depends more on which patients are selected than on which custom-designed panel is used. Previous studies have focused on early-onset epileptic encephalopathy patients, who may be at the severe end of the phenotypic spectrum (4, 12). Recent epilepsy gene panel testing studies have analyzed large numbers of patients with broad epilepsy phenotypes (8, 9, 11, 13, 14). Since most studies report the results of referral-based tests, they include large variability in seizure onset, epilepsy type, familial occurrence, and the presence of development delay or encephalopathy. This variability might lead to lower diagnostic yields, which are generally <20% of the tested patients. One finding common among these studies has been the suggestion that patients with early-onset epilepsy, especially neonatal or early infantile onset, tend to have higher diagnostic rates (8, 9, 11, 14). However, few epilepsy gene panel studies have specifically targeted infantile-onset epilepsy patients and analyzed the diagnostic rate.

Since pathogenic CNVs and somatic mosaicism variants have been reported in a small proportion of epilepsy patients (16, 17), epilepsy gene panel testing capable of identifying these variants would also increase the diagnostic rate. Pathogenic structural variants and low-frequency variants could be readily identified by epilepsy gene panel testing, since targeted testing would ensure deep coverage of a target region.

In the present study, we applied our customized epilepsy gene panel test to a group of epilepsy patients whose seizure onset was before they were 1 year old. We analyzed the diagnostic yield in relation to clinical variables. We also tested the extended applicability of epilepsy gene panel testing by investigating the structural and low-frequency variants in this patient group.

MATERIALS AND METHODS

Patients

The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (1804-052-936), and the study was conducted in accordance with relevant guidelines and regulations. One hundred and twelve epilepsy patients who met the following criteria were included retrospectively: seizure onset before 12 months of age, no structural abnormality on brain magnetic resonance imaging, and no suspected single genetic cause from history and metabolic studies. We only included patients who had initially presented with febrile seizure if they experienced subsequent, afebrile seizures. The following clinical variables were collected: seizure onset, seizure type(s), presence of developmental delay or encephalopathy before and after seizure onset, family history of epilepsy within first-degree relatives, and response to antiepileptic drugs. We tried to classify electroclinical syndromes according to the International League Against Epilepsy proposal (18). Among these patients were 22 with Dravet syndrome, 11 with benign familial infantile epilepsy, 9 with benign infantile epilepsy, 4 with benign familial neonatal epilepsy, 4 with Ohtahara syndrome, and 1 with benign myoclonic epilepsy of infancy. All Dravet syndrome patients had been previously screened with *SCN1A* sequencing and were reported to have no pathogenic variants. We excluded the West syndrome cohort from this study, despite its high prevalence among early-onset epileptic encephalopathy patients, for two reasons. First, whole-exome sequencing studies of large groups of West syndrome patients are already available (19, 20). Second, the West syndrome cohort in our institution was included in another whole-genome-based trio analysis. The detailed clinical features of all 112 patients are summarized in **Table S1**.

Epilepsy Panel Design and Sequencing

A custom-designed SureSelect Target Enrichment System Kit (Agilent Technologies, CA, USA) was used to assess epilepsy and epilepsy-associated genes. The capture kits were updated twice to include newly identified genes. Thirty-one patients were sequenced with the first kit (79 genes), 61 with the second kit (119 genes), and 20 (127 genes) with the third kit (**Table S2**). Library preparation was completed as recommended in the manufacturer's instructions (Agilent Technologies). The library was paired-end sequenced on an Illumina HiSeq 2500 sequencing system.

Sequence Analysis

We aligned paired-end sequencing reads with a read length of 101 base pairs to Genome Reference Consortium human genome build 37 (patch release 13) using BWA-0.7.15. Picard software (v.2.1.1), SAMtools (v.1.3.1), and the Genome Analysis Toolkit (v.3.8) best-practice pipelines were used for data analyses. Variant calling was performed using HaplotypeCaller. We used ANNOVAR for variant annotation. Using the Exome Aggregation Consortium database, for further analysis we selected only variants with zero frequency in the database for autosomal dominant genes and with a frequency lower than 0.01% for autosomal recessive genes. For low-frequency variant detection, we also used MuTect2 (21) to search for variants with a variant allele frequency from 0.05 to 0.25. We selected only the low-frequency variants with a variant allele count above 30.

For CNV analysis, we calculated reads per kilobase per million mapped reads (RPKM) using CoNIFER (22). Only those reads with mapping quality above 15 were included in the RPKM values. Due to coverage fluctuations among samples in targeted sequencing, we calculated Z-scores twice: within single samples and among multiple samples sequenced in the same panel. With the normalized Z-score values, we calculated the interquartile ranges (IQRs) for each sample. The standards used for identifying CNVs were:

$$\text{deletion}: \text{Z-score} < \text{q25} - 2.5 \times \text{IQR}$$
$$\text{duplication}: \text{Z-score} > \text{q75} + 2.5 \times \text{IQR}$$

where q25 and q75 were the 25th and 75th percentiles of the Z-score values of each sample in each exon. Prominent outlier samples were removed from the analyses for more accurate CNV detection. If more than half of the exons in a gene were amplified or deleted, they were considered for further analysis and testing.

Variant Interpretation and Validation

All selected sequence variants were further confirmed with Sanger sequencing, which was also conducted for available

family members. We classified sequence variants according to the international guidelines of the American College of Medical Genetics (ACMG) (23). Variants classified as "pathogenic" or "likely pathogenic" were considered causative for the phenotype. Low-frequency variants were further validated with amplicon sequencing, in which six nucleotide barcode sequences unique to each sample, along with adaptor sequences (AGAT), were added to forward PCR primer to identify individual samples. Then the same amounts of PCR products for each sample were pooled using an Illumina dual-indexed PCR free library preparation kit and sequenced on an Illumina HiSeq 2500 sequencing system. During sequence analysis, each paired-end read was assigned to an individual by barcode sequences and read numbers, with or without the variant for each sample being counted. To validate CNVs, we conducted chromosomal microarray analysis testing using Agilent Human Genome oligonucleotide comparative genomic hybridization microarrays 4 × 180 K or 8 × 60 K (Agilent Technologies). All experimental procedures and data analyses were performed according to the manufacturer's guidelines (Agilent Technologies).

RESULTS

The overall coverage of targeted genes was reasonably consistent and deep. The mean coverage depth of 112 patient samples was 1,337×, with 98.6% of the target region above 100×. A more detailed sequencing summary of all samples in the three different panels is in **Table S3**. After adjusting the filtering criteria described in the Materials and Methods, ∼0–3 single-nucleotide variants were found in each patient. The pathogenic or likely pathogenic variants were found in 53 of 112 patients (47.3%), including five pathogenic CNVs.

Spectrum of Pathogenic and Likely Pathogenic Variants

Sequence Variants

Eighteen genes were identified as harboring pathogenic or likely pathogenic sequence variants (**Figure 1**). The most frequently found genes were *PRRT2* in 10 patients, *SCN1A* in 6 patients, *KCNQ2* in 5 patients, and *SCN2A* in 4 patients. Family studies were done in 33 patients. All 6 patients with *SCN1A* pathogenic variants had been previously reported as *SCN1A* mutation negative. This type of missed *SCN1A* mutation has been reported in many studies, indicating the technical limitations of the Sanger sequencing method (24). Seventeen patients were confirmed as harboring *de novo* mutations. Nine variants were inherited from one of the affected parents, and six variants were inherited from one of the asymptomatic parents (**Figure S1**). We classified these seven variants as pathogenic or likely pathogenic despite the inconsistent familial segregation. The mothers in Case 5 and Case 29 had a mosaic form of the pathogenic variants. Although there is no specific guideline on the interpretation of mosaic variants, asymptomatic parents harboring a mosaic variant of the proband have frequently been interpreted as carriers for the variant (17, 25). The other four variants were either null variants (Case 2, Case 68, Case 78) or a previously reported pathogenic variant (Case 57), which could suggest incomplete penetrance. **Table 1** summarizes the pathogenic or likely pathogenic sequence variants.

Structural Variants

We identified five pathogenic CNVs encompassing genes that were included in the present target panel (**Table 2**). All of these variants were separately validated with chromosomal microarray testing.

Low-Frequency or Mosaic Variants

Two patients (2/112, 1.8%) were suspected of carrying low-frequency variants in *KCNQ2* and *SCN8A*, respectively (**Table 3**). The *KCNQ2* and *SCN8A* variants were separately validated with amplicon sequencing. These variants were not found in the parents. Although the *de novo* mosaic status of *KCNQ2* and *SCN8A* variants was demonstrated, we did not classify these variants as pathogenic. The parents of two patients were suspected of having mosaic status for the pathogenic and likely pathogenic variants of *ARHGEF9* and *GABRA1* in the Sanger sequencing results. The *GABRA1* p.Lys339Glu variant was further validated with amplicon sequencing, and it confirmed the mother's mosaic status (**Table 3**).

Yield by Subgroups

Age of Onset

We classified patients into three groups according to age of seizure onset—neonatal, early infantile (1–6 months), and late

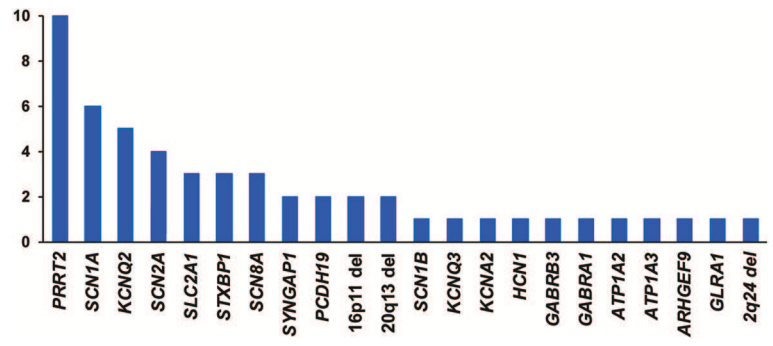

FIGURE 1 | Frequency (y-axis) of genes or copy number variations with pathogenic or likely pathogenic variants.

TABLE 1 | Profile of 49 pathogenic or likely pathogenic sequence variants.

Case	Gene	Variant (RefSeq:DNA base: amino acid)	Inheritance	ACMG criteria	ACMG classification	ClinVar	HGMD
Case 29	*ARHGEF9*	NM_001173479:c.1355G>A:p.Trp452*	From mosaic carrier mother	PVS1, PM2, PP1	Pathogenic		
Case 75	*ATP1A2*	NM_000702:c.1096G>T:p.Gly366Cys	*De novo*	PS2, PM1, PM2, PP2, PP3	Pathogenic		
Case 8	*ATP1A3*	NM_152296:c.1088T>C:p. Ile363Thr	Not evaluated	PM1, PM2, PM5, PP2, PP3	Likely Pathogenic		
Case 5	*GABRA1*	NM_001127648:c.1015A>G:p.Lys339Glu	From asymptomatic mosaic mother	PM2, PM6^{+}, PP2, PP3	Likely Pathogenic		
Case 66	*GABRB3*	NM_001191320:c.577C>T:p.Leu193Phe	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic		
Case 2	*GLRA1*	NM_001292000:c.494_495insAC:p.Met165fs	From asymptomatic father	PVS1, PM2	Likely Pathogenic		
Case 82	*HCN1*	NM_021072:c.1171G>A:p.Gly391Ser	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic		
Case 73	*KCNA2*	NM_004974:c.971G>A:p.Ser324Asn	*De Novo*	PS2, PM2, PP2, PP3	Likely Pathogenic		
Case 85	*KCNQ2*	NM_004518:c.727C>G:p.Leu243Val	*De novo*	PS2, PM2, PP2, PP3	Pathogenic		
Case 14	*KCNQ2*	NM_004518:c.766G>T:p.Gly256Trp	*De novo*	PS2, PM1, PM2, PP2, PP3, PP4	Pathogenic		
Case 11	*KCNQ2*	NM_004518:c.997C>T:p.Arg333Trp	Not evaluated	PS2, PS4, PM2, PP2, PP3	Pathogenic	Pathogenic	DM
Case 57	*KCNQ2*	NM_004518:c.998G>A:p.Arg333Gln	From asymptomatic father	PS3, PS4, PM2, PP1, PP2	Pathogenic	Pathogenic	DM
Case 68	*KCNQ2*	NM_004518:c.1130dupC:p.Pro377fs	From asymptomatic father	PVS1, PM2, PP1	Pathogenic		
Case 39	*KCNQ3*	NM_001204824:c.590T>C:p.Ile197Thr	From symptomatic father	PM1, PM2, PP1, PP2, PP3	Likely Pathogenic		DM
Case 56	*PCDH19*	NM_001105243:c.595G>T:p.Glu199*	Not evaluated	PVS1, PM2	Likely Pathogenic	Pathogenic	
Case 96	*PCDH19*	NM_001105243:c.1105G>C:p.Ala369Pro	From asymptomatic father	PM2, PP1, PP2, PP3, PP4	Likely Pathogenic		
Case 51	*PRRT2*	NM_001256442:c.649delC:p.Ala217fs	From asymptomatic father	PVS1, PS4, PM1	Pathogenic	Pathogenic	
Case 43	*PRRT2*	NM_001256442:c.649dupC:p.Ala217fs	From symptomatic father	PVS1, PS4, PM1, PP1	Pathogenic	Pathogenic	
Case 58	*PRRT2*	NM_001256442:c.649dupC:p.Ala217fs	Not evaluated	PVS1, PS4, PM1	Pathogenic	Pathogenic	
Case 71	*PRRT2*	NM_0012564c.649dupC:p.Ala217fs	Not evaluated	PVS1, PS4, PM1	Pathogenic	Pathogenic	
Case 77	*PRRT2*	NM_001256442:c.649dupC:p.Ala217fs	Not evaluated	PVS1, PS4, PM1	Pathogenic	Pathogenic	
Case 78	*PRRT2*	NM_001256442:c.649dupC:p.Ala217fs	From asymptomatic father	PVS1, PS4, PM1	Pathogenic	Pathogenic	
Case 81	*PRRT2*	NM_001256442:c.649dupC:p.Ala217fs	From symptomatic mother	PVS1, PS4, PP1	Pathogenic	Pathogenic	
Case 83	*PRRT2*	NM_001256442:c.649dupC:p.Ala217fs	From symptomatic father	PVS1, PS4, PP1	Pathogenic	Pathogenic	
Case 105	*PRRT2*	NM_001256442:c.649dupC:p.Ala217fs	From symptomatic mother	PVS1, PS4, PP1	Pathogenic	Pathogenic	
Case 34	*PRRT2*	NM_001256442:c.796_797insGG:p.Arg266fs	From symptomatic father	PVS1, PS4, PP1	Pathogenic		

(Continued)

TABLE 1 | Continued

Case	Gene	Variant (RefSeq:DNA base: amino acid)	Inheritance	ACMG criteria	ACMG classification	ClinVar	HGMD
Case 26	*SCN1A*	NM_001165963:c.596_602del:p.Thr199fs	Not evaluated	PVS1, PM2, PP4	Pathogenic		
Case 101	*SCN1A*	NM_001165963:c.2244G>A:p.Trp748*	Not evaluated	PVS1, PS4, PM2, PP4	Pathogenic		
Case 48	*SCN1A*	NM_001202435:c.2947-1G>A	Not evaluated	PVS1, PM2, PP4	Pathogenic	Likely Pathogenic	
Case 40	*SCN1A*	NM_001165963:c.4201G>C:p.Glu1401Gln	Not evaluated	PM1, PM2, PP2, PP3, PP4	Likely Pathogenic		
Case 13	*SCN1A*	NM_001165963:c.4219C>T:p.Arg1407*	Not evaluated	PVS1, PM2, PP4	Pathogenic	Pathogenic	DM
Case 54	*SCN1A*	NM_001165963:c.5288T>A:p.Ile1763Asn	From symptomatic mother	PS4,PM2, PP1, PP2, PP3, PP4	Pathogenic		DM
Case 100	*SCN1B*	NM_001037:c.373C>T:p.Arg125Cys	Not evaluated	PS3, PM2, PP1, PP2, PP3	Pathogenic	Pathogenic	DM
Case 25	*SCN2A*	NM_001040143:c.466A>G:p.Lys156Glu	*De novo*	PS2, PM2, PP2, PP3, PP4	Likely Pathogenic		
Case 45	*SCN2A*	NM_001040143:c.605C>T:p.Ala202Val	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic	Uncertain Significance	
Case 33	*SCN2A*	NM_001040143:c.1879C>T:p.Gln627*	Not evaluated	PVS1, PM2	Likely Pathogenic		
Case 31	*SCN2A*	NM_001040143:c.2932T>C:p.Phe978Leu	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic		
Case 104	*SCN8A*	NM_001177984:c.3820G>A:p.Val1274Met	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic		
Case 111	*SCN8A*	NM_014191:c.4423G>A.:p.Gly1475Arg	Not evaluated	PM2, PP2, PP3, PP4, PP5	Likely Pathogenic	Pathogenic/Likely Pathogenic	
Case 79	*SCN8A*	NM_001177984:c.5491C>T:p.Arg1831Trp	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic	Pathogenic	DM
Case 47	*SLC2A1*	NM_006516:c.223C>A:p.Gly75Arg	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic		
Case 12	*SLC2A1*	NM_006516:c.940G>C:p.Gly314Arg	Inherited from symptomatic mother	PM2, PM5, PP1, PP2, PP3	Likely Pathogenic		
Case 93	*SLC2A1*	NM_006516:c.1255G>C:p.Gly419Arg	*De novo*	PS2, PM2, PP2, PP3	Likely Pathogenic		
Case 17	*STXBP1*	NM_001032221:c.703C>T:p.Arg235*	*De novo*	PVS1, PS2, PM2	Pathogenic	Pathogenic	DM
Case 109	*STXBP1*	NM_001032221:c.1099C>T:p.Arg367*	Not evaluated	PVS1, PM2	Likely Pathogenic	Pathogenic	DM
Case 64	*STXBP1*	NM_001032221:c.1212A>C:p.Lys404Asn	*De novo*	PS2, PM2, PP2, PP3	Pathogenic		
Case 1	*SYNGAP1*	NM_006772:c.2116-1G>A	*De novo*	PVS1, PS2, PM2	Pathogenic		
Case 36	*SYNGAP1*	NM_006772:c.3718C>T:p.Arg1240*	*De novo*	PVS1, PS2, PM2	Pathogenic		

*ACMG, American College of Medical Genetics; DM, Disease causing Mutation; HGMD, Human Gene Mutation Database; *Indicates stopgain.*

TABLE 2 | Profile of five pathogenic microdeletions.

Case	Chromosomal position (hg19)	Size (Mb)	Involved epilepsy genes	Onset	Electroclinical syndrome
Case 92	Chr2:165755330-168986256	3.23	*SCN2A, SCN1A, SCN9A*	2 months	Dravet syndrome
Case 16	Chr16:29652999-30198600	0.54	*PRRT2*	4 months	Unclassified
Case 32	Chr16:29673954-30119759	0.44	*PRRT2*	4 days	Benign infantile epilepsy
Case 18	Chr20:61472348-62281707	0.80	*CHRNA4, KCNQ2*	2 months	Unclassified
Case 80	Chr20:61845191-62065069	0.21	*CHRNA4, KCNQ2*	1 day	Benign infantile epilepsy

TABLE 3 | Validation results from amplicon sequencing for the mosaic variants found in patients and parents.

Case	Gene	Variant	Epilepsy panel sequencing			Amplicon sequencing		
			References	Variant	% of variant	References	Variant	% of variant
Case 61	*KCNQ2*	c.643G>A:p.Gly215Arg	1920	170	8.7%	54190	9933	15.45
Case 61 (Mo)						59508	137	0.23
Case 61 (Fa)						55091	128	0.23
Case 42	*SCN8A*	c.2105G>C:p.Ser702Thr	2427	167	6.4%	19035	4535	19.2
Case 42 (Mo)						15401	129	0.83
Case 42 (Fa)						17973	110	0.61%
Case 5	*GABRA1*	c.1015A>G:p.Lys339Glu	1238	1242	50.1%	59872	53604	47.2%
Case 5 (Mo)						100118	18074	15.3%
Case 5 (Fa)						118255	1269	1.06%

Fa, father; Mo, mother.

infantile (6–12 months)—and analyzed the diagnostic yield for each group. The diagnostic yield was higher in the neonatal (61.5%, 16/26) and early infantile (50.0%, 29/58) groups than in the late infantile group (28.5%, 8/28) (**Figure 2A**). The variants most frequently found in the neonatal group were *KCNQ2* (five patients) and *SCN2A* (three patients), while in the early infantile group, *PRRT2* (nine patients), and *SCN1A* (four patients) were the most frequently found (**Figure 2A**).

Electroclinical Syndromes

The diagnostic rates of patient groups with or without specific electroclinical syndromes were comparable: classified (51.9%, 27/52) vs. unclassified (43.3%, 26/60). The patient group with no specified electroclinical syndromes was more frequently associated with developmental delay or intellectual disability and pharmaco-resistance (**Table S4**). The diagnostic rate for specific electroclinical syndromes varied and is presented in **Figure 2B**. In the Dravet syndrome cohort, *SCN1A* sequence variants were found in six patients, although Sanger sequencing performed before panel testing was negative in these patients. The other variants were found in *ARHGEF9* (p.Trp452*), *GABRA1* (p.Lys339Glu), *HCN1* (p.Gly391Ser), *PCDH19* (p.Glu199*), and 2q24.3 microdeletion. The single *PRRT2* variant (c.649dupC) was found in five patients with benign familial infantile epilepsy. Three of the four benign familial neonatal epilepsy patients revealed pathogenic or likely pathogenic variants in *KCNQ2* (two patients) and *KCNQ3* (one patient). Three of the four Ohtahara syndrome patients showed pathogenic or likely pathogenic variants in *KCNQ2* (p.Gly256Trp), *SCN2A* (p.Lys156Glu), and *STXBP1* (p.Arg235*). The remaining Ohtahara syndrome patient also harbored a novel *SCN2A* variant (p.Leu769Thr), classified as a variant of unknown significance due to the absence of a family study.

Genotype to Phenotype Correlation

Besides the six patients with *SCN1A* variants who could all be classified as having Dravet syndrome, large phenotypic heterogeneity was noted among patients with *PRRT2*, *KCNQ2*, and *SCN2A* variants. Both severe epileptic encephalopathy and self-limited epilepsies were associated with *KCNQ2* and *SCN2A* variants. Two patients with *PRRT2* variants also showed intellectual disability and behavioral problems that could not be classified as self-limited or benign. The phenotypic spectra of the patients with *PRRT2*, *KCNQ2*, or *SCN2A* variants are presented in **Table 4**. In addition to *PRRT2*, which was implicated in both epilepsy and other paroxysmal disorders, we found four variants in genes that cause paroxysmal disorders other than epilepsy: *ATP1A2* (familial hemiplegic migraine, p.Gly366Cys), *ATP1A3* (alternating hemiplegia, p.Ile363Thr), *GLRA1* (hyperekplexia, p.Met165fs), and *ARHGEF9* (hyperekplexia, p.Trp452*). These four patients showed varying degrees of developmental delay or intellectual disability and pharmaco-resistance. However, no paroxysmal disorder other than epilepsy was reported in these patients.

DISCUSSION

In the present study, a genetic etiology for nearly half of the patients (47.3%) with infantile-onset epilepsy was identified. The higher diagnostic yield in this age group was recently

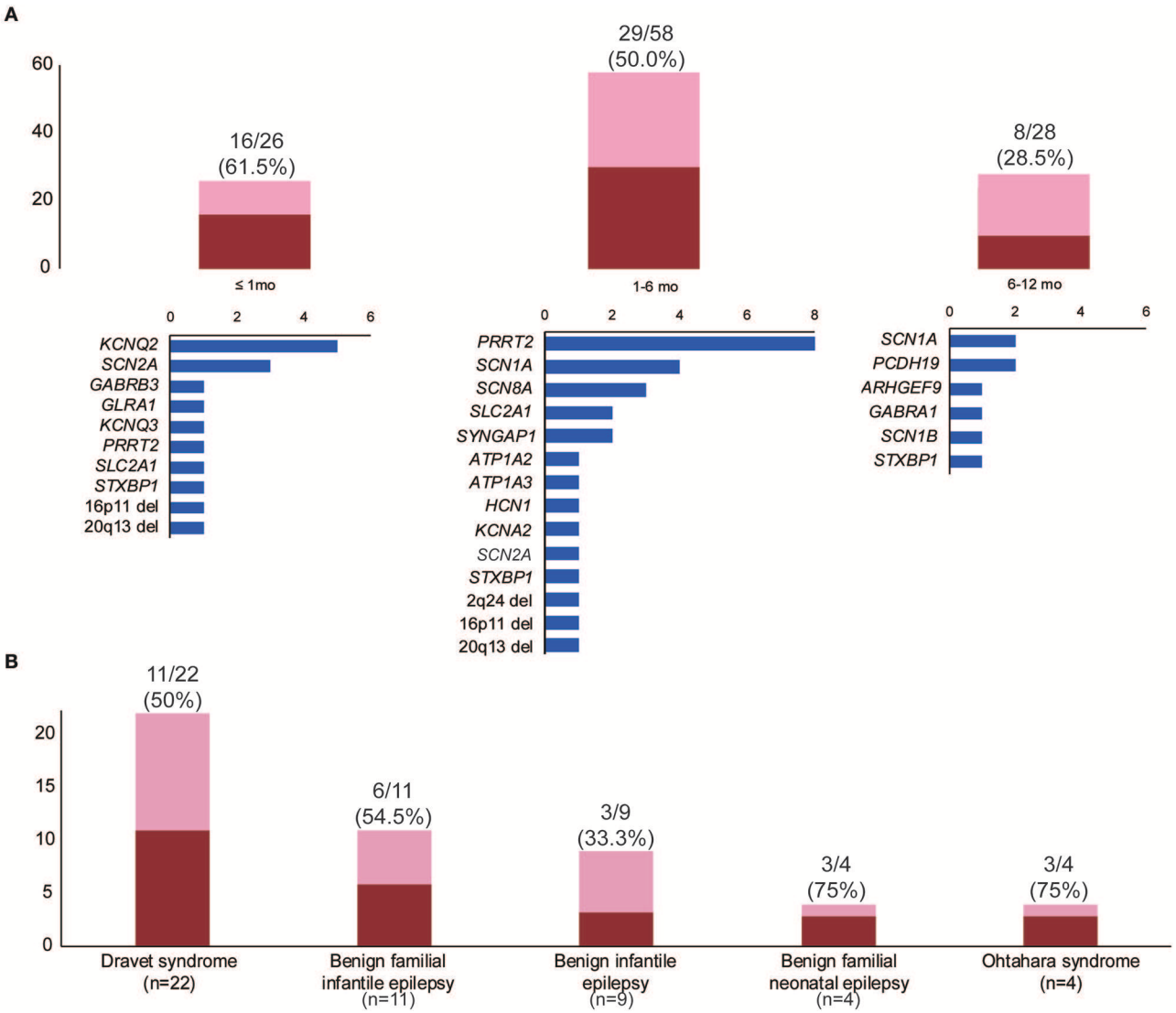

FIGURE 2 | Diagnostic yields by subgroups. **(A)** Diagnostic yields and gene frequencies according to the time of seizure onset: neonatal (≤1 mo), early infantile (1–6 mo), and late infantile (6–12 mo). **(B)** Diagnostic yields according to electroclinical syndrome. The blue bars in **(A)** indicate the number of patients in each group, with the pathogenic or likely pathogenic variants in the genes on the left. The red bar indicates the number of patients with pathogenic or likely pathogenic variants within each group. The pink bar indicates the number of patients without putative variants within each group.

demonstrated in a prospective population-based study by Symonds et al. (26). They prospectively recruited patients whose seizure onset was before 36 months of age. In this study, earlier seizure onset (<6 months) resulted in higher genetic diagnostic yield (45.9%, 34/74) regardless of seizure type and presence of encephalopathy. Thus, these results clearly show the important role of genetic etiology in epilepsy patients with onset in the first year of life.

Infantile-onset epilepsy has several unique features to support the important role of genetic testing. The incidence in this age group is frequently reported to be higher than in all other age groups (27, 28). Moreover, except for West syndrome—in which a structural and metabolic etiology accounts for two-thirds of patients (29)—most of the electroclinical syndromes in infancy had well-characterized genetic profiles as the sole contributing etiological factor. However, we found that patient groups with no recognizable epilepsy syndrome also showed high diagnostic rates (43.3%, 26/60). Thus, age at seizure onset could be the most important factor in genetic diagnosis using epilepsy gene panel testing. We assert that this age-focused approach has an additional advantage over targeting only specific patient groups (e.g., epileptic encephalopathy or drug-resistant epilepsy), insofar as we cannot confidently determine at seizure onset the presence of drug resistance, developmental delay, or encephalopathy. Even self-limited epilepsy syndrome in infancy can only be reliably classified after clinical follow-up beyond infancy. Given that this patient group would benefit greatly from genetic diagnosis at initial presentation to guide treatment and genetic counseling,

TABLE 4 | Phenotypic spectrum of patients with *KCNQ2, SCN2A*, or *PRRT2* pathogenic variants.

	***PRRT2* (*n* = 10)**	***KCNQ2* (*n* = 5)**	***SCN2A* (*n* = 4)**
Epilepsy syndrome			
	Benign familial infantile (*n* = 5)	Benign familial neonatal (*n* = 2)	Ohtahara syndrome (*n* = 1) Unclassified (*n* = 3)
	Benign infantile (*n* = 3)	Ohtahara syndrome (*n* = 1)	
	Unclassified (*n* = 2)	Unclassified (*n* = 2)	
Drug responsiveness			
Self-limited	8	3	1
Drug responsive	2	1	1
Drug resistant		1	2
Developmental disability			
Normal	8	3	0
Intellectual disability	1	2	3
ADHD*/ASD*	1	0	1

**ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder.*

the age of onset, especially if it is within the first year, should be regarded as the most important indicator for considering genetic testing.

