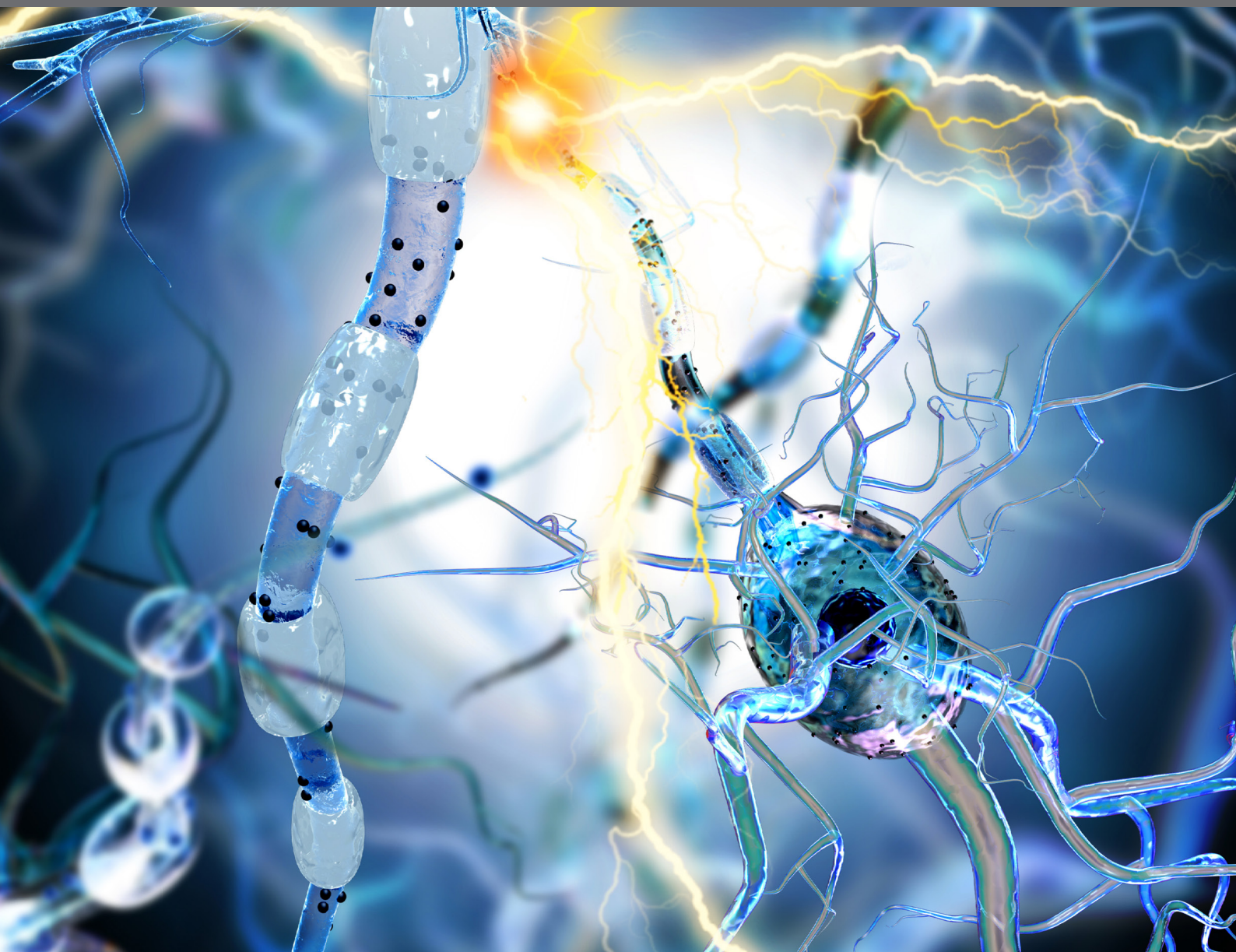# SUDDEN DEATH IN EPILEPSY: BASIC AND TRANSLATIONAL RESEARCH

EDITED BY: Christopher M. DeGiorgio
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SUDDEN DEATH IN EPILEPSY: BASIC AND TRANSLATIONAL RESEARCH

Topic Editor:
Christopher M. DeGiorgio, UCLA David Geffen School of Medicine, Olive View-UCLA Medical Center, United States
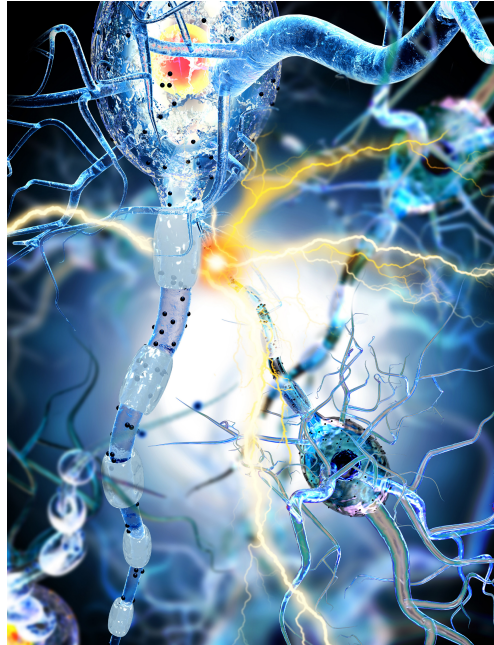
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Sudden Death in Epilepsy (SUDEP) is a major cause of death in people with epilepsy, accounting for up to 15% of all deaths. The incidence of SUDEP averages 1.2 per 1000 persons per year. Research interest is exploding, focusing on epidemiology, basic mechanisms, risk factor stratification and prevention. New wearable technologies are approved or in development. These incorporate accelerometers and advanced heart rate detection, which are linked to smart phones. The advent of FDA approved detection devices now allows immediate intervention by family and loved ones. The next frontier for SUDEP remains effective prevention strategies, which will likely include new devices and pharmacologic interventions.

This volume is organized into three sections: Basic and Physiologic Mechanisms; Clinical Risk Factors and Inventories; and Very Early Research into Pharmacologic Interventions.

It is our hope that this eBook will inform clinicians of key advances in the field, and to foster and stimulate basic and translational research with one purpose: To prevent SUDEP in those at risk.

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Editorial: Sudden Death in Epilepsy: Basic and Translational Research

Christopher M. DeGiorgio *

UCLA Department of Neurology, Olive View-UCLA Medical Center, David Geffen-UCLA School of Medicine, Los Angeles, CA, United States

Editorial on the Research Topic

Sudden Death in Epilepsy: Basic and Translational Research

Sudden Unexpected Death in Epilepsy (SUDEP) is an important cause of death in people with epilepsy, especially those with chronic epilepsy. In a UK cohort, SUDEP accounted for 17% of deaths in those with chronic epilepsy (1). The incidence of SUDEP is 0.2 per 1,000 persons per year in children, and 1.0 per 1,000 persons per year inadults (2). The incidence of SUDEP in chronic epilepsy is substantially higher, ranging from 2.64 to as high as 5.95 per 1,000 persons per year (1, 3). In this book genetics, functional imaging, autonomic physiology and clinical risk factors for SUDEP are explored in depth.

GENETICS

Bagnall et al. provide an elegant and comprehensive review of current knowledge of the genetics and basic mechanisms of SUDEP. Several candidate genes are associated with the risk for SUDEP, especially genes associated with long QT interval and cardiac arrhythmias. These include mutations in potassium channels, specifically KCNQ1 and KCNH2 (see Table 2, Bagnall et al.). Other channelopathies associated with SUDEP are caused by defects in sodium channels, especially SCN1A, SCN2A, SCN5A, and SCN8A (Bagnall et al.). Dravet's syndrome, a form of catastrophic childhood onset epilepsy, is associated with the highest recorded SUDEP incidence (9.23 per 1,000 person years) (Bagnall et al.). Over 80% of Dravet's patients express a defect in a subunit of the voltage gated sodium channel SCN1A (Bagnall et al.). Ten percent of SUDEP cases studied expressed a missense or nonsense variant in the gene DEPDC5 (Bagnall et al.). DEPDC5 encodes the protein Egl-10, which regulates the target of rapamycin complex I (mTORC1) (Bagnall et al.). Mutations in DEPDC5 may impart a higher risk of SUDEP, and cause an increase in mTORC1 activity, which is associated with focal cortical dysplasia (Bagnall et al.).

CLINICAL RISK FACTORS

Since the 1990's, researchers have identified risk factors which are associated with an increase in risk for SUDEP. (3) "Ranking the Leading Risk Factors for Sudden Unexpected Death in Epilepsy" systematically analyzes the major SUDEP risk factors published to date (DeGiorgio et al.). The top 10 risk factors from multiple published cohort studies are ranked using the weighted log of the adjusted Odds Ratio [adjusted log OR/Standard Error], which adjusts the Odds ratio for the size of each study and the confidence interval (DeGiorgio et al.). The top 10 risk factors ranked by the weighted log of the adjusted Odds Ratio are, in order: 1: > 3 GTC seizures per year; 2: > 13 seizures in the last year; 3: No AED treatment in a patient with at least one seizure in the last year 4: > 3 concomitant AED's; 5: > 3 GTCs in the past year; 6: 11–20 GTC seizures in the last 3-months; 7: onset of seizures between birth and 15

years old; 8: IQ < 70; 9: 3 to 5 AED changes in the last year; 10: > 3 concomitant AEDs (DeGiorgio et al.). Note that three or more seizures per year and three or more AED's appear twice in the list, indicating that these factors were validated in more than one study cohort (DeGiorgio et al.). Table 1 by DeGiorgio et al. provides detailed information about each of the top 10 leading risk factors associated with SUDEP.

In another chapter, Dlouhy et al. report a case of SUDEP in a 21-month old little girl who suffered febrile seizures. This tragic case should cause us to take a closer look at any association between febrile seizures and SUDEP, and should remind all SUDEP researchers that behind every SUDEP case reported, there is a human person and a family who suffers (Dlouhy et al.).

ABNORMAL AUTONOMIC AND CARDIAC PHYSIOLOGY

Hampel et al. and Novak et al. explore abnormalities of autonomic function in patients at risk for SUDEP. Hampel et al. examined baroreflex reflex sensitivity, which simultaneously measures beat-to-beat heart rate and blood pressure. Impaired baroreflex sensitivity is often seen in myocardial infarction and heart failure, and is associated with a significantly higher risk of sudden cardiac death (Hampel et al.). High baroreflex sensitivity, expressed as ms/mmHg, is associated with reduced risk for cardiac arrhythmias, and lower baroreflex sensitivity is associated with higher risk (Hampel et al.). Hampel et al. measured baroreflex sensitivity before, during and after seizures in 26 patients with chronic epilepsy hospitalized for epilepsy video telemetry (Hampel et al.). Immediately after seizures, in the post ictal period, baroreflex sensitivity decreased 79% from a pre-ictal value of 15.0–3.1 ms/mmHg, $p < 0.0001$. This important discovery may help explain why most cases of SUDEP are observed in the postictal period, when hypoxia coupled with reduced baroreflex sensitivity increases the risk for lethal cardiac arrhythmias [Hampel et al., (4)].

Novak et al correlated SUDEP risk, as estimated by the SUDEP-7 inventory, with RMSSD, a measure of high-frequency vagus-mediated heart rate variability (Novak et al.). They found that RMSSD is inversely correlated with scores on the SUDEP-7 risk inventory (Pearson $r = -0.43$, $p = 0.035$, Novak et al. Figure 3) (Novak et al.). Subjects with the highest SUDEP-7 scores (higher risk for SUDEP, SUDEP-7 scores between 5 and 7) had significantly lower RMSSD values than subjects with low SUDEP-7 scores (scores < 1). RMSSD values for those with the highest SUDEP-7 scores averaged 17.6 msec SD 5.1, while RMSSD values for the those with the lowest SUDEP-7 scores averaged 32 msec SD 12.5, $p = 0.03$, trend test (Novak et al.). Interestingly, RMSSD values in those at highest risk for SUDEP are similar to values recorded in patients with heart failure, which is consistent with Hampel et al's findings of reduced baroreflex sensitivity following seizures (Dlouhy et al. and Novak et al.). Together, the articles by Hampel at al. and Novak et al. provide evidence that patients at risk for SUDEP have abnormal autonomic function (Dlouhy et al. and Novak et al.).