An increasing number of genes are now known to cause both self-limited and severe epilepsies (30). We clearly identified this tendency in the present study, especially for three genes: *SCN2A*, *KCNQ2*, and *PRRT2*. We expect that an age-focused, unbiased approach to drug resistance and developmental status will reveal this tendency more clearly. Another notable finding regarding the phenotypic spectrum in the present study was that genes related to paroxysmal disorders other than epilepsy could also be associated with epilepsy as a separate phenotype. Benign familial infantile epilepsy is a well-known phenotype of *PRRT2* (OMIM 605751) in addition to paroxysmal dyskinesia (OMIM 128200). *ATP1A2* and *ATP1A3* have previously been implicated in familial hemiplegic migraine (OMIM 602481) and alternating hemiplegia (OMIM 614820). Although the association of infantile epilepsy with these genes has not yet been separately determined, the pathogenic variant in each gene was found in two of our participants, whose epilepsy phenotype could be characterized as developmental epileptic encephalopathy. Additional infantile-onset epilepsy patients linked with *ATP1A2* and *ATP1A3* are found in the literature (8, 9, 15, 31). The independent occurrence of epilepsy and other paroxysmal disorders in a single gene was also reported for *CACNA1A*, an epileptic encephalopathy that has been recognized as a separate phenotype in addition to episodic ataxia and familial hemiplegic migraine (32).

Detection of these five pathogenic CNVs improved the diagnostic rate. All of these CNVs have been reported in infantile-onset epilepsy patients. The phenotypic spectrum in our study was diverse, from self-limited epilepsy to epileptic encephalopathy, even in patients with similar sizes of pathogenic CNVs. Notably, CNV size, which was confirmed by chromosomal microarray, was relatively small, so we could not identify any other genes that may have affected the patient's phenotype other than epilepsy. Since there is no consensus on whether patients with self-limited epilepsy or without dysmorphic features should be tested with a chromosomal microarray, epilepsy gene panel testing could play an important role in identifying epilepsy patients with these phenotypes. In addition to pathogenic CNVs, we found and validated the mosaic variants of *SCN8A* and *KCNQ2* in each patient. Without the mosaic status, these variants might have been interpreted as pathogenic according to the ACMG guidelines. The p.Gly215Arg variant in *KCNQ2* was previously reported in a patient with severe neonatal-onset epilepsy (33). However, we could not confidently classify these variants as pathogenic, since the parents' mosaicism for pathogenic variants in their proband was frequently reported to be asymptomatic (17, 25). Thus, even though the variant could be classified as pathogenic, whether it could result in a clinical phenotype with mosaic status requires separate experimental validation or additional evidence in an unrelated patient. Considering the high frequency of mosaic variants in epilepsy and neuro-developmental disorders (17), more data should be obtained to interpret and validate mosaic variants. Epilepsy gene panel sequencing with deep coverage could be uniquely advantageous for this purpose.

Despite the many advantages discussed above, the limitations of the epilepsy gene panel testing approach need to be addressed. We updated our panel design twice during our study to include newly discovered epilepsy genes. This inevitably leaves a patient group that was not tested for the updated genes. A genome-wide approach, such as whole-exome or whole-genome sequencing, would have a clear advantage over gene panel testing in this situation, because reanalysis could identify additional cases with pathogenic variants in the newly discovered epilepsy genes. However, the benefits and limitations should be weighed carefully based on a head-to-head analysis of cost and diagnostic yield within a specific cohort.

In conclusion, we provided a comprehensive analysis of epilepsy gene panel testing in a group of infantile-onset epilepsy patients, which will contribute to refining the indication of epilepsy gene panel testing by providing a specific test candidate group and expected diagnostic yields.

DATA AVAILABILITY

We submitted all of the sequenced paired-end reads to the EBI European Nucleotide Archive database with the accession number PRJEB26566 (direct access: https://www.ebi.ac.uk/ena/data/view/PRJEB26566).

ETHICS STATEMENT

The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 1804-052-936), and the study was conducted in accordance with relevant

guidelines and regulations. Informed consent was obtained from a parent and/or legal guardian.

AUTHOR CONTRIBUTIONS

SJ, BL, J-IK, and JC designed and conceived the study. SK, HK, HH, JC, KK, and BL collected samples, clinical features/data, and ethical statements permitting us to perform the research. SJ and SK analyzed and interpreted the data. SJ and BL reviewed the literature and drafted the manuscript. SK, HK, HH, KK, and JC revised the manuscript for intellectual content.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00988/full#supplementary-material

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Efficacy and Tolerance of Synthetic Cannabidiol for Treatment of Drug Resistant Epilepsy

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Objective: Controlled and open label trials have demonstrated efficacy of cannabidiol for certain epileptic encephalopathies. However, plant derived cannabidiol products have been used almost exclusively. Efficacy of synthetically derived cannabidiol has not been studied before. The objective of this study was to evaluate tolerability and efficacy of synthetic cannabidiol in patients with pharmacoresistant epilepsy.

Methods: In this prospective, open-label study (DRKS00013177), patients with pharmacoresistant epilepsy received synthetic cannabidiol in addition to their previously stable anticonvulsive treatment. Starting dose was 5 mg/kg/day, up-titrated to a maximum of 50 mg/kg/day. Primary efficacy endpoint was monthly frequency of motor seizures at 3 months.

Results: Between April 2017 and May 2019, 35 patients were enrolled in the study. Mean age was 19.7 years (SD 14.6). Median motor seizure frequency decreased from 21.8 (IQR 7.5–52.5) seizures per month at baseline to 8.5 (IQR 3.7–28.3, $p < 0.001$) at 3 months, effect not influenced by AED changes and drop-outs. Adjusted percentage reduction was 40.0% (IQR 18.2–58.5). Adverse events (AE) were reported in 25 patients (71.4%), most frequently somnolence (40%), diarrhea (34.3), and loss of appetite (20%). Two patients (5.7%) discontinued treatment due to AE. Median (range) of treatment duration was 321 days (range 36–824). With ongoing treatment up to date in 21 patients (60%).

Conclusion: Efficacy and tolerance in our study of synthetic CBD treatment in pharmacoresistant epilepsy is similar to open label studies using plant derived CBD. Regarding economic and ecological aspects, synthetic cannabidiol might be a reasonable alternative to plant derived cannabidiol.

Keywords: epilepsy, cannabidiol, open label, pharmacotherapy, adverse events, cannabinoids, antiepileptic drug

INTRODUCTION

Over the last decade, the therapeutic use of cannabidiol (CBD) in intractable epilepsies has increased considerably (1). Its anticonvulsant properties have been shown in several animal models for acute and chronic epilepsy (2). Recent randomized, controlled trials have demonstrated that CBD is superior to placebo in seizure reduction in children with Dravet syndrome and patients with Lennox-Gastaut syndrome (3–5). In addition, open label studies indicate that cannabidiol has anticonvulsive properties in a broader range of epilepsy syndromes and etiologies (6). In most studies, 10% solutions of purified CBD are used, in some with additional small amounts of delta-9-tetrahydrocannabinol (THC) (7). Regardless of compositions, all studied preparations contain plant derived CBD (8). Recently, the first pharmaceutical formulation of highly purified, plant derived CBD has been approved by the US Food and Drug Administration (9). Single molecule cannabinoid drug development is a different approach where pharmaceutical-grade synthetically derived substances are used (10). Easier quality control, unlimited production possibilities and reduced environmental impact are advantages of synthetically derived cannabinoids and support further investigations of its therapeutic use. Synthetic CBD is a (+)-enantiomer of the (–)-natural CBD. Since the chemical structure is otherwise identical, similar efficacy and tolerance are to be expected (11). However, besides one phase II study and one study using transdermal application, to our knowledge no studies using synthetic CBD in pharmacoresistant epilepsies have been published (12, 13).

The objective of this study was to evaluate the long-term safety, tolerability, and efficacy of synthetic cannabidiol in children and adults with pharmacoresistant epilepsy.

MATERIALS AND METHODS

Patients

Patients with pharmacoresistant epilepsy as defined by the International League Against Epilepsy, medicated with at least one anticonvulsive drug (AED) at a stable dose for 4 weeks pre-intervention, stable ketogenic diet/vagal nerve stimulation device settings for at least 4 weeks pre-intervention and willingness of patients/caregivers to comply with seizure diary were eligible for inclusion. Exclusion criteria were current treatment with cannabis-based products, pregnancy or unstable hepatic, or renal disease.

Standard Protocol Approvals, Registrations, and Patients Consent

The trial was approved by the institutional research ethics board (397/17) and registered (DRKS00013177). All patients or parents/legal representatives provided written informed consent and assent according to patients' physical and mental capability before trial onset.

Trial Design

This prospective, open-label, observational study was conducted at the University Epilepsy Center in Freiburg, Germany since November 2017; data cut was August 2019. Patients were prospectively followed by a pediatric neurologist or by a neurologist. Visits were scheduled at baseline and at 3, 6, and 12 months of treatment. Patients received a pharmaceutical formulation of synthetic CBD, manufactured by THC Pharm GmbH/Germany, in a 100 mg per mL MCT-oil-based oral solution, according to national drug-preparation regulations. An internal quality control at final solution level was performed at our center. CBD was administered orally in addition to the baseline antiepileptic drug regimen at a starting dose of 5 mg/kg/days divided into two daily doses. Patients were advised to take CBD with fatty meals. Dosage was up-titrated by 2–5 mg/kg/days up to 18–20 mg/kg/days over 14–21 days. If no effect was observed, dosage could be increased further up to 50 mg/kg/days. Concomitant AEDs were reviewed at each clinic visit. For the first 3 months of cannabidiol treatment, efforts were made to keep concomitant doses of antiepileptic drug constant. However, if addition of cannabidiol led to relevant increase of serum levels of concomitant AED, those antiepileptic drugs were decreased as clinically indicated. In case of study withdrawal, CBD was tapered down over 2–4 weeks.

Efficacy Assessments

Terminology of patient's seizure types was synchronized between physicians and patients/caregivers prior to enrollment. Seizure frequency was recorded for all patients based on a prospective paper diary during a baseline of 4 weeks and during treatment. Parents were asked to document all visible seizures, but series of spasms were documented as one seizure. Motor seizures were defined as either focal or generalized seizures with a clear motor component of $\geq$3 s. Seizure diaries were reviewed by the study team at each clinic visit. A calculation of mean monthly seizure frequency was performed for baseline period and at each visit. In addition, the impact of CBD on seizures was measured by objective data from video-EEG at baseline and after 3 months of therapy, in all patients able to participate in monitoring. Video EEG was performed according to international guidelines over 24–72 h (14). During video-EEG monitoring, seizures were counted individually even if occurring in series. The number of seizures per 24 h was calculated accordingly.

The aim of the study was to establish safety and tolerability of synthetic cannabidiol. The primary endpoint was frequency of motor seizures at 3 months. For the main secondary efficacy analysis we assessed the monthly frequency of motor seizures at 6 and 12 months, as well as monthly frequency of all other seizure types including countable focal non-motor seizures but excluding myoclonic seizures and absences due to difficulties in counting them reliably. Those patients that did not characteristically exhibit countable seizures were not included in the primary endpoint analysis. We also examined the response rates for motor seizures and for all countable seizures, defined as patients whose reduction in mean monthly seizure frequency was >50%.

Tolerability Assessments

Adverse events were documented by patients/caregiver, including epilepsy-related hospital admissions and emergency department

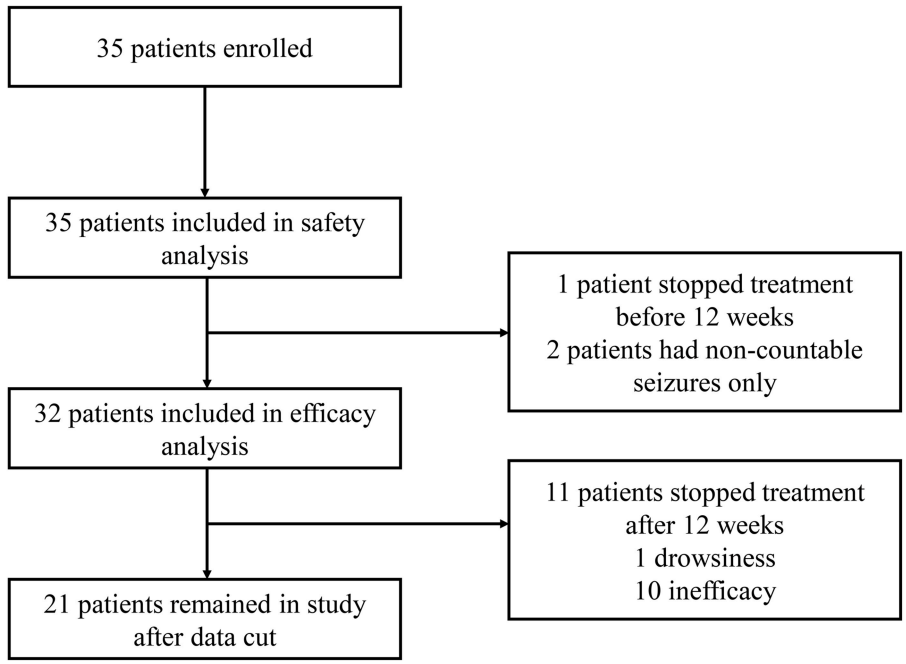

FIGURE 1 | Study participants flow charts.

visits and assessed at each visit and phone contact. Laboratory studies for hematology, electrolytes, liver and kidney function, and concentrations of antiepileptic drugs were conducted at baseline, and at every study visit.

Statistical Analysis

The study sample size was not predetermined but based on patient enrollment. Statistical software GraphPad Prism, Inc. version 8.1.1 (California, USA, www.graphpad.com) as well as IBM SPSS Statistics version 23 were used for statistical analyses. As most of the seizure parameters were not normally distributed, non-parametric statistical procedures were applied. For the analysis across all assessment points, Friedman tests were conducted. Single comparisons across two assessment points were calculated using Wilcoxon tests. Comparisons of the dosages and responder rates or absolute change in seizure frequency from baseline to end point between adults and children were conducted with a Mann-Whitney *U*-test. The threshold of statistical significance was $p < 0.05$. Patients who exited the study were not included in any calculations for the upcoming visit. Main analyses were repeated using the last-observation-carried-forward (LOCF) principle. Furthermore, as some changes in medication occurred during the study period, separate analyses were conducted excluding patients with any change in concomitant anticonvulsive medication. Correlation of seizure reductions in VEEG and in patients' diary was assessed using a Spearman correlation.

RESULTS

Study Population

Between November 2017 and May 2019, 35 patients were enrolled in the study. All 19 children and all 16 adults were included in the safety analysis. In the efficacy analysis, 32 patients were included; two patients with uncountable seizures only; and one patient who withdrew due to adverse events after 4 weeks were excluded (**Figure 1**). Patient characteristics are shown in **Table 1**. Over 80% ($n = 29$) of the participants were taking a combination of at least two AED. The most commonly used medications were valproate (42.9%), lamotrigine (25.7%), and levetiracetam (22.9%). Clobazam was used by 4 patients (11.4%) in our study. By the time of data cut, 21 (60%) patients had ongoing treatment; the median CBD treatment duration was 321 days (range 36–824).

Dosing

By the 3 months follow up visit, titration to the target dose of 18 mg/kg/day CBD was achieved in 28 patients (80%). The remaining seven participants did not reach the target dose due to diarrhea ($n = 4$), excessive somnolence ($n = 2$), and elevated liver enzymes ($n = 1$). Titration to a maximum dose above 25 mg/kg per day at any time during the study was done in 12 children (63.2%) and one adult (6.3%). Six patients (17.1%) reduced their dose of CBD at any time during follow-up. The mean actual doses attained are shown in **Figure 2**. The median dose in children was significantly higher compared to adults at 3 months ($p = 0.006$) and at 6 months ($p < 0.001$).

Efficacy

Median motor seizure frequency decreased from 21.8 (IQR 7.5–52.5) seizures per month at baseline to 8.5 (IQR 3.7–28.3, $p < 0.001$) at 3 months. Total seizure frequency decreased from a median of 22.3 (IQR 7.7–53.0) seizures per month at baseline to 11.85 (IQR 4.3–30.3, $p < 0.001$) at 3 months. Seizure reductions of motor and all countable seizures were statistically significant across all assessment points (both $p < 0.001$) and across individual assessment points (**Table 2**). Adjusted median reduction of motor seizures was 40.0% (IQR 18.2–58.5) and of all seizures 38.4% (IQR 18.6–58.9) at 3 months (**Figure 3**). One patient was free of all motor seizures during the 12 months treatment period. No patient reported an increase in countable seizure frequency, but one patient reported a subjective increase of absences.

Results of the LOCF analysis showed that the observed reductions in seizure frequency were not affected by dropouts with a median reduction of monthly motor-seizure frequency compared to baseline of 40% (IQR 18.2–58.5, $p < 0.001$) after 3 months and 47.8% (IQR 16.4–76.8, $p < 0.001$) after 6 months. Also, the result of primary-endpoint result did not change when patients with any change in baseline AED therapy ($n = 9$) were excluded from the analysis ($p < 0.001$). Patients younger than 18 years at treatment onset had a significantly higher reduction in motor seizures than adults at 3 months (53.9 vs. 34.5%, $p = 0.01$). The percent reduction in motor seizures after 3 months was similar between those patients with Lennox-Gastaut or Dravet syndrome ($n = 10$) and those patients with other epilepsies ($n = 22$) (median 36.7 vs. 42.3%, $p = 0.64$). The ≥50% responder rate for motor seizures after 3 months was 43.0% and after 6 months 56.1%. Treatment response rates were generally similar in the LOCF analysis, with ≥50% responder rate for motor seizures of 43.8 after 3 months and 60% after 6 months. In those patients receiving video-EEG monitoring, seizure reduction at 3 months compared to baseline was statistically significant, both for frequency calculated from seizure diary ($p = 0.001$) and as recorded during monitoring ($p = 0.01$) (**Table 3**). There was, however, no clear correlation between seizure frequency measured by VEEG and by seizure diary at baseline ($R = 0.407$, $p = 0.15$) and at 3 months ($R = 0.494$, $p = 0.075$). The long-term retention rate after 6 and 12 months was 78.1 and 73.1% respectively.

TABLE 1 | Demographic and baseline characteristics.

	Total ($n = 35$)	Children ($n = 19$)	Adults ($n = 16$)
Male *n* (%)	19 (54.3)	10 (52.6)	9 (56.3)
Age in years mean (SD)	19.7 (14.6)	9.1 (4.7)	32.2 (12.2)
Age at epilepsy beginning median (range)	2 (0.08–52)	1 (0.08–11)	7 (0.33–52)
Epilepsy syndrome *n* (%)			
Focal/multifocal	15 (42.9)	5 (26.3)	10 (62.5)
Epileptic encephalopathy*	6 (17.1)	5 (26.3)	1 (6.25)
Lennox-Gastaut syndrome	6 (17.1)	4 (21.1)	2 (12.5)
Dravet syndrome	5 (14.3)	2 (10.5)	3 (18.75)
Doose syndrome	2 (5.7)	2 (10.5)	0
Generalized epilepsy	1 (2.9)	1 (5.3)	0
Etiology *n* (%)			
Genetic	18 (51.4)	11 (57.9)	7 (43.8)
Structural	15 (42.9)	8 (42.1)	7 (43.8)
Unknown	2 (5.7)	0	2 (12.4)
Therapy			
Concomitant AED median (range)	2 (1–4)	2 (1–3)	2 (1–4)
Previous AED median (range)	7 (2–23)	5 (2–12)	10 (2–23)
Previous ketogenic diet *n* (%)	11 (31.4)	9 (47.4)	2 (12.5)
Previous steroid treatment *n* (%)	10 (28.6)	10 (52.6)	0
Previous epilepsy surgery *n* (%)	2 (5.7)	1 (5.3)	1 (6.25)

**Not otherwise specified. AED, anticonvulsive drug(s).*

Adverse Events and Withdrawals

Twenty-five patients (71.4%) reported at least one adverse event (**Table 4**). Most adverse events were mild and transient. Possibly treatment related serious adverse events were reported in 2 (5.7%) patients and included one adult patient with drowsiness and one adult with extrapyramidal symptoms both requiring hospitalization. The latter occurred shortly after onset

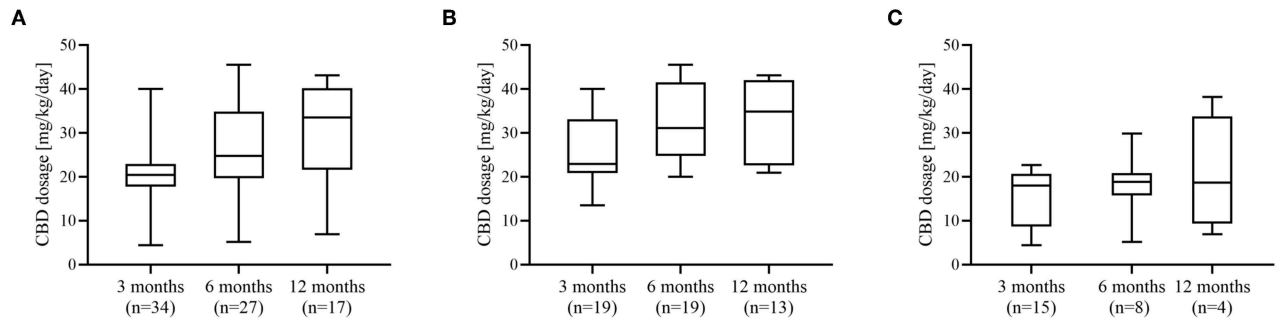

FIGURE 2 | Dosage of cannabidiol. Dosage of CBD (mg/kg/day) administered at 3, 6, and 12 months of treatment in **(A)** whole cohort, **(B)** pediatric cohort, and **(C)** adult cohort.

TABLE 2 | Frequency of motor seizures and of all countable seizures at baseline and at 3, 6, and 12 months of cannabidiol treatment in whole cohort, pediatric, and adult cohort.

Seizure types and cohort	Visit	*N*	Median	Range	*p*
Motor seizures					
All patients	Baseline	32	21.8	1.7–330	
	3 months	32	8.5	0–225	<0.001
	6 months	25	7.0	0–124.7	<0.001
	12 months	15	8.5	0–89.5	=0.008
Adults	Baseline	14	10.2	1.7–53.0	
	3 months	14	7.0	0.8–33.0	<0.001
	6 months	7	3.4	0.3–14.0	=0.06
	12 months	3	3.0	0–12.0	=0.05
Children	Baseline	18	42.0	1.7–330	
	3 months	18	13.0	0–225	<0.001
	6 months	18	11.7	0–224.7	<0.001
	12 months	12	7.5	0–89.0	=0.005
All countable seizures					
All patients	Baseline	32	21.3	1.7–330	
	3 months	32	11.9	1–225	<0.001
	6 months	25	11.7	0.7–124.7	<0.001
	12 months	15	7.5	0.5–89.0	=0.008
Adults	Baseline	14	12.2	2.7–53.0	
	3 months	14	7.2	1.0–33.0	=0.016
	6 months	7	15.0	2.0–28.7	=0.31
	12 months	3	3.0	0.7–18	=0.25
Children	Baseline	18	42.0	1.7–330	
	3 months	18	14.7	1.0–225.0	<0.001
	6 months	18	11.7	0.7–124.7	=0.005
	12 months	12	10	0.5–89.0	=0.001

Significance level of seizure frequency at each visit compared to baseline.

of omeprazole therapy and declined after omeprazole was stopped. Somnolence was reported in 14 patients (40%), of those only three patients received concomitant clobazam. Nine participants recovered without intervention; the cannabidiol dose was reduced, or titration interrupted in four patients, and in one patient clobazam dose was reduced. A significant weight loss of more than 5% from baseline was seen in four participants ranging from 7.5 to 20% of body weight. A significant weight gain of more than 5% from baseline was also observed in eight patients, ranging from 5 to 26.3% of body weight. There were no clinically significant changes in white or red blood cell counts, thrombocytes counts or renal function. In five patients occurred elevated ALT, AST, or GGT levels >3 times the upper limit of normal. Of those, three patients were taking valproate. Increased ALT/AST/GGT levels had resolved in four patients spontaneously and in one patient following treatment discontinuation. Altogether, two patients (5.7%) left the study due to adverse events after 4 and 12 weeks, respectively. Cumulatively, 10 (28.6%) patients withdrew due to lack of efficacy, of those, 6 between 3 and 6 months and 4 between 6 and 12 months.

Co-medication and Interaction

In 9 patients, AED levels over the upper therapeutic range or increase of levels >10% were detected. In three of four patients on clobazam, the active metabolite desmethylclobazam (D-CLB) increased by 20–468%. The only patient on clobazam with no change in D-CLB level was also on primidone. Of seven patients on brivaracetam (BRV), four patients showed an increase of brivaracetam plasma levels by 107–280%. In the remaining three patients on BRV, the levels were not available. In one patient on eslicarbazepine, the level increased by 23%, whereas in the only other patient on eslicarbazepine, no change was seen.

During the whole observation period, three patients stopped one of the concomitant AED, three patients started a new AED, and 30 patients remained on the initial comedication. Of those 30 patients, 26 also remained on the initial dose, whereas in two patients, dose of concomitant AED was reduced due to an increase in plasma levels and in two patients, dosage was increased as an effort to optimize treatment.

DISCUSSION

In our prospective open-label study, add-on treatment with synthetic cannabidiol led to a clinically meaningful reduction in seizure frequency in many patients and had an adequate safety profile in this patient population of children and adults with highly treatment-resistant epilepsy. Eighty percent of our patients were treated with two or more anticonvulsive drugs at baseline but still had a high seizure frequency with a median of almost 22 motor seizures per month.

Median modal dose after 3 months of treatment was similar for adults and children about 20 mg/kg/days and comparable with dosages in studies using plant derived cannabidiol (15). Whereas, adults remained on a stable median dose over the whole treatment period, dose was increased in most children between 3 and 6 months, resulting in a broader dose range. We believe this reflects a higher metabolism and a higher tolerance in children, rather than a secondary loss of efficacy. Other studies with longer observation periods also reported maintenance of long term efficacy (16, 17).

The present study showed a statistically significant reduction in motor seizures. Primary endpoint was frequency of monthly motor seizures at 3 months, which decreased significantly compared to baseline by an adjusted percentage reduction of 40%. At 6 months the median percentage change in the monthly frequency of motor seizures was 49.3%. The increase in treatment response is not explained by drop-outs, but rather reflects a higher fraction of children in the 6 months cohort and might also be related to a better dose-finding after 6 months compared to 3 months. As this is an ongoing study, numbers of patients receiving treatment for more than 12 months are too small to assess the long-term efficacy. Nevertheless, retention rates at 6 and 12 months are 78.1 and 73.1%, respectively, and comparable with other open label studies using plant-derived CBD (18). The role of clobazam-cannabidiol interaction in seizure reduction has been discussed before and is not fully understood (1). In our study, the rate of clobazam comedication (11%) was much lower but responder rates and median percent reduction of seizures similar compared to other open label studies (6). Seizure frequency reduction was significantly higher in children than in adults. This difference could be based on a shorter disease duration, but numbers were too small to assess confounding

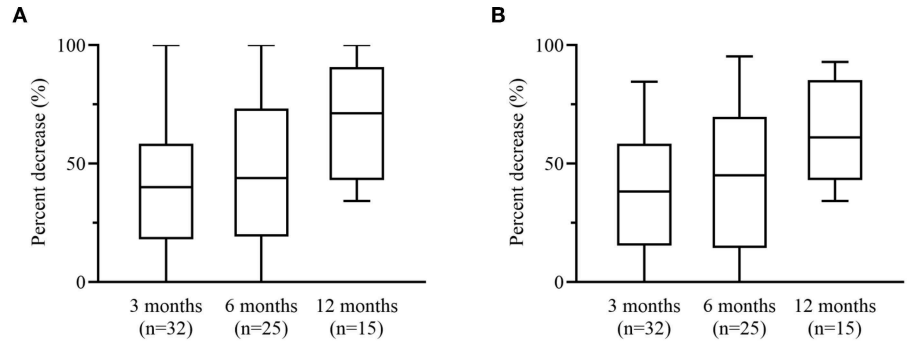

FIGURE 3 | Percentage change of motor and all countable seizures. Median percentage change at 3, 6, and 12 months in **(A)** motor seizures and **(B)** all countable seizures. Boxplots show median value with 25th and 75th percentile. Whiskers denote minimum and maximum.

TABLE 3 | Percent reduction in seizures recorded during 48 h of video-EEG monitoring at baseline and after 3 months of CBD treatment compared to percent reduction if seizures as calculated from seizure diary in relation to patient's habitual seizures.

Subject	Percent reduction of all seizure types		Patient's seizure types
	Recorded in VEEG	Calculated from diary	
01	0	75.7	DS
02	40.5	57.1	ES, TS
03	66.7	NA	AS
04	38.6	54.9	ES
05	+78.0*	64.0	BTCS
06	15.3	7.7	BTCS, MS, AS
07	100	16.4	BTCS
08	100	72.5	Focal unaware non-motor
09	0	84.5	DS
10	85.6	19.9	ES, nocturnal TS
11	53.8	41.6	ES, focal unaware non-motor
12	0	38.4	Focal unaware non-motor
13	100	65.5	ES, nocturnal MS, BTCS
14	46.3	0	ES, TS
15	82.8	98.6	TS, focal non-motor, HKS

*AS, absences; BTCS, bilateral tonic clonic seizures; DS, drop seizures; ES, epileptic spasms; HKS, hyperkinetic seizures; MS, myoclonic seizures; NA, not applicable; TS, tonic seizures; VEEG, video-EEG monitoring. *Increase in seizure frequency.*

TABLE 4 | Adverse events observed during whole observation period.

Adverse events	Total *n* (%)	Children *n* (%)	Adults *n* (%)
At least one AE	25 (71.4)	16 (84.2)	9 (56.3)
Somnolence	14 (40)	11(57.9)	3 (18.8)
Diarrhea	12 (34.3)	7 (38.8)	5 (31.3)
Weight gain >5%	8 (22.9)	8 (42.1)	0 (0)
Loss of appetite	7 (20)	5 (26.3)	2 (12.5)
Irritability	7 (20)	6 (31.6)	1 (6.3)
Increased appetite	5 (14.3)	4 (21.1)	1 (6.3)
Weight loss >5%	4 (11.4)	2 (10.5)	2 (12.5)
Others*	4 (11.4)	4 (21.1)	0 (0)

**drowsiness (n = 3), extrapyramidal symptoms (n = 1).*
AE, adverse event.

factors in treatment response in our study. In larger open label studies, efficacy for children and adults is not reported separately (6). The better treatment response might also be explained by the much higher monthly seizure frequency at baseline in children (median 42) compared to adults (median 12.2). Etiology might be another important point in treatment response since more than 60% of the children had epileptic encephalopathy whereas more than 60% of adults had focal/multifocal epilepsy. Besides the randomized controlled trials, only few studies assessed CBD efficacy in specific epilepsy etiologies or syndromes, but data are not sufficient to support the perception of a better treatment response in children with epileptic encephalopathy (19). In our cohort as well, we could not see statistically significant differences in a direct comparison of patients with Lennox-Gastaut or Dravet syndrome vs. patients with other epilepsies.

Synthetic cannabidiol was generally well-tolerated. As in previous open label studies using plant-derived CBD, adverse events were reported frequently but remained mainly mild or moderate; only two patients discontinued treatment due to adverse events. The most common side effects in our study were similar to those reported previously: somnolence (40%) and diarrhea (34.3%) (20). Another frequently described side effect in long term open label studies is loss of appetite, resulting in weight loss in some patients (16). Interestingly, whereas loss of appetite was also reported in our study, overall more patients showed a significant weight gain (22.9%) than a significant weight loss (11.4%). Especially in children with epileptic encephalopathies and a high seizure burden, the general condition improved considerably, permitting an improved nutrition and likely explaining a weight gain in 40% of those children. As reported before, significant increase of liver enzymes was more frequent in patients taking valproate, but also observed in patients without any comedication (20). In our cohort, patients and parents did not report any significant changes in behavior or concentration.

However, long-term effects on cognition is one major concern with cannabinoid treatment (21). Longer observational periods and studies with a more detailed neurocognitive test protocol are needed.

Overall, 25% of patients in our study showed a relevant increase of their concomitant AED's plasma levels, in particular desmethylclobazam. Increased sedation in those patients is in line with previous reports, but occurred in patients without clobazam also (22). The only patient with no increase in D-CLB level was also on primidone, a potent inducer of Cytochrome P450 (CYP) enzymes, counteracting the CYP-enzyme inhibiting effect of CBD. However, we also found interaction with other AED, such as BRV that is described in detail elsewhere, and potential interaction with other comedications as omeprazole (23). Cannabidiol is known for its high interaction potential but further pharmacokinetic studies are needed to understand clinically relevant interactions with AED and other drugs in mono-and polytherapy (24).

There are several limitations in this study. As an open-label study of a drug with high public interest, the placebo-effect may be augmented compared to other anticonvulsive drugs. However, the overall median reduction of motor-seizures in our study was comparable to other open label studies as well as randomized controlled trials using plant-derived CBD (15). Although doses and number of AED remained stable in the majority of patients, another limitation is that concomitant AED were not strictly controlled. In any case, the calculations that include only patients with stable AED regimen still revealed statistically significant reductions in seizures, making a relevant contribution of AED changes to the outcome in this study unlikely. Also, our cohort was very heterogeneous, limiting the assessment of efficacy in this population.

Even though it is generally accepted to calculate primary outcome variables from patients' seizure diaries, there is distinct evidence that patient-reported seizure-counts lack validity (25). In our study, seizure reduction as observed by video-EEG (video-efficacy) and seizure reduction as calculated from seizure diaries (diary-efficacy) showed discrepancy in 5 of 15 patients. As expected, mainly nocturnal seizures and seizures without prominent motor signs were present in those patients. Reduction of video-EEG-recorded seizures was statistically significant after 3 months compared to baseline ($p = 0.01$), showing efficacy of cannabidiol in our cohort when established in an objective manner. Reduction of seizures of those patients as calculated from seizure diary was still highly significant ($p = 0.001$). The difference between video-efficacy and diary-efficacy could be based on a placebo effect in CBD treatment. However, the comparison is limited by the different time frames of both methods and there is no clear statistical correlation between these two measures. An additional benefit of video-EEG monitoring was the possibility to reveal a significant seizure reduction in one patient who only exhibited non-countable seizure and was therefore not included in the primary outcome measurement. Counting seizure frequency in an objective manner during video-EEG is time- and cost-consuming and not possible in all patients but might be a sensitive approach in patients with subtle and nightly seizures at least.

The exact mechanism of action of CBD has been only partially elucidated and involves a series of different mechanisms but has been shown to be mainly independent of the endocannabinoid system (26). For other indications than epilepsy, combinations of cannabinoids have been used and the entourage effect, i.e., the positive synergistic effect of different cannabis compounds has been discussed. Despite some metanalytic evidence of potential clinical benefits of CBD extracts over purified CBD, informative value is extremely limited by quality of the included studies (27). In almost all prospective open label studies with larger cohorts purified plant derived CBD has been used. Since pharmaceutical-grade synthetically derived cannabidiol is chemically identical to the cannabidiol found naturally in the cannabis plant, we did not expect any relevant differences in efficacy and tolerance (10). Nevertheless, this study is the first to report efficacy and tolerance of synthetic cannabidiol in epilepsy treatment to be comparable to plant derived CBD. The production of plant derived CBD is economically and ecologically challenging, and it is unclear if will be possible to meet increasing global demand for the long term. Therefore, synthetic CBD might be a worthwhile alternative.

CONCLUSION

In summary, the results of this study provide class III evidence of efficacy and safety of synthetic cannabidiol in children and adults with pharmacoresistant epilepsy. Additional studies investigating efficacy and tolerance of synthetic CBD in larger cohorts are needed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethik-Kommission der Albert-Ludwigs-Universität Freiburg, Germany. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KK designed and conceptualized study, analyzed the data, and drafted the manuscript for intellectual content. DG played major role in the acquisition of data. BM interpreted the data, revised the manuscript for intellectual content, and performed statistical analysis. MH, AS-B, and JJ interpreted the data and revised the manuscript for intellectual content.

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Personalizing Heart Rate-Based Seizure Detection Using Supervised SVM Transfer Learning

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Objective: Automated seizure detection is a key aspect of wearable seizure warning systems. As a result, the quality of life of refractory epilepsy patients could be improved. Most state-of-the-art algorithms for heart rate-based seizure detection use a so-called patient-independent approach, which do not take into account patient-specific data during algorithm training. Although such systems are easy to use in practice, they lead to many false detections as the ictal heart rate changes are patient-dependent. In practice, only a limited amount of accurately annotated patient data is typically available, which makes it difficult to create fully patient-specific algorithms.

Methods: In this context, this study proposes for the first time a new transfer learning approach that allows to personalize heart rate-based seizure detection by using only a couple of days of data per patient. The algorithm was evaluated on 2,172 h of single-lead ECG data from 24 temporal lobe epilepsy patients including 227 focal impaired awareness seizures.

Results: The proposed personalized approach resulted in an overall sensitivity of 71% with 1.9 false detections per hour. This is an average decrease in false detection rate of 37% compared to the reference patient-independent algorithm using only a limited amount of personal seizure data. The proposed transfer learning approach adapts faster and more robustly to patient-specific characteristics than other alternatives for personalization in the literature.

Conclusion: The proposed method allows an easy implementable solution to personalize heart rate-based seizure detection, which can improve the quality of life of refractory epilepsy patients when used as part of a multimodal seizure detection system.

Keywords: epilepsy, transfer learning, seizure detection, personalization, heart rate analysis, SVM

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1. INTRODUCTION

Epilepsy is one of the most common neurological disorders, which affects around 1% of the population worldwide (1). Anti-epileptic drugs provide adequate treatment for about 70% of epilepsy patients (2). The remaining 30% of the patients continue to have seizures, which drastically affects their quality of life. This can be improved by an automated warning system that alarms the

parents or caregivers when the patient experiences a seizure. By using such a system, the patients and relatives feel more at ease knowing someone will be able to help the patient out when a seizure would occur. Quick intervention can then lead to a decrease in injuries and avoid (post-)ictal complications, including sudden unexplained death in epilepsy (SUDEP). In addition, a seizure diary, automatically generated from the alarms, can be used for a follow-up of the disease and evaluation of the treatment. A seizure diary, kept by the patients or their families, has proven to be unreliable, which leads to bad treatment follow-up (3). An automatically generated seizure diary could lead to a more objective seizure count and an improved treatment selection.

The key element of such a warning system is the automated seizure detection algorithm. In the literature, these algorithms are typically based on full electroencephalography (EEG). EEG recordings mostly require wet electrodes on the scalp, which is uncomfortable for a long-term monitoring solution (4). More easily obtainable biomedical signals used to detect epileptic seizures include accelerometry (ACC), electromyography (EMG), electrocardiography (ECG) and galvanic skin response (5). The most suitable modality or combination of modalities depends on the seizure type. ECG-based seizure detection, for instance, is ideal for the detection of focal impaired awareness seizures (FIAS) arising from the temporal lobe, as they are not accompanied by typical motor components, but they are associated with ictal tachycardia (6–8). Therefore, this study focuses on patients with temporal lobe epilepsy, as for this type of focal seizures, ECG-based seizure detection is of most added value compared to other wearable modalities. It should however be noted that ECG-based seizure detection algorithms can be used for a wider range of seizure types, including focal seizures with non-temporal seizure onsets and generalized tonic-clonic seizures (9). It is also a very important modality for long-term monitoring applications as it allows to assess the patient's general health status (e.g., sleep and general heart conditions).

Most ECG-based seizure detection systems from the literature are based on patient-independent models (10–13). For this type of models, no patient-specific data is required, making them directly usable in practice. However, due to the large inter-patient differences in heart rate characteristics, performance is too low for practical use.

In order to increase the performance, models can be adapted to the patient heart rate characteristics (14, 15). Different options are possible. A first option is the manual setting of some parameter thresholds per patient (15, 16). This requires manual screening of previous patient data, and it only works well if the parameters are easily understandable. Simple thresholding approaches are however too simple to grasp the large complexity of the problem. Automated personalization is therefore advised, but it normally requires a lot of patient-specific data in order to find a robust algorithm for a specific patient (17). Often, only a limited amount of accurately annotated patient-specific data is available, hence a lot of complex approaches are not useful for making fully patient-specific classifiers. Heuristic automated algorithms allow a low-complex and fast personalization, but might lead to suboptimal results (9).

A more robust and optimal solution can be found through transfer learning (18, 19). In transfer learning, the solution to a classification problem is found by using the solution from a similar problem ("source task") as a starting point (see **Figure 1**). This way, less data for the new problem ("target task") is required in order to get a robust solution as a part of the knowledge is already contained in the reference model. In this paper, a patient-specific heart rate-based seizure detection model is trained through transfer learning by using a patient-independent classifier as a reference model. Therefore, only a limited amount of patient-specific heart rate data is required in order to get a robust patient-specific model, obtained with a relative limited complexity. This paper proposes a new enhanced transfer learning solution that is also able to deal with the large class imbalance and allows to give more importance to seizure samples during classifier training for each individual.

The novelty of this study is threefold. First, a new transfer learning procedure is proposed. An existing transfer learning method is enhanced in order to deal with class imbalance and allows to give increased importance to sensitivity rather than specificity. The latter is a crucial aspect for automated seizure detection. Secondly, the proposed transfer learning approach is applied in order to efficiently personalize heart rate-based seizure detection with a limited amount of patient data. To the best of our knowledge, it is the first time transfer learning is used to personalize automated non-EEG-based seizure detection. Unimodal seizure detection systems based on heart rate typically

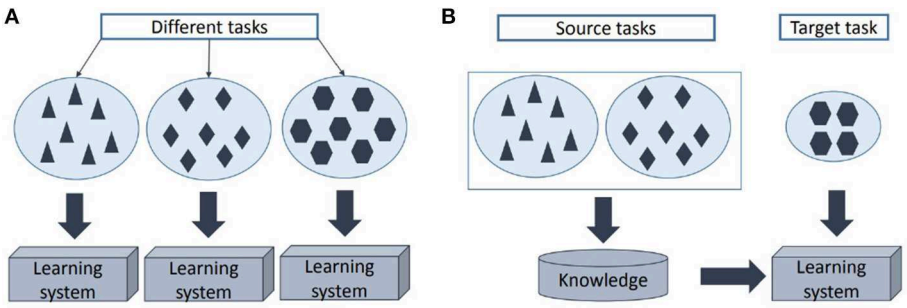

FIGURE 1 | Illustration of the learning procedure using **(A)** traditional machine learning and **(B)** transfer learning.

lead to inaccurate results that are insufficient to be used in practice (20). In this context, the proposed methodology offers a novel solution that can be used as part of a multimodal system, which allows to adapt the detection system to each patient. As a result, a more accurate and usable solution could be achieved (21). These multimodal systems are currently getting close to sufficiently high accuracy for practical usage, but are typically restricted to a certain seizure type (22). The added value of using heart rate in a multimodal system is that it is the ictally most activated modality besides EEG, which allows detection of a wide range of seizure types. Improving the unimodal heart rate-based seizure detection using the proposed method will also improve the multimodal seizure detection, closing the gap to practical usage for a wide range of seizure types. Finally, an in-depth analysis is performed in order to indicate the added value of the proposed transfer learning approach compared to the state-of-the-art literature of heart rate-based seizure detection.

2. MATERIALS AND METHODS

2.1. Data Acquisition

The dataset contains recordings from refractory epilepsy patients, who underwent presurgical evaluation at the University Hospitals Leuven (UZ Leuven), Belgium and had at least five FIAS originating from the temporal lobe during the evaluation. The patients were recorded with 10–20 scalp EEG with 1 bipolar ECG channel (lead II) with a sampling frequency of 250 Hz in a fully wired system. The data was recorded continuously and contains both day and night data of the patients within a hospital room. The single-lead ECG signal was continuously unreadable due to noise during 4.6 (patient 13), 8.8 (patient 16), and 7.4 (patient 18) hours. Those segments were removed from the analysis. The remaining data consists of 24 patients with 2,172 h of data. In total, 228 seizures were recorded (see **Table 1**).

A clinical expert annotated the seizure onsets and offsets with the use of video-EEG data, without considering the ECG. Afterwards, a neurologist validated the annotations. The seizure duration was defined as the time between EEG seizure onset and offset. However, during 30% of the seizures, the offsets could not be determined. The ethical committee of the UZ Leuven approved the study (approval number S59662). All patients signed the informed consent for their participation in this study.

2.2. Preprocessing

Single-lead ECG was used as input for the proposed seizure detection algorithm. First, the heart rate was extracted from the

TABLE 1 | An overview of the dataset.

Patient	# Seizures	RD (h)	Hemisphere	Origin	Age	Gender	Mean SD (s)	Range SD (s)
1	10	26	B	T	49	M	31	[24–39]
2	9	63	L	F-T	41	F	13	[13–13]
3	13	71	R	T	27	M	71	[28–96]
4	10	25	B	T	18	M	19	[14–26]
5	11	47	R	T	29	F	50	[40–60]
6	7	148	L	T	26	M	63	[32–116]
7	30	67	R	F-T	19	M	50	[17–90]
8	11	114	L	T	38	M	39	[19–75]
9	8	64	L	T (7), O (1)	28	M	23	[11–31]
10	6	111	EEG not readable		35	M	126	[69–183]
11	6	100	B (5), R (1)	T	67	F	26	[21 31]
12	9	91	R	F-T	24	F	47	[33–85]
13	8	109	R	T	32	M	46	[25–61]
14	5	100	L	T	19	F	56	[29–83]
15	13	110	EEG not readable		49	M	16	[8 30]
16	5	96	L	T	45	M	64	[11–95]
17	7	102	UC (4), R (3)	UC (4), T (3)	18	F	33	[33–33]
18	5	84	L (3), R (2)	T	62	M	125	[89–187]
19	15	113	UC		40	F	23	[6–52]
20	5	113	L	T	41	F	74	[58 83]
21	8	103	L (3), R (5)	T (3), F-T (5)	43	M	67	[29–99]
22	12	115	L (4), R (3), B (5)	T	35	M	27	[17–38]
23	6	101	R	T	23	F	80	[51–108]
24	8	99	R	T (1), O-T (5)	24	F	42	[17–84]
Total	227	2172			[18–67]	14M/10F	50 ± 30	[6–187]

RD, Recording Duration; SD, Seizure Duration; L, Left; R, Right; B, Bihemispheric; UC, Unclear; T, Temporal; O, Occipital; F, Frontal.

ECG using an approach that used a real-time R peak detection algorithm. It detected the R peaks based on the derivative signal and an adaptive threshold T_t that changed based on the maximal derivatives P_t of the previously detected R peaks ($T_t = 0.9 * T_{t-1} + 0.1 * P_t$). Next, strong heart rate increases (HRI) caused by sympathetic activations were detected by automated slope analysis on the tachogram. HRI extraction was performed on a filtered tachogram, using a median filter with an order of 15 heart beats. If a heart rate gradient was larger than 1 bpm/s, a strong HRI was assumed. The beginning and end of this HRI was found by analyzing when this heart rate gradient became negative again. The HRI was then assumed to be a strong HRI if thresholds on the length of the HRI ($> 8\,s$), the achieved peak heart rate during the HRI (>60 bpm) and the (percentual) increase in heart rate during the HRI were exceeded (>10 bpm absolute heart rate increase, >25% percentual increase). These thresholds are based on the findings presented in De Cooman et al. (10).

2.3. Feature Extraction

Features were extracted whenever such a strong HRI was detected. In De Cooman et al. (10), it was shown that four features extracted from the HRI led to optimal patient-independent results: the peak heart rate, the heart rate at the start of the HRI, the baseline heart rate (extracted from the minute before the HRI) and the standard deviation of the baseline heart rate period. As the primary goal of this study was to lead to optimal patient-specific results with a limited amount of patient-specific data, only the first two features were used in this study. The reason for this was that most of the performance of the system was already accomplished by those two features. Adding more features to the system requires more training data for robust personalization through transfer learning. Choosing these two features led to an optimal balance between performance and limited requirement of patient-specific data. These features were then classified with either the patient-independent (PI) classifier or the patient-specific (PS) transfer learning classifier.

2.4. Patient-Independent Classification

Let $\{x_i, y_i\}_{i=1}^N$ be the training data points extracted from patients different than the ones used for testing the algorithm, with $x_i \in \mathbb{R}^2$ the data samples and $y_i \in \{-1, +1\}$ the corresponding labels. Let class -1 correspond to seizure samples and class +1 to non-seizure samples. Support vector machines (SVM) will map data points to a higher dimensional space using a (non-)linear transformation $\varphi(x)$, so that the data points can be separated in this space by the hyperplane $w^T\varphi(x) + b$, with w the unknown weight vector and b an unknown constant.

The solution for weighted SVM can be found by solving the following optimization problem

$$\begin{aligned} &\min_{w,b,\xi} \frac{1}{2}||w||^2 + C\sum_{i=1}^{N} c_i\xi_i \\ s.t. \quad &\begin{cases} y_i(w^T\varphi(x_i) + b) \geq 1 - \xi_i \\ \xi_i \geq 0 \end{cases} \quad ,\forall i \in [1, N] \end{aligned} \tag{1}$$

with ξ_i the error of the model on x_i and C a tunable hyperparameter. A modification of the typical SVM is used here to remove the class imbalance from the dataset (23). The values of c_i are defined as

$$c_i = \begin{cases} \gamma\frac{(N^+ + N^-)}{2N^-} & : y_i = -1 \\ \frac{N^+ + N^-}{2N^+} & : y_i = +1 \end{cases} . \tag{2}$$

The parameter γ gives more importance to the correct classification of seizure samples compared to non-seizure samples during classifier training. The Lagrangian of (1) becomes

$$\begin{aligned} \mathcal{L}(w, b) = &\frac{1}{2}||w||^2 + C\sum_{i=1}^{N} c_i\xi_i - \sum_{i=1}^{N} \nu_i\xi_i \\ &- \sum_{i=1}^{N} \alpha_i \left(y_i \left(w^T\varphi\left(x_i\right) + b\right) - 1 + \xi_i\right) \end{aligned} \tag{3}$$

with $\alpha_i, \nu_i \geq 0$ the Lagrange multipliers, leading to the dual problem

$$\begin{aligned} &\min_{\alpha} = \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N} y_i y_j \alpha_i \alpha_j K\left(x_i, x_j\right) - \sum_{i=1}^{N} \alpha_i \\ &s.t. \quad \begin{cases} \sum_{i=1}^{N} \alpha_i y_i = 0 \\ 0 \leq \alpha_i \leq Cc_i \end{cases} \quad ,\forall i \in [1, N] \end{aligned} \tag{4}$$

with $K(x_i, x_j) = \varphi(x_i)^T\varphi(x_j)$ the kernel function. The values of the hyperparameter C, γ and the Gaussian kernel parameter σ are taken as reported to be optimal in De Cooman et al. (10). The classifier is trained using the leave-one-patient-out crossvalidation (LOPO-CV) approach, training the classifier on all patients except the one used for evaluating the algorithm.

2.5. Personalized Classification Through Transfer Learning

The goal of this study is to personalize the seizure detection classification in order to get an optimal patient performance with a limited amount of patient-specific data. One solution for this is to use transfer learning (TL), which allows to train a new classifier for a problem with a limited amount of data by using a reference classifier that solves a similar problem. The previously trained patient-independent classifier discussed in section 2.4 is used as the reference classifier. This way, the classifier can be personalized with a limited amount of patient-specific data by using the knowledge already incorporated in the patient-independent classifier. An overview of the proposed procedure for personalizing the heart rate-based seizure detection is given in **Figure 2**.

The newly proposed transfer learning approach is based on the concept described in Yang et al. (24). It states that the weight vector of the new SVM solution should be sufficiently similar compared to the weight vector of the reference SVM solution, while also minimizing the misclassification error on the data of the new problem. In this use case, it means that the weight vector of the new patient-specific and reference

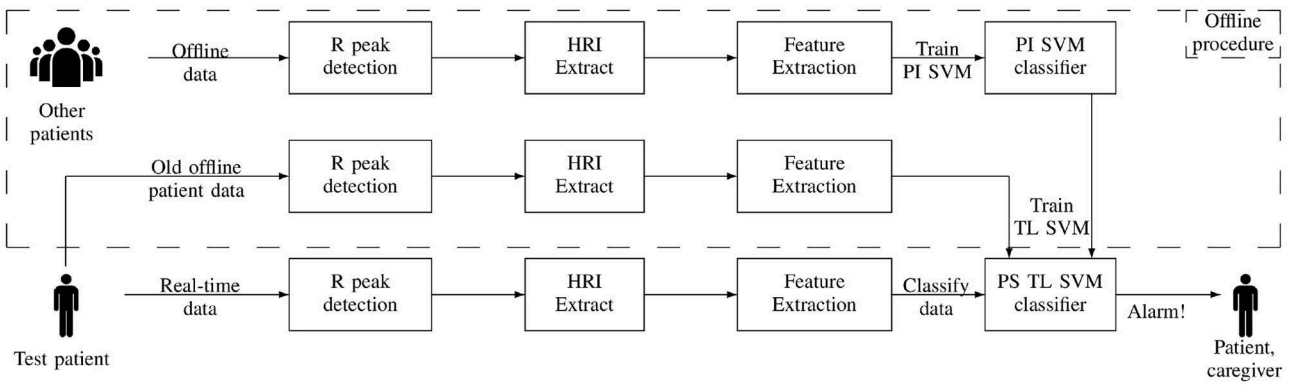

FIGURE 2 | Overview of the proposed transfer learning approach for personalized heart rate-based seizure detection. HRI, heart rate increase; PI SVM, patient-independent support vector machine; PS TL SVM, patient-specific transfer learning support vector machine.

patient-independent classifier should be sufficiently similar. The minimization problem proposed in Yang et al. (24) is enhanced here in order to also be able to deal with class imbalance and the increased importance of the sensitivity in seizure detection algorithms. The following minimization problem to create an SVM classifier for the patient-specific data $\{\tilde{x}_k, \tilde{y}_k\}_{k=1}^{M}$ is proposed

$$\begin{aligned} &\min_{\tilde{w},\tilde{b},\tilde{\xi}} \frac{1}{2}||\tilde{w}-w||^2 + D\sum_{k=1}^{M} \tilde{c}_k\tilde{\xi}_k \\ &s.t. \quad \begin{cases} \tilde{y}_k(\tilde{w}^T\varphi(\tilde{x}_k)+\tilde{b}) \geq 1-\tilde{\xi}_k \\ \tilde{\xi}_k \geq 0 \end{cases} \quad ,\forall k \in [1,M] \end{aligned} \tag{5}$$

with $\tilde{\xi}_k$ the error of the model on data point $\tilde{x}_k$ and w the weight vector obtained from the patient-independent classifier trained using (1), defined as

$$w = \sum_{i=1}^{N} \alpha_i y_i \varphi(x_i) \tag{6}$$

by the original SVM optimization problem. The same transformation function φ and corresponding kernel K used in the reference classifier are used here. This minimization problem contains weights $\tilde{c}_k$ for each $\tilde{x}_k$, which allows to also take care of the class imbalance and sensitivity importance in the optimization. These weights are defined as

$$\tilde{c}_k = \begin{cases} \widetilde{\gamma}\frac{(M^+ + M^-)}{2M^-} & : \tilde{y}_k = -1 \\ \frac{M^+ + M^-}{2M^+} & : \tilde{y}_k = +1 \end{cases}, \tag{7}$$

with M^+ and M^- indicating the number of patient-specific non-seizure and seizure data points. The introduction of these weights $\tilde{c}_k$ are crucial as they ensure that the personalized classifier is sufficiently stable and targets a sufficiently large sensitivity, which is required for real-life seizure detection systems. Hyperparameter D allows to balance between minimizing the errors for the patient-specific data points and minimizing the difference compared to the reference patient-independent classifier (defined by w). Parameter $\widetilde{\gamma}$ is experimentally initialized to a value of 1.5. The initial value for hyperparameter D depends on the amount of seizures available in the training set, and is set to 0.1 for patients with less than 10 seizures and set to 100 for patients with more than 10 seizures. These values are based on the findings reported in De Cooman et al. (25). Nevertheless, these parameters should ideally be optimized per patient, based on their validation performance, but it is very challenging to get robust hyperparameter optimization results due to the low amount of patient training data. Therefore, a more heuristic method is applied, which lowers the values of $\widetilde{\gamma}$ (linear decrease of 0.25) and D (exponential decrease by factor 1/10) if the resulting classifier leads to 50% more false detections on the patient training data than the available patient-independent approach. This reduction is repeated until the false detection rate (FDR) is dropped below this threshold or minimal values for $\widetilde{\gamma}$ (=0.25) and D (=0.01) are reached. This procedure is required to avoid that the personalized approach would overtrain on a limited amount of abnormally small ictal heart rate increases, which could lead to a drastic increase in FDR compared to the reference patient-independent classifier.

An optimal solution for (5) is found for the saddle point in the Lagrangian $\mathcal{L}$

$$\begin{aligned} &\max_{\tilde{\alpha},\tilde{\beta}} \min_{\tilde{w},\tilde{b},\tilde{\xi}} \mathcal{L}(\widetilde{w},\tilde{b},\widetilde{\xi};\widetilde{\alpha},\widetilde{\beta}) \\ &= \max_{\tilde{\alpha},\tilde{\beta}} \min_{\tilde{w},\tilde{b},\tilde{\xi}} \frac{1}{2}||\widetilde{w}-w||^2 + D\sum_{k=1}^{M}\widetilde{c}_k\widetilde{\xi}_k - \sum_{k=1}^{M}\widetilde{\beta}_k\widetilde{\xi}_k \\ &\quad - \sum_{k=1}^{M}\widetilde{\alpha}_k\left(\tilde{y}_k\left(\widetilde{w}^T\varphi(\widetilde{x}_k)+\tilde{b}\right)-1+\widetilde{\xi}_k\right) \end{aligned} \tag{8}$$

with $\widetilde{\alpha}_k, \widetilde{\beta}_k \geq 0$ the Lagrangian multipliers. This leads to

$$\begin{cases} \frac{\partial\mathcal{L}}{\partial\widetilde{w}} = 0 & \rightarrow \tilde{w} = w + \sum_{k=1}^{M}\widetilde{\alpha}_k\tilde{y}_k\varphi(\widetilde{x}_k) \\ \frac{\partial\mathcal{L}}{\partial\tilde{b}} = 0 & \rightarrow \sum_{k=1}^{M}\tilde{y}_k\widetilde{\alpha}_k = 0 \\ \frac{\partial\mathcal{L}}{\partial\tilde{\xi}_k} = 0 & \rightarrow \widetilde{\alpha}_k + \widetilde{\beta}_k = D\tilde{c}_k, \forall k \in [1,M] \end{cases} \tag{9}$$

so that the dual problem of (5) is defined as

$$\min_{\widetilde{\alpha}} \sum_{i=1}^{N}\sum_{k=1}^{M} y_i\widetilde{y}_k\alpha_i\widetilde{\alpha}_k K\left(x_i,\widetilde{x}_k\right) - \sum_{k=1}^{M}\widetilde{\alpha}_k + \frac{1}{2}\sum_{k=1}^{M}\sum_{l=1}^{M}\widetilde{y}_k\widetilde{y}_l\widetilde{\alpha}_k\widetilde{\alpha}_l K\left(\widetilde{x}_k,\widetilde{x}_l\right)$$
$$s.t. \quad \begin{cases} \sum_{k=1}^{M}\widetilde{\alpha}_k\widetilde{y}_k = 0 \\ 0 \le \widetilde{\alpha}_k \le D\widetilde{c}_k \end{cases}, \forall k \in [1, M] \quad . \tag{10}$$

Note that

$$\widetilde{w} = w + \sum_{k=1}^{M}\widetilde{\alpha}_k\widetilde{y}_k\varphi(\widetilde{x}_k) \tag{11}$$

indicates that the patient-specific $\tilde{w}$ is a combination of patient-independent and patient-specific information. A new data point $\tilde{x}_n$ is then classified using

$$y(\widetilde{x}_n) = sign\left(\sum_{k=1}^{M}\widetilde{\alpha}_k\widetilde{y}_k K(\widetilde{x}_k,\widetilde{x}_n) + \widetilde{b} + \sum_{i=1}^{N}\alpha_i y_i K(x_i,\widetilde{x}_n)\right) \quad . \tag{12}$$

The TL classifier is trained and tested using a 5-fold crosstesting scheme, in which 4 folds of patient-specific data are used for training and 1 for testing. This is then repeated 5 times so that each fold is used once as test set.