Polytherapy has been implicated as a risk factor for SUDEP, but polytherapy as an independent risk factor is controversial (5). In an *in-vivo* study, Hulbert et al. found that simultaneous exposure of multiple antiepileptic drugs (carbamazepine, lamotrigine, and levetiracetam) impaired electromechanical coupling in cardiac myocytes. Impaired electromechanical coupling in cardiac myocytes may lead to cardiac arrhythmias (Novak et al.). This finding should lead to a closer evaluation of the risk of multiple AED's in people at risk for SUDEP.

FUNCTIONAL MRI AND FUNCTIONAL CONNECTIVITY

In an elegant functional MRI study (fMRI), Allen et al explored which autonomic structures and networks are abnormal in 32 patients with chronic epilepsy (Allen et al.). Subjects were stratified for risk of SUDEP by age of onset, duration, frequency of generalized tonic–clonic seizures, and nocturnal seizures (Allen et al.). Fourteen subjects were classified as high risk, and 18 were classified as low risk. Those subjects at high risk for SUDEP demonstrated significantly reduced functional connectivity in the thalamus, midbrain, anterior cingulate, putamen and amygdala (Allen et al.). This report provides key evidence of an anatomic and functional defect in key structures which regular sympathetic and parasympathetic activity in patients at risk for SUDEP (Allen et al.).

INFLAMMATION

Inflammation is believed to contribute to epileptogenesis and excitotoxicity (6). For example, the pro-inflammatory cytokine Interleukin Ib (IL-1b) can interact with the NR2B subunit of the NMDA receptor, resulting in calcium influx across the neuronal membrane and increased excitability (6). A causal role of inflammation in SUDEP is unknown (7). Nejm et al. explored whether fish oil containing the anti-inflammatory n-3 fatty acids DHA and EPA could reduce inflammation in the hearts of rats in a pilocarpine model. Their group found that long-term supplementation with fish oil significantly reduced cardiac levels of IL-6 compared with controls (Nejm et al.). The study did not explore the effect of fish oil on IL-6 levels in the brain, but these findings may provide insight into the mechanism of n-3 fatty acids in reducing seizures (8).

SUMMARY

This topic adds to current knowledge of the role of genetics, autonomic networks, autonomic physiology and clinical risk factors for SUDEP. It is our hope that this work will encourage basic and clinical scholarship to advance understanding of the mechanisms that underlie SUDEP, and to spur clinical interventions that can prevent SUDEP.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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Dysfunctional Brain Networking among Autonomic Regulatory Structures in Temporal Lobe Epilepsy Patients at High Risk of Sudden Unexpected Death in Epilepsy

Luke A. Allen[1,2,3]*, Ronald M. Harper[3,4,5], Rajesh Kumar[3,5,6,7,8], Maxime Guye[9], Jennifer A Ogren[3,4], Samden D. Lhatoo[3,10], Louis Lemieux[1,2], Catherine A. Scott[1,3], Sjoerd B. Vos[2,3,11], Sandhya Rani[3,10] and Beate Diehl[1,2,3]

[1] Institute of Neurology, University College London, London, United Kingdom, [2] Epilepsy Society, Chalfont St. Peter, United Kingdom, [3] The Center for SUDEP Research, National Institute of Neurological Disorders and Stroke, Bethesda, MD, United States, [4] Department of Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [5] UCLA Brain Research Institute, Los Angeles, CA, United States, [6] Department of Anaesthesiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [7] Department of Radiological Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [8] Department of Bioengineering, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [9] Aix Marseille University, CNRS, CRMBM UMR 7339, Marseille, France, [10] Epilepsy Centre, Neurological Institute, University Hospitals Case Medical Centre, Cleveland, OH, United States, [11] Translational Imaging Group, University College London, London, United Kingdom

Background: Sudden unexpected death in epilepsy (SUDEP) is common among young people with epilepsy. Individuals who are at high risk of SUDEP exhibit regional brain structural and functional connectivity (FC) alterations compared with low-risk patients. However, less is known about network-based FC differences among critical cortical and subcortical autonomic regulatory brain structures in temporal lobe epilepsy (TLE) patients at high risk of SUDEP.

Methods: 32 TLE patients were risk-stratified according to the following clinical criteria: age of epilepsy onset, duration of epilepsy, frequency of generalized tonic–clonic seizures, and presence of nocturnal seizures, resulting in 14 high-risk and 18 low-risk cases. Resting-state functional magnetic resonance imaging (rs-fMRI) signal time courses were extracted from 11 bilateral cortical and subcortical brain regions involved in autonomic and other regulatory processes. After computing all pairwise correlations, FC matrices were analyzed using the network-based statistic. FC strength among the 11 brain regions was compared between the high- and low-risk patients. Increases and decreases in FC were sought, using high-risk > low-risk and low-risk > high-risk contrasts (with covariates age, gender, lateralization of epilepsy, and presence of hippocampal sclerosis).

Results: High-risk TLE patients showed a subnetwork with significantly reduced FC ($t = 2.5$, $p = 0.029$) involving the thalamus, brain stem, anterior cingulate, putamen and amygdala, and a second subnetwork with significantly elevated FC ($t = 2.1$, $p = 0.031$), which extended to medial/orbital frontal cortex, insula, hippocampus, amygdala, subcallosal cortex, brain stem, thalamus, caudate, and putamen.

Conclusion: TLE patients at high risk of SUDEP showed widespread FC differences between key autonomic regulatory brain regions compared to those at low risk. The altered FC revealed here may help to shed light on the functional correlates of autonomic disturbances in epilepsy and mechanisms involved in SUDEP. Furthermore, these findings represent possible objective biomarkers which could help to identify high-risk patients and enhance SUDEP risk stratification *via* the use of non-invasive neuroimaging, which would require validation in larger cohorts, with extension to patients with other epilepsies and subjects who succumb to SUDEP.

Keywords: graph theory, resting state, functional connectivity, hippocampus, insula

INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) is the most common cause of premature death among people with epilepsy (1), for whom the risk of sudden death is over 20 times that of the general population (2, 3). Patients at higher risk of SUDEP are individuals who experience ongoing and frequent generalized tonic–clonic seizures [GTCS (4)]. Although the underlying mechanisms remain elusive, seizure-induced autonomic (cardiac arrhythmia or hypotension) or respiratory (hypoventilation, apnea or apneusis) dysfunction, or a fatal combination of these have been postulated as likely causes (5–7). Other precipitating processes, including metabolic, hormonal, and genetic actions may contribute to SUDEP (8). However, clear pathophysiological mechanisms linking epilepsy with SUDEP remain lacking.

A recent evaluation of cardiorespiratory arrests in epilepsy monitoring units—the MORTEMUS project (9)—showed severe respiratory and cardiac alterations (apnea/hypoventilation and bradycardia/asystole) to occur terminally in cases of SUDEP. However, whether the fatal respiratory or cardiac observations in SUDEP cases are secondary to profound inhibition of central respiratory or autonomic regulatory sites (10) is unclear. All but one of the SUDEP cases reviewed in the MORTEMUS project were preceded by a GTCS and experiencing frequent GTCS (>3 per year) has been identified as a major risk factor for SUDEP (11, 12). Other key factors related to increased SUDEP risk include the occurrence of nocturnal seizures, a longer disease duration, and an earlier age of disease onset (11, 13).

Recent efforts to identify structural neuroimaging biomarkers of SUDEP have revealed volume differences within key autonomic regulatory brain structures. Voxel-based morphometry (VBM) procedures show significantly reduced bilateral posterior thalamic (pulvinar) gray matter (GM) volumes and increased right hippocampal and amygdala GM volumes in high- vs. low-risk SUDEP subjects (14). A similar volumetric approach found severe volume loss in the dorsal mesencephalon among SUDEP cases compared to epilepsy and healthy controls (15). Resting-state functional magnetic resonance imaging (rs-fMRI), a technique used to identify at-rest functional connectivity (FC) between brain areas (16), shows reduced connectivity between several key regions, including the pons, thalamus, and anterior cingulate in high- vs. low-risk SUDEP patients (17). However, it is not known how the FC involving other forebrain, limbic, and basal ganglia regions, sites that regulate autonomic and respiratory functions, is affected in patients at high risk of SUDEP. For example, FC investigations involving the hippocampus and medial and orbital frontal cortices are lacking despite their known role in blood pressure regulation (18). Since SUDEP likely involves processes incorporating failure or dysfunction of respiratory or autonomic regulation, an objective of this study was to focus on FC between areas related to autonomic and respiratory processes.

Central regulation of both autonomic and respiratory control is represented through multiple structures at several levels of the neuraxis, and extends far beyond the usually designated areas in the medulla and pons. The final pathways for respiratory as well as sympathetic and parasympathetic control are mediated through medullary areas, but multiple cortical, diencephalic, midbrain, and especially cerebellar structures contribute to activation, inhibition, and timing of both respiratory and autonomic control. Cerebellar structures include the deep fastigial nuclei, important for influences on breathing, while the cerebral cortex includes the bilateral insulae, the ventral medial prefrontal gyri, and the cingulate cortex. Subcortical structures such as the hippocampus, hypothalamus, amygdala, thalamus, and basal ganglia (particularly the caudate and putamen) are also heavily involved. These structures have repeatedly been shown to respond to autonomic or respiratory challenges and structural changes in breathing and cardiovascular conditions (19–21), send projections between each other, and many project directly to nuclei regulating respiratory and autonomic action in the brain stem (22–25).

Of particular concern is that epileptic seizures arising in, or rapidly propagating to, central autonomic control sites within the limbic system (26) result in damage to or dysregulation of critical autonomic and other regulatory structures (27). The majority of seizures are accompanied by symptoms of autonomic nervous system activation and, in some cases, dysfunction (28). Cardiac alterations, particularly increased heart rate, are found in almost all seizures (29), with some suggesting more often so in temporal lobe epilepsy (TLE) patients (30). Interictal heart rate variability (HRV) reflects autonomic imbalances in patients with poorly controlled epilepsy (31) and among those who experience GTCS (32) and reduced HRV has shown to correlate with increased SUDEP risk (33). The most severe autonomic and respiratory alterations are observed during and after GTCS (9). Ongoing GTCS could exert a profound impact on critical brain areas, potentially disrupting vital processes by which respiratory, cardiac, and blood pressure functions are regulated (34).

Little is known about how, or to what extent, brain regions involved in autonomic and respiratory regulation are affected in TLE patients as a consequence of increased SUDEP risk (which includes a higher frequency of GTCS). The main objective of the current study was to investigate potential differences in FC among a subnetwork of key structures related to autonomic and respiratory regulation. We investigated this subnetwork using rs-fMRI, and applying the network based statistic [NBS (35)] to compare FC between high and low risk of SUDEP patients. The NBS is a graph theory-based approach to FC analysis which exploits the clustering structure of between-group differences in network topology. That is, connections within a network which significantly differ across groups often form a connected subnetwork or "component." Similar to conventional neuroimaging analysis (36), whereby clusters are identified among voxels in physical space, the NBS identifies clusters in topological space and possesses greater power to detect strength-based differences as opposed to methods which ignore such a topological structure. We hypothesized that TLE patients at high risk of SUDEP would exhibit altered FC among the subnetwork of selected autonomic regions of interest (ROIs) compared to patients at low risk of SUDEP.

MATERIALS AND METHODS

Subjects and Risk Stratification

Sixty patients with TLE underwent rs-fMRI scanning (34 left TLE; 26 right TLE). Of these subjects, 28 were excluded from further analyses due to the presence of large lesions (9), interictal epileptic discharges (IEDs) recorded during the rs-fMRI scan acquisition (8), excessive head movement [9: 4 max head-motion (exceeding 2 mm), 5 scrubbing; see fMRI preprocessing], and two cases who suffered SUDEP. We excluded patients who suffered from SUDEP, so as not to mix potential pathological differences which may be present in these cases.