2.6. Alternative Automatic Personalization Solutions

The proposed transfer learning approach is also compared to two different alternatives for personalization. The first alternative includes a fully PS approach which is trained with only PS data using the SVM classifier defined by (1) as in De Cooman et al. (14). The other alternative is a so-called mixed model, in which both PI and PS data are used for training an SVM classifier defined by (1), but adapting the values of c_i in (2) into c_i^{MIX}:

$$c_i^{MIX} = \begin{cases} s_i\gamma\frac{N^+ + N^- + M^+ + M^-}{2(N^- + M^-)} & : y_i = -1 \\ s_i\frac{N^+ + N^- + M^+ + M^-}{2(N^+ + M^+)} & : y_i = +1 \end{cases} \tag{13}$$

with

$$s_i = \begin{cases} 4 & : i \in PSdata \\ 1 & : i \notin PSdata \end{cases} \tag{14}$$

such that misclassification of PS data is more critical during training than misclassification of non-PS data. The value 4 is chosen as recommended in De Cooman et al. (14).

2.7. Algorithm Evaluation

In order to compare the different seizure detection algorithms, four performance metrics were used. The first two metrics correspond to the sensitivity (Se, percentage of detected seizures) and false detection rate (FDR, expressed in false positives/hour, FP/h). A seizure is detected if a detection is generated between 1 min prior to the seizure onset and 2 min after the seizure onset. All other detection were classified as false detections. False detections within 1 min of each other were counted as one false detection. In order to combine the Se and FDR in one metric, the F_β-score with $\beta = 3$ is defined as:

$$F_\beta = \frac{(1+\beta^2)TP}{(1+\beta^2)TP + \beta^2 FN + FP} \tag{15}$$

with TP, FN, and FP the number of true positives (detected seizures), false negatives (missed seizures) and false positives (false detections). The F_3-score is chosen for this application because it gives more importance to Se compared to FDR. As last metric, the detection delay was determined, which indicates the time difference between the moment of detection and the EEG seizure onset. Average measures over the entire dataset can be expressed as patient average performance (Pat.-Av.), which is the average of the performance of each patient, or overall average (Tot.-Av.), computed on the total number of seizures or recording duration. The first average measure is used, unless specifically mentioned. To prove the significant differences between the algorithms, paired *t*-tests were performed. All results were obtained retrospectively in a simulation which replicated a real-time setting.

2.8. Impact of Number of Seizures in Training

One of the advantages of transfer learning is that it allows to train a new classifier with a relative limited amount of data by using a reference classifier (18). In this simulation, the number of required training seizures needed to gain sufficient added value in seizure detection performance was evaluated. Instead of using the full training set, only a few seizures (0–4) from the patient-specific training set were used for training (using the crosstesting scheme described in section 2.5). A model trained with zero seizures is equivalent to a patient-independent model. These training seizures were chosen randomly in 100 simulations for each selected amount of training seizures. This simulation was done for the proposed transfer learning approach and the alternatives for personalization.

2.9. Comparison With the Literature

In order to compare the proposed algorithms against the state-of-the-art literature, three algorithms (11, 15, 26) were also implemented and evaluated on the same dataset. The algorithms were implemented based on the corresponding publications. Both Osorio (11) and van Elmpt et al. (15) were based on an algorithm that requires two moving windows: one short window indicating the current heart rate behavior and one longer window indicating the reference/baseline heart rate. Both approaches were described to be patient-independent algorithms, but no preferred threshold values were described in the papers. Therefore, optimal patient-independent threshold values leading to the highest F_3-score were automatically chosen using a LOPO-CV procedure on each training set. The algorithm described in Jeppesen et al. (26) was originally designed to be a patient-specific algorithm (based on non-seizure patient data), but also a patient-independent variation of the algorithm is evaluated here. The

used threshold on the parameter value is also optimized in a LOPO-CV procedure.

Also new patient-specific versions of these algorithms from the literature were constructed in an automated fashion. This is done using 5-fold crosstraining, where the threshold values are automatically chosen based on the optimal F_3-score found in the 4 folds of training. Also the test mentioned in section 2.8 is performed for the different state-of-the-art algorithms to compare how fast these can adapt to a patient's characteristics.

3. RESULTS

The preprocessing procedure discussed in section 2.2 identified 84.7% of the seizures, which gives an indication of the amount of seizures with ictal heart rate increases. **Table 2** gives an overview of the results of the patient-independent (PI), fully patient-specific (PS), mixed (MIX) and transfer learning (TL) approaches. The PI algorithm results in an average Se of 75% with 3.0 FP/hour and an F_3-score of 0.22. By adapting the model to the patient characteristics with the TL algorithm, a similar Se is observed (71%) with 37% less false positives (1.9 FP/hour). The average F_3-score is increased to 0.30. **Figure 3** shows the results of the proposed TL approach and the reference PI approach for each patient. It illustrates that personalization allows to strongly increase the performance for most patients.

The alternative mixed approach results in a similar Se, but with on average 0.7 FP/h more than the proposed TL approach. The fully PS approach results in a decreased Se, with a slightly increased FDR (2.2 FP/h) compared to the TL approach. The TL approach did not only result in a decreased average FDR, but also decreased FDR variability over the different patients compared to the personalization alternatives (see **Figure 4**).

By performing a two-sided paired t-test, the sensitivity of the PI and MIX algorithm were found to be not statistically different from the TL algorithm [$p = 0.29$ (PI vs. TL) and $p = 0.82$ (MIX vs. TL)]. However, the FDR of the PI and MIX algorithm are different compared to the TL algorithm [$p < 0.001$ (PI vs. TL), $p = 0.03$ (MIX vs. TL)]. The fully PS approach has a lower Se than the TL algorithm ($p = 0.01$), whereas the FDRs are not significantly different ($p = 0.29$). The F_3-score of the PI, fully PS and MIX algorithm are lower than the TL algorithm [$p < 0.001$ (PI vs. TL), $p = 0.01$ (PS vs. TL), $p < 0.01$ (MIX vs. TL)]. This shows that the proposed transfer learning method is indeed statistically better than the other evaluated approaches for personalization. The average detection delay for the proposed transfer learning approach was 21 s.

Table 2 also shows the results of both the patient-independent and personalized versions of the algorithms from the literature. The algorithms from van Elmpt et al. (15) and Jeppesen et al. (26) result in clearly lower sensitivity with a comparative FDR as the proposed patient-independent approach. The algorithm from Osorio (11) leads to nearly the same average result as the proposed patient-independent approach, with a slightly lower sensitivity, FDR and F_3-score. Both algorithms from Osorio (11) and Jeppesen et al. (26) show an increase in performance by personalizing the thresholds, but result in a lower performance than the proposed TL approach. The personalized version of van Elmpt et al. (15) leads to a strongly increased sensitivity, but also the FDR increases.

As explained in section 2.8, the influence of the number of training seizures on the TL algorithm performance was investigated. **Figure 5A** shows the impact on the F_3-score performance in function of the number of seizures available during training. The F_3-score for the TL approach strongly improves compared to the PI performance if only one patient-specific seizure is available in the training set. When two seizures are available, the variation between the results decreases, while the average performance increases. The performance further increases by including additional seizures to the training set, while still maintaining a similar variation in results. **Figure 5A** also shows the effect of the number of seizures in the training set for the other personalization methods, which results in clearly lower performances for each number of available seizures.

The same simulation results for the state-of-the-art algorithms are shown in **Figure 5B**. The algorithms from Osorio (11) and van Elmpt et al. (15) lead to a decent increase with one available patient-specific seizure, but only increases in accuracy slowly by adding more seizures. This increase is stronger for Jeppesen et al. (26) by adding more seizures, although the results with four available seizures are still worse than the proposed patient-independent algorithm. Although the algorithm from Osorio (11) had a similar patient-independent F_3-score, the proposed personalized TL approach outperforms the personalized version of Osorio (11).

TABLE 2 | Results for the patient-independent (PI), fully patient-specific (PS), mixed (Mix) and transfer learning (TL) approach.

	Se (%)		FDR (FP/h)		F_3-score	
	P-Av	**T-Av**	**P-Av**	**T-Av**	**P-Av**	**T-Av**
Proposed method						
PI	**75 ± 22**	**76**	3.0 ± 1.3	3.0	0.22 ± 0.14	0.20
PS	58 ± 27	59	2.2 ± 1.7	2.3	0.24 ± 0.20	0.19
MIX	72 ± 24	74	2.6 ± 1.5	2.5	0.25 ± 0.17	0.22
TL	71 ± 27	73	**1.9 ± 1.1**	**2.0**	**0.30 ± 0.22**	**0.26**
Jepessen (26)						
PI	51 ± 32	52	2.8 ± 1.6	3.0	0.16 ± 0.17	0.13
PS	46 ± 23	49	2.0 ± 1.5	1.9	0.23 ± 0.21	0.18
Osorio (11)						
PI	71 ± 25	72	2.8 ± 1.2	3.0	0.22 ± 0.15	0.19
PS	75 ± 23	76	2.3 ± 0.8	2.3	0.27 ± 0.18	0.24
VanElmpt (15)						
PI	59 ± 35	61	3.4 ± 2.4	3.5	0.17 ± 0.15	0.14
PS	76 ± 20	77	4.9 ± 3.4	5.2	0.19 ± 0.17	0.13

Both patient average (P-Av) ± standard deviation and overall average (T-Av) are shown in the table.

Bold values indicate the best result for all proposed algorithms for a specific metric.

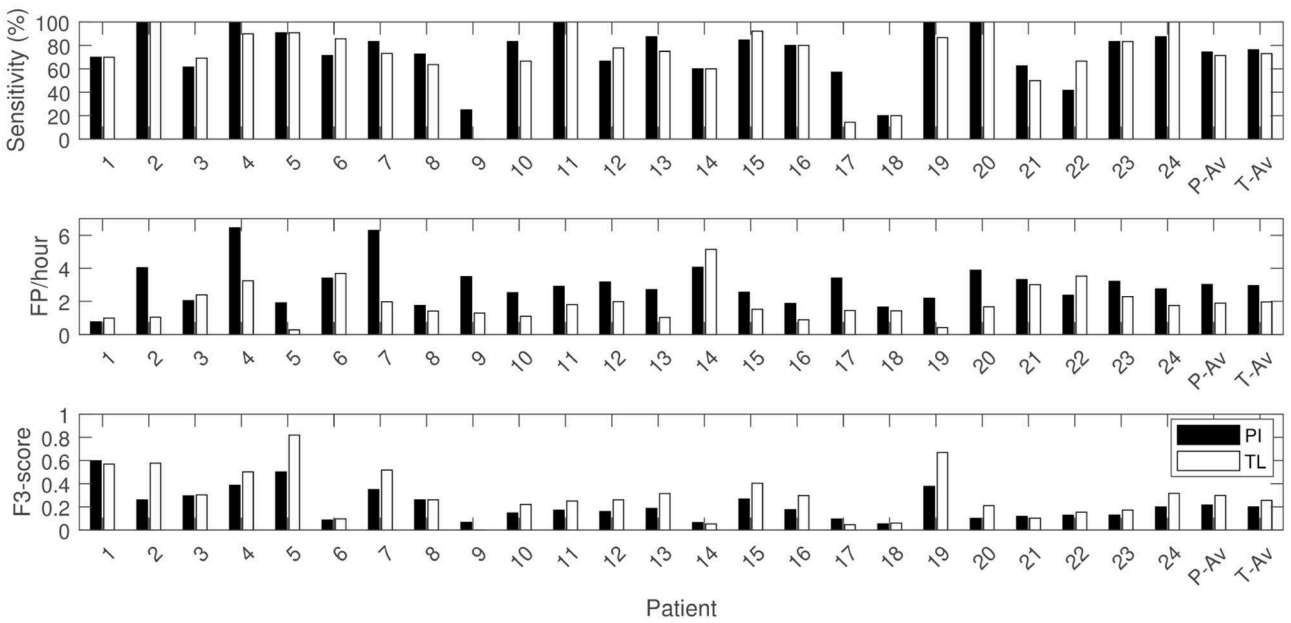

FIGURE 3 | Sensitivity, FDR and F_3-score per patient with patient average (P-Av) and overall average (T-Av) performances for the patient-independent (PI) and transfer learning (TL) algorithm.

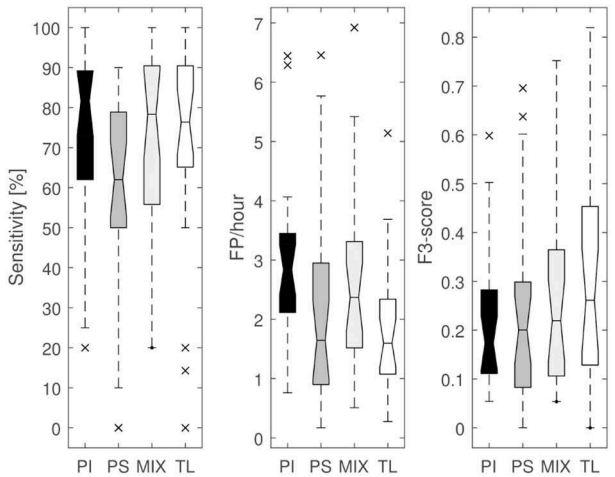

FIGURE 4 | Boxplots of sensitivity, FDR and F_3-score for the patient-independent (PI), fully patient-specific (PS), mixed (MIX) and transfer learning (TL) algorithm.

4. DISCUSSION

4.1. Performance Comparison of the PI and TL Approach

Table 2 and **Figure 4** show that the mean and median sensitivity of the PI and TL approaches are similar, whereas the FDR decreases and the F_3-score increases. By looking at the patients individually (**Figure 3**), it can be observed that the TL approach clearly reduced the FDR for some patients without decreasing the sensitivity (e.g., patients 2, 5, and 20). The transfer learning model adapts well to the patient-specific heart rate characteristics. In these patients, the ictal heart rate changes are often very stereotypical, showing little intra-patient variability, which leads to a strong decrease in FDR. **Figure 6** illustrates the impact of the number of seizures on the performance for some patients. For patient 5, the personalized approach already gets most performance increase by only including 1 patient-specific seizure. There is also limited variability in the results of the different simulations, showing that the model is accurate and robust for this patient, and little intra-patient variability is found in the ictal HRIs. For patient 2, also a fast personalization can be obtained, but more variability is found between the results of the simulations. This is due to a larger intra-patient ictal variability, so that the selection of seizures in training has a bigger impact on the performance. This impact however reduces if more patient-specific seizures are added to the model, leading to a more robust personalized model. Patient 15 had a lot of seizures during the recording, but these seizures showed a large intra-patient variability in terms of ictal HRIs. This intra-patient variation was mostly caused by the variation of seizure duration, which were typically to short to be strongly differentiable from non-epileptic HRIs. The ictal HRIs are also harder to differentiate from non-ictal HRIs compared to patients 2 and 5, leading to a slower learning curve than those from patients 2 and 5. In general, the steepness of the learning curve and amount of seizures before convergence depend on both ictal and inter-ictal heart rate behavior of each patient.

For some patients, however, the sensitivity dropped slightly. This is due to the fact that the model adapts to the patient characteristics. However, the heart rate characteristics during

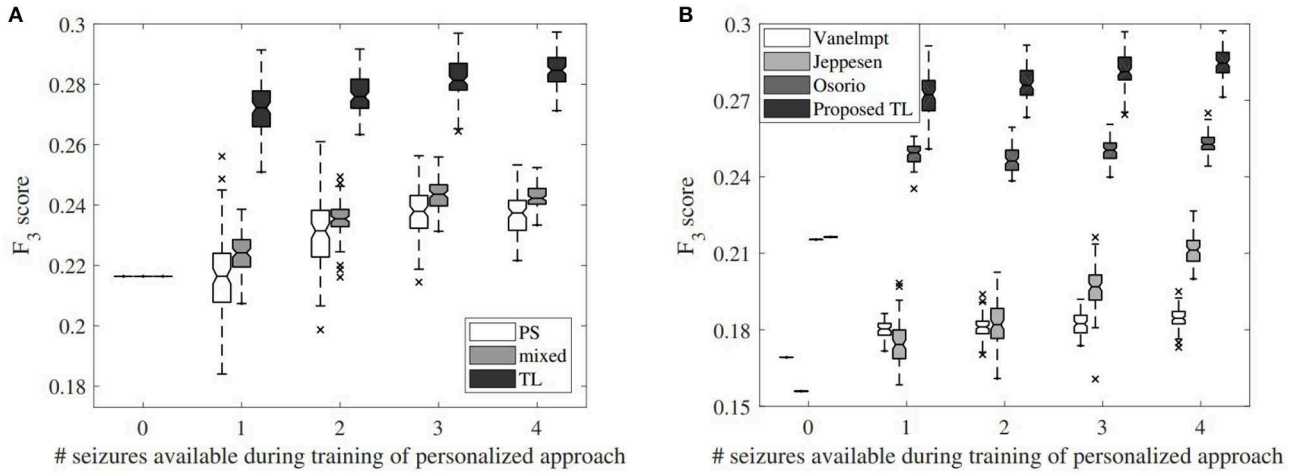

FIGURE 5 | Impact of the number of seizures on the F_3-score performance for different alternatives. **(A)** Effect for the different proposed personalization approaches, including the full patient-specific (PS) approach, mixed approach (mixed) and transfer learning (TL) approach. In case 0 seizures are available for training, the performance of the patient-independent algorithm is shown. **(B)** Effect for the different algorithms implemented from the literature, compared to the proposed transfer learning approach.

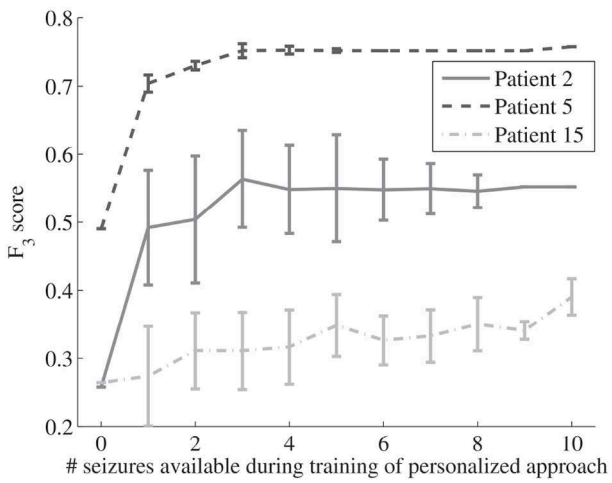

FIGURE 6 | Impact of the number of seizures on the average F_3-score performance (including standard deviations for the performed simulation test) for the proposed personalized transfer learning approach for patients 2, 5, and 15.

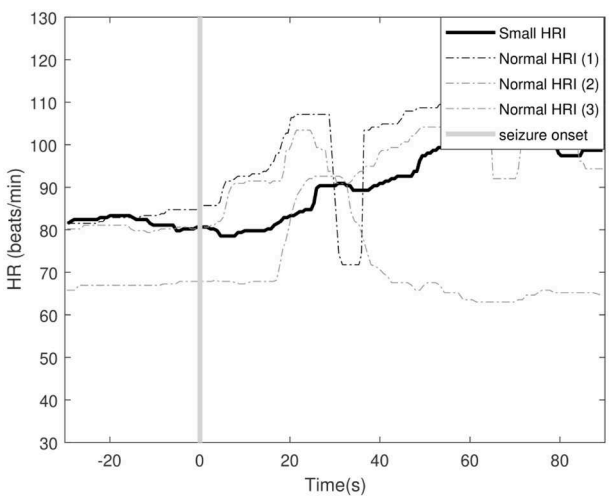

FIGURE 7 | Example of an ictal small heart rate increase (HRI) and 3 normal ictal heart rate increases for patient 8. The vertical line indicates the seizure onset of the different seizures. The shown tachogram signals are filtered using the median filter discussed in section 2.2.

some seizures were atypical (different from other seizures) for that patient. Less severe seizures were typically accompanied with smaller heart rate increases. Those seizures were sometimes not detected with the TL model. An example of a smaller ictal heart rate increase compared to normal ictal heart rate behavior for a particular patient is illustrated in **Figure 7**. The proposed TL approach adapts to the majority of seizures, and therefore might lead to a missed detection of these atypical seizures. However, this small decrease in sensitivity is often accompanied with a strong decrease in FDR for those patients. The reverse also occurred in some patients, where borderline seizures that were missed by the PI approach are detected by the proposed TL approach (e.g., patient 24). The results above show that, although personalization in general allows to improve the performance, it still has difficulty to counter unpredictable intra-patient variability in seizure behavior. If five seizures within a patient are stereotypical and used for training, an atypical sixth seizure will not be detected accurately using supervised personalization. This is however typically not the case for stronger seizure type (e.g., focal to bilateral seizures), as they are typically easier to detect with heart rate-based seizure detection, even if the algorithm is not specifically trained for this seizure type.

This increase in performance was, however, not achieved for all patients, for example in patient 1. For those patients, rather

atypical ictal HR increases are observed, or the HRIs are too weak in magnitude, making it difficult to differentiate them from non-epileptic heart rate activity. If the seizure activity is too similar to non-seizure activity for that patient, the model cannot be improved by means of personalization. Other ECG-based features or features from other modalities might have to be included then in order to achieve better performance for these patients.

Only 84.7% of seizures had ictal HRIs in the analyzed dataset, which is a similar percentage as in the literature (6–8). Personalizing the algorithm will not help to detect these seizures without ictal heart rate changes. Also false detections or missed seizures caused by too strong ECG noise cannot be avoided by the personalization as they can occur both ictally and inter-ictally (27). Other approaches should be used to further improve the performance, such as improved noise removal techniques.

An example of the obtained classifier boundaries for patient 11 are illustrated in **Figure 8**. The seizure and non-seizure data points are shown together with the PI, PS and TL boundary for two different values of hyperparameter D (0.01 and 1), with a fixed $\widetilde{\gamma}$ value of 1.5. The PI boundary gives a good general indication, but lacks adaptation to the patient characteristics. The TL approach adapts to these characteristics with a limited amount of available patient-specific data. By choosing a low value for D (i.e., 0.01) the TL model will be similar to the PI model because a relative low weight is given to the errors obtained for the patient-specific data compared to the first term in (5), which quantifies how different the new model is to the original one. With a higher value of D the model adapts more to the patient-specific data (and the corresponding errors $\tilde{\xi}_k$) and shows less similarity to the PI boundary. The PS boundary is less optimal, compared to the TL solution. It can be seen from the TL boundary that it still contains information gathered in the PI classifier (especially for low values of D), indicating the added value of this approach. This way, the FDR is strongly decreased, without affecting the sensitivity.

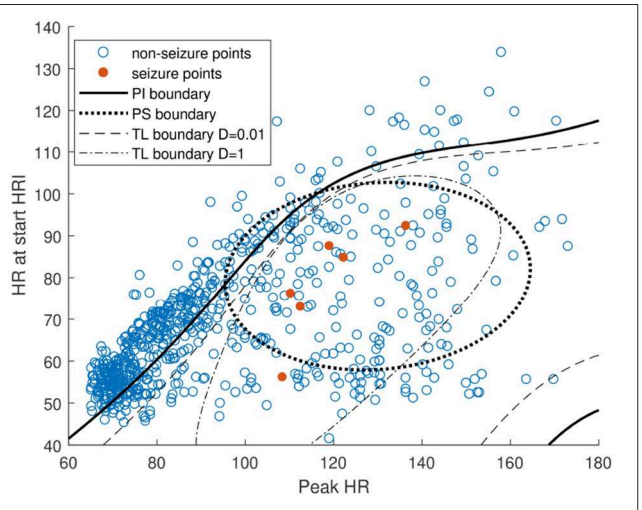

FIGURE 8 | Visualization of SVM boundary of the patient-independent (PI), fully patient-specific (PS), and personalized transfer learning (TL) classifier for different D-values (0.01 and 1).

4.2. Comparison of Alternative Personalization Solutions

Different alternatives for personalization were implemented and evaluated in order to compare with the proposed transfer learning approach (see **Table 2** and **Figure 4**). The naive approach only uses patient-specific data points for training a normal SVM classifier. Despite all patients had at least 5 seizures, this was still an insufficient amount of data for most patients in order to get a robust patient-specific classifier. In Cogan et al. (17), it was mentioned that at least 6–8 seizures were required in order to make a personal algorithm, and the reason for this was to better include the inter-seizure variability of autonomic changes within a patient. Due to the relative low amount of patient-specific data, it often occurred that the classifier was overtrained on a limited amount of seizure data, not taking into account potential fluctuations between different seizures from a patient. This led to a strong decrease in sensitivity, although the FDR was not so much higher than the proposed TL approach. The TL approach is able to better take this inter-seizure variation into account by holding on to the knowledge described by the reference PI classifier.

The mixed model (MIX) uses a mixture of patient-specific data with data from other patients in the training set of a standard SVM training procedure. It produced more robust results than the fully patient-specific approach without strong negative outliers. However, it generated, on average, around 0.5 FP/h more than the proposed TL approach. Applying transfer learning to the reference classifier allows to better take over the information of the PI classifier and translate it to the patient-specific model, whereas the mixed approach would try to create a new model without this prior model knowledge. The proposed transfer learning method not only leads to a better performance, but is also trained faster with an optimization problem which contains less data to analyze.

4.3. Impact of Number of Seizures on Personalization

Transfer learning allows to train a personalized classifier for heart rate-based seizure detection. It is however important to have an idea of how much data is actually needed for this. In seizure detection, the amount of seizures is often the restricting factor as some patients have a low seizure frequency. In section 2.8, a simulation study was described to evaluate the impact of the number of seizures in training on the personalized performance (see **Figure 5**).

The proposed TL approach already leads to a strong increase in F_3 performance by only including 1 seizure in the training set. This shows that with only 1 seizure, the algorithm can already be personalized. There is a lot of variation in the results, which is caused by the heterogeneity of the seizures in the training set. There is also a lot of variation in ictal heart rate changes between different seizures within a patient, so if an atypical seizure is used in training, this will lead to suboptimal results for that patient. By using 2 seizures during training, the F_3 performance increases slightly, and also the variability between results of different simulations decreases. However, from 3 seizures onwards, the

variability is no longer strongly decreased with the proposed method. The average performance increases further up until 4 available seizures, and is expected to increase further if more seizures are included in training (17). From a certain amount of seizures, it is however expected that the fully PS approach would lead to a better performance than the proposed TL method. Nevertheless, it is currently not known how many seizures are required for this with the proposed procedure.

Figure 5 also shows the effect of the number of seizures on the alternative personalization approaches. It can be seen that by only having 1 patient-specific seizure in the training data, the TL performance strongly increased, whereas this increase is less evident for the other approaches. For the PS approach, the median performance is increased, but a large portion of the results were actually worse than for the reference PI approach. This is due to the fact that the PS method is often strongly overfitting on data from 1 seizure, which is not a robust way for training a classifier. The average F_3 scores for the fully PS approach increase by adding more seizures, and the variation on performance decreases. The results however remain lower than those from the proposed TL approach when using 4 seizures. The mixed model is more robust than the fully PS approach for a limited amount of seizures, but it slightly loses its added value when more seizures are added to the training set.

4.4. Comparison to the Literature

Different algorithms from the literature were also implemented both patient-independently and patient-specifically and evaluated on the dataset described in section 2.1. **Table 2** shows that the patient-independent version of the proposed algorithm clearly outperforms the algorithms from van Elmpt et al. (15) and Jeppesen et al. (26), but has a similar result as Osorio (11). A simplified patient-independent algorithm from De Cooman et al. (10) is used here as described in section 2.3, which has shown to outperform the literature. Due to the simplification, the added value of this algorithm over (11) is reduced, but its added value compared to van Elmpt et al. (15) and Jeppesen et al. (26) remained similar.

However, when patient-specific alternatives for these three algorithms were made using the automatic procedure described in section 2.9, the proposed personalization procedure clearly outperforms all these algorithms (see **Figure 5B** and **Table 2**). The algorithm from Osorio (11) shows a big increase by personalizing the algorithm, even for only one available seizure, but the added value is much smaller compared to the proposed transfer learning approach. Smaller increase in performance is found in the algorithm of van Elmpt et al. (15), with only a very slow learning curve with increasing number of annotated seizures per patient. A much steeper learning curve is found for the algorithm from Jeppesen et al. (26). Although the algorithm has the lowest patient-independent performance, the performance increases very fast with increasing number of seizures. The algorithm was originally meant for patient-specific evaluation. However, even with four seizures available per patient, it performs worse than the proposed personalized transfer learning method. The proposed personalization method using transfer learning thus not only performs more accurately in general, it also allows a much faster training if only a limited amount of patient-specific seizures are available. This is crucial in practice as some patients have a very low seizure frequency, and thus the personalized algorithm can reach much faster a desired level of accuracy than state-of-the-art algorithms.

4.5. Limitations of the Study

There are however some limitations to the performed study that have to be taken into account. First of all, the data is recorded in the hospital, where the patients were restricted to move within their room. This leads to a limited activity of the patient, which can lead to an underestimation of the amount of false detections in practice. However, it is compared to state-of-the-art algorithms from the literature on the same dataset, and has proven to outperform these on this dataset. However, currently no study in the literature has shown results of such heart rate-based algorithms for full day-and-night monitoring in a real home environment.

Furthermore, during the presurgical evaluation, drug treatment can be altered or completely removed. This can influence the results in two ways. First, certain drugs can alter the heart rate variability of the patient, which might lead to different (stronger) ictal and inter-ictal heart rate changes during the presurgical evaluation compared to the home situation. In some patients, indeed small changes in heart rate variability could be found during the first and last day of monitoring. However, the ictal heart rate changes between the first and last day of monitoring were found to be limited compared to the variability that has to be taken into account for the circadian fluctuation of the heart rate features. Therefore, this did not lead to extra missed seizures or a large increase in false detection rate in this study. A second influence is the fact that stronger seizures can occur during the presurgical evaluation compared to the typical home situation. However, only a couple of focal to bilateral seizures were found in our dataset, and the large majority of seizures were perceived to be typical seizures for that patient in their home environment. Other drugs not applied in this study could however have stronger influence on the heart rate variability and the ictal changes. This should be further investigated in future work, as it could influence the usability of data from the presurgical evaluation as training data for such algorithms when used in a home environment.

No dedicated wearable ECG derived device is used in this study. Currently, a large portion of false detections and a small percentage of missed seizures is caused due to poor ECG quality, largely due to the wiring. A previous study has shown that better performance can be obtained by using a wearable ECG device rather than the standard wired hospital ECG (27). This would however be the case for all evaluated algorithms in this study, including those from the literature tested in this study on the discussed dataset.

4.6. General Discussion and Future Work

The proposed method allows a fully automated personalization of heart rate-based seizure detection. In literature, personalization was often reached after adjusting thresholds manually after visual inspection of the data (15, 16). This is however a very costly and non-scalable solution. In Jeppesen et al. (26), the personalization was done automatically by adjusting the threshold per patient

based on data from a non-epileptic segment of 30 min. Although this is a more scalable solution, only 30 min of non-seizure data does not contain sufficient information to grasp the full complexity of the heart rate variability of that patient (e.g., the circadian rhythm). The proposed solution does take into account much more complexity in a fully automated way, which allows a better implementation in practice.

Despite the increased performance caused by personalization through transfer learning, still a too high FDR is obtained for usage in practice. Personalization allows to solve some of the issues leading to a too high FDR, but is not able to solve all issues related to heart rate-based seizure detection. Some seizures do not contain ictal heart rate changes, and still a large portion of non-epileptic heart rate changes cannot be differentiated from epileptic HRIs with the current techniques. The proposed method should, however, be used as part of a multimodal algorithm, where it is combined with another modality. Such multimodal combination has shown to lead to FDR decreases with factors 5–10 compared to unimodal performance (21). Similar to the seizure detection algorithms, also the used modalities should be chosen for each individual patient based on its typical ictal changes and seizure type. Accelerometers and EMG sensors could lead to an increased performance for the detection of motor seizures (21, 28). For the detection of non-motor focal seizures, behind-the-ear EEG could be used (29). The advantage of heart rate-based seizure detection over other modalities is that ECG is often monitored as well during video-EEG monitoring in the hospital, which allows to get accurately annotated heart rate data to personalize the algorithm. It is also ictally the most activated modality (apart from full EEG), so it is ideal to increase the detection performance of a wide range of seizure types. If a late integration approach is used for combining information from different modalities, the proposed personalized method can be easily integrated and further improve the multimodal performance with a similar accuracy increase as in an unimodal setting.

The proposed transfer learning approach is a supervised approach, which means that annotated data was required. In practice, these annotations can be made in the hospital during, for example, presurgical evaluation, but they could also be made by the patient or their caregivers/family. Extra procedures should then be added to avoid a too big impact of incorrectly annotated data as patients are not always aware about whether they actually had a seizure or not (3, 14). Ideally, an unsupervised approach could be used (9, 25, 26), which indicates that epileptic heart rate activity can be seen as an outlier to normal heart rate activity. This is however only the case during the night (30) or in certain severe seizure types, which makes this approach only sufficiently successful for nocturnal monitoring of severe seizures. Supervised approaches are thus still required for personalizing full day monitoring applications. Due to the supervised approach, still at least one patient-specific seizure is required in order to adapt to the patient characteristics. In patients who have a very low seizure frequency, this might still be a problem for a fast personalization. For these patients, it is then advised to have a large pool of patients, and seizures from patients with similar HRV parameters as the test patient. Then, these seizures could be added to the patient-specific data in order to be able to apply this method.

5. CONCLUSION

Transfer learning allows to personalize heart rate-based seizure detection in a fast and robust way by using only a limited amount of annotated patient-specific data. The false detection rate dropped by 37% compared to the patient-independent approach while maintaining a similar sensitivity. The novel automated personalization approach proposed in this study outperforms the state-of-the-art patient-independent algorithms while also being less prone to overfitting than manual state-of-the-art patient-specific approaches. The proposed method can be used as part of a multimodal algorithm in order to increase the performance and make real-time epileptic seizure warning systems clinically feasible.

DATA AVAILABILITY STATEMENT

The datasets for this study will not be made publicly available because the patients and healthy participants in the study have not given their approval for the data to be made public. Requests to access the dataset can be sent to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the International Conference on Harmonization guidelines on Good Clinical Practice with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Medical Ethics Committee of the University Hospitals KU Leuven.

AUTHOR CONTRIBUTIONS

TD, KV, CV, BH, EC, WV, and SV designed the experiments, wrote and revised the manuscript. WV and EC collected the data for the experiments. TD and KV carried out all experiments.

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METHODS
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Deep Convolutional Neural Network-Based Epileptic Electroencephalogram (EEG) Signal Classification

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Electroencephalogram (EEG) signals contain vital information on the electrical activities of the brain and are widely used to aid epilepsy analysis. A challenging element of epilepsy diagnosis, accurate classification of different epileptic states, is of particular interest and has been extensively investigated. A new deep learning-based classification methodology, namely epileptic EEG signal classification (EESC), is proposed in this paper. This methodology first transforms epileptic EEG signals to power spectrum density energy diagrams (PSDEDs), then applies deep convolutional neural networks (DCNNs) and transfer learning to automatically extract features from the PSDED, and finally classifies four categories of epileptic states (interictal, preictal duration to 30 min, preictal duration to 10 min, and seizure). It outperforms the existing epilepsy classification methods in terms of accuracy and efficiency. For instance, it achieves an average classification accuracy of over 90% in a case study with CHB-MIT epileptic EEG data.

Keywords: epileptic EEG signal classification, power spectrum density energy diagram, deep convolutional neural networks, electroencephalogram, EEG

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1. INTRODUCTION

Epilepsy is a chronic disease involving sudden and repeated seizures of brain dysfunction. Due to different starting locations and transmission modes of abnormal electrical activities in brains, there are various complex clinical manifestations of epilepsy, including transient sensory disorders, limb convulsions, loss of consciousness, behavioral disorders, etc. Such clinical manifestations of epilepsy can cause severe physical damage and mental trauma to patients (1). Monitoring electrical activities in the brain and identifying progressing epileptic states and the possible occurrence of seizures can be helpful to mitigate the adverse effects of seizures (2).

The electroencephalogram (EEG) has been a prevalent approach for examining brain activities in epilepsy. For patients with epilepsy, the EEG signals of their brain activities can be categorized into interictal, preictal, and seizure states. When a seizure occurs, the EEG signals exhibit certain unusual patterns. Moreover, the EEG signals of the preictal state and the interictal state also show distinctive patterns. Therefore, these patterns in the EEG signals can be used to differentiate epileptic states, enabling the identification of the progress and the potential occurrence of a seizure and the mitigation of damaging impacts on the patients.

Seizure detection by using EEG signals has been investigated for decades (3–5). For instance, Gotman (6) proposed time-domain feature extraction from the EEG waveform for seizure detection in 1982. In 2006, Jahankhani et al. (7) used a discrete wavelet transform (DWT) to extract EEG features and combined the multilayer perceptron network (MLP) and radial basis function network (RBF) for classification. Wang et al. (8) recognized seizures with different parameters of wavelet coefficients in each frequency band from EEG signals. Acharya et al. (9) decomposed EEG signals into sub-band signals by wavelet packet transform, then took the high-order cumulants of sub-band signals as EEG features, and combined these with a support vector machine (SVM) classifier to complete epilepsy detection. Song et al. (10) used approximate entropy and sample entropy as EEG features, respectively, and combined these with an extreme learning machine (ELM) for automatic detection of epileptic seizures. Based on pattern recognition, a novel method for detecting seizures was presented and tested using the Freiburg database. The method was applied for symbolic data analysis of the EEG signals based on N-gram modeling, a probabilistic pattern recognition technique that identifies the occurrence of symbolic data sequences within data (11). The authors proposed a method based on the mean phase coherence (MPC). MPC was originally proposed by Mormann et al. as a measure of phase synchronization and was found to decrease before seizure onset (12). Williamson et al. proposed a method combining patient-specific machine learning and multivariate features (13). The features were based on the eigenspectra of space delay correlation and covariance matrices computed at multiple time delays.

In recent years, deep learning has started to gain popularity for medical image analysis and bioelectric signal processing. With a large amount of data, it outperforms traditional feature extraction and machine learning methods in pattern detection and image recognition in terms of classification accuracy (14). Deep learning algorithms, especially the convolutional neural network (CNN), are also gradually being adopted for seizure detection. For example, Acharya et al. (15) used a 13-layer depth CNN with EEG signals to detect epileptic seizures and achieved an accuracy of 88.7%. Hu et al. (14) generated a mean amplitude spectrum (MAS) map from EEG signals and incorporated CNN and SVM for feature extraction and classification. The method identified seizure with an accuracy of 86.25%. Besides classification accuracy, sensitivity (i.e., probability of detection) is also used to evaluate the classification performance. Truong et al. (16) applied CNN to learn features from time-frequency energy maps of EEG signals and realized classification with a sensitivity of 89.8%. Khan et al. (17) also used a CNN architecture with six convolutional layers to extract features from the wavelets of EEG signals and achieved seizure detection with an average sensitivity of 87.8%.

However, most of these studies applied domain knowledge to select a specific channel from multichannel EEG signals for analysis, while the data-driven analysis with multichannel epileptic EEG signals remains unexplored. Moreover, there is still room left to further improve the accuracy and efficiency of seizure classification from EEG signals with advanced signal processing and deep learning algorithms. This paper focuses on enhancing the accuracy of classification by analyzing EEG signals of different epileptic states in the brain, which would be helpful for the potential detection of seizures in future study. The epileptic states include an interictal state, a preictal state, and a seizure state. The preictal state can be further divided into two durations: preictal duration to 30 min (denoted as "preictal I") and preictal duration to 10 min (denoted as "preictal II"). These four categories of epileptic states can be determined from EEG signals. Usually, the small differences in the features of EEG signals between the interictal and preictal states and between the "preictal I" and "preictal II" states are hardly visible or not discernable by eye. However, the dissimilarity in these epileptic states can be captured by deep learning with superior computational power. Therefore, we aim to achieve accurate epileptic state classification by proposing a deep learning-based classification methodology for multichannel EEG signals, named "epileptic EEG signal classification (EESC)." It adopts wavelet transform and power spectrum density (PSD) to preprocess the multichannel EEG signals and incorporates three deep convolutional neural network (DCNN) models for feature extraction and epileptic state classification.

2. EPILEPTIC EEG SIGNAL DATA

Epileptic EEG signal data in the CHB-MIT database (an open-source public database) are used to verify the effectiveness of the proposed EESC methodology in the case study. Extensive comparisons with the existing epilepsy classification algorithms are implemented. The CHB-MIT database contains child scalp electroencephalogram (sEEG) data from 23 cases (18) that were recorded continuously for 844 h with 163 epileptic seizures. The majority of the sEEG signals are collected through 23 channels with a sampling rate of 256 Hz. Electroencephalographers require EEG abnormalities to persist and evolve for at least 6–10 s before they consider the abnormality to be a seizure, so only the data of patients with seizures of more than 6–10 s were included (16). In our analysis, the EEG signals from 11 patients are used; the seizure duration for these 11 patients are listed in **Table 1**.

As mentioned in the Introduction, the EEG signals can be divided into interictal, preictal, and seizure states. Moreover, classifying epileptic seizures 10 to 30 min in advance can help prevent and mitigate the adverse effects of possible seizure occurrence. Up to now, there is no consensus on the earliest detection time before the seizure occurrences. In our analysis, we choose the latest detection time as 10 min, since the EEG signals can show certain peculiar signals when close to the occurrence of seizure (for instance, 5 min before the seizure). It is of use to further divide the preictal state into two durations: preictal duration to 30 min (denoted as "preictal I") and preictal duration to 10 min (denoted as "preictal II") for differentiating the importance of duration to the epilepsy progress. Therefore, the EEG signals are divided into four categories: interictal, preictal I, preictal II, and seizure state.

The final dataset for the analysis includes the EEG signals from 11 patients with a total of 56 min of EEG signals in the seizure state and 110 min in the preictal I, preictal II, and interictal states

TABLE 1 | Seizure duration of eleven selected patients from the CHB-MIT database.

Patient	Gender	Age	Seizure number	Seizure duration (seconds)
Chb01	F	11	7	402
Chb02	M	11	3	172
Chb03	F	14	7	402
Chb07	F	14.5	3	325
Chb10	M	3	7	447
Chb17	F	12	3	293
Chb18	F	18	6	323
Chb19	F	3	3	236
Chb20	F	8	8	294
Chb21	F	4	4	199
Chb22	F	3	3	294

respectively for classification. The EEG signals are divided into frames, with a length of 4 s. Since the EEG signals in the seizure state are shorter than other states, we overlap the consecutive frames of the EEG signals in the seizure state by 2 s. For the classification algorithm, we hold out 70% of the data as the training set and use the remaining 30% as the testing set.

3. CLASSIFICATION METHODOLOGY

In this paper, an epileptic EEG signal classification (EESC) methodology based on deep convolutional neural networks is proposed to classify four critical epileptic states with multichannel EEG signals. The overall framework of the proposed methodology is summarized in **Figure 1**, and it proceeds in two primary steps: (1) data preprocessing and feature extraction: to denoise multichannel EEG signals and transform them with power spectral density analysis; (2) epileptic EEG signal classification: to classify epileptic states with deep convolutional neural networks (DCNNs) and transfer learning.

3.1. Multichannel EEG Signal Preprocessing and Feature Extraction

Multichannel epileptic EEG signals are used to classify four categories of epileptic states, namely the interictal state, preictal I, preictal II, and seizure state. The features of these multichannel EEG signals are represented by characterizing the energy variation of the signals in the frequency domain. To obtain the effective characteristics of multichannel EEG signals, they are first denoised by wavelet transform and then analyzed by power spectrum density (PSD). The two-dimensional images generated from PSD, called power spectrum density energy diagrams (PSDEDs), reflect the energy information of different frequency bands of the EEG signals. PSDEDs are used as features for the subsequent classification since they reveal the differences among the four categories of epileptic states.

3.1.1. EEG Signal Denoising

The original EEG signals are collected on human scalps, so they are inevitably full of noise (such as EEG artifacts, minor interference) and have a low signal-to-noise ratio. In order to reveal the characteristics of EEG signals, they first undergo a denoising procedure (19). For this paper, a wavelet threshold denoising method is used. Particularly, the Daubechies wavelet of order 6 (dB6) is chosen as the mother wavelet for applying discrete wavelet transform (DWT) in denoising (20). The denoised EEG signals are able to highlight the information in different epileptic states, particularly the interictal state, for analysis.

3.1.2. Power Spectrum Density Analysis and Power Spectrum Density Energy Diagram

Power spectrum density (PSD) analysis is used on the denoised multichannel EEG signals for feature extraction. The main idea here is to extract the corresponding EEG features by characterizing the energy variation of the signal in the frequency domain (21). PSD can represent the distribution of signal power in the frequency domain (22). As mentioned in the previous section, the EEG signals are segmented into 4-s frames. PSD analysis is implemented on these 4-s frames of the EEG signals, and the resulting periodograms are shown in **Figure 2A**. It is noticed that the power spectrum density (or energy) of the EEG signals is different among the epileptic seizure, preictal, and interictal states. Therefore, PSD analysis is a viable way to extract features for different epileptic states.

Furthermore, the EEG signals can be transformed into two-dimensional images called power spectrum density energy diagrams (PSDEDs). For instance, when multichannel EEG signals have n channels, the periodogram is obtained for each channel of the EEG signals. If the EEG signals are divided into 32 frequency bands, and PSD functions of different frequency bands can be integrated. A two-dimensional matrix with n rows and 32 columns is then formed and is then normalized to generate a PSDED. The power spectrum densities in the interictal, preictal I, preictal II, and seizure state for one of the patients are shown in **Figure 2B**. Deep learning will capture such visible differences. Therefore, PSDED is a suitable feature of multichannel EEG signals for the subsequent classification of different epileptic states (23).

3.2. Epileptic EEG Signal Classification

Here, epileptic EEG signal classification (EESC) is used for classifying four different epileptic states by using deep convolutional neural networks (DCNNs) and transfer learning with the PSDEDs from the original multichannel EEG signals. The proposed method is shown in **Figure 3**. It integrates three DCNNs: Inception-ResNet-v2, Inception-v3, and ResNet152. They will be introduced in the following sections. In a transfer learning framework, these three DCNNs are loaded with corresponding pre-trained weights from ImageNet (24). Two fully connected layers and an output classification layer with softmax are concatenated to the DCNNs. The PSDEDs from the multichannel EEG signals are used to train and fine-tune these

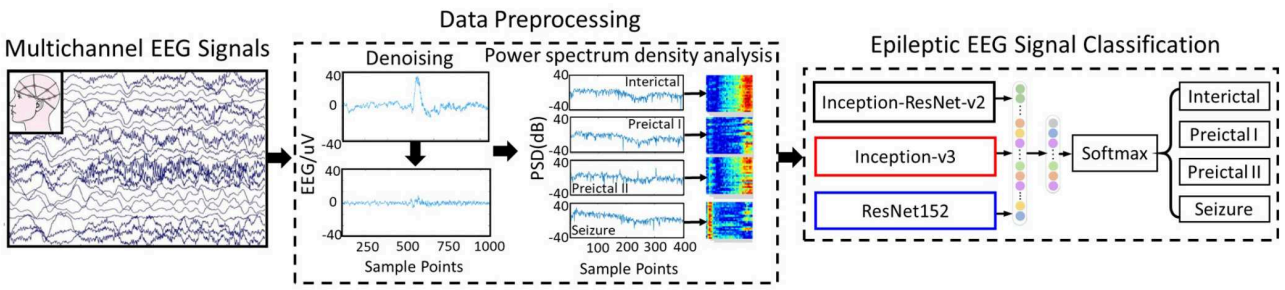

FIGURE 1 | The overall framework of the proposed epileptic EEG signal classification methodology.

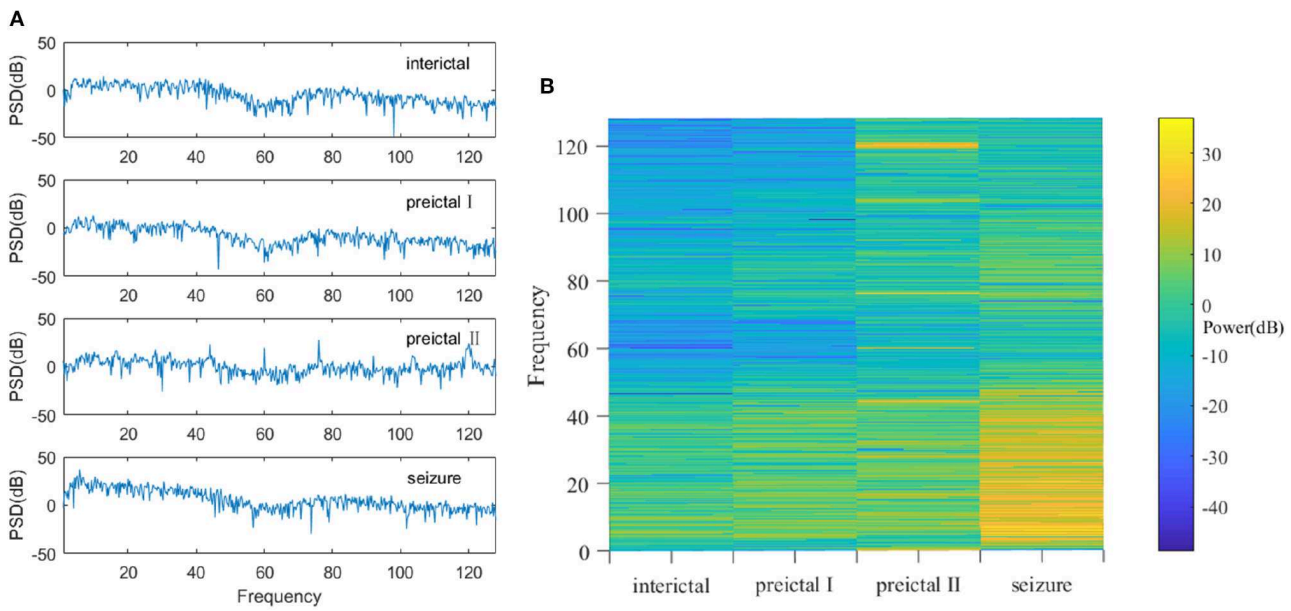

FIGURE 2 | (A) The PSD at interictal, preictal I, preictal II, and seizure states. The amplitude of the PSD signal varies greatly among different states. **(B)** Power spectra in interictal, preictal I, preictal II, and seizure states. They are quite different, especially in the low frequency areas (i.e., the region of interest in EEG).

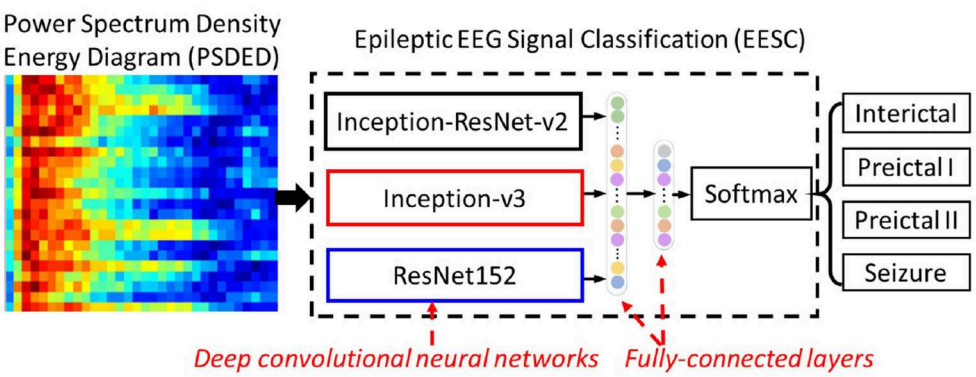

FIGURE 3 | The proposed epileptic EEG signal classification (EESC) integrates three deep convolutional neural networks (DCNNs) and fully connected layers.

deep neural networks. Finally, the proposed EESC is ready for classifying the different epileptic states for seizure classification.

3.2.1. Model Structure of the Proposed Epileptic EEG Signal Classification

3.2.1.1. Inception-v3

The architecture of Inception-v3 (25) has been greatly improved on Googlenet. In Inception-v3, the large convolution kernels are decomposed into small convolution kernels to reduce computational complexity and enhance the non-linear expression of features. In the proposed EESC, Inception-v3 has input images with a size of 299 × 299 × 3 and outputs 2,048-dimensional feature vectors.

3.2.1.2. ResNet152

ResNet can alleviate the problem of gradient vanishing in the training of DCNN by adjusting the traditional network structure. Its key structure is to propose the basic network unit, the residual block, by adding a shortcut connection. Residual blocks are used in the whole network as the basic units of ResNet as

$$y = f(x, w) + x \tag{1}$$

where x,y,$f(x, w)$ represent the input, output, and residual mapping of the block, respectively. By transforming the output y into the residual $f(x, w)$, the network is more sensitive to the small fluctuations between output y and input x than a plain network structure like VGGNet. In the proposed EESC, ResNet152 transforms the input image with a size of $224 \times 224 \times 3$ into a feature vector of 2,048-dimensions.

3.2.1.3. Inception-ResNet-v2

Inception-ResNet-v2 (26) combines the advantages of the Inception network and ResNet. The residual block is applied to the Inception block, which greatly improves performance and especially accelerates the convergence speed. Such improvement makes the deep network easier to train. In the proposed EESC, Inception-ResNet-v2 transforms the input image with a size of 299 × 299 × 3 into a 1,536-dimensional feature vector.

For each image, the feature vectors extracted from three network features are concatenated into a 5,632-dimensional feature vector. Two fully connected layers with 1,024 and 512 neurons are added to reduce the dimensions, and a dropout layer (0.5) is set behind each fully connected layer to prevent over-fitting. The softmax of the output layer is expressed as:

$$P(S_i) = \frac{e^{g_i}}{\sum_{k}^{n} e^{g_i}} \tag{2}$$

where k represents the number of categories, i represents a category in k, g_i represents the calculated value of that category, and softmax converts the calculated values into the output probability for each category.

3.2.2. Training Procedure of the Proposed Epileptic EEG Signal Classification

3.2.2.1. Transfer learning

Training the three DCNNs in the proposed EESC requires large amounts of data and time. Transfer learning (27) can be used to optimize network initialization by loading pre-trained weights. It inherits the trained network characteristics and increases training efficiency. There are two ways to apply transfer learning for training classification networks (28): (1) Loading the pre-trained weight, freezing the parameters before the fully connected layer, and only training the fully connected layer. (2) Loading pre-trained weights, and updating the parameters of the whole network during training.

When the current datasets differ greatly from the datasets used in pre-trained weight training, the second approach above is usually adopted. Transfer learning with the pre-training model facilitates the training of classification networks and enables a superior fine-tuning effect. First, the pre-trained weights of ImageNet are loaded to the proposed EESC deep neural networks, and then the weights are updated by the PSDED images from the original multichannel EEG signals. The PSDED images share similar basic features, such as lines, edges, etc., with images from ImageNet. Therefore, transfer learning can still learn important information on the weights of the EECS networks from networks trained with ImageNet data. For the classification algorithm, we hold out 70% of the data as the training set and use the remaining 30% as the testing set.

3.2.2.2. Loss function for EESC

The cross-entropy loss function is widely used in classification problems. Its formula is shown in Equation (3).

$$L_i = -[y_i log\hat{y}_i + (1 - y_i) log(1 - \hat{y}_i)] \tag{3}$$

$$L_{batch} = \frac{-\sum_{i}^{n} L_i}{n} \tag{4}$$

where y_i is the label and $\hat{y}_i$ is the predicted probability. During training, samples of a batch are fed to the network each time, and the mean value of the loss of samples in the batch is considered as the loss of the batch. Despite its simplicity, it cannot differentiate the losses from different samples in a batch during training and further improve the training accuracy.

An online hard example mining (OHEM) (29) loss function is used to replace the commonly used cross-entropy loss function; its expression is as Equation (5). In OHEM, the loss of a batch sample is sorted in descending order, and the largest k (top_k) values are averaged as the final loss. It prioritizes ambiguous samples with large loss values during training and improves the classification accuracy on those.

$$L_{ohem} = \frac{-\sum_{i}^{top_k} L_i}{top_k} \tag{5}$$

4. RESULTS AND ANALYSIS

4.1. Evaluation Metrics

Accuracy, sensitivity, and specificity are the metrics most widely used in the literature for evaluating model performance. They are derived from the correctness of prediction, including true positive (TP: correctly predicts the positive class), true negative (TN: correctly predicts the negative class), false positive (FP: incorrectly predicts the negative class as positive), and false negative (FN: incorrectly predicts the positive class as negative). They can be calculated as follows:

$$accuracy = \frac{TP + TN}{TP + TN + FP + FN} \tag{6}$$

$$sensitivity = \frac{TP}{TP + FN} \tag{7}$$

$$specificity = \frac{TN}{TN + FP} \tag{8}$$

Besides, the confusion matrix is a systematic way to illustrate the classification accuracy for the four categories of epileptic states in the case study. The classification rate displayed in the diagonal of the confusion matrix represents the accuracy of each category, while other values represent the percentage of misclassified samples. This paper focuses on the analysis of four epileptic states in EEG signals of patients to classify the four epileptic states accurately. We can classify the preictal, interictal, and seizure states, and this could potentially help detection.

4.2. Performance of the Proposed EESC Methodology

Epileptic EEG signals are first used by the three individual deep convolutional neural networks (ResNet152, Inception-v3, and Inception-ResNet-v2) for epileptic state classification. Four states can be classified and heat maps can be generated, as shown in **Figure 4**. The confusion matrices of their individual performance are shown in **Figures 5A–C**, respectively. It can be seen that: (1) the Inception-ResNet-v2 model outperforms the other two models except for preictal states; (2) all three models have higher classification accuracy for the interictal state and seizure state than for preictal I and preictal II.

Epileptic EEG signals are then used by the proposed EESC methodology. The confusion matrix of EESC is shown in **Figure 6A**. Compared with the three individual DCNN models above, the classification accuracy of all the four epileptic states is improved in the proposed EESC methodology, but preictal I and preictal II are still not classified as accurately as the other two states.

When integrating the OHEM loss function mentioned in section 3.2 into the EESC methodology, the classification accuracy of preictal I and preictal II is increased by 3 and 4%, respectively, as shown in **Figure 6B**. The improvement can be attributed to the strength of the OHEM loss function, which prioritizes samples with large losses during training and therefore increases the classification performance of the EESC methodology.

We summarize the classification accuracy of all the aforementioned models for epileptic EEG signal classification in **Figure 7**. It is shown that: (1) All three individual DCNNs models have decent classification accuracy, yet the proposed EESC methodology performs even better. (2) The integration of the EESC methodology with the OHEM loss function has superior performance in seizure classification. (3) For all models, the classification accuracy of the seizure state and interictal state is higher than that of preictal I and preictal II.