Of 32 patients remaining for further analysis, 17 had left TLE (9 females) and 15 right TLE (7 females). Subjects were classified as being at high or low risk of SUDEP based on clinical factors (11, 14, 17) as follows: An odds ratio (OR) score was generated for each patient using duration of epilepsy > 15 years (OR = 1.95), epilepsy onset < 16 years (OR = 1.72), >3 GTCS per year (OR = 15.46), and nocturnal seizures present (OR = 3.9). Patients with >3 GTCS per year (OR = 15.46) or nocturnal seizures (OR = 3.9) were classified as high risk. The OR cutoff value of 3.9 for the high-risk label was selected based on a previous SUDEP neuroimaging study (14), in which 90% of SUDEP cases were correctly identified as high risk if their summed OR score was at least 3.9 (presence of nocturnal seizures). Therefore, any patients above 3.9 were classed as "high risk" and any below were classed as "low risk." In our cohort, this classification resulted in 14 high-risk (8 L TLE, 7 females) and 18 low-risk (9 L TLE, 9 females) subjects. Patient characteristics are shown in **Table 1**. There were no significant differences in the number of patients using multiple antiepileptic drugs (AEDs) (polytherapy) or one AED (monotherapy) between the high- and low-risk group. The average number of AEDs per high- and low-risk group was 2.4 and 2.2 respectively. AED dosages per high- and low-risk

TABLE 1 | Summary of patients at low and high risk of SUDEP.

Characteristics	Low risk (n = 18)	High risk (n = 14)	p
Mean age at scan (years) ± SD	30.0 ± 7.1	33.5 ± 9.1	0.332
Gender (M:F)	9:9	7:7	1
Epilepsy lateralization (L:R)	9:9	8:6	0.693
Mean age epilepsy onset (years) ± SD	12.9 ± 9.5	12.4 ± 8.5	0.203
Mean epilepsy duration (years) ± SD	17.6 ± 10.3	21.2 ± 12.3	0.068
>3 GTCS per year	0	14	<0.001
Mean number of GTCS per year	0.3 ± 0.6	62 ± 58	<0.001
Nocturnal seizures	0	6	0.002
Hippocampal sclerosis	7	9	0.161
Polytherapy	13	12	0.367
Monotherapy	5	2	0.367
Mean SUDEP risk (OR) score ± SD	1.6 ± 1.7	19.2 ± 2.2	<0.001

SUDEP, sudden death in epilepsy; L, left; R, right; GTCS, generalized tonic–clonic seizures; OR, odds ratio; AED, antiepileptic drug.

patients can be found in supplementary material (Table S1 in Supplementary Material).

Functional MRI

All subjects underwent a 20-min resting-state electroencephalogram-functional magnetic resonance imaging (EEG-fMRI) scan (3.0 T scanner, Signa Excite HDX, GE Medical Systems), during which they were instructed to lay idly with eyes closed. The echo planar imaging-based blood oxygen level-dependent (BOLD) functional MRI scans were acquired with the following parameters: repetition time = 3,000 ms, echo time = 30 ms; flip angle = 90°, matrix size = 64 × 64, field of view = 24 cm × 24 cm, slice thickness = 3 mm, number of slices = 44. Simultaneous EEGs with 32 channels recorded with MRI compatible electrodes were acquired (Brain Products, Munich, Germany). The EEG recordings were used to exclude patients with epileptiform activity during the scan. The study was approved by the National Research Ethics Committee (04/Q0512/77 and 14/SW/0021) and all patients gave written informed consent.

Data Preprocessing

The rs-fMRI time-series data were preprocessed in MATLAB 2016a (MathWorks Inc.) with DPARSFA (data processing assistant for resting state fMRI (37)) software, which calls functions from the software packages REST (38) and SPM12 (statistical parametric mapping; http://www.fil.ion.ucl.ac.uk/spm). The following steps were carried out: slice time correction, realignment, coregistration of structural and functional MRI images, segmentation *via* diffeomorphic anatomical registration through exponential lie algebra [DARTEL (39)], and spatial normalization to Montreal Neurological Institute space.

To reduce the effects of physiological noise, and to improve the specificity of signals pertaining to GM, the white matter (WM), and cerebrospinal fluid (CSF) signals were regressed out using the components analysis-based noise correction method, CompCor (40), in which five principal components derived from noise ROIs based on each subject's segmented WM and CSF mask (mask threshold = 0.99) were removed (41).

The six motion realignment parameters calculated by SPM12 were also regressed out. Four patients from the original cohort were excluded due to maximum head motion exceeding 2 mm in any given direction of the rotation or translation parameters computed during realignment. For the remaining subjects included for further analysis, maximum head motion was 1.9 and 1.7 (mm) in the high- and low-risk groups, respectively. There were no significant differences in any motion parameters between the high- and low-risk cohorts ($p > 0.05$) as evaluated with independent samples t-tests. Head motion "scrubbing" was implemented, using DPARSFA's built-in functions, to account for small but excessive head movements which are known to effect interregional correlations despite routine motion correction (42–46). For every scan in a given time series, the framewise displacement (FD), an index of head-movement from one volume to the next, was calculated as the sum of the absolute values of the realignment estimates relative to the preceding scan (42). Mean FD in the high- and low-risk cohorts was 0.15 ± 0.09 and 0.17 ± 0.08, respectively, and did not significantly differ ($t = 0.586$, $p = 0.562$). Scans to be scrubbed were defined as those for which FD exceeded 0.25 mm; for each of those, the preceding 1 and subsequent 2 scans were replaced *via* linear interpolation. In 5 of the original 60 patients, this procedure resulted in 75%, or more, of the scans being scrubbed—these patients were excluded from further analysis. In the remaining datasets, the proportion of scrubbed scans was below 50%. Finally, the linear trend was removed, and a bandpass filter of 0.01–0.08 Hz was applied, which is consistent with the frequency range most relevant to BOLD signal fluctuations. Spatial smoothing was not applied to not extend the BOLD signal between nearby ROIs.

ROI Selection

The Harvard-Oxford (HO) cortical and subcortical atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html) was used to extract ROI-averaged time-series from the processed fMRI time series. We selected 11 bilateral brain regions (22 total) from the HO atlas based on their known involvement in the central control of autonomic regulation (see **Figure 1**) These regions included structures belonging to the limbic system: hippocampus, amygdala, anterior cingulate cortex (ACC), and subcallosal cortex (SC); the insulae, thalamus, orbitofrontal cortex (OFC), frontal medial cortex (FMC), brain stem, and two regions of the basal ganglia: caudate and putamen.

Resting-State FC Analysis and Network Based Statistic (NBS)

After extracting the time-series belonging to each ROI (network node), the absolute value of the Pearson r correlation coefficient was calculated for every possible ROI pair (each ROI pair defining a network edge or "path" between two structures) and a Fisher Z-transform normalization applied, yielding a 22 × 22 FC matrix for every subject. We then used the Network Based Statistic [NBS (35)] to compare the FC strength of every edge in the matrices between high risk and low risk of SUDEP patients. We sought to identify increased and decreased FC (contrasts: high risk < low risk; high risk > low risk) using analysis of
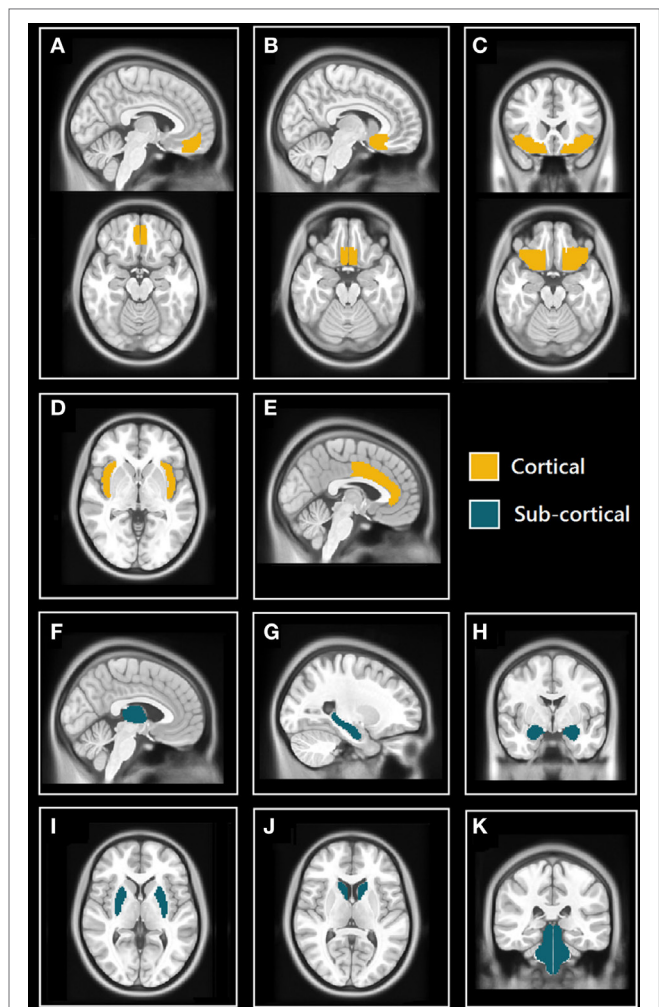
FIGURE 1 | Selected cortical and subcortical regions of interest (ROIs) masks from Harvard-Oxford (HO) atlas. **(A)** Sagittal (top) and axial (bottom) views of the frontal medial cortex (FMC); **(B)** sagittal (top) and axial (bottom) views of the subcallosal cortex (SC); **(C)** coronal (top) and axial (bottom) views of orbitofrontal cortex (OFC); **(D)** axial view of insulae (Ins); **(E)** sagittal view of anterior cingulate cortex (ACC); **(F)** sagittal view of thalamus; **(G)** sagittal view of hippocampus; **(H)** coronal view of amygdalae; **(I)** axial view of putamen; **(J)** axial view of caudate; and **(K)** coronal view of brain stem (includes midbrain, pons, and medulla).

covariance, with the following covariates: age, gender, lateralization of epilepsy, and presence of hippocampal sclerosis (HS). In addition to using presence of HS as a covariate, we also performed analyses whereby hippocampal GM volume of the epileptogenic hemisphere was regressed out (see Methods in Supplementary Material) in order to quantitatively control for differences in connectivity which may arise from changes in brain structure—namely, those resulting from HS.