Furthermore, we compare the classification results from the aforementioned models in terms of sensitivity and specificity. In this case study, sensitivity is the percentage of correct classification of a particular epileptic state, while specificity is the percentage of correct classification for other epileptic states. They are summarized in **Figures 8**, **9**, respectively. The proposed EESC method with an OHEM loss function outperforms other methods in terms of both sensitivity and specificity. It has high sensitivity (97.8, 93.6, 92.3, and 95.8%) and specificity (99.2, 97.1, 97, and 99.3%) in classifying the four epileptic states (interictal, preictal I, preictal II, and seizure).

Finally, state-of-the-art research in epileptic EEG signal classification is applied to the dataset from the CHB-MIT database to implement a comparison with the proposed EESC methodology. The results are summarized in **Table 2**. It is noted that the proposed EESC methodology outperforms other methods in terms of classification accuracy on preictal duration (31) in epileptic EEG signals.

5. DISCUSSION

5.1. Effective EEG Features Represented by PSDED

The PSDED obtained from PSD analysis can represent the energy level at each frequency of the epilepsy EEG signals. It is noted from **Figures 2B**, **4** that compared to the other three epileptic states, the PSDED of the seizure state has an increasing energy level at low frequencies but a decreasing energy level at high frequencies. In contrast, the PSDED of the interictal state has a high energy level at high frequencies but a low energy level at low frequencies. Since EEG operates at low and medium frequencies, it can better capture the seizure occurrence at low frequencies with high energy level. Moreover, the analysis of PSDEDs in preictal I and preictal II shown in **Figures 2B**, **4** demonstrates that there are many similarities between these two states. Both PSDEDs show a high energy level at high frequencies and a low energy level at low frequencies. The subtle differences in the PSDEDs of these two states can be effectively classified by features extracted by DCNNs, as shown in **Figure 4**.

The heat map of PSDEDs in **Figure 4** is an effective illustration of differences between the four epileptic states. It is obtained through the training of DCNNs and can highlight the unique features in these four categories. It is a useful indication of seizure occurrence. The increasing energy level at low frequencies in the heat maps indicates a looming seizure occurrence. The heat maps in **Figure 4** also highlight certain EEG channels such as T8-P8, F3-C3, and FP2-F4, which are verified to be important EEG channels for seizure detection (32, 33). Therefore, the PSDED is

TABLE 2 | Comparison with the state of the art in the literature for the preictal duration of EEG signal classification.

Authors	Feature	Classifier	Accuracy	Sensitivity	Specificity	Preictal duration (min)
Truong et al. (16)	STFT spectral images	CNN	–	89.1	–	5
Chu et al. (30)	Phase locking value	SVM	–	82.44	82.76	5
Khan et al. (17)	Wavelet transform coefficients	CNN	–	83.33	–	10
Hu et al. (14)	MAS	CNN	75.28 73.29	–	–	20 40
Our proposed work	PSDED	EESC	92.6 92.5	92.3 92.6	97 97.1	10 30

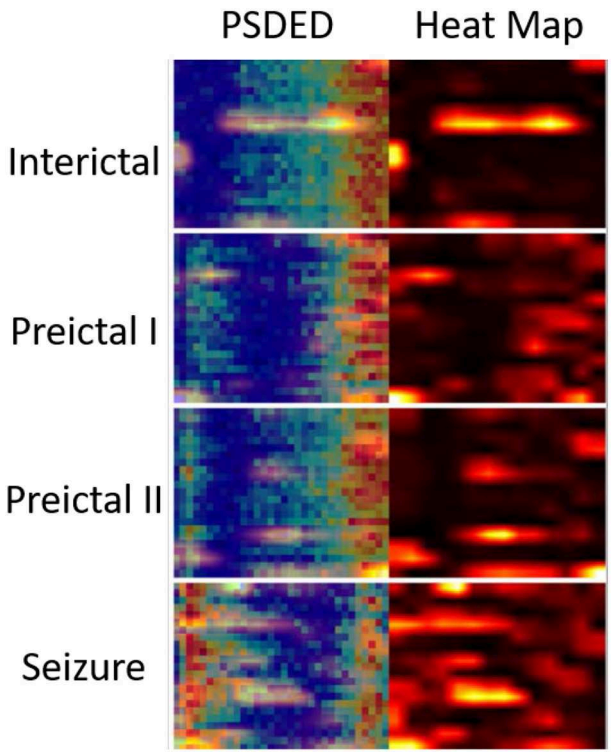

FIGURE 4 | PSDED and its heat map are juxtaposed together for different epileptic states. The heat maps highlight certain important EEG channels for seizure detection.

once again proved to be an appropriate and effective method to extract EEG signal features for seizure classification.

5.2. Comparison Between Conventional Models and the Proposed EESC Methodology

Essentially, this proposed EESC methodology is a multi-classification algorithm, mainly for four categories of epileptic EEG classification. The purpose of this study is to accurately classify EEG signals of different states.

Conventional models for epileptic seizure classification use wavelet transform (WT), short-time Fourier (STF), and other methods to extract features from EEG signals and then use machine learning to classify them. For comparison, three popular machine learning algorithms, i.e., support vector machine (SVM) (34), extreme learning machine (ELM) (35), and linear discriminant analysis (LDA) (36) are used as benchmark models for the classification of different EEG states. The EEG signals are decomposed by wavelet transform, and the reconstructed wavelet coefficients are used as features to classify the epileptic states with these selected machine learning algorithms.

The results of these conventional models with different machine learning algorithms are summarized in confusion matrices in **Figure 10**. The classification rates presented in the diagonal represent the correct accuracy of each category. It is noted that the classification accuracy of conventional methods with SVM, ELM, and LDA is only 63.85, 42.8, and 50.14%, respectively. Compared with the performance of the proposed EESC methodology shown in **Figure 7**, they underperform significantly. The reason for poor classification performance could be that wavelet transform does not preserve some important information in the features from the EEG signals, and traditional machine learning algorithms are not sensitive enough to discover the patterns in the weak features.

We augment the feature extraction in those conventional models with the proposed ensemble of DCNNs on the power spectrum density energy diagram (PSDED) obtained from original EEG signals. With the PSDED images, the three machine learning classifiers (SVM, ELM, and LDA) are still used to classify epileptic states. The performance metrics obtained are represented by the confusion matrices in **Figure 11**. It is found that the classification performance is improved by using features extracted by DCNNs from PSDEDs. In this case, the accuracy of conventional methods with SVM, ELM, and LDA are 92.25, 79.75, and 61.30%, respectively. It can be inferred from the comparison that DCNNs can extract more informative features from PSDED to increase classification accuracy for these traditional machine learning algorithms.

Furthermore, by comparing the classification results with the proposed EESC methodology in **Figure 6**, we can conclude the proposed EESC methodology has significantly

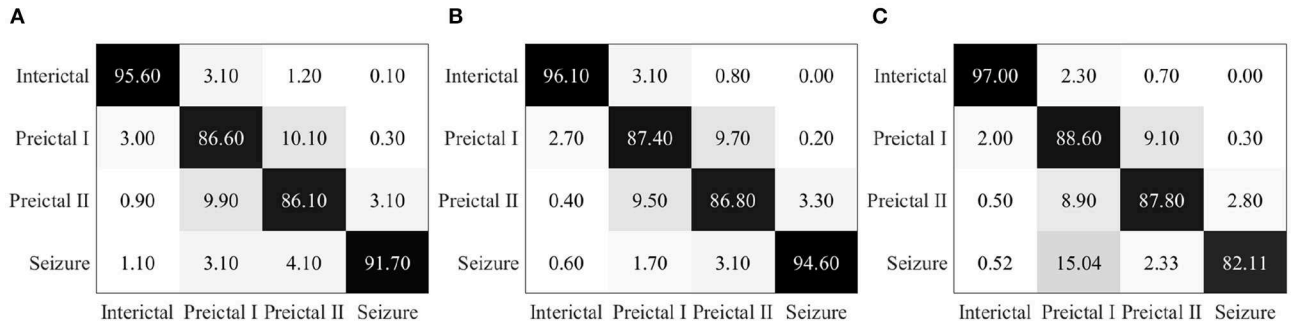

FIGURE 5 | (A) Confusion matrix for seizure prediction by using ResNet152 and PSDED. **(B)** Confusion matrix for seizure prediction by using Inception-v3 and PSDED. **(C)** Confusion matrix for seizure prediction by using Inception-Resnet-v2 and PSDED.

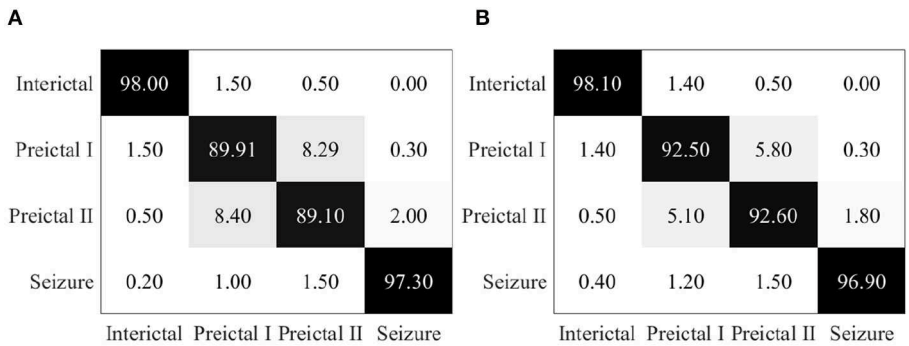

FIGURE 6 | (A) Confusion matrix for seizure prediction by using the proposed EESC. **(B)** Confusion matrix for seizure prediction by using the proposed EESC and the OHEM loss function.

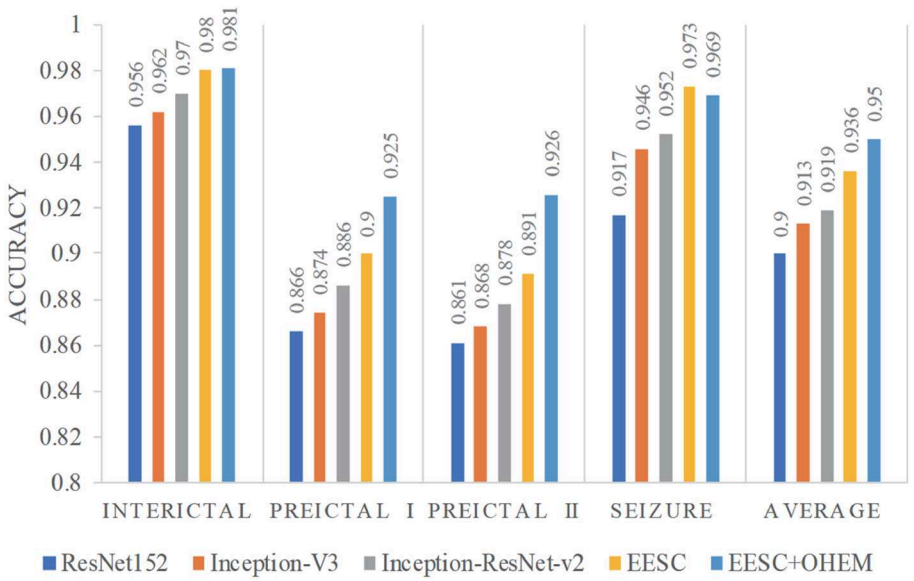

FIGURE 7 | Accuracy of different models for epileptic EEG signal classification.

better performance in classifying epileptic EEG signals than the conventional methods with machine learning classifiers. Through confusion matrices, we can see that the challenges in classification are mainly in preictal I and preictal II. This is usually due to the similarity between the EEG signal features of the preictal states. With DCNNs, the proposed EESC

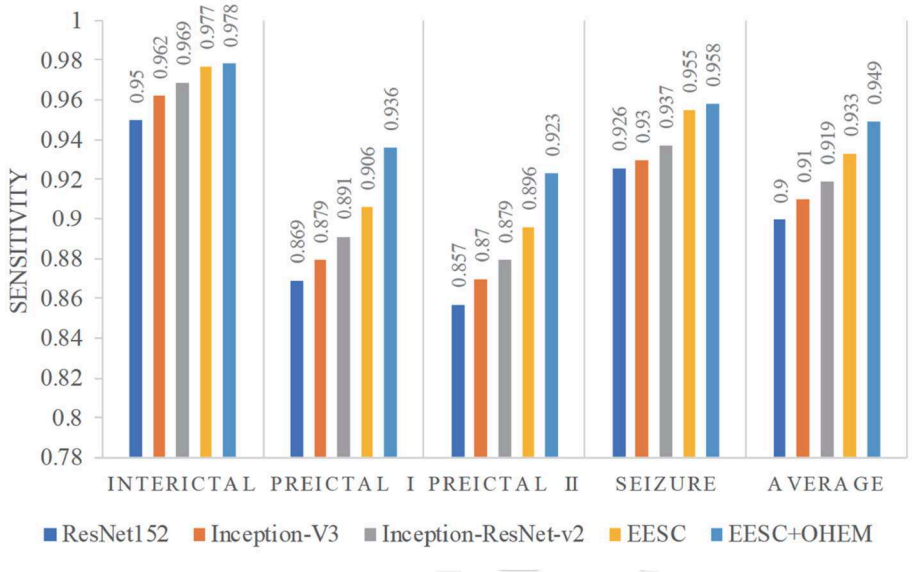

FIGURE 8 | Sensitivity of different models for epileptic EEG signal classification.

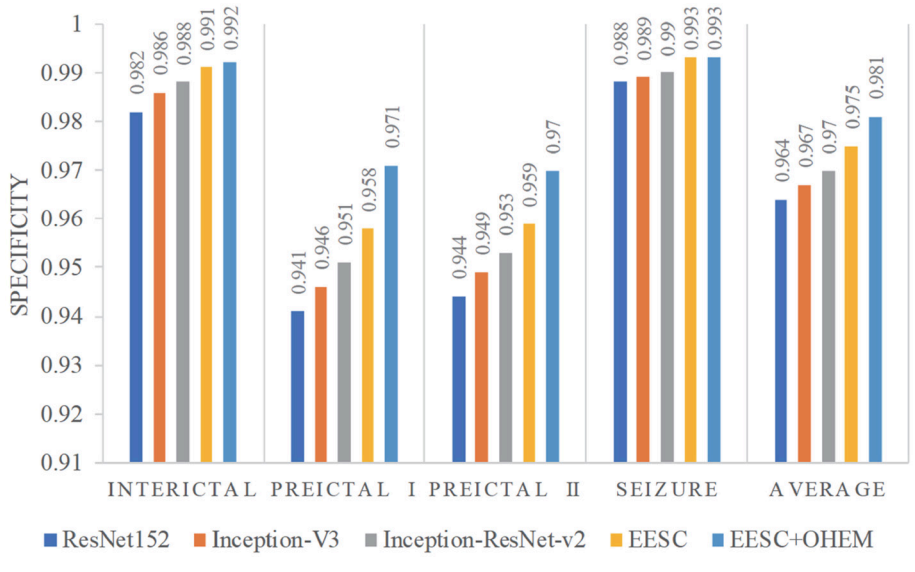

FIGURE 9 | Specificity of different models for epileptic EEG signal classification.

methodology can learn the subtle differences in the features of EEG signals, enabling better differentiation between preictal I and preictal II.

5.3. Performance Evaluation for the Proposed EESC Methodology

Accuracy, sensitivity, and specificity are used in this paper to evaluate the performance of the classification of epileptic states. For instance, as illustrated in **Table 1**, the proposed EESC methodology integrating the OHEM loss function can achieve 92.6% sensitivity and 97.1% specificity in classification. This means that the methodology has 92.6% correct seizure classification and 97.1% correct non-seizure classification. On the other hand, it means we fail to detect 7.4% of the seizure occurrences and make 2.9% false classification of seizure occurrence. Here, we discuss the potential impacts of the missed classification and false classification.

6. CONCLUSIONS

Accurate classification could potentially reduce damage caused by seizure occurrence. In this paper, we propose a novel

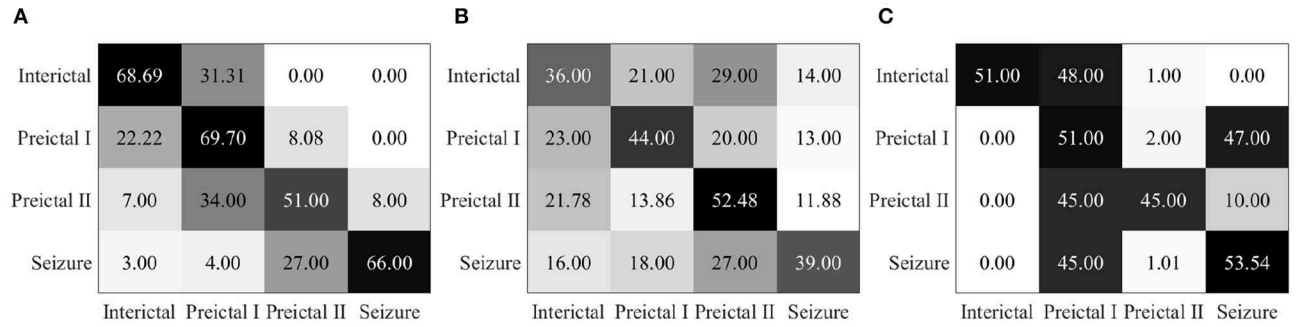

FIGURE 10 | (A) Confusion matrix for seizure prediction by using wavelet transform and SVM. **(B)** Confusion matrix for seizure prediction by using wavelet transform and ELM. **(C)** Confusion matrix for seizure prediction by using wavelet transform and LDA.

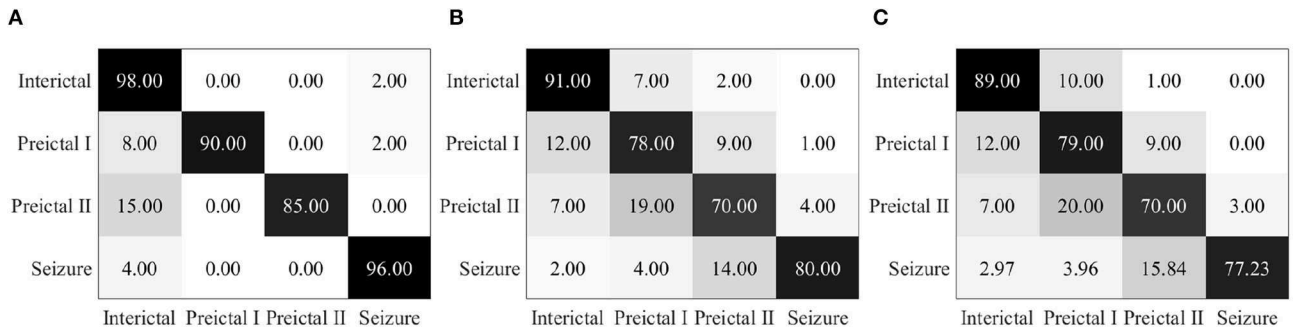

FIGURE 11 | (A) Confusion matrix for seizure prediction by using DCNNs and SVM. **(B)** Confusion matrix for seizure prediction by using DCNNs and ELM. **(C)** Confusion matrix for seizure prediction by using DCNNs and LDA.

epileptic EEG signal classification (EESC) methodology using DCNNs based on transfer learning and the power spectrum density energy diagrams (PSDED) to classify different epileptic states (i.e., interictal, preictal I, preictal II, and seizure). The methodology is verified by the multichannel EEG signals in the CHB-MIT database. It can be concluded through the study that (1) the proposed EESC methodology outperforms other benchmark models in classifying different epileptic states; (2) DCNNs have excellent feature extraction ability from the power spectrum density energy diagram (PSDED) of multichannel EEG signals; (3) the model trained with an OHEM loss function prioritizes samples with large loss and achieves high classification accuracy. In medical practice, the proposed EESC methodology could have important practical impacts on epilepsy diagnosis and treatment. For instance, to patients, the high classification accuracy of preictal states (i.e., preictal I, preictal II) of EESC can enable reliable and timely warning; to doctors, the high classification accuracy of EESC can facilitate their understanding of the categories of epilepsy in patients, enabling effective epilepsy prevention and treatment.

Consequently, this work addresses one of the significant challenges for accurate epileptic state classification with multichannel EEG signals. As part of our future research, we aim to improve the EESC methodology in the following ways in order to better serve epilepsy prevention and treatment: (1) to design precise tags for EEG signals in the preictal state to further improve the classification performance; (2) to utilize the proposed classification of EEG to detect and/or predict seizures; (3) to further reduce the false detection of seizure occurrence, for instance, by incorporating temporal correlation among frames of EEG signals; (4) to enable the diagnosis of different categories of epilepsy by locating the focus of epileptic seizures.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: http://physionet.mit.edu/physiobank/database/chbmit/.

AUTHOR CONTRIBUTIONS

YG: substantial contributions to the conception or design of the work. YZ: provide approval for publication of the content. JL: revising it critically for important intellectual content. QC and BG: analysis and interpretation of data for the work.

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REVIEW
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Psychogenic Non-epileptic Seizures and Pseudo-Refractory Epilepsy, a Management Challenge

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Psychogenic nonepileptic seizures (PNES) are neurobehavioral conditions positioned in a gray zone, not infrequently a no-man land, that lies in the intersection between Neurology and Psychiatry. According to the DSM 5, PNES are a subgroup of conversion disorders (CD), while the ICD 10 classifies PNES as dissociative disorders. The incidence of PNES is estimated to be in the range of 1.4–4.9/100,000/year, and the prevalence range is between 2 and 33 per 100,000. The International League Against Epilepsy (ILAE) has identified PNES as one of the 10 most critical neuropsychiatric conditions associated with epilepsy. Comorbidity between epilepsy and PNES, a condition leading to "dual diagnosis," is a serious diagnostic and therapeutic challenge for clinicians. The lack of prompt identification of PNES in epileptic patients can lead to potentially harmful increases in the dosage of anti-seizure drugs (ASD) as well as erroneous diagnoses of refractory epilepsy. Hence, pseudo-refractory epilepsy is the other critical side of the PNES coin as one out of four to five patients admitted to video-EEG monitoring units with a diagnosis of pharmaco-resistant epilepsy is later found to suffer from non-epileptic events. The majority of these events are of psychogenic origin. Thus, the diagnostic differentiation between pseudo and true refractory epilepsy is essential to prevent actions that lead to unnecessary treatments and ASD-related side effects as well as produce a negative impact on the patient's quality of life. In this article, we review and discuss recent evidence related to the neurobiology of PNES. We also provide an overview of the classifications and diagnostic steps that are employed in PNES management and dwell on the concept of pseudo-resistant epilepsy.

Keywords: PNES, functional neurological disorder, pseudo-refractory epilepsy, dual diagnosis, PNES psychopathology, PNES Imaging, PNES treatment

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INTRODUCTION

Psychogenic non-epileptic seizures (PNES) are relatively common disorders managed by epilepsy centers (1) and consist of paroxysmal motor, non-motor, or behavioral alterations that resemble epileptic seizures without EEG correlates. These disorders are considered to reflect the response to distress or behavioral problems (2). According to the DSM 5, PNES are a subgroup of conversion

disorders (CD) or, as indicated by the ICD 10, a dissociative disorder (3). Patients with PNES exhibit a high percentage of psychiatric comorbidities like personality and post-traumatic stress disorders, anxiety, and major depressive disorders (4). A childhood history of abuse, psychiatric comorbidities, and the female gender are all risk factors for CD (5). Trauma, brain injury, surgical procedures (6), or learning disability (7) have also been considered to facilitate the ensuing of PNES. For a long time, PNES have been considered disorders generated in the absence of biological and organic substrates. Thus, most of the attention has been focused on the psychosocial correlates of the condition (8, 9) and PNES patients have been mainly investigated and treated with psychoanalytic/psychodynamic approaches. The psychosocial origins of PNES have been largely endorsed by specialists as well as patients who often find it difficult to reconcile themselves with the idea of suffering from a disorder that lacks an organic basis (10, 11). However, over the last two decades, the use of neuroimaging techniques and functional connectivity studies have provided evidence to further understanding of the neurobiological underpinnings of this condition (12–15).

The current systematization favors the notion that PNES results from the convergence of genetic, neural, and environmental factors that synergistically act in the context of permissible psychological conditions/disorders (16). The management of PNES patients is different compared to what employed for epileptic patients, and accurate diagnosis of PNES is essential. The lack of prompt identification of PNES in epileptic patients can lead to potentially harmful increases in the dosage of anti-seizure drugs (ASD) as well as erroneous diagnoses of drug-resistant epilepsy. Epilepsy and PNES can coexist in a "dual diagnosis" condition. This condition mandates accurate discrimination between real epileptic seizures from PNES as the lack of pharmacological response to ASD of PNES events may lead to a diagnosis of a (pseudo)pharmacoresistant epilepsy.

In this article, we review and discuss recent evidence related to the neurobiology of PNES; we provide an overview of classifications and diagnostic steps that are employed in PNES management. Finally, we stress the concept of "pseudo-refractory epilepsy" which represents a central issue in the treatment and management of epileptic patients who are also presenting PNES.

EPIDEMIOLOGY OF PNES

PNES are relatively common disorders that are managed by neurologists, particularly in epilepsy clinics. The incidence of PNES is estimated to be in the range of 1.4–4.9/100,000/year, and the prevalence range is between 2 and 33 per 100,000 (2, 17). Five to 10% of the outpatients of epilepsy clinics and 20–40% of the inpatients of epilepsy monitoring units exhibit PNES. PNES usually begin young adulthood, although the disorder can occur at any age (18–20). A confirmed diagnosis is often significantly delayed, thereby leading patients to receive unnecessary treatments for years. Neurobiological, social, and vulnerability factors may explain why PNES are predominantly seen in females (16). Intriguingly, the prevalence of epilepsy in patients with PNES has been estimated to vary in a wide range from 5.3 to 73% (21). Although previous studies did not report the exact figure of this condition, a recent review has shown a prevalence of epilepsy among PNES patients around 22%, whereas the prevalence of PNES among epilepsy patients is 12% (22). This higher incidence has brought specialists to speculating that epilepsy may be a contributing risk factor for developing PNES not only because of predisposing biological mechanisms but also because, in subjects affected by genuine epilepsy, the experience of epileptic seizures may provide an opportunity for model learning (11, 23).

PNES CLASSIFICATIONS

A practical semiological classification of PNES must address proper diagnosis, the etiological systematization as well as help the management of patients. Experts have provided several classification systems based one the age, semiology, or video-EEG analysis (19, 24–26). At the beginning of the century, Gröppel and colleagues (27), taking into account the semiology of the disorder, classified PNES in: (1) "Major motor," a form characterized by the association of clonic and exaggerated motor movements of the upper and/or lower extremities, pelvic thrusting, head movements, and tonic posturing of the head; (2) "Minor motor or trembling," a form characterized by trembling of the upper and/or lower extremities; and (3) "Atonic psychogenic seizures," a form characterized by falls as the only symptoms. In the same years, Selwa and collaborators (28) proposed six types: (1) "Catatonic PNES"; (2) "Trashing PNES"; (3) "Automatisms"; (4) "Tremor"; (5) "Intermittent PNES"; and (6) "Subjective PNES". Later on, Seneviratne and colleagues (29) offered a new classification structured in six categories: (1) "Rhythmic motor PNES"; (2) "Hypermotor PNES"; (3) "Complex motor PNES"; (4) "Dialeptic PNES"; (5) "Non-epileptic auras" or (6) "Mixed PNES". Hubsch and colleagues (26) have then proposed a more detailed cluster analysis that identified five subtypes, based on the clinical features of the attacks, as (1) "Dystonic attack with primitive gestural activity"; (2) "Paucikinetic attack" with preserved responsiveness; (3) "Pseudosyncope"; (4) "Hyperkinetic prolonged attack with hyperventilation and auras" or (5) "Axial dystonic prolonged attack". Dhiman and colleagues (19) have recently modified a previous classification employed in children with PNES, and proposed five subtypes: (1) "Abnormal motor" (hypermotor movement of the whole body or only of the head and neck); (2) "Affective/emotional behavior phenomena"; (3) "Dialeptic Coma-like state"; (4) "Aura"; or (5) "Mixed".

All these past classifications shared a complex structured organization based on an accurate clinical video-EEG description of PNES. According to some studies (28, 30), outcome of PNES may vary among different clinical types, and it was also believed that different psychopathologic aspects underpinned all these manifestations. In fact, psychologists and psychiatrists documented a variety of different personality profiles and psychological etiologies including conversion disorders, depression, post-traumatic stress disorder, anxiety, emotional trauma, dissociative disorders, psychosis, and impulse

TABLE 1 | PNES classifications.

Gröppel et al. (27)	1. Major motor 2. Minor motor or trembling 3. Atonic psychogenic seizures
Selwa et al. (31)	1. Catatonic 2. Trashing 3. Automatisms 4. Tremor 5. Intermittent 6. Subjective
Seneviratne et al. (29)	1. Rhytmic motor 2. Hypermotor 3. Complex motor 4. Dialeptic 5. Non-epileptic auras 6. Mixed
Hubsh et al. (26)	1. Dystonic attack with primitive gestural activity 2. Paucikinetic attack (with preserved responsiveness) 3. Pseudosyncope 4. Hyperkinetic prolonged attack with hyperventilation and auras 5. Axial dystonic prolonged attack
Dhiman et al. (19)	1. Abnormal motor 2. Affective emotional behavior phenomena 3. Dialeptic coma-like state 4. Aura 5. Mixed
Magaudda et al. (25)	1. Hypermotor 2. Akinetic 3. Focal motor 4. PNES with "subjective symptoms"

control problems have been implicated in the pathogenesis of different clinical types of PNES. However, complex classifications encounter some limits in the daily clinical routine application especially if they are far different from the classification of true seizures.

Finally, in 2016, Magaudda et al. (25) proposed a classification based on the notion that all the PNES subtypes are similar to the subtypes of true seizure, and have, therefore, offered four categories corresponding to the ones most frequently found in their clinical experience as (1) "Hypermotor"; (2) "Akinetic"; (3) "Focal motor"; or (4) PNES with "Subjective symptoms". This latest classification was considered useful and practical, providing a good classification tool that can allow standardization across future studies (24).

A synopsis of all the classifications is provided in **Table 1**. Of note, it is indisputable that in the end all these classifications can be simply reconfigured in terms of "motor" vs. "non-motor" PNESor PNES with or without "unresponsiveness".