In summary, NBS consists of the following steps: independently test the null hypothesis at every connection in the network using a two-sample t-test, endowing each edge with a t-statistic. A t-statistic threshold is required and must be specified prior to testing. Any edges for which the t-statistic threshold is exceeded are defined as suprathresholded connectivity. Clusters, or any set
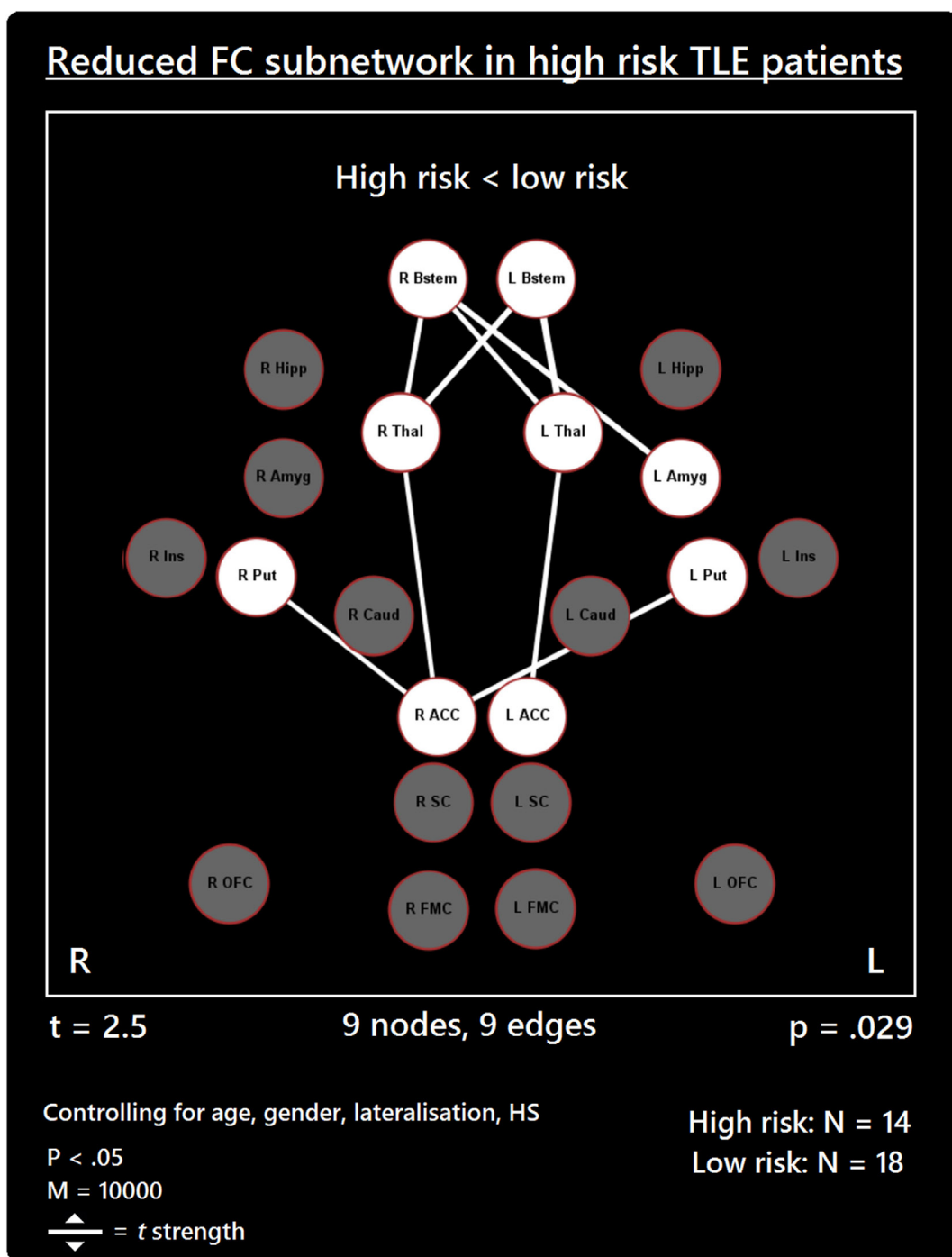
FIGURE 2 | Reduced functional connectivity (FC) subnetwork in high risk over lower risk of sudden unexpected death in epilepsy (SUDEP) patients. Subnetwork of reduced FC involving the bilateral brain stem (Bstem), bilateral thalamus (Thal), bilateral putamen (Put), bilateral ACC, and left amygdala (Amyg). L, left; r, right; HS, hippocampal sclerosis; t, t-statistic threshold; M, number of permutations; p value was set at <0.05, family-wise error rate (FWER) corrected. Nodes in white are those which were involved in the significant subnetwork. Red node outline represents search for reduced connectivity (high < low). Visualization using Gephi (https://gephi.org/).

of nodes between which a path can be found, are then identified among the suprathresholded connectivity. The main assumption of the NBS is that any suprathresholded edges which form a cluster are not isolated from each other and therefore comprise a connected component, or subnetwork, differentiating the two groups (35). Finally, a family-wise error rate (FWER)-corrected p value is calculated using permutation testing (47). For each permutation, members of the two samples are randomly permuted,

and the size of the extended cluster is calculated—in the current study, the number of permutations used was 10,000. These calculations yield an empirical null distribution of the maximal supra-threshold cluster size. Significance level was set at $p < 0.05$.

RESULTS

The comparison between high- and low-risk SUDEP patients revealed a subnetwork of significantly reduced FC ($t = 2.5$, $p = 0.029$) and one subnetwork of significantly enhanced FC ($t = 2.1$, $p = 0.033$). The reduced FC subnetwork consisted of nine edges between the following nine nodes: bilateral ACC, bilateral thalamus, bilateral brain stem, left amygdala, and bilateral putamen (**Figure 2**; **Table 2**). The subnetwork of enhanced FC consisted of 16 nodes (bilateral FMC, bilateral SC, bilateral OFC, bilateral insula, bilateral hippocampus, bilateral amygdala, right caudate, right putamen, right brain stem, and left thalamus) and 24 edges (**Figure 3**; **Table 3**). Comparable significant subnetworks emerged following regression of hippocampal GM volume (instead of "presence of HS"). The high-risk < low-risk contrast revealed a significantly reduced subnetwork of 11 nodes (bilateral brain stem, bilateral thalamus, left amygdala, right insula, bilateral ACC, bilateral putamen and right SC) and 14 edges ($t = 2.5$, $p = 0.035$). The high-risk > low-risk contrast showed a significantly enhanced subnetwork comprising 15 nodes (bilateral hippocampus, amygdala, putamen, insula, SC, orbitofrontal cortex, medial frontal cortex, and right caudate) and 27 edges ($t = 2.5$, $p = 0.028$) (Results and Figures S1 and S2 in Supplementary Material).

DISCUSSION

We examined whether, and to what extent, the FC between a group of structures associated with autonomic and respiratory regulation differs between TLE patients at high and low risk of SUDEP. We found that high-risk TLE patients exhibit highly altered FC among important brain regions known to be involved in autonomic regulation, when compared with low-risk patients. A subnetwork of reduced FC became apparent and involved several areas previously linked to increased SUDEP risk (17), including the thalamus, brain stem, and ACC. However, here we show involvement of additional brain regions which have not been previously linked to FC investigations into SUDEP, including the bilateral putamen and left amygdala. Additionally, we show a subnetwork of enhanced FC in TLE patients at high risk of SUDEP, the connections of which extended to many of the regions in the subnetwork. A large proportion of enhanced connections involved medial/orbital frontal cortices, the insulae and limbic areas (amygdalae and hippocampus). The ACC was not involved in enhanced FC in high-risk TLE patients. These findings prompt the need for further investigations into these structures, given their known involvement in cortical/subcortical autonomic control functions, particularly those pertaining to blood pressure regulation.

Reduced FC Subnetwork
Our findings further support the importance of altered ACC-thalamus and thalamus–brain stem connectivity in patients at

TABLE 2 | Reduced subnetwork.

Connection	t
L ACC–L thalamus	2.91
L brain stem–L thalamus	3.76
L brain stem–R thalamus	3.38
R ACC–R thalamus	2.66
R ACC–L putamen	3.04
R ACC–R putamen	2.59
R brain stem–L amygdala	3.23
R brain stem–L thalamus	2.87
R brain stem–R thalamus	2.89

List of decreased connections belonging to the subnetwork of reduced connectivity found (high risk < low risk), with a threshold of $t = 2.5$.
t, t-test statistic.

high risk of SUDEP (17). The thalamus relays extensive information to and from cortical and subcortical brain sites. The posterior thalamus plays significant roles in oxygen sensing (19, 48) and in relaying afferent activity essential for breathing. A disruption of the thalamic-brain stem link, as shown here in high-risk SUDEP patients, is particularly concerning given the apparent involvement of respiratory failure in SUDEP (9). The reduced thalamic connectivity bares resemblance to a VBM study showing reduced GM volumes in the thalamus among high-risk subjects and SUDEP victims (14). The Wandschneider study, and others [e.g., Ref. (49)], revealed however, that injury to the posterior thalamus is common in epilepsy, and disease duration potentiates the extent of that damage. Such injury may predispose to a failure to recover from hypoxia accompanying ictal episodes.

Anterior cingulate cortex involvement in autonomic regulation is well documented with early stimulation studies demonstrating its role in blood pressure regulation (50). Neuroimaging studies corroborated these findings (51) and show consistent fMRI activation and deactivation patterns of ACC in association with heart rate changes (52, 53), and cold pressor and hand-grip responses (20). In human epilepsy, thalamic-cingulate circuitry alterations were previously described (17, 54). Upon stimulation of the cingulate, asystole—a potential SUDEP mechanism—has been observed (55). The reduced thalamic–ACC connectivity among patients at high risk for SUDEP reflects a disruption of key pathways involved in central modulation of cardiorespiratory and blood pressure mechanisms, which may be implicated in SUDEP (34).

Our data reveal for the first time a role of the putamen in the reduced connectivity subnetwork found in high-risk SUDEP patients. The putamen serves significant autonomic regulatory behaviors, and has major projections to insular and limbic sites (56, 57). The putamen also serves to integrate sensory information for preparation of movements (58, 59). Reduced connectivity between the putamen and ACC could alter communication between autonomic and motor regulatory pathways in patients at high risk of SUDEP. Furthermore, we show reduced FC of the bilateral putamen with the right ACC only. The right ACC is preferentially involved in baroreflex-mediated autonomic cardiovascular function in humans (60). Patients with congenital central hypoventilation syndrome (CCHS), who are also at high risk of sudden death, show BOLD signal reductions within the putamen when compared with controls (61).
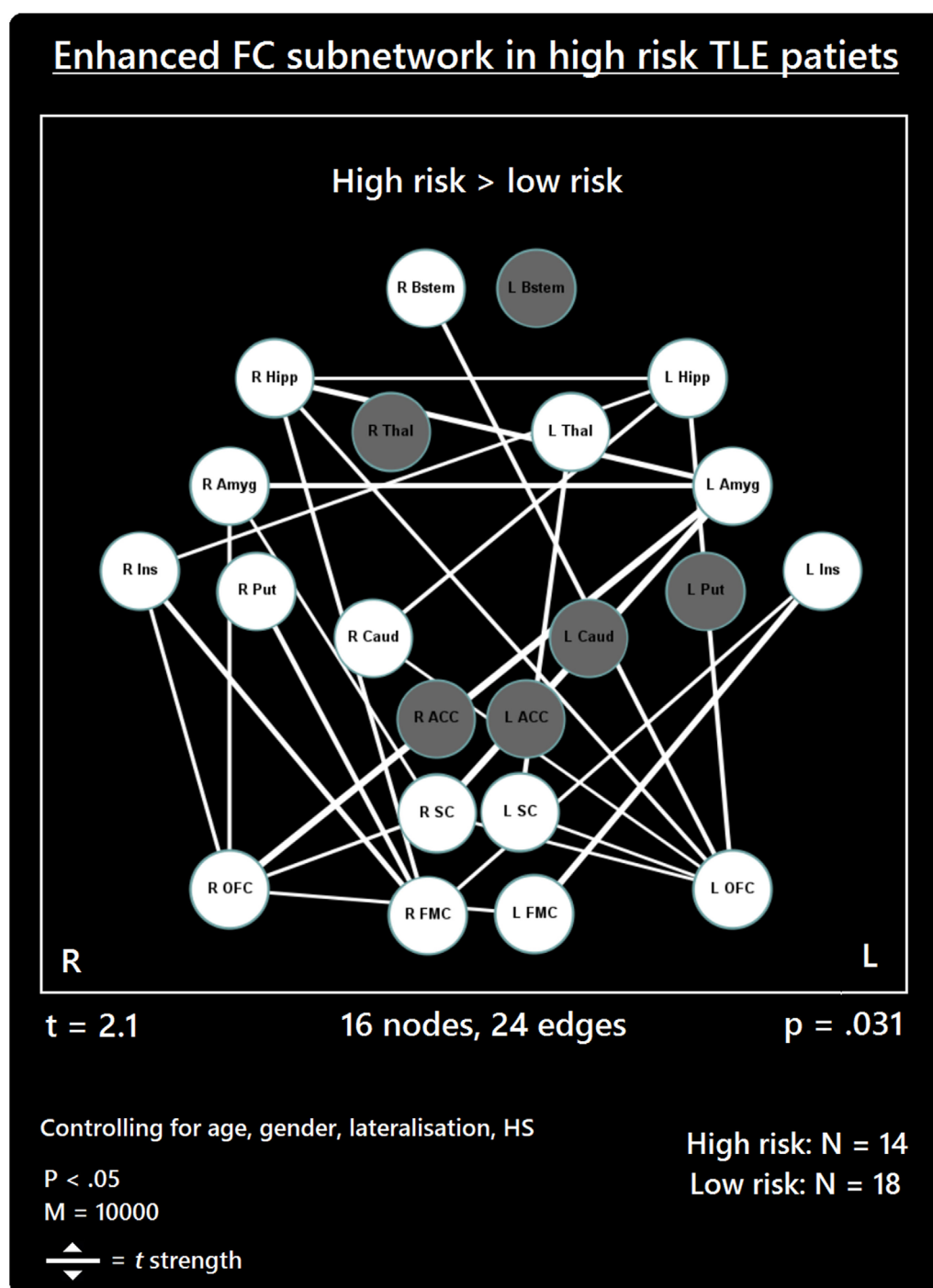
FIGURE 3 | Increased functional connectivity (FC) subnetwork in high risk over lower risk of sudden unexpected death in epilepsy (SUDEP) patients. Subnetwork of enhanced FC in high-risk temporal lobe epilepsy (TLE) patients when compared with low-risk TLE patients. Regions include: bilateral amygdala (L Amyg, R Amyg), right brain stem (R Bstem), right caudate (R Caud), bilateral frontal medial cortex (L FMC, R FMC), bilateral hippocampus (L Hipp, R Hipp), bilateral insula (L Ins, R Ins), bilateral orbitofrontal cortex (L OFC, R OFC), right putamen (R Put), bilateral subcallosal cortex (L SC, R SC), and the left thalamus (L Thal). L, left; R, right; HS, hippocampal sclerosis; *t*, *t*-statistic threshold; *M*, number of permutations; *p* value was set at <0.05, family-wise error rate (FWER) corrected. White nodes represent ROIs involving significant connections. Blue node outline represents search for increased connectivity (high > low). Visualization using Gephi (https://gephi.org/).