PNES PATHOPHYSIOLOGY

Psychopathology Aspects

As PNES is a group of symptoms and not a disease or a syndrome, the underlying etiology is expected to be heterogeneous. The psychopathologic aspects of PNES and other conversion disorders (CD) have been documented for centuries and summarized in a statement by Stone and colleagues (32) as "patients who show difficulty in expressing conflicts verbally, sometimes express distress somatically." Despite the presence of a wealth of studies that have described the functional and structural neuroimaging correlates as well as the serologic, cardiac, and electrophysiological features occurring in patients affected by functional neurologic disorders and CD (33), a unifying neurophysiological model for these conditions is still missing. *Dissociation* is considered by many specialists a key mechanism of the disorder, and people who experience PNES often exhibit a variety of dissociative symptoms (34). According to the DSM-5, dissociation is defined as "a disruption and/or discontinuity in the normal integration of consciousness, memory, identity, emotion, perception, body representation, motor control, and behavior" (35). Dissociation is considered a defense mechanism that helps the individual in coping with traumatizing events. In that sense, PNES often follow stressful or traumatic events that are generating a dissociation of the mental organization (36).

Four PNES etiological models are available. The first model is based on the Freudian construct (37) and posits that PNES is a physical manifestation of emotional stress. The second model, proposed by Moore and Baker (38), had suggested that PNES results from learned behavior and operant conditioning. Two more recent models have been centered on the presence of dissociative mechanisms. Bowman (39) has proposed that PNES results from dissociated memories or mental functions that are set in motion by traumatic events. Baslet (40) has instead proposed that PNES is an acute dissociative response to a threat or a state of high arousal. We have not achieved a "*one size fits all*" model, and, realistically, each one of the four can only partly explain the underlying mechanisms of PNES. However, the new integrated cognitive model (ICM) put forward by Brown and Reuber (3) appears to be a step in the right direction toward the identification of a unitary explanation. According to the model (**Figure 1**), PNES results from the consequences produced by altered stimuli on the activation of memory networks. The model is based on the alteration of physiological functioning in which the response to a stimulus depends on the familiarity with it. Accordingly, a familiar stimulus, already represented and stored in memory networks, generates an automated execution of a motor program (41) while if the stimulus is unfamiliar and memory networks have not been primed, a non-automated response is generated. The physiological model takes into consideration also the activation of secondary attention systems that are in charge of the "go" for responses to be executed. Action is, therefore, perceived as voluntary and self-controlled.

Upon PNES, an altered pattern of automatic responses that do not match or are rooted in reality is generated. PNES are, therefore, caused by a "rogue representation," a distorted perception of a prior or unfamiliar stimulus. At difference with physiological functioning, the automatic response is experienced as involuntary and unwanted. According to the model, PNES production is influenced by the patient background of life experiences that include memories of seizures (experienced or witnessed) as well as by an intrinsic repertoire of automatic responses to emotions like anger, fear, or disgust. In summary, while in healthy people, automated behavior, even when stereotypical, is not elicited by emotional triggers, PNES patients

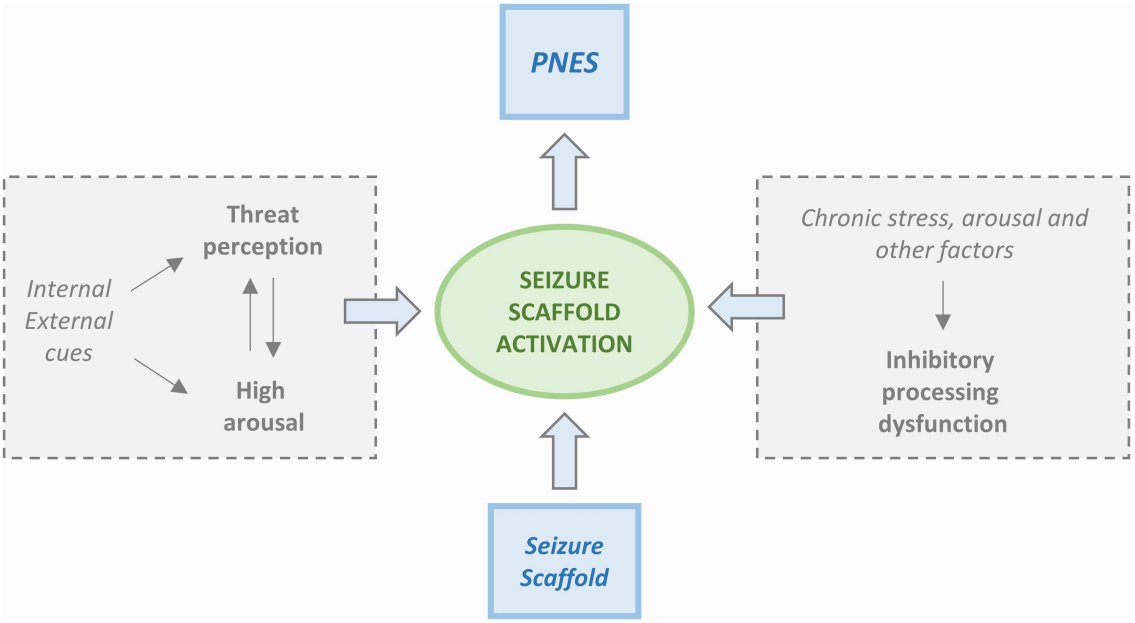

FIGURE 1 | Simplified scheme of the Integrated Cognitive Model (ICM). PNES result from the automatic execution of acquired mental representations of seizures (i.e., the enacting of a *"seizure scaffold"*). The seizure scaffold consists of a sequence of perceptions and motor activities shaped by experiences such as inherent reflexes (i.e., freezing movements, startle, wandering) or physical symptoms (i.e., of pre-syncope, dissociation, hyperventilation, head injury). Seizure scaffolds can be triggered by a range of internal or external stimuli. The process often occurs in response to increases in autonomic arousal. However, the seizure scaffold is more likely to be triggered in the presence of dysfunctional inhibition that can be due to chronic stress but also driven by "physical" causes like concurrent illness, effects of medication, etc. Patients usually experience the enactment of the seizure scaffold as non-volitional, although they may be able to inhibit it voluntarily.

exhibit an abnormal coupling between emotional triggers and the production of automatic behavioral responses that take the form of pseudo-seizure attacks. This sequence of perceptions and actions is relatively stable but not completely uniform. As such, the pathophysiological setting has much in common with the essential constituents of classical conditioning. Furthermore, these patients are unaware of the connection between the emotional state that has acted as a trigger and the resulting dysfunctional automatic behavior. **Table 2** summarizes the main psychopathologic theories of PNES.

TABLE 2 | PNES models.

Freudian model	PNES is a physical manifestation of emotional stress
Moore and Baker model	PNES results from learned behavior and activated via operant conditioning
Dissociative models by *Bowman and Baslet*	PNES results from dissociated memories or mental functions that are set in motion by a traumatic event (Bowman)
	PNES is an acute dissociative response to a threat or a state of high arousal (Baslet)
Integrated Cognitive Model (ICM) by *Brown and Rewber*	PNES results from an altered stimulus that in physiological conditions would have caused the activation of memory networks; the response to the stimulus depends on the familiarity with it. A familiar stimulus, already represented and stored in the networks, generates the automated execution of a motor program. If the stimulus is unfamiliar and memory networks are not primed, no-automated responses are generated. A secondary attention system that selects responses to be executed is also involved. Action is perceived as voluntary and self-controlled.

Neurobiology of PNES

A wealth of studies has provided insights into the psychosocial features of PNES (8, 41), but the biological underpinnings of the disorder have received much less attention and are still poorly understood. However, an emerging and growing body of evidence has finally started to unravel the neurobiological basis of PNES (42–44). Structural and functional connectivity studies (42) have shown that PNES patients exhibit network instability and distinct alterations of functional connectivity patterns (12, 43, 45–52). This evidence provides the missing neurophysiological correlates of dissociative mechanisms that let emotions to influence executive control and produce symptoms. However, it is still unclear whether these findings are specific to PNES or are instead tied to other comorbidities like depression, traumatic brain injury, etc., conditions that, it should be underlined, are frequently found in PNES patients (11, 12). Functional connectivity studies investigating CD patients have found distinct connectivity patterns that link the emotional and executive systems (53). These findings indicate that PNES patients exhibit a distinct activation of patterns of functional connectivity that occurs between the insula and the parietal associative areas that are involved in motor planning. These data support the presence of functional connections between regions that control emotional processing and areas in charge

of motor planning, a process that occurs while bypassing the conscious motor control (54). This hypothesis is in support of the ICM model and the one proposed by Baslet (40) that postulates that PNES can be interpreted as the paroxymal occurrence of episodes of dysfunctional behavior that are facilitated by the presence of unstable cognitive-emotional-attention systems.

Morphological Brain Changes in Patients With PNES

To date, only two morphological studies have examined the structural changes occurring in the brain of individuals with PNES. One study by Labate et al. (46) indicated that PNES patients, when compared to healthy controls, show significant gray matter volume reductions in the cerebellum, the right precentral and middle frontal gyrus, the right anterior cingulate cortex, and the right supplementary motor area as well as signs of cortical thinning in the right precentral gyrus, the right superior frontal gyrus, the right precuneus, and the right paracentral gyrus. A second, surface-based morphometric study by Ristić et al. (47), differed somewhat from the findings reported by Labate et al. (46) and indicated that, compared to healthy subjects, PNES patients exhibit increased cortical thickness in the left insula, the left and right medial orbitofrontal, and left orbitofrontal regions, as well as the decreased cortical thickness of the right precentral gyrus, the right entorhinal, the right lateral occipital, and left precentral areas. Both studies revealed the presence of decreased regional cortical thickness in PNES patients; however, the study by Labate et al. (46) indicates decreases that occur only in the right hemisphere while the study by Ristić et al. (47), has shown bilateral decreases as well as increases in limbic and orbitofrontal regions (47). It should be pointed out that morphometric changes may also occur for non-pathological reasons (55).

Structural and Functional Connectivity Patterns in PNES Patients

Another way to look at the structural brain changes that more closely match brain functioning is through the investigation of the strength and integrity of the connectivity that spans across distinct brain regions. A study (48), had employed diffusion tensor imaging (DTI) indices to examine the white matter based structural connectivity of the uncinate fasciculus of PNES patients. The uncinate fasciculus is a critical tract for the connection of the medial prefrontal regions with limbic areas that play an essential role in the production and modulation of emotion and memory processes. The study revealed the presence of lateralization of the connectivity of the uncinate fasciculus. In PNES patients, the authors found significantly higher numbers of streamlines (the visual and statistical DTI-based representations of white matter tracts) in the right uncinate fasciculus, a lateralization that is not present in healthy controls. This connectivity pattern suggests that individuals with PNES exhibit preferential and stronger connections between the prefrontal and limbic regions in the right hemisphere. The study also suggested that the right lateralization has detrimental effects on emotion regulation. However, another DTI-based study (49) found the presence of increased connectivity only in the left uncinate fasciculus and superior temporal gyrus. The study also reported increased connectivity in the corona radiata, and internal and external capsules, areas that are critically associated with motor functions. Thus, DTI-related data are, to date, somewhat contradictory. Stronger structural connectivity between the prefrontal and limbic regions may predispose to PNES by favoring emotion dysregulation; however, it is not clear whether the enhanced connectivity of the uncinate fasciculus potentiates the ability to downregulate emotional responses rather than cause emotion dysregulations. Furthermore, given the intrinsic complexity of the structural connectivity of the white matter and the large number of subcortical connections, it is reasonable to consider that other tracts are involved in the process.

The use of fMRI offers additional evidence for the brain-related features of PNES. To date, only one study, employing DTI as well as resting-state fMRI (rs-fMRI), had simultaneously evaluated the structural and functional connectivity features exhibited by PNES patients (42). The study found that PNES patients exhibit significantly decreased strength of structural and functional connectivity occurring in brain regions that are involved in attention and sensorimotor processing as well as areas that are part of the Default Mode Network (DMN). A follow-up study (43), employing functional connectivity density mapping based on the same rs-fMRI data, found that PNES patients show bilateral differences in the long-range and short-range functional connectivity that involves the frontal, sensorimotor, cingulate, insular, and occipital regions. A study (56), focused on the distinct functional connectivity patterns of the insula and comparing PNES patients with healthy controls has shown that functional connectivity maps relative to the left ventral anterior insula, the right dorsal anterior insula, and the right posterior insula exhibit significant differences in connectivity values in the patient group. A follow-up rs-fMRI study by the same group (50) re-analyzed the dataset and found that, compared to healthy controls, PNES patients show increased synchronous activity mainly occurring in the dorsolateral prefrontal cortex, parietal, and motor regions. PNES patients also show decreased activity in the right triangular inferior frontal gyrus, an area that is part of the ventrolateral prefrontal cortex and associated with the modulation of response inhibition (50). These findings suggest that alterations of the functional connectivity of brain regions associated with attention, memory, emotion processing, sensory, and motor functions are compromised in PNES patients. These alterations, likely resulting from life experiences, generate aberrant sensorimotor interactions that escape the conscious control of the individual. Moreover, it can be hypothesized that the inability to inhibit behavioral outputs in response to emotional stimuli (50) results from the dysfunctional hyper-connectivity that occurs between subregions of the insula and selected sensorimotor, parietal, and occipital regions (56). The process can be at the basis of maladaptive long-term enhanced vigilance to external stimuli (43). In summary, these findings provide support to the idea that PNES is produced by alterations in cognitive-emotional-behavioral mechanisms that result from

adverse life experiences and/or maladaptive experiential learning (3, 50, 57).

Positron Emission Tomography (PET) Findings in PNES Patients

Fluorodeoxyglucose (FDG) Positron-Emission Tomography (PET)-based evidence indicates that compared to healthy subjects, PNES patients exhibit significant hypometabolism in the right inferior parietal/central brain regions as well as, bilaterally, in the anterior cingulate (44). These findings provide support for two pathophysiological mechanisms involved in PNES: the emotion dysregulation that involves the anterior cingulate hypometabolism and dysfunctional processes associated with self-awareness/consciousness of oneself and the environment that are associated with the hypometabolism of the right inferior parietal cortex. Although intriguing, this study has significant limitations related to the employed exclusion criteria set to exclude co-existing psychopathologies in the recruited patients, a key confounding factor especially when considering the role of the anterior cingulate cortex in the production of anxiety and post-traumatic stress disorders (PTSD) (58, 59).

Single-Photon Emission Computed Tomography (SPECT) Findings in PNES Patients

Epileptic patients, evaluated with SPECT scans during ictal events, show hyperperfusion of the epileptic focus while, in the interictal period, the region is hypoperfused (60). Thus, computerized quantifications of the ictal, inter-ictal, and postictal changes in regional cerebral blood flow may be useful to differentiate epileptic from non-epileptic episodes (61–63). Some studies have indicated the possibility of abnormal SPECT findings in the post-ictal phase exhibited by PNES patients (61, 63). A note of caution is required, as most authors concur in the conclusion that solid SPECT-based evidence is still missing in PNES patients. It should also be underlined that these findings are difficult to interpret, given the small sample size and the presence of psychiatric comorbidity in most of the investigated PNES patients (64).

Genetic and Other Intrinsic Factors

Genetics of PNES is growing. However, the identification of specific mutations is still missing. Some evidence can be inferred by the analysis of single nucleotide polymorphisms that have been associated with a range of psychiatric disorders (i.e., autism spectrum disorders, attention deficit hyperactivity disorder, bipolar disorder, major depressive disorder, schizophrenia). These studies have provided the first genome-wide based evidence that many distinct psychiatric disorders share individual and aggregate genetic risk factors. To date, the only acknowledged genetic risk factor for the development of PNES is gender (11, 65). The evaluation of this risk factor goes along with the growing field of gender-based neurology, as recent evidence indicates the presence of distinct sex-dependent differences that shape the functional connectivity of regions involved in emotional and cognitive processing (66).

DIAGNOSIS

The Importance of an Early Diagnosis

About one-quarter of patients who are sent for video-EEG monitoring in cases of suspected pharmaco-resistant epilepsy are then found to suffer from PNES (21). PNES patients commonly experience delays in the diagnosis and/or receive inappropriate treatment. Physicians often fail to communicate and explain the condition to patients. As ASD are of no use in PNES and may exacerbate the disorder (67), early and accurate diagnosis, as well as the exclusion of epileptic seizures and other paroxysmal disorders, is of paramount importance. According to the National Association of Epilepsy Centers (NAEC) Guidelines (68), if PNES are suspected, prompt referral to an epilepsy center is required as early diagnosis of PNES is associated with better outcomes. Recent evidence shows that delays in PNES diagnosis are common. Some factors may contribute to the delay and include demographic (i.e., young age), clinical variables (i.e., the association of PNES with trauma and body injury, or physician-related variables (i.e., ASD history).

Differential Diagnosis Between PNES and Epileptic Seizures Based on Clinical Features

Clinical and semiology information can help in distinguishing PNES from epileptic seizures. Avbersek and Sisodiya (69), for instance, state that the criterion: "occurrence from sleep" has a 100% specificity for epileptic seizures. Unfortunately, approximately half of the PNES patients has a positive history of ictal events occurring "upon arising from sleep" (70), thereby indicating that the sleep-related criterium cannot be taken as good evidence for epilepsy unless the events occur only upon sleep (70). The reduced semiotic congruency of PNES episodes, when compared to genuine seizures, is another criterium that has been employed to differentiate the two disorders, but also a matter of controversy among experts (71). A recent retrospective semiotic study concluded that neither the stereotypic quality of the ictal episodes nor the variability of clinical presentations should be used as a valid criterion to differentiate PNES from epilepsy (28). Another discriminating criterion concerns the length of ictal events. As a rule of thumb, it is assumed that episode duration in PNES is longer than what occurring in genuine seizures (31). Real seizures exhibit a well-characterized onset, reach the peak of the clinical manifestations within 70 s after the onset (72), and are followed, within a few minutes, by the ensuing of the ictal offset. In the case of tonic-clonic seizures, motor manifestations that last longer than 2 min strongly indicate the need for differential diagnosis with PNES (31). A ictal episode lasting more than 10 min is most likely due to PNES (69). Epilepsy and PNES can be differentiated by a broad array of distinct symptoms and signs. It is true that PNES patients commonly exhibit asynchronous limb movements, out-of-phase clonic activity, rhythmic shaking movements with episodes of inactivity, side-to-side head movements, pelvic movements, dystonic body posturing, closure of the eyes during the event as well as enacting of non-stereotypical seizure patterns.

TABLE 3 | Differential diagnostic features PNES and epileptic seizures.

	PNES	Epileptic seizures
Aura	Less frequent	More frequent
Length of ictal events	>10 min	<70 s (<2 min for tonic-clonic seizures)
Seizure patterns	Non-stereotypical, less organized spatial patterns, variable rhythmicity, and amplitude of movements	Stereotypical and organized progression
Clinical findings	Asynchronous limb movements, out-of-phase clonic activity, rhythmic shaking movements with episodes of inactivity, side-to-side head movements, pelvic movements, dystonic body posturing, closure of the eyes during the event	Bilateral adduction and external rotations of limbs followed by tonic extension of all four limbs, then the production of diffuse clonic jerking movements before the ictal offset
Vocalization	Present not only at the beginning of the event, can fluctuate, persist and be present, with different pitch intensities, throughout the whole course of the ictal episode	At the beginning of the seizures
Subjective symptoms	Less frequent	More frequent
Urinary incontinence	Less frequent	More frequent
Occurrence at night	Less frequent	More frequent
Ictal self-injury	Less frequent	More frequent

A word of caution is required as, according to recent evidence, the prevalence of aura, subjective symptoms, urinary incontinence, night occurrence of ictal events, and self-injury in PNES patients is higher than what previously researches reported, thereby making challenging to discriminate PNES and epileptic seizures only based on clinical signs.

However, it should be remembered that none of these signs are pathognomonic for PNES. In the case of generalized tonic-clonic seizures (GTCS), the differential diagnosis is eased by the fact that a genuine GTCS evolves through a stereotyped, structured progression, typically beginning with an ictal vocalization, followed by the bilateral adduction and external rotations of the limbs, the tonic extension of all four limbs, and then the production of diffuse clonic jerking movements before the ictal offset. By contrast, patients with PNES exhibit vocalization not only at the beginning of the event but also throughout the whole ictal episode. The vocalization can fluctuate, persist, and be present, with different pitch intensities, throughout the whole course of the "ictal" episode. Moreover, movements produced in hypermotor PNES are usually showing less organized spatial patterns and characterized by movements of variable rhythmicity and amplitude.

Focal-to-bilateral tonic-clonic seizure can be frequently preceded by a focal seizure described as "epileptic aura" according to the past nomenclature. Auras are also common in PNES and cannot be considered a hallmark of epilepsy. In a recent study (73), the authors investigated the incidence of aura in patients with PNES, a clinical sign often present in these patients. Unfortunately, PNES auras are not different from the ones exhibited by epileptic patients.

Subjective symptoms, urinary incontinence, at night, and ictal self-injury are often associated with genuine seizures; however, again, none of these signs is pathognomonic for epilepsy as more than one-third of the PNES patients reported the same symptoms (74). Thus, these symptoms cannot be used as different and discriminating features of the two conditions.

Table 3 depicts the distinct clinical features of PNES and epilepsy that can help to discriminate the two conditions.

Video-EEG Monitoring: The Diagnostic Gold Standard

Prolonged video-EEG monitoring with ictal recording is considered the optimal test for the diagnostic ascertainment of PNES. However, unfortunately, some types of seizures either do not exhibit ictal EEG abnormalities, or EEG changes are concealed by the movements (i.e., frontal lobe seizures), thereby making the clinical differentiation with PNES difficult. The diagnosis of PNES should, therefore, take into account a combination of data. To that aim, the combination of the patient history, witness reports, clinician observations, ictal and interictal EEG as well as ictal video-EEG can be used (70, 75). Nowadays, home video recording is available and significantly helps the diagnostic process. While accurate evaluation of clonic movements, tremors, or thrashing movements is difficult when assessed only on what referred by eyewitnesses, the examination of video recording by expert clinicians significantly helps in producing a correct differential diagnosis (31). One caveat on the use of home-based EEG recording concerns the fact that they rarely capture the beginning of the ictal event. It is also important to note that the postictal phase of some epileptic seizures may look like PNES.

In summary, an accurate diagnosis is produced when (1) the patient history is compatible with PNES; (2) the semiology is coherent with the distinct features of PNES, as assessed by an expert clinician employing video-EEG monitoring; and (3) the episode unmistakably lacks epileptiform activity in all the phases (i.e., immediately before, during or after the ictal event). A useful rule of thumb to suspect PNES is "the rule of 2 s" that indicates that likely PNES patients, subjects exhibiting at least two normal interictal EEG along with at least two episodes per week and resistance to two antiepileptic drugs. The rule yields an 85% positive predictive value for PNES (76).

Also, seizure-related induction procedures exhibit good sensitivity and excellent specificity for PNES and help to shorten the length of the hospitalization time required for the diagnosis (77). These techniques are not universally accepted in clinical practice and currently employed by about 39–73% of the US epilepsy centers (78). A critical issue concerns the fact that there are no standardized induction techniques. The induction encompasses an array of triggers that range from simple verbal suggestions to the employment of placebos like saline injections, perfumes and olfactive stimulants, sham application of EEG-electrodes, the use a soaked pad on the patient's neck or a vibrating tuning fork on the forehead as well as the use of standard activation procedures employed in EEG like hyperventilation and photic stimulations. The use of saline injections has, for instance, a diagnostic sensitivity in the range of 60 to 90% (79). While clinically useful, the employment of induction procedures raises ethical concerns and is a matter of debate (80–88). A major ethical issue is posed by the levels of deception involved in the information provided to patients. In that regard, communication strategies have been commonly divided into three categories: (1) "explicitly deceptive," a situation in which an untruthful statement is madelike when a patient is told that "*a seizure will be produced [. . .] by placing a patch on the arm*"; (2) "truthful but omissive"when the information is technically truthful and the patient, for example, is told "*we will inject an IV drug that will perhaps help in inducing the usual spell"* but the words "epileptic seizure" are omitted to avoid lying to the patient (89); or (3) "explicitly open" when the provided information is technically correct, the psychological origin of the condition is introduced as a possibility before the induction, and the patient is made aware of the possible occurrence of both epileptic and psychogenic seizures (like during hyperventilation and photic stimulations). A recent review by Stoyan Popkirov and colleagues (89) has analyzed changes in communication methods over the years and indicates a predominant tendency toward the use of more honest strategies. Some of the ethical concerns can be circumvented by using only activation procedures that are routinely employed in EEG. Hyperventilation and photic stimulation, in fact, exhibit a diagnostic power comparable with the induction with placebo (83).

The PNES diagnosis is possible, probable, clinically established, or documented:

- **Possible PNES**: cases in which a witness or the patient reports ictal events, and the interictal EEG is normal. An abnormal interictal EEG can also be consistent with a diagnosis of possible PNES.
- **Probable PNES**: cases in which the ictal events with a semiology indicative of PNES are witnessed by an expert clinician or assessed by video-EEG recording that also indicate no ictal epileptiform activity. Situations in which the observation of the onset of the ictal episode is missing or the evaluation is made by a clinician who lacks experience in ictal assessments make PNES "probable."
- **Clinically established PNES**: cases in which an epilepsy specialist witnesses the episodes, and the semiotic and objective findings are compatible with PNES. That includes situations in which, for instance, there is resistance to the opening of the eyes, the interaction with the patient during the episode is possible as he/she maintains some level of consciousness and partial responsiveness, or the ictal episode ceases as the physician persuades the patient to terminate it. No epileptiform activity in interictal or ictal EEG can be found.
- **Documented PNES**: cases in which the diagnosis produced by an epilepsy specialist taking into account typical PNES semiology and no EEG-related epileptiform activity is found in any phase of the ictal event, or before and after it.

Clinicians have also tested the patient's responsiveness during PNES using different more or less invasive procedures. Old reports have indicated patients being pinched, stuck with a needle, splashed with water, or forced to inhale noxious chemical substances such as ammonia during or around a psychogenic attack in order to test the level of consciousness. However, there is no evidence that any of these invasive procedures are more effective than an intranasal tickle with a cotton swab (90).

SUPPLEMENTARY DIAGNOSTIC PROCEDURES

As mentioned above, only ictal EEG can be used to differentiate a subject suffering from PNES from a person affected by real epilepsy. However, many neurophysiologic, neuro-humoral, and neuropsychological tools can be used to identify at-risk subjects for PNES. These can help in conjunction with a thorough medical history, mental status, and neurologic examination.

Blood Markers

Several serologic measures have been used to differentiate epilepsy from PNES. One of the most useful markers concerns the analysis of prolactin (PRL) levels (88, 91). Many studies have shown that the absence of postictal increases of PRL predicts PNES with an average sensitivity of 89% (92, 93). False-positive are usually due to the undergoing use of dopamine antagonists or tricyclic antidepressants as well as breast stimulation and the occurrence of syncope (93). PRL levels may also fail to rise after frontal lobe seizures. The American Academy of Neurology Therapeutics and Technology Assessment Subcommittee concluded that in samples collected 10–20 min after the onset of the ictal even, doubling of relative or absolute serum PRL levels (taking into account pre-ictal values) significantly helps to discriminate generalized tonic-clonic epilepsy from PNES (94). The analysis of serum levels of cortisol at baseline or after the dexamethasone suppression test does not reliably allow the differentiation between PNES, depression, or epilepsy (95). Increases in peripheral white blood counts, creatine kinase, and neuron-specific enolases have shown little discriminative power between PNES and true epilepsy (96). Compared to age-matched healthy controls, levels of the Brain-Derived Neurotrophic Factor (BDNF) are lower in patients with PNES but do not differ from patients with epilepsy (97).

Neuroimaging Markers

As discussed above, structural imaging studies in patients with PNES have documented changes in the cortex and cerebellum (46). fMRI studies have also revealed changes in the functional connectivity that occurs between emotional, cognitive, and motor regions (42, 54). However, neuroimaging-related findings are of modest diagnostic value at the present time. It remains unclear whether these findings are specific to PNES, or can instead be tied to other comorbidities like depression, traumatic brain injury, etc., conditions that, it should be underlined, are frequently found in PNES patients. To date, only one fMRI study has examined the functional connectivity changes that are produced in PNES patients in response to external stimuli (54). The study suggested that PNES subjects exhibit a higher tendency to dissociate, a phenomenon that reflects the presence of hyperconnectivity between brain regions involved in emotion processing like the insula and motor regions adjacent to the precentral sulcus. The model is intriguing because it is the first to hypothesize a network-based mechanism for PNES. A recent investigation (98), using machine learning (ML), highlighted the role of selected cerebral areas that appear to be primarily involved in the clinical expression of PNES. The ML-based analysis revealed that the inferior frontal cortex (IFC), posterior cingulate cortex (PCC), and medial orbitofrontal cortex (OFC) are selectively activated in PNES patients. These findings are in line with the increased functional connectivity and reduced cortical thickness observed in these regions. OFC alterations have been consistently reported. It is conceivable that the altered communication between brain key regions involved in emotion regulation like the cingulate cortex, OFC, and frontal regions represents the neurobiological root of the dissociation process, by generating the disruption of information processing and aberrant sensorimotor activities. According to the author, these findings can be useful in distinguishing patients with PNES from controls at the individual level.

Neuropsychological Tests

Neuropsychological tests can help to isolate distinct cognitive, emotional, and personality features of PNES patients, but have limited value for the differential diagnosis with epilepsy (99). PNES patients exhibit deficits in several cognitive domains (100). Many studies have examined the emotional factors associated with PNES and psychiatric comorbidity. PNES patients show a high presence of personality disorders. Studies aimed at differentiating PNES from epilepsy patients have made use of interview methods like the Structured Clinical Interview for DSM Diagnosis (SCID) or the Mini-International Neuropsychiatric Interview (MINI) as well as the Minnesota Multiphasic Personality Inventory (MMPI) or the MMPI-2. These studies have indicated that personality traits may differ when comparing patients with PNES top patients with PNES and epilepsy (100).

Personality traits, type of abuse, and age of onset of trauma vary as a function of the CD subtype. A recent study has shown that patients with PNES exhibited high scores in Neuroticism and low in Conscientiousness. Neuroticism-related features like anxiety, anger, hostility, depression, excessive self-consciousness, and vulnerability can be directly and specifically associated with the type of trauma reported by the patients. The Neuroticism domain describes a persistent, life-long tendency to experience life events negatively and has been associated with mood disorders (101). Neuroticism may represent a "distress proneness." Thus, the higher neuroticism found in PNES patients indicates that they may be more sensitive to stressful events. Other studies have shown difficulties in coping with stress. Conscientiousness is frequently associated with higher levels of well-being and productivity, but can also predispose to experience more significant distress and difficulties in matching demanding tasks or situations. Conscientiousness has also been associated with self-oriented perfectionism (i.e., the tendency to set excessively high standards for oneself) as opposed to socially-oriented perfectionism (i.e., the tendency to believe that acceptance by others requires excessively high standard performances), a condition often associated with Neuroticism (102).

Research on the effects of traumatic experiences upon early childhood trauma indicates that severe early-life stress generates higher sensitivity of the hypothalamic-pituitary-adrenal axis in response to stressing situations that occur upon adulthood. Early traumatic experiences also produce greater vulnerability to depression (103). Moreover, PNES patients exhibit high rates of alexithymic personality traits (104). In a study focused on "psychosomatic" patients, Sifeos described a new personality trait which he, "for lack of a better term," named alexithymic, a term derived from old from Greek that means "no words for mood" (105). Alexithymia is defined as the failure or difficulty in mentalizing, recognizing, and verbally describing emotional states, and is a well-documented risk factor for the development of depression (106). Thus, alexithymia is a relative constriction in emotional functioning, poverty of fantasy life, and inability to recognize and verbally describe one's emotions with appropriate words. The presence of alexithymia in PNES was investigated for the first time by Bewley and colleagues (107) who indicated higher levels of alexithymia in PNES patients when compared to epileptic patients. The developmental or biological etiology of alexithymia is still largely unknown. While some authors indicate that the disorder can develop as a maladaptive coping mechanism in response to trauma, only some scant neuroimaging-based evidence supports the notion that structural changes in the corpus callosum and frontal lobes are the anatomical substrate for alexithymia (108, 109).

EPILEPSY AND PNES: THE "DUAL DIAGNOSIS"

The PNES diagnosis is often complicated by the fact that epilepsy is a recognized risk factor for the development of PNES. About 10% of patients with PNES (68) also exhibit genuine epileptic seizures, a number likely higher when assessments are made by specialized centers. This condition is known as in epilepsy circles as "dual diagnosis." Patients with "dual diagnosis" have similar demographic of PNES and epileptic patients (22). Mechanisms

speculated to be at the basis of the development of PNES in epilepsy patient include (1) psychiatric comorbidities correlated to epilepsy, (2) the presence of a "seizure scaffold" on which PNES ensues; and (3) the development of substitute symptoms (in particular in patients recovering from epilepsy) to obtain secondary gains like caregiver attention, monetary compensation or work avoidance. Patients with pharmaco-resistant epilepsy are at higher risk of developing PNES, and vice versa. The dual diagnosis must be taken into account in cases of epilepsy patients showing unexpected patterns of seizures in terms of features and frequency. A dual diagnosis is harder than isolated PNES. No neurobiological or neuropsychological feature can be employed to differentiate these subjects from epileptic patients or PNES patients. Very few studies have attempted to assess the outcomes of these patients. Some data showed that the dual diagnosis predisposes to worse outcomes. However, once the correct diagnosis is made, the number of events and the use of ASD level off, thereby emphasizing the importance of a timely diagnosis of PNES in patients already affected by epilepsy.

PHARMACORESISTENCY AND PSEUDO-REFRACTORY EPILEPSY

The International League Against Epilepsy (ILAE) has identified PNES as one of the ten key neuropsychiatric conditions associated with epilepsy (1). Pharmacoresistency and pseudo-refractory epilepsy represent the other, critical, side of the PNES coin. One out of four to five patients admitted to video-EEG monitoring units with a diagnosis of pharmaco-resistant epilepsy is later found to suffer from non-epileptic events, the majority of which are of psychogenic origin (110, 111). The diagnostic differentiation between pseudo-refractory and true refractory epilepsy is essential to avoid unnecessary treatment, ASDs related side effects, and a negative effect on the quality of the patient life. Pharmaco-resistant epilepsy is defined as a neurological condition characterized by the failure to achieve a sustained seizure-free period in response to two courses of ASD (either as monotherapies or in combination) that are tolerated, appropriately chosen, and used with accurate titrations. The pseudo-intractability, instead, relates to the resistance to treatment that is caused by diagnostic errors. Pseudo-intractability is a condition relatively easy to manage but often underestimated and unrecognized in clinical practice. It should be stressed that not all patients with intractable epilepsy are truly pharmacoresistant (112, 113). Pseudo-intractability in epilepsy is still present, even at times in which sophisticated diagnostic and therapeutic options are available.

Future research will be needed to explore in more detail the clinical aspects as well as the psychopathological features of pseudorefractory epilepsy. The process will be helped by recruiting groups of subjects who exhibit selected types of psychopathology and different levels of trauma exposures.

MANAGEMENT

The management of PNES is still largely unclear. A 2014 Cochrane review concluded that there is insufficient robust evidence to support any specific treatment for PNES (109). A 2017 study by Carlson and Perry (114) also indicated the absence of specific treatments and suggested the implementation of personalized approaches. Recent evidence suggests that psychological approaches may be the most effective way (115).

The management process should be divided into three stages. The initial stage concerns the communication of the diagnosis, a key step. Communication is facilitated by the presence of family members, a strategy that increases the understanding of the condition by patients and loved ones (70). The diagnosis must always be communicated with a tactful, empathic, and positive approach (70). The choice of the most appropriate words to be employed is a matter of a lively debate. Specific communication strategies have been published to maximize the efficacy of the process (38, 116). Terms like "hysterical seizures" and "pseudoseizures" are to be avoided and considered offensive. It is, however, questionable whether terms like "attack" (that can be associated with traumatic events sustained by the patient) or "seizure" (possibly generating confusion with real epileptic seizures) are more suitable (117). A small study of 13 PNES patients suggested that both terms were felt as problematic (118). A shared communication of the diagnosis increases the insight of the patient regarding his/her condition. Sometimes, communication of the PNES diagnosis can act as a therapeutic intervention. Recent studies have stressed out that most patients became PNES-free with time and after receiving a definite and clear diagnosis (119, 120).

The second stage of treatment involves acute therapeutic intervention. Detail psychiatric assessments should be arranged as psychiatric comorbidities are the rule and not the exception in PNES patients. Only 5% of patients do not exhibit the presence of comorbid psychiatric disorders or stressors (121). Predisposing, precipitating, and perpetuating factors must be investigated. Individualized psychotherapeutic and psychopharmacological treatment plans must be then set in place. PNES may be confused with panic attacks or associated with other CD like psychogenic movement disorders (122). Continued involvement of the epileptologist who has established the diagnosis is necessary to allow a safe tapering of ASD and treatment of any comorbid neurological condition. The treatment plan should include early tapering and discontinuation of ASD unless the patient showed specific beneficial effects by the use of ASD like, for instance, the antidepressant activity of lamotrigine or the mood-stabilizing effects of valproate. Sertraline or venlafaxine can be used to treat mood, anxiety, or psychotic disorders (70). There are no guidelines on the duration of any pharmacological and/or non-pharmacological treatment (123).

The final stage consists of the implementation of long-term interventions. The stage can make use of personalized interventions that include a long-term course of psychotherapy, case management as well as long-term pharmacological management of psychiatric comorbidity (70). Psychotherapy is considered the treatment of choice (124). Cognitive-behavioral therapy currently exhibits the most robust experimental and clinical evidence of efficacy (125–127). Individual or group psychodynamic therapy can also be considered (128–131). Psycho-educational approaches hold some promises

(131). Unfortunately, compliance with psychotherapy and specifically CBT, of PNES patients is poor compared to patients affected by other psychiatric conditions (115). This reduced therapeutic compliance may be due to the scarcity of mental health services and mental health professionals, as well as the low confidence that patients exhibit toward this therapeutic approach (115).

CONCLUSIONS

A better understanding of the complexity of PNES requires the concerted and coordinated efforts of neuroscientists, neurologists, psychiatrists, psychologists as well as, in our opinion, the creation of a multidisciplinary, multicultural/international study group set to develop a coherent research agenda and the promotion of large-size collaborative projects.

AUTHOR CONTRIBUTIONS

FA and FD contributed to the conception and design of the review. FA, FD, GE, MD, SS, and MO wrote the manuscript. All authors contributed to manuscript revisions, read and approved the submitted version.

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Causes and Effects Contributing to Sudden Death in Epilepsy and the Rationale for Prevention and Intervention

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Sudden unexpected death in epilepsy (SUDEP) claims the lives of one in every thousand epileptic patients each year. Autonomic, cardiac, and respiratory pieces to a mechanistic puzzle have not yet been completely assembled. We propose a single sequence of causes and effects that unifies disparate and competitive concepts into a single algorithm centered on ictal obstructive apnea. Based on detailed animal studies that are sometimes impossible in humans, and striking parallels with a growing body of clinical examples, this framework (1) accounts for the autonomic, cardiac, and respiratory data to date by showing the causal relationships between specific elements, and (2) highlights specific kinds of data that can be used to precisely classify various patient outcomes. The framework also justifies a "near miss" designation to be applied to any cases with evidence of obstructive apnea even, and perhaps especially, in individuals that do not require resuscitation. Lastly, the rationale for preventative oxygen therapy is demonstrated. With better mechanistic understanding of SUDEP, we suggest changes for detection and classification to increase survival rates and improve risk stratification.

Keywords: apnea, laryngospasm, SUDEP, airway obstruction, respiratory arrest

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INTRODUCTION: PHYSIOLOGICAL BACKGROUND AND KEY CONCEPTS

Reviews of preclinical and clinical data on autonomic, cardiovascular, and respiratory contributions to sudden unexpected death in epilepsy (SUDEP) have captured the progress made toward understanding this important aspect of epilepsy (1–7). Whereas, the mechanisms for SUDEP remain unknown, the main categories of potential mechanism are (1) autonomic derangements, as these are the critical link between seizure activity and the rest of the body (2) lethal cardiac events, which can link epileptogenesis and cardiac risk among the channelopathies, and (3) apnea, which may result from seizure spread to brainstem or a catastrophic failure of brainstem circuits. The key challenge has been to demonstrate which of these is/are responsible for SUDEP given the "unexpected" nature of cases and the limitations on physiological monitoring during events.

Humans and animals have extensive pathways involving insular cortex, subiculum, and amygdala that permit seizure spread to reach hypothalamus and brainstem autonomic preganglionic and premotor neurons and thus impact all body systems with autonomic innervation (8). Changes in cardiac, respiratory, gastrointestinal, and genitourinary function before, during and after a seizure are well-known from clinical and preclinical data (2, 9–15). Significant autonomic effects of seizures occur more commonly in association with generalized tonic-clonic seizures or partial seizures originating in the temporal lobe (16–18).

In contrast to the direct ictal activation of the autonomic nervous system (ANS), there can be autonomic activity that is secondary to other ictal phenomena (e.g., hypoxemia from obstructive apnea) (13, 19, 20). Such autonomic activity is a "normal" response to protect core blood flow during a survival threat.

Repeated organ stress caused by recurring seizures or parallel pathophysiological processes as in the heritable channelopathies can lead to sustained autonomic abnormalities that impact the direct or indirect responses to seizure activity. Some have argued that an abnormal autonomic baseline is essential for the extreme physiological events leading to sudden death (21–26).

Pathways exist for seizure activity to impact respiratory rhythm generation and motor output (27–30). Reports of ictal tachypnea, bradypnea, and apnea all point to an impact of seizure activity on respiratory physiology (3, 31–35) and thereby a role in ictal oxygen desaturation (36–39).

Ictal airway obstruction has been reported in humans (40–44), and our group demonstrated laryngospasm as the basis for ictal obstructive apnea (defined as periods of no airflow with evidence of inspiratory effort) using continuous laryngoscopy, recurrent laryngeal nerve recordings, plethysmography, ECG, and EEG in a rat model (34). Obstructive apnea (OA) was accompanied by pronounced hypoxemia, followed by bradycardia, respiratory arrest, and eventually death (34, 45). Further evidence of airway obstruction as part of the SUDEP mechanism is the fact that pulmonary edema is often found at autopsy in SUDEP cases (46–50).

Ictal central apnea (defined as periods of no airflow and no evidence of respiratory effort) has been demonstrated with recordings that can distinguish central apnea from OA or respiratory arrest (34, 51–53). During ictal central apnea, the central respiratory rhythm generation continues and the respiratory motor output is inhibited in the same manner as during the apnea that occurs with voluntary breath holding or the diving response, a complex reflex that includes apnea and co-activation of the divisions of the ANS (51, 54–56). A remarkable example is the "central" apnea associated with amygdala stimulation (57, 58). The absence of "stress" during amygdala-evoked apnea and the minimal oxygen desaturation is consistent with spontaneous ictal central apnea events having resemblance to the diving response. Mouse deaths from audiogenic seizures have been suspected to involve central apnea or respiratory arrest due to brainstem disruption, particularly brainstem circuits involving serotonergic neurons (27, 29, 32, 59–64), but we showed deaths to include obstructive apnea leading to respiratory arrest (65).

Lethal arrhythmias appear to be less common. Cases of ventricular fibrillation (VF) arising from seizure activity (66, 67) or seizure-induced takotsubo cardiomyopathy (68) have been reported. Whereas, the most common cause of VF in humans is regional cardiac ischemia in the setting of myocardial infarction, global hypoxemia, such as may occur during asystole or apnea, has also been implicated in severe tachyarrhythmias (69, 70). We have shown in rats that entry into ventricular tachycardia and ventricular fibrillation could occur spontaneously under narrow conditions of moderate, but not severe hypoxia, sympathetic overdrive, and minimal vagal activity (71, 72). Whereas, VF is certainly one path to SUDEP, the existing literature indicates that it is uncommon.

PROPOSED SUDEP MECHANISM ACCOUNTS FOR CAUSES AND EFFECTS

Two recent lines of research enable us to propose a comprehensive mechanistic sequence for the majority of SUDEP cases (**Figure 1**). The first was the report of results from the MORTEMUS study (1), which summarized the range of autonomic, cardiac, and respiratory data between seizure onset and death from the rare human SUDEP cases that could be clearly identified as such and at the same time were accompanied by recordings of vital signs. This critical consensus established the sequence of clinical "landmarks" in SUDEP cases. The second was extensive work with invasive and non-invasive monitoring in rodents that showed how OA occurs during seizures, how OA serves as the link between a seizure and respiratory arrest (RA), and how non-invasive measures can be used to interpret human data. Demonstrations that ictal OA can be due to laryngospasm (34), that inspiratory effort can be detected with EMG (45) or inductance plethysmography (73), and even that a surrogate airway protects against death in a widely-studied mouse model of SUDEP (65) collectively argue that OA is part of a common mechanism for SUDEP. These data permit events associated with a seizure to be defined as causes or effects.

In our opinion, the sequence begins with a generalized seizure. Seizure generalization to brainstem autonomic and respiratory areas is the cause of "first level" autonomic co-activation, irregular ventilation, and laryngospasm producing partial airway occlusion. Autonomic co-activation is a source of physiological variance. Heart rate, for example, will be altered and the observed increase or decrease in ictal rate depends upon the relative levels of the autonomic components [as well as the baseline heart rate (74)]. Laryngomotor neurons are driven by seizure spread (34) and the resulting "convulsive" movement of the vocal folds (laryngospasm) occurs throughout the seizure, but an adequate airway is usually maintained. As the seizure ends spontaneously, the drive to alter autonomic and respiratory activity is eliminated (12, 74).

Occasionally laryngospasm is sufficient to cause OA (34, 52). OA is associated with intense effort to inspire, rapid

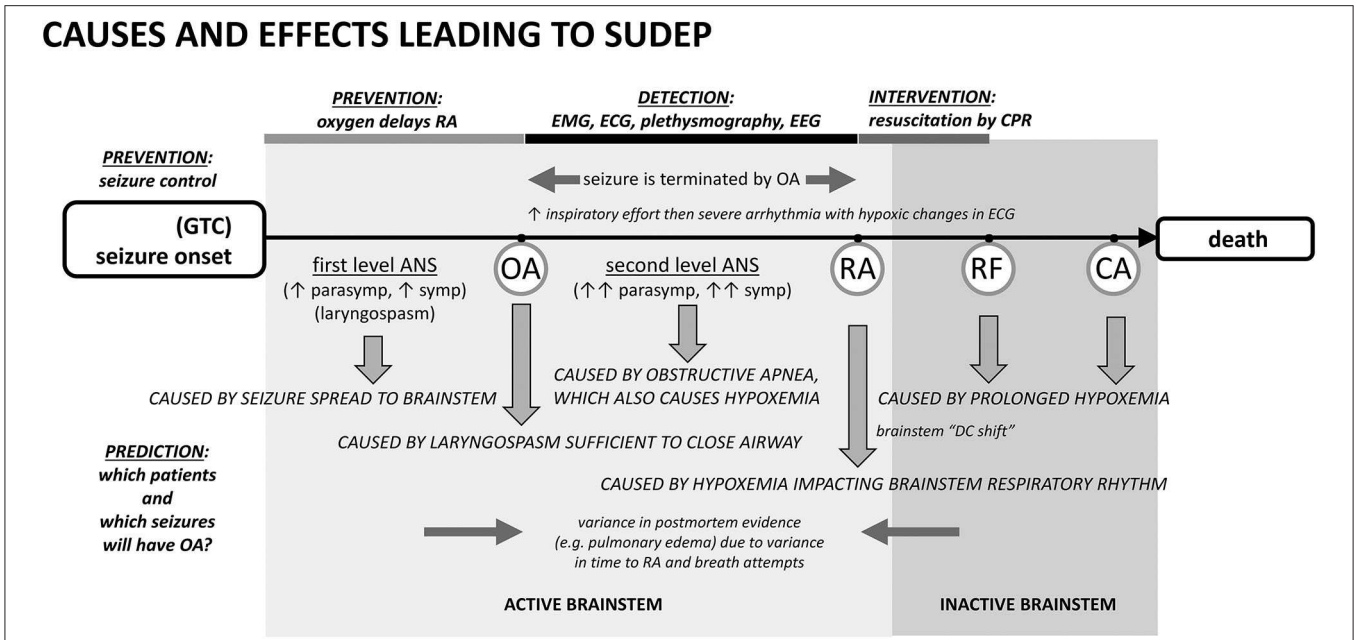

FIGURE 1 | Proposed cascade of autonomic, cardiac, and respiratory causes and effects leading to sudden death following a seizure with points of prevention, detection, and intervention. Seizure spread to brainstem laryngomotor neurons causes laryngospasm, which can be sufficient in a minority of cases for obstructive apnea (OA). OA drives additional autonomic co-activation and is associated with a rapid oxygen desaturation. Desaturation leads to respiratory arrest (RA), respiratory failure (RF), and ultimately cardiac arrest (CA). The best-established form of prevention is seizure control. An alternative prevention is to provide oxygen at the beginning of the seizure or earlier. Oxygen can delay the time to RA long enough to permit the seizure to end spontaneously even if the airway is transiently occluded by ictal laryngospasm. In the detection period, EMG or inductance plethysmography can provide evidence of inspiratory exertion during obstructive apnea. EEG can show that the seizure was aborted. Pre- and post-mortem evidence of airway occlusion is variable because consequences such as pulmonary edema will depend upon the amount of time the airway was obstructed and the frequency and amplitude of inspiratory attempts. ANS, autonomic nervous system.

desaturation, and a significant "second level" autonomic co-activation to protect core blood flow. EMG evidence of the unproductive inspiratory effort can appear in ECG and EEG records (1, 45). This measure has been used in studies of obstructive sleep apnea (75–77) and can been seen in recordings from elite apneists (e.g., breath-holding divers) (78), where stertorous breathing is not a confound. Inductance plethysmography in epilepsy patients also shows the inspiratory effort (73).

Seizures can end in two different ways after a period of OA has started. In the first, most common way, seizures end spontaneously, i.e., on their own. The stimulus for laryngospasm ends as the seizure ends. Alternatively, the hypoxemia and decreased cardiac output associated with the autonomic changes can abort the ongoing seizure activity (34, 45, 79, 80) by starving it of blood flow and oxygen. Seizure termination by asystole has been specifically noted in the clinical literature (81–83). Once aborted, recovery of baseline autonomic, cardiac, and respiratory function occurs because the seizure stimulus was removed. The full set of outcomes is illustrated in **Figure 2**. Based on our experience with verified OA (34), controlled airway occlusion (45), or asystole (80), the EEG can differentiate between seizures that end spontaneously and those that are aborted [see (84) for a mechanistic example in a different context]. Aborted seizure activity ends with a decrease in EEG amplitude and modest increase in EEG frequency, not the typical increase in amplitude and associated decrease in frequency.

In the second, rarer, but more dangerous way, seizure activity can persist and thus OA can last to the point of respiratory arrest (RA), defined as the point at which attempts to inspire cease (45, 53). This is a critical concept because it is the point at which spontaneous recovery is in jeopardy, and intervention by personnel other than the person experiencing the seizure may be necessary. Based on our work, the point of RA corresponds to the onset of "terminal apnea" as described in the MORTEMUS data (1). RA is distinct from respiratory failure (RF), which is defined as the point at which attempts to inspire are no longer possible. If the airway opens after the point of RA, but before RF, spontaneous recovery of respiration can sometimes occur (53). Apneic oxygenation is possible once the airway re-opens due to glottic relaxation (85), and this may account for the spontaneous recovery of respiration. Postural or positional factors can contribute to a compromised airway and block spontaneous recovery (86, 87). Based on this conceptual framework, we suggest that any case involving OA should be identified as a near-miss case, irrespective of whether the seizure ended spontaneously or was aborted, or whether the individual required resuscitation for recovery (**Figure 2**).

Cardiopulmonary resuscitation is known to be effective within a short time after the onset of terminal apnea/respiratory arrest (1). In our experience, a majority of cases that reach the point

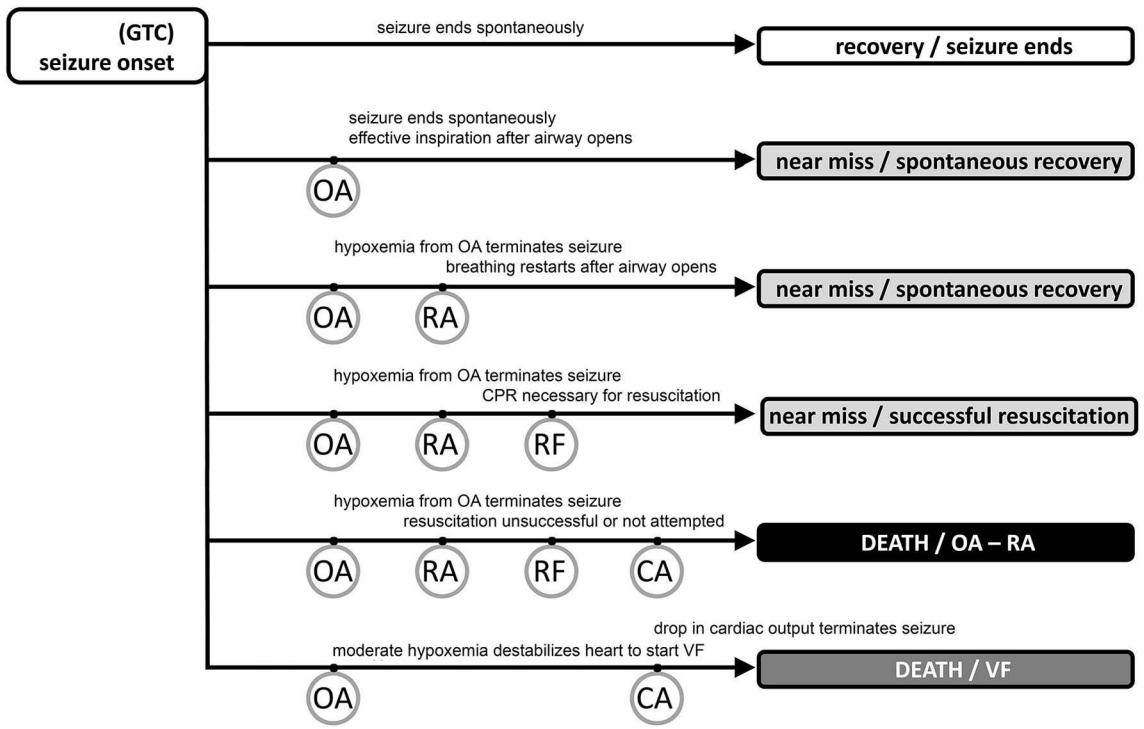

FIGURE 2 | Possible outcomes following generalized tonic clonic seizure activity with major "landmarks." The vast majority of seizures are associated with a spontaneous end of the seizure, no evidence of obstructive apnea, and a rapid return of autonomic, cardiac, and respiratory function to baseline levels (track 1). Obstructive apnea (OA) can occur, resulting in three types of near miss outcome (tracks 2, 3, 4) or sudden death (track 5). If the seizure ends spontaneously during OA (track 2) there may be evidence of inspiratory effort, but the EEG will show a normal pattern of decreasing frequency/increasing amplitude associated with the seizure ending on its own. Preventative oxygen treatment will successfully move any case to track 2. Seizure activity will be aborted in tracks 3 and 4 evidenced by EEG frequency increase/amplitude decrease due to a lack of brain blood flow and oxygen. The difference between tracks 3 and 4 is whether resuscitation is necessary (track 4) because of the inability to spontaneously recover breathing, i.e., respiratory failure (RF). SUDEP results from the events in tracks 5 and 6 where cardiac arrest (CA) is the endpoint. Track 5 is the sequence described in the text and shown in greater detail in **Figure 1**. CA is hypoxic cardiac failure in track 5. Track 6 is the outcome of seizure-induced ventricular fibrillation and a global hypoxemia trigger due to OA or asystole with or without OA.

of RA, without resuscitation attempts, progress to respiratory failure and death (34). Cases progressing to respiratory failure will show hypoxic cardiac failure as the final sign of life.

Postictal EEG suppression (PGES) or brain shutdown, suggested as a cause of brainstem dysfunction and death (26, 88, 89) is not a cessation of brain activity (90, 91). Rather, it reflects the termination of seizure activity by hypoxemia and decreased cardiac output (12, 34, 80). The work on brainstem depolarization (92) demonstrates that this form of spreading depression likely accounts for the development of respiratory failure after reaching the point of respiratory arrest. The time to develop, the time of occurrence, and the time that would be necessary for resolution all point to brainstem depolarization occurring as a result of hypoxemia and after the point of respiratory arrest (52, 93).

Lastly, any seizure-driven cascade of events is further complicated by the possibility that (and predictive opportunity that arises when) seizures impact an abnormal background physiology due to repeated seizures, genetic variation (30, 94–97), pharmacotherapy, or other causes. None of our proposed sequence of events from seizure onset to death depends upon an abnormal background physiology.

DISCUSSION: APPLICATION IN PREVENTION, INTERVENTION, AND CLASSIFICATION

Two forms of prevention have been discussed in the literature. The most straightforward prevention is seizure control, which avoids the sequence of life-threatening events (98). A second strategy is to expose the individual to oxygen as early as possible. Oxygen, even for a short time prior to the onset of OA, delays the time to RA (53, 99, 100) and thus permits spontaneous seizure termination even if OA is present. Based on our data and proposed sequence of events, a critical preventative intervention

is to enrich the inspired oxygen as near the onset of a seizure as possible, without waiting for evidence of obstructive apnea (oxygen will NOT prevent obstructive apnea, but will help the individual to survive an episode of OA). This should delay RA and prevent SUDEP (**Figure 2**). Oxygen would also minimize the potential for VF. We propose that any case with evidence of OA should be counted as a near miss so that in these higher risk patients, oxygen will be applied earlier, potentially preventing SUDEP.

CPR has been shown to be an effective for resuscitation within an adequate time window (1). During OA, artificial ventilation will be possible only after the laryngospasm relaxes to permit airflow. Chest compressions are important because cardiac contractility is minimal after the point of respiratory arrest (34). There is a vital race to start CPR before irreversible respiratory failure.

Risk stratification and prediction of life-threatening events remain a challenge. Post convulsive central apnea (PCCA) (70, 101) has been suggested as a predictive biomarker to stratify risk of SUDEP among epilepsy patients (102). PCCA in our framework would describe the period of time from respiratory arrest to spontaneous or assisted recovery of respiration. Prior to RA, OA accounts for the absence of airflow. PCCA is clear evidence of near miss status and this explains its predictive value.

Still missing is an answer to the question of why only some seizures have laryngospasm sufficient to cause OA. Fortunately, indicators of OA exist (e.g., our biomarker or inductance plethysmography) and can be used for risk stratification even if oxygen is delivered at the start of every seizure to prevent RA. Further complicating the classification of cases and prediction, but not complicating our mechanistic sequence, is whether the individual experiencing the seizure that causes sudden death is considered epileptic (103), i.e., what if the first seizure you have is the one that causes sudden death?

In summary, (1) we propose that obstructive apnea is the critical mechanistic link between seizure activity and respiratory and cardiac failure in the majority of SUDEP cases, (2) we recommend modifying the "near SUDEP" definition (104) to include any individuals with a near miss event because these indicate that the patient is prone to seizure-induced obstructive apnea and thus at increased risk for SUDEP, and (3) we argue that early oxygen exposure is a rational preventative step that can significantly reduce SUDEP rates. Based on our improved mechanistic understanding, we suggest changes for detection and classification of SUDEP patients to increase their survival and enhance their risk stratification.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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