Reduced FC between the right brain stem and left amygdala also occurred in high-risk SUDEP patients. The final common path nuclei for cardiac, respiratory, and blood pressure control lie within the brain stem. The involvement of the amygdala in cardiovascular and respiratory activities has been described (62, 63), as are the afferent and efferent pathways through which

TABLE 3 | Enhanced subnetwork.

Connection	t
L amygdala–R amygdala	2.68
L FMC–R OFC	2.21
L OFC–L hipp	2.51
L OFC–R brain stem	2.58
L OFC–R caudate	2.10
L OFC–R hipp	2.42
L hipp–L hipp	2.16
L ins–L FMC	2.99
L ins–R FMC	2.29
L SC–L OFC	2.14
L SC–L thalamus	2.62
R caudate–L hipp	2.46
R FMC–R hipp	2.49
R FMC–R putamen	2.69
R OFC–L amygdala	3.19
R OFC–R amygdala	2.39
R hipp–L amygdala	2.78
R ins–L hipp	2.24
R ins–R FMC	2.66
R ins–R OFC	2.35
R SC–L amygdala	3.17
R SC–L OFC	2.15
R SC–R amygdala	2.18
R SC–R OFC	2.35

List of enhanced connections found using high-risk > low-risk contrast, with a threshold of $t = 2.1$.
t, t-test statistic.

the amygdala projects to the midbrain, pons, and brain stem (22, 64–66). Single-pulse stimulation of the amygdala central nucleus will trigger state-dependent inspiration (67). Animal models reveal a vital role of the amygdala in the propagation of seizures from the brain stem to the forebrain (68). Upon stimulation of the amygdala in patients with epilepsy, apnea and oxygen desaturation are observed (8, 63, 69), demonstrating the significant influences of this structure on brain stem respiratory nuclei in humans. The reduced FC we found between the amygdala and brain stem in high-risk patients is of considerable concern, especially given the occurrence of terminal apnea in the majority of SUDEP cases (9). We speculate that the FC reduction may contribute to a failure of amygdala influences to trigger inspiratory efforts and recover from possible hypoventilation or apnea during seizures, or possibly result in sustained apneusis. Reduced FC may provide a marker for the respiratory alterations in epilepsy and mechanisms underpinning SUDEP.

Enhanced FC Subnetwork

As well as reduced connectivity, an enhanced FC subnetwork emerged in high-risk SUDEP patients involving 24 increased functional links in 16 of the 22 regions investigated (**Figure 1**). The majority of enhanced connectivity patterns found in high-risk patients were represented by connections from the frontal medial or orbital frontal cortex to the insula and limbic cortices (hippocampus and amygdala).

The enhanced FC of orbital frontal and frontal medial cortices found here is of particular interest given their involvement in blood pressure modulation (62, 70). Portions of the medial

prefrontal cortex also influence key areas involved in modulating cardiac sympathetic and parasympathetic responses and baroreflex activity (71–73). Hand grip tasks, which induce heart rate changes, are associated with reduced activity within the hippocampus, orbitofrontal and medial prefrontal cortex in human subjects (52, 60, 74), demonstrating the largely inhibitory role of the medial and orbital frontal cortices in autonomic regulation (21, 25, 60). The enhanced connectivity observed between medial prefrontal cortices and limbic structures in high-risk SUDEP patients could reflect an imbalance in the medial prefrontal–hippocampal circuitry involved in blood pressure regulation (18, 19). A recent rs-fMRI study showed that increased vagal modulation, as measured by postexercise HRV, is accompanied by increased FC between the right anterior hippocampus and the ventral medial prefrontal cortex (75). Of interest, enhanced FC between the right hippocampus and the right FMC emerged in our study, and not in the left in high-risk patients at rest. One possibility, therefore, is that this increased connectivity is linked to resting elevated sympathetic tone, which is exhibited among poorly controlled epilepsy patients (31).

The increased connectivity between the insulae and the OFC/FMC revealed in the enhanced subnetwork may be linked with blood pressure regulation during ictal periods. A role of the insula in autonomic regulation has long been known (76), with neuroimaging studies confirming earlier stimulation studies (77). The right insula exerts major influences over sympathetic control, while the left insula more-prominently influences parasympathetic outflow (76, 78, 79). The inhibitory role of the medial prefrontal cortex is well known for both autonomic processes and somatic reflexes (80). Increased activation of the insula, coupled with observed decreases in the medial prefrontal cortex (60, 74) during autonomic challenges implies a dynamic relationship between these regions which, along with the hippocampus, forms part of a major network concerned with blood pressure regulation (21). Projections from the insular cortex to the medial frontal cortex, if exaggerated by seizure discharge, could lead to enhanced suppression of blood pressure levels, with the potential for hypotension. Such an imbalance at rest provides evidence of dysfunctional networking among these structures which may alter their ability to recover following a significant disturbance, such as a seizure.

The current data also demonstrate increased FC between the left and right hippocampus and left and right amygdalae in high-risk patients. Human electrophysiological studies demonstrate homotopic connectivity of bilateral mesial temporal structures in drug-resistant focal epilepsy (81). Stimulation of the fornix results in contralateral hippocampal responses without involvement from the neocortex, establishing a link between bilateral mesial temporal structures (82). These findings also demonstrate that temporal lobe seizures likely propagate between the hemispheres *via* the limbic system. The high functional interconnectivity among high-risk patients between the bilateral amygdalae and bilateral hippocampi poses a risk of exaggerated descending influences on both breathing and blood pressure. The role of the amygdala in both sustaining inspiration (67) with the potential for apneusis or generating apnea has been described earlier

(8, 69). If both amygdalae combine to exert influences to the phase-switching brain stem areas, the risk for apneusis or apnea is raised. The hippocampus plays an essential role in the diencephalic blood pressure regulatory circuitry (21, 62, 70). Safe constraints may exist with unilateral influences, but bilateral extreme activation, as may happen by recruitment in ictal discharge, may pose overwhelming drives to lower blood pressure final common path structures. Resting interictal imbalances as shown here could result in erroneous and disturbed autoregulation during extreme circumstances, such as during ictal or postictal periods. Given the much higher frequency of seizures experienced by the high-risk cohort, it is plausible to suggest that these enhanced connections may be evidence of long-term seizure-induced hyperconnectivity of these structures. However, further work is required to establish whether and how seizure frequency influences the homotopic connectivity of these, and other, structures in TLE and other epilepsies.

The SC exhibited increased FC with the amygdala, OFC, and thalamus. Recent investigations involving direct cortical stimulation of the SC in human epilepsy patients demonstrate its role in cortical control of blood pressure. Significant hypotensive changes were observed upon stimulation of the bilateral SC (Lacuey et al., Unpublished).[1] These findings confirm subcallosal involvement in cortical blood pressure control, and implicate this region in the genesis of peri-ictal hypotension in epilepsy patients (see footnote text1). The increased connections found in high-risk patients indicate further hyperconnective imbalances among autonomic regions, particularly those involved in blood pressure regulation.

The basal ganglia participate heavily in autonomic regulation, which likely follows from its prominent projections to the lateral hypothalamus and nuclei in the brain stem (83). Deterioration of the basal ganglia has been linked to cardiovascular disturbances, particularly relating to blood pressure, observed in Parkinson's disease (57, 84, 85). As well as receiving strong thalamic input, portions of the basal ganglia, particularly the caudate, share connectivity with cortical sites, including the medial/orbital frontal cortices and hippocampal and amygdala structures (86, 87). The basal ganglia sites are involved in the complex circuitry responsible for modulation of signaling between cortical and subcortical structures, and are linked with many other processes, including those related to arousal- (88), sensory- (89), and cognitive-based (90) functions. The enhanced connections involving the putamen and caudate among the subnetwork provide further evidence of the potential for disturbed communication between cortical and subcortical systems to exert profound autonomic distortions in patients who are at high risk of SUDEP.

Results after Regression of Hippocampal GM Volume, Not Presence of HS

As well as using presence/non-presence of HS as a covariate, we conducted further analyses using a more quantitative approach

to control for connectivity changes related to morphological differences of the mesial temporal structures (hippocampus) between high- and low-risk patients. Similar reduced and enhanced subnetworks were revealed following this approach and, importantly, the core effects observed using presence of HS as a covariate were mirrored in this analysis. In summary, these were: reduced connectivity of the brain stem, thalamus, amygdala and putamen; and enhanced connectivity involving medial and orbital frontal cortices, the insulae, hippocampi and amygdalae, putamen, and caudate (see Results and Figures S1 and S2 in Supplementary Material). Additional edges were revealed in the reduced subnetwork and comprised connections from the brain stem to the insula, putamen, and SC, and from the subcallosal to the ACC. In the high-risk > low-risk contrast, a greater number of connections involving the left medial frontal cortex emerged, and enhanced bilateral homotopic connectivity of the frontal medial and SC is shown. These connections highlight further altered connectivity in relation to increased SUDEP risk which must be explored in future studies. These data also demonstrate the importance of taking into account volumetric alterations in connectivity analyses, which should be considered in future studies.

Neuroimaging Findings in Other Cohorts at Risk of Sudden Death

Alterations in brain structure, function, and connectivity are not unique to subjects with epilepsy at risk for SUDEP. Neuroimaging studies of other syndromes in which risk of sudden death is high reveal both structural and functional brain alterations between autonomic regulatory brain areas in patient groups compared with controls. Heart failure (HF) patients show damage to cortical autonomic regions, including the insulae, anterior cingulate, subgenu, and the ventromedial prefrontal cortex (VMPFC) (91), as well as volume loss in the putamen (92). Obstructive sleep apnea (OSA) subjects show significant amplitude and phase changes in functional MRI signals of autonomic and respiratory regulatory structures to blood pressure and ventilator challenges [for reviews see Ref. (93) or (94)], as well as highly altered FC of the insular cortices in patients (95) and volumetric alterations of the putamen (96). Patients with congenital central hypoventilation syndrome (CCHS), a syndrome accompanied by severe disturbances in both autonomic and respiratory function (97), show cortical thinning of the insular cortex, cingulate, and VMPFC (19, 98–100), as well as injury to hippocampal and other limbic structures (19). These syndromes, especially heart failure and CCHS, share a risk for sudden, unexpected death with the epilepsy group studied here, especially during sleep. Moreover, both structural injury and fMRI signal responses to challenges were lateralized in these other conditions.

Known Interictal Autonomic Disturbances in Epilepsy and Relation to the Current Findings

Temporal lobe epilepsy patients show highly altered interictal HRV (101) which reflect imbalances in sympathetic and

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[1]Lacuey N, Hampson JP, Theeranaew W, Zonjy B, Vithala A, Loparo K, et al. Brodmann area 25 is a cortical structure for human blood pressure control.

parasympathetic control over cardiorespiratory actions, and is particularly disturbed in refractory epilepsy patients and those who experience GTCS (31, 32). Increased SUDEP risk has been associated with such alterations in HRV (33), particularly reductions in root-mean square differences of successive R-R intervals (102)—a measure of HRV which reflects vagus nerve-mediated autonomic control of the heart (33). The findings outlined in the current study may shed light on the underlying neural correlates of such autonomic imbalances in TLE patients at high risk of SUDEP.

Limitations and Future Work
ROI Issues and Considerations
A potential drawback of the current study is the incomplete parcelation of the template used to define ROIs. Many of the structures investigated here contain subdivisions which may be important for interpreting the relevance of our findings with respect to their specific autonomic function. For example, the insular cortices are large structures, the subdivisions of which have differential roles in autonomic function (79). The lack of insular subdivisions in the current study hampers interpretation of enhanced connections found involving this structure. Similarly, subdivisions of the hippocampus also serve different functions (103), and future brain stem studies should include, at least, separation of the midbrain, pons, and medulla. The thalamus also contains multiple subdivisions, each with specialized functions and which project to different sites (104). Portions of the posterior thalamus, for example, play a critical role in oxygen and CO_2 regulation (19, 48), and the region shows reduced GM volume among high-risk patients and SUDEP victims (14) and is also damaged in CCHS patients (19).

The current study did not consider the cerebellum among the selected ROIs due to inadequate scan coverage. The cerebellum has been extensively linked to autonomic and respiratory functions, and especially with its role in dampening extremes of blood pressure changes (19), and is another structure which exhibits damage in HF patients, who are at considerable risk of sudden death (105). Exploring functional interactions between the cerebellum and other brain structures in epilepsy, and with particular respect to SUDEP, is of significant interest. Future studies investigating structural and functional changes in this setting should include both cerebellar cortex and deep "autonomic" nuclei in the evaluation.

Network-Based Statistic Limitations and Choice of Statistical Significance Threshold
The NBS enables detection of cluster-based differences (components) among a set of connections (in a network), enabling differentiation of two group-based significant subnetworks. Thus, the NBS has reduced power to detect stand-alone connections as belonging to the significant detected component. Furthermore, identification of a cluster relies first, on detection of edges which surpass a given threshold (t), which must be specified *a priori*. One drawback of this approach is that it is rarely known which

t should be used in practice, resulting in an unavoidable level of subjectivity. To limit this bias here, we chose the minimum threshold at which a significant subnetwork for each contrast was revealed. The threshold required to reveal the reduced subnetwork (high risk < low risk) was $t = 2.5$, while $t = 2.1$ was required to reveal the enhanced subnetwork. The relatively higher threshold used in the high-risk < low-risk contrast reflects the discovery of a smaller, but more intense subnetwork of reduced FC, while the slightly lower threshold used for the high-risk > low-risk contrast explains the more extended yet less intense subnetwork of increased FC found (35).

Cohort
Future neuroimaging studies investigating SUDEP would benefit from applying network-based FC approaches to larger samples involving more epilepsy subtypes and patients who are subsequent victims of SUDEP. Furthermore, comparisons involving a group of healthy subjects are also necessary to evaluate findings in patients with reference to the healthy brain. Further sampling issues relate to inclusion of left and right TLE patients in the same group which, although controlled in statistical analysis, does not offer the opportunity to independently explore high-risk vs. low-risk differences in each subgroup separately. Such an investigation would be of interest, given the lateralization of autonomic brain circuitry (20) and the known whole-brain network differences between left and right TLE patients (106).

Neuroimaging studies have demonstrated altered FC of mesial temporal structures, including the hippocampus and amygdala (107), and medial prefrontal regions, including the SC (108), among patients with depression. Given the overlap involving epilepsy and psychiatric complications such as depression and anxiety (109), future efforts should include methods to partition variance due to the incidence and severity of psychiatric diagnoses.

Finally, while the current study offers insight into connectivity differences among cortical and subcortical autonomic regions at rest, it would be of interest to evaluate functional responses in relation to task-based fMRI assessments of autonomic and respiratory brain function in epilepsy patients. Particularly important would be comparisons between patients who with and without GTCS. Investigating activation patterns in direct association with autonomic and respiratory challenges could shed light on affected autonomic brain function as a function of GTCS frequency—the most significant SUDEP risk factor (11).

CONCLUSION

Alterations in FC observed indicate a dysfunctional network of critical cortical and subcortical brain regions involved in autonomic and respiratory regulation. Resting-state FC imbalances among these regulatory structures may predispose such a network to fail to recover from extremities caused by seizures, particularly GTCS. Our results build on existing findings and shed further light on interactions between affected structures

related to increased SUDEP risk and underline the importance of laterality considerations on connectivity, and the need to consider integration from multiple brain sites in evaluating autonomic or breathing outcomes in SUDEP mechanisms.

ETHICS STATEMENT

Informed written consent was obtained from all participants. The study was approved by the Health Research Authority (HRA)—NHS England.

AUTHOR CONTRIBUTIONS

LA prepared and analyzed data. LA, RH, RK, LL, and BD wrote the manuscript. All authors contributed editorially. RH, RK, JO, and MG advised on ROI selection, imaging analysis and interpretation of findings. RH, BD, SL, and SR helped to refine the clinical and physiological interpretation of findings. CS advised on clinical and neurophysiological issues. MG, SV, and LL advised on imaging and methodological issues.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fneur.2017.00544/full#supplementary-material.

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Genetic Basis of Sudden Unexpected Death in Epilepsy

Richard D. Bagnall[1,2]*, Douglas E. Crompton[3,4] and Christopher Semsarian[1,2,5]

[1]*Agnes Ginges Centre for Molecular Cardiology, Centenary Institute, Sydney, NSW, Australia,* [2]*Sydney Medical School, University of Sydney, Sydney, NSW, Australia,* [3]*Department of Neurology, Northern Health, Melbourne, VIC, Australia,* [4]*Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Melbourne, VIC, Australia,* [5]*Department of Cardiology, Royal Prince Alfred Hospital, Sydney, NSW, Australia*

People with epilepsy are at heightened risk of sudden death compared to the general population. The leading cause of epilepsy-related premature mortality is sudden unexpected death in epilepsy (SUDEP). Postmortem investigation of people with SUDEP, including histological and toxicological analysis, does not reveal a cause of death, and the mechanisms of SUDEP remain largely unresolved. In this review we present the possible mechanisms underlying SUDEP, including respiratory dysfunction, cardiac arrhythmia and postictal generalized electroencephlogram suppression. Emerging studies in humans and animal models suggest there may be an underlying genetic basis to SUDEP in some cases. We will highlight a mounting body of evidence for the involvement of genetic risk factors in SUDEP, with a particular focus on the role of cardiac arrhythmia genes in SUDEP.

Keywords: sudden unexpected death in epilepsy, genetics, sudden death, cardiovascular disease, tonic–clonic

INTRODUCTION

People with epilepsy have a twofold to threefold increased risk of premature mortality compared to the general population, which is attributed to factors both related and unrelated to epilepsy (1, 2). The most common cause of death that is related to epilepsy is sudden unexpected death in epilepsy (SUDEP), defined as "a sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death in patients with epilepsy with or without evidence for a seizure, and excluding documented status epilepticus, in which postmortem examination does not reveal a structural or toxicologic cause of death" (3). The definition "probable SUDEP" applies when a postmortem is not performed, and "possible SUDEP" for people with limited information regarding the cause of death or with a competing cause of death. Cases in which cardiorespiratory arrest is reversed by resuscitation with subsequent survival for more than 1 h are termed "near SUDEP" (3).

Estimates in studies of the incidence of SUDEP vary widely owing to differences in the age range and epilepsy populations studied. In general populations of people with epilepsy, approximately 1 SUDEP occurs annually per 1,000 people, whereas the incidence is lower in studies limited to children with epilepsy. In hospital or clinic-based studies, up to 7 SUDEP cases occur annually per 1,000, reflecting the higher proportion of more severe and treatment refractory cases (2–5). Because the incidence of SUDEP is common in young adulthood, the public health burden in terms of years of potential life lost ranks second only to stroke among neurological conditions in the United States (6).

Case–control studies analyzing clinical variables associated with SUDEP have highlighted generalized tonic–clonic seizures as the major risk factor. In addition, a long history of epilepsy, young age at diagnosis, early adulthood (aged 20–40 years), intellectual disability, and male gender, are also associated with elevated risk (4–7). While awareness of the health burden and risk factors of SUDEP is increasing among patients, doctors, and the community, the underlying causes of SUDEP are unknown.

Because many SUDEP cases are unwitnessed, the exact sequence of events is not known for the majority of cases. However, when witnessed, SUDEP almost always occurs in the aftermath of a generalized tonic–clonic seizure, and people with genetic epilepsies associated with this seizures type may be at heightened SUDEP risk. During the seizure, depressed autonomic control of respiratory drive may result in severe oxygen desaturation and a rise in blood carbon dioxide levels. The adverse effects of cessation of breathing may be exacerbated by airway obstruction in people sleeping face down, as is a common circumstance in SUDEP. Furthermore, depressed activity of serotonin neurons may blunt the natural arousal response to increased blood carbon dioxide levels. A seizure may also have various effects on the cardiac rhythm, including shortening or prolongation of the QT interval and changes in the QT interval are associated with sudden death in the general population. SUDEP cases with pathogenic variants in genes that cause the long QT syndrome (LQTS) may have a cardiac arrhythmogenic basis. Profound postictal suppression of central nervous system function may leave the patient immobile and unable to respond to hypoxia and hypercapnea, with a failure to auto-resuscitate. Thus, SUDEP likely results from an unfortunate coincidence of precipitating factors that makes one seizure a terminal event following a lifetime of seizures with little or no impact on life.

MECHANISMS OF SUDEP

Recent data obtained from human studies and animal models have implicated severe alterations to respiratory, cardiac, and brain function as three possible mechanistic areas in SUDEP (**Figure 1**) (8–10).

Respiratory Dysfunction

Inpatient video electroencephalogram (EEG) recordings of SUDEP occurring in epilepsy monitoring units show a fairly consistent sequence of respiratory and cardiac events preceding death. In the landmark MORTEMUS study of 16 SUDEP and 9 near SUDEP cases, generalized tonic–clonic seizures preceded an immediate and short phase of rapid breathing, then marked reduction in respiration rate with bradycardia and EEG suppression, followed by terminal apnea and asystole (4). In two-thirds of cases, there was a brief restoration of cardiac function, followed by progressive deterioration of respiration, apnea and asystole. Rarely, SUDEP may also occur without preceding epileptic seizures and follows a similar sequence of cardiorespiratory collapse as that seen in seizure-associated SUDEP (11). While the causes of respiratory dysfunction in SUDEP remain unresolved, physiological recordings of oxygen saturation and end-tidal carbon

dioxide levels in people living with epilepsy have shown that generalized tonic–clonic seizures, which are the predominant terminal seizure type preceding SUDEP, can lead to transient respiratory arrest and apnea (12). One-third of seizures in 56 people with intractable localization-related epilepsy were associated with oxygen desaturation below 90%, and 11 seizures had severe oxygen desaturation below 70%, whereas the interictal baseline of oxygen desaturation in these people never fell below 90% (12). Furthermore, the extent of oxygen desaturation was correlated with seizure duration and was accompanied by an increase in end-tidal carbon dioxide levels in people with available data (12).

Centrally mediated apnea is more common than obstructive apnea during seizures. The forebrain sites underlying seizure-evoked hypoventilation were explored in three people with intractable epilepsy undergoing intracranial electrode and respiratory monitoring (13). When seizure activity spread to the amygdala, or on electrical stimulation of the amygdala, the respiratory rate immediately declined from a baseline of 17 down to 1 breath per minute, and was accompanied by oxygen desaturation (13, 14). The people were awake and vigilant, but unaware of the collapse of respiratory rate, even when the duration of amygdala stimulation was increased to 47 s. Collectively, these studies suggest that seizures can lead to centrally mediated hypoventilation, causing hypoxemia and hypercapnia, and that seizure-related respiratory compromise is a key early event in SUDEP.

Prone sleeping and pulmonary edema are common findings in SUDEP and may further exacerbate the effects of seizure-induced hypoventilation. SUDEP occurs most often at night with people lying in bed in the prone position (4, 5, 15, 16), and people with

FIGURE 1 | Possible mechanisms of sudden unexpected death in epilepsy (SUDEP). PGES, postictal generalized EEG suppression.
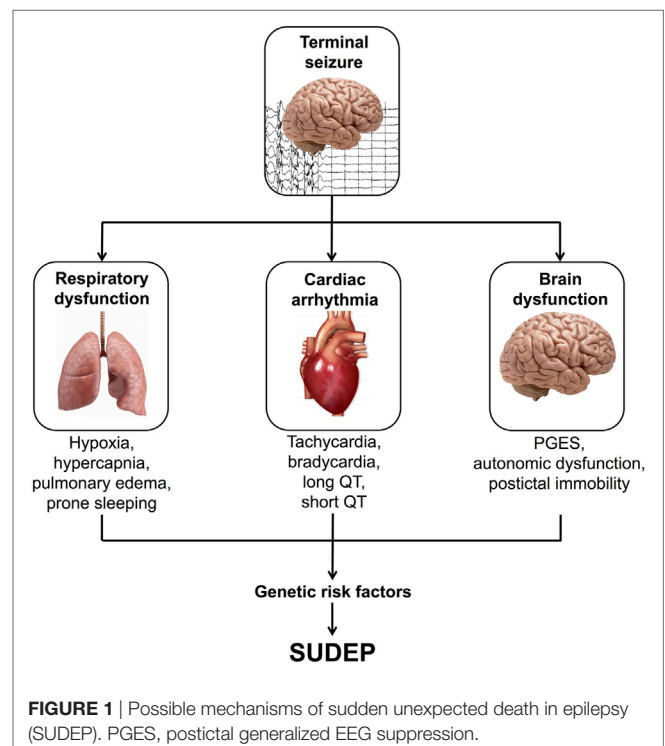
a history of nocturnal seizures have a heightened risk of SUDEP (17). The majority of "near SUDEP" events of the MORTEMUS study occurred during the day, with cardiopulmonary resuscitation occurring within 3 min of cardiorespiratory arrest, whereas no SUDEP cases received such intervention within 10 min of apnea (4). Nocturnal supervision is a proposed protective factor in SUDEP (18), and this raises the possibility that SUDEP occurs more often at night simply because they are less promptly attended, and tending to these people may be sufficient to restore cardiorespiratory function. Pulmonary edema and congestion of the lungs is found in the majority of SUDEP (19–21). Chest X-ray examination of 11 people following a generalized tonic–clonic seizure showed postictal pulmonary edema in 7, and the extent of edema was associated with seizure duration (22). However, although the degree of pulmonary edema is considered to be mild in SUDEP, it may be a further compounding factor on the adverse effects of seizure-related respiratory insufficiency and prone sleeping.

Cardiac Arrhythmia

Epilepsy and seizures can have adverse affects on cardiac function, which may play an important role in the pathophysiology of SUDEP. Seizures can induce cardiac changes, such as tachycardia, bradycardia, and prolongation of the QT interval, possibly due to seizure-related effects on the autonomic nervous system. Ictal tachycardia, generally defined as >100 beats per minute, preceding, during, or following the onset of seizures, is common, occurring in an average of 82% of patients studied (23). Ictal tachycardia leading to ventricular fibrillation has been documented in two "near SUDEP," both of which required defibrillation (4, 24). Slowing of the heart rate during seizures, or ictal bradycardia, is much less common than ictal tachycardia and has mostly been observed in people with temporal lobe epilepsy (25, 26). Postictal bradycardia followed by apnea was a consistent finding among monitored SUDEP of the MORTEMUS study. Why some seizures lead to SUDEP is unresolved, but it is apparent that heart rate changes may vary from seizure to seizure in some people, whereas others may have a consistent pattern.

The QT interval is a measure of the duration of ventricular depolarization and repolarization, and a prolonged or shortened QT interval is associated with sudden death risk in the general population (27, 28). The QT interval may become prolonged or shortened in people with refractory epilepsy (29), following generalized tonic–clonic seizures (30), or in association with seizure-associated oxygen desaturation (31), and seizure-related QT changes have been proposed to be involved in SUDEP (32, 33). However, in one retrospective study of 19 people with epilepsy who later died of SUDEP, the proportion of people with seizure-related prolongation of the QT interval was similar to those who had not died (34).

Long QT syndrome is an autosomal dominant disorder which affects 1 in 3,000 of the general population and is characterized by prolongation of the corrected QT interval on the electrocardiogram (35). Approximately 75% of LQTS is caused by pathogenic variants in the potassium ion channel subunits *KCNQ1* (LQT1, 35%) and *KCNH2* (LQT2, 30%), and the sodium ion channel subunit *SCN5A* (LQT3, 10%). Importantly, familial LQTS can lead to syncope, seizures, and in the most severe cases, sudden cardiac

death (36). When LQTS is compared to SUDEP, there exist some parallels in the circumstances of death (**Table 1**). Of note, both in SUDEP and familial LQTS, the sudden death event is unexpected, frequently occurs at rest, or in bed, and the postmortem findings are identical in that no cause is identified and the heart appears structurally and histologically normal. Emerging evidence suggests that there may be an association between LQTS and epilepsy (37–40). Key causal genes of LQTS encode ion-channels that are expressed in the heart and the brain. *KCNQ1* transcripts are found in the adult human brain, and kcnq1 protein in the mouse is found in pyramidal neurons in CA1 to CA3, granule cells of the dentate gyrus, and hilar interneurons (41). Full-length *KCNH2* transcripts, and a primate-specific brain isoform, are found in human hippocampus, and *KCNH2* protein expression was confirmed in human hippocampus and frontal cortex using western analysis (42). *SCN5A* transcripts are found in human brain, and scn5a protein expression in the rat is detected in the ventral medial, dorsal medial, and posterior hypothalamic nuclei (43). Seizure episodes are common in LQTS, and particularly in LQTS type 2; however, while seizures in LQTS may be related to arrhythmia-mediated cerebral hypoxia, 1.6% of people with LQTS have EEG-documented seizure activity (37). As discussed below, a subset of SUDEP cases have rare variants in common genes responsible for LQTS and mice with a mutation in the LQTS type 1 gene, *kcnq1*, have seizures and sudden death (41).

EEG Suppression

Recordings from monitored SUDEP have shown that the EEG often shows global, persistent attenuation after seizures end, i.e., postictal generalized EEG suppression, suggesting that cerebral compromise could be a key mechanism of SUDEP. Generalized tonic–clonic seizures are the major risk factor for SUDEP across case–control studies and are associated with postictal generalized EEG suppression (44). In a case–control study of 10 SUDEP and 30 people with epilepsy, the duration of postictal generalized EEG suppression was longer in the seizures of SUDEP cases, and for each 1-s increase in duration, the odds of SUDEP increased by a factor of 1.7% (45). It was postulated that the profound and prolonged electrical shutdown of the brain might result in a tendency to central apnea. However, in another study of 17 presurgical patients who died with SUDEP and matched alive control patients, postictal generalized EEG suppression was not an independent risk factor for SUDEP (44). Furthermore, while postictal generalized EEG suppression was linked to the duration and extent of oxygen desaturation, there

TABLE 1 | Comparison of sudden unexpected death in epilepsy (SUDEP) with familial long QT syndrome (LQTS).

Clinical feature	SUDEP	LQTS
Male predominance	Yes	Yes
Heart at postmortem	Normal	Normal
QT interval changes on ECG	Yes	Yes
Circumstances of death	Often unwitnessed, in bed	LQTS3 often at rest
Cause of death at postmortem	Unascertained	Unascertained

LQTS3—long QT syndrome type 3 caused by SCN5A mutations.

was no link with the coincidence of apnea (46). This has lead to an alternative proposal, in which postictal generalized EEG suppression is related to the severity of seizure-related intrinsic pulmonary dysfunction, rather than central apnea. This notion was supported in a study of 70 people with generalized tonic–clonic seizures in whom postictal generalized EEG suppression and postictal immobility were associated with respiratory dysfunction, and in which there was only a short duration of ictal apnea (47).

Most people who fit the high-risk profile of SUDEP do not die, and it is not clear why some seizures lead to SUDEP while others do not. Case reports and case–control studies of SUDEP have enhanced our understanding of SUDEP and its risk factors. However, inconsistencies between clinical associations likely relate to confounding factors such as the ascertainment of highly selected clinical or presurgical groups and the different seizure types, anti-epileptic medications, and study methodologies. Furthermore, the number of cases studied tends to be limited by the paucity of SUDEP cases with available phenotype data. Most likely, cardiorespiratory rates vary between seizures and patients; whether a seizure leads to SUDEP probably involves an unfortunate and interrelated combination of mechanisms, with additional environmental and genetic risk factors.

EVIDENCE FOR GENETIC RISK FACTORS IN SUDEP

While SUDEP does not show a familial tendency, with two possible exceptions (48, 49), genetic analysis of SUDEP cases has been performed in a small number of studies to search for possible genetic risk factors, with a number of genes implicated (**Table 2**). With the availability of low cost, high throughput DNA sequencing, studies have progressed from single "candidate gene" -based studies (16, 50), to sequencing all 22,000 protein-coding genes, i.e., the exome (51), or the whole genome (52). A limiting factor is the availability of sufficient DNA from SUDEP cases, which is either collected during life of the patient or extracted from postmortem blood. There is increasing awareness of the importance of collecting a blood sample in the setting of unexplained sudden cardiac death, as postmortem genetic testing of cardiac arrhythmia genes, i.e., the molecular autopsy, can identify additional causes of sudden cardiac death over autopsy alone (53, 54). Most recently, DNA extracted from formalin-fixed, paraffin-embedded postmortem tissue that was stored for up to 14 years has been successfully exome sequenced (55). This study included the fixed postmortem tissue of one SUDEP in a person with Dravet syndrome in which the molecular autopsy identified a pathogenic SCN1A variant. This raises the exciting prospect that historical collections of postmortem fixed tissue may hold untapped pools of SUDEP cases suitable for genetic analysis and for which phenotyping data are available. Furthermore, this study offers encouragement that postmortem fixed tissue may provide an alternative source of DNA for exome sequencing-based molecular autopsy when postmortem blood or high quality extracted DNA is not available. Nevertheless, we recommend that postmortem blood should be kept in all SUDEP cases as a source of high quality DNA for genetic analysis.

TABLE 2 | Genes associated with sudden unexpected death in epilepsy (SUDEP).

Gene	OMIM disease	Evidence for association with SUDEP
KCNA1	Episodic ataxia/myokymia syndrome	Animal model; variant found in SUDEP case
SCN1A	Dravet syndrome	Animal model; de novo variants found in SUDEP cases
SCN2A	Early-infantile epileptic encephalopathy 11	De novo variants found in SUDEP cases
SCN8A	Early-infantile epileptic encephalopathy 13	Animal model; de novo variants found in SUDEP cases
DEPDC5	Familial focal epilepsy with variable foci	De novo variants found in SUDEP cases
KCNQ1	Long QT syndrome type 1	Variants found in SUDEP cases
KCNH2	Long QT syndrome type 2	Variants found in SUDEP cases
SCN5A	Long QT syndrome type 3	De novo variant found in SUDEP case

Inherited Cardiac Arrhythmia Genes

The first support for involvement of LQTS gene variants in SUDEP was on finding a novel SCN5A Arg523Cys variant following sequencing of five LQTS-associated genes in four SUDEP cases (56). The female patient had experienced generalized tonic–clonic seizures from age 17 years, involving bilateral synchronous epileptogenic activity on EEG; she was found dead in bed at age 25 years. Variants in SCN5A, encoding sodium voltage-gated channel alpha subunit 5, cause Brugada syndrome and LQTS type 3, which is associated with arrhythmias during rest or sleep (57).

Further support for LQTS variants in SUDEP was provided with a larger retrospective review of postmortem reports at a single forensic center between 1993 and 2009, which identified 22 SUDEP and 46 possible SUDEP cases, with DNA available on 48 cases (16). Sequencing of the three common LQTS genes, KCNQ1, KCNH2, and SCN5A, identified two rare nonsynonymous variants. These were a KCNH2 Arg176Trp variant, which has been reported as a founder mutation associated with a prolonged QT interval in Finnish LQTS families, and alters ion-channel activity in vitro, and an SCN5A Pro1090Leu, which has been reported previously in LQTS and in a case of sudden cardiac death (16). Further analysis of the same cohort was performed with sequencing of the family of four hyperpolarization-activated cyclic nucleotide-gated cation channel genes (HCN1-4) (50). HCN channels are voltage-gated ion channels primarily involved in the generation of spontaneous rhythmic activity in both cardiac pacemaker and neuronal cells, and genetic variants in HCN1, HCN2, and HCN4 genes have been reported to account for familial sinus bradycardia and familial epilepsy syndromes. Three rare non-synonymous variants were identified; Phe738Cys and Pro802Ser in HCN2 and Gly973Arg in HCN4, all of which were located in the cytoplasmic tail region of the proteins (50). Since the ascertained cases were de-identified, there was no opportunity to contact the surviving family to perform clinical phenotyping or co-segregation studies. The finite source of DNA and Sanger sequencing approach limited the analysis of additional genes in these people with SUDEP.
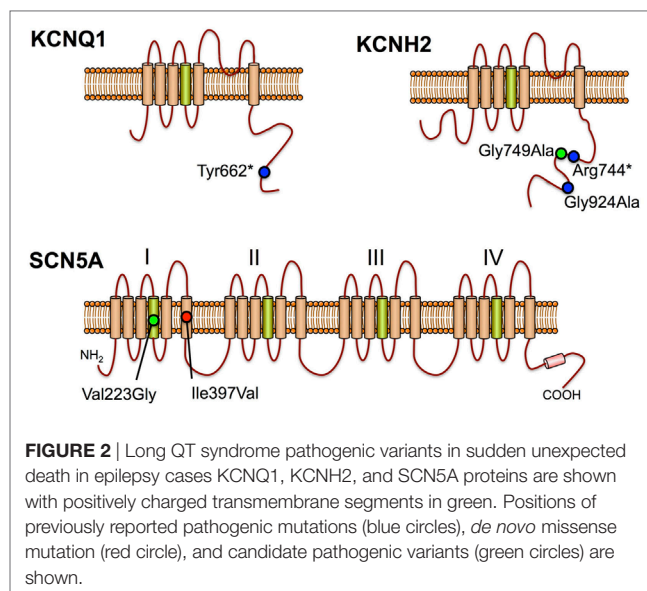
The largest SUDEP cohort with genetic investigation involved exome sequencing-based analysis of cardiac arrhythmia genes and

epilepsy genes in 61 SUDEP cases (51). Importantly, the majority ($n = 54$) were classified "definite SUDEP" and 27 were previous participants of the Epilepsy Research Program, Melbourne, with detailed epilepsy phenotyping data and access to parental DNA for six. Of the remaining SUDEP, 15 were prospectively collected and 19 retrospectively collected coronial cases. The cohort comprised 56% males and the mean age at SUDEP was 28 years, with 27 out of 28 people found dead in bed in the prone position for which this information was available. There was a range of epilepsies phenotyped during life from the Melbourne cohort, but none had a diagnosis of cardiac disease. Analysis of exome sequencing data focused on the 3 common LQTS genes, 29 additional cardiac arrhythmia genes, 5 genes involved in central control of ventilation, and 72 epilepsy genes. Of particular note in cardiac arrhythmia genes, four pathogenic and two candidate pathogenic variants were found in the three key LQTS genes, including a *de novo SCN5A* Ile397Val variant, a Gly924Ala and Arg744* nonsense variant in *KCNH2*, and a Tyr662* nonsense variant in *KCNQ1* (**Figure 2**). The *KCNQ1* and *KCNH2* variants had been previously reported in people with LQTS and were absent in over 60,000 population controls, whereas the *de novo SCN5A* variant occurred in a highly conserved transmembrane domain. These four variants were regarded as highly likely to be pathogenic for LQTS and were found in one coronial SUDEP case and three SUDEP from the Melbourne Epilepsy Research Centre cohort.

In a further analysis of the exome data, the number of rare variants in each one of 22,000 genes from 58 European SUDEP cases and 2,936 European people who did not have epilepsy were compared, to test for genes enriched with rare variants. Although no gene reached genome-wide significance after correcting for multiple testing, the LQTS type 2 gene, *KCNH2* ($p = 0.0037$), was among the top 30 genes with the greatest number of rare variants in SUDEP compared to controls. People with LQTS type 2 are more likely to have a personal diagnosis of epilepsy history and a seizure phenotype compared to other types of long QT (37, 38), and this may account for the higher number of *KCNH2* variants in this cohort of SUDEP.

The finding of LQTS variants in SUDEP raises a number of questions: do variants in LQTS genes expressed in the brain and heart cause epilepsy as well as arrhythmias and sudden cardiac death? Is the sequence of cardiorespiratory events preceding SUDEP in people with epilepsy and LQTS variants different from those without LQTS variants? Does finding a LQTS variant in a person with epilepsy and sudden unexpected death following a molecular autopsy mean that the death should be reclassified as a sudden cardiac death? The finding of LQTS variants in SUDEP also has important implications for the surviving family members, both with and without epilepsy, who risk inheriting the variant and, therefore, being at increased risk of arrhythmias and sudden death. If an LQTS variant is found in a person with epilepsy during life there may be important clinical implications, including avoidance of medications that may prolong the QT interval, selection of antiarrhythmic drugs such as beta-blockers, and interventions for potentially lethal cardiac arrhythmias, such as implantable cardioverter defibrillator therapy to prevent sudden death. Importantly, SUDEP in

FIGURE 2 | Long QT syndrome pathogenic variants in sudden unexpected death in epilepsy cases KCNQ1, KCNH2, and SCN5A proteins are shown with positively charged transmembrane segments in green. Positions of previously reported pathogenic mutations (blue circles), *de novo* missense mutation (red circle), and candidate pathogenic variants (green circles) are shown.

people with epilepsy and LQTS variants may be predictable and preventable.

Genetic Epilepsies with Increased SUDEP Risk

Some genetic epilepsies have a high incidence of SUDEP and the associated pathogenic variants may represent convenient biomarkers of SUDEP risk. In Dravet syndrome, >80% of people have a variant in *SCN1A*, encoding a neuronal voltage-gated sodium channel alpha subunit 1, with 95% of 80 tested variants arising *de novo* (58). There is a high mortality rate and SUDEP is the most common cause of death, with 59% of deaths due to SUDEP in a cohort of 100 Dravet syndrome who were followed for 1,073 person-years (59). This constitutes a Dravet-specific SUDEP rate of 9.32 per 1,000 person-years, which is among the highest SUDEP incidence rate reported in selected groups. Risk factors of SUDEP are common features of Dravet syndrome, including generalized tonic–clonic seizures, early seizure onset, and polytherapy. Furthermore, heart rate variability, a measure of sino-atrial node activity, was decreased in two cohorts of 15 and 20 Dravet syndrome compared to people with other epilepsies and healthy people, and this may increase susceptibility to cardiac conduction disease (60, 61). Mice heterozygous for a partial deletion of *scn1a,* or an *scn1a* nonsense variant, recapitulate the features of Dravet syndrome, including sudden death, and have been proposed as models of SUDEP (62, 63). In both of these mouse models, the circumstances of spontaneous or provoked seizures preceding death are similar to SUDEP in humans, in that generalized tonic–clonic seizures are followed by marked bradycardia and death. Mice that died had a higher number of seizures in the preceding 24 h compared to mice that did not die. Mice with global deletion of *scn1a,* or selective deletion of *scn1a* from the forebrain, have reduced heart rate variability, as in human Dravet syndrome (61), whereas *scn1a* deletion from the ventricles causes increased heart rate variability.

Variants in the brain and cardiac expressed voltage-gated sodium channel alpha 8 gene, *SCN8A*, cause early-infantile encephalopathy, with seizure onset between birth and 12 months of age (64). SUDEP occurs in 10% of reported cases, and SUDEP risk factors, such as generalized tonic–clonic seizures and intellectual disability, were present in most of these people (64, 65). Close to 1% of epileptic encephalopathy have *SCN8A* missense variants, typically located in the highly conserved transmembrane segments, and more than 60 have arisen as *de novo* mutations (65). Functional studies suggest that *SCN8A* missense mutations may cause impaired channel inactivation and persistent sodium current, which may increase neuronal excitability and firing (64). A *de novo SCN8A* Asn1768Asp variant found in a child with ataxia, intellectual disability, early-onset epileptic encephalopathy and SUDEP, was incorporated into a mouse model (52). Mice heterozygous for this variant show ataxia, as found in the child with this variant, and up to three seizures per day from age 2–3 months, which progress to SUDEP within the following month (66). There was incomplete penetrance, with half of the heterozygous mice showing no seizures after 6 months, whereas all mice homozygous for the missense variant have SUDEP, consistent with a gene dosage effect. The mice have increased persistent sodium current and neuronal hyperexcitability, in keeping with functional studies of *SCN8A* missense variants in transfected cells. *SCN8A* is expressed in the heart, and myocytes from mice with the *SCN8A* Asn1768Asp variant showed a lowered threshold for action potential firing and an increased incidence of delayed after-depolarizations. Two of three mice that had monitored death showed severe bradycardia preceding asystole (67).

As previously mentioned, exome-wide variant burden analysis of 59 SUDEP and almost 3,000 people without epilepsy was performed to identify genes with an excess of rare variants (51). Epilepsy genes showing a high number of variants with this analysis included another of the neuronal voltage-gated sodium channel genes, *SCN2A,* whereas the familial focal epilepsy gene, *DEPDC5,* was ranked first when considering variants that appear only once in the entire dataset. Variants in *SCN2A* cause Ohtahara syndrome and unclassified early-onset epileptic encephalopathies (68). At least three SUDEP with variants in *SCN2A* have been reported, including two with *de novo* mutations (51, 69). It remains to be determined why the early onset epileptic syndromes caused by variants in voltage-gated sodium channels, *SCN1A*, *SCN2A*, and *SCN8A* should have a high risk of SUDEP, but may be directly related to their effects on cardiorespiratory function, or their association with known SUDEP risk factors, such as generalize tonic–clonic seizures, young age at seizure onset, and intractable epilepsy.

Six out of 61 SUDEP had a rare or novel variant in *DEPDC5*, including 4 with nonsense variants, which accounted for the higher number of *DEPDC5* variants in SUDEP compared to people without epilepsy. Further *DEPDC5* nonsense variants have been reported in two brothers with SUDEP (48) and in one individual from a large French family with focal epilepsy (70). *DEPDC5* encodes disheveled, Egl-10, and plekstrin domain-containing protein 5, a negative regulator of the mammalian target of rapamycin complex I (mTORC1), and nonsense mutations lead to increased mTORC1 activity.

These insights into possible genetic underpinnings of SUDEP highlight the value of the molecular autopsy of SUDEP and the possible value of genetic biomarkers of SUDEP risk. In particular, the finding of LQTS gene variants has important clinical implications for the surviving family members who may also have inherited the variant and also be at risk of sudden death. The high number of *de novo* variants in SUDEP suggests that sequencing analysis of family trios can reveal SUDEP high-risk alleles. We advocate postmortem genetic testing of SUDEP with analysis of previously implicated genes (**Table 2**) as an adjunct to the autopsy investigation.

Respiratory Genes with SUDEP Risk

The link between genes associated with neuronal regulation of respiratory function and SUDEP stem primarily from studies of animal models. Serotonin and other neuropeptides modify excitability of the respiratory network, and excitation or inhibition of serotonin neurones can increase or reduce respiratory drive, respectively. Serotonin neurones are stimulated by increased carbon dioxide concentrations and dysfunction of serotonin neurons may impair the ventilatory response to hypercapnea, predisposing to SUDEP. Mice with a nonsense mutation in the serotonin receptor gene, *htr2c*, are susceptible to rare spontaneous and audiogenic tonic–clonic seizures, respiratory arrest and sudden death (71, 72). The DBA/2 mouse is also considered a useful model of SUDEP, since the mice exhibit convulsive audiogenic seizures followed by respiratory arrest and sudden death (73). DRBA/2 mice have reduced expression of specific serotonin receptors in the brain (74), and treatment with the serotonin reuptake inhibitor, fluoxetine, showed a dose-related reduction in the incidence of seizures and respiratory arrest (75).

In humans, seizure-related respiratory compromise during sleep, followed by bradyarrhythmia and apnea, is a common finding in monitored SUDEP. Congenital central hypoventilation syndrome is a potentially lethal autonomic nervous system disorder characterized by hypoventilation and impaired ventilatory response to hypercapnia and hypoxemia during sleep. An increased frequency of bradyarrhythmias has been reported in children with congenital central hypoventilation syndrome. Expansion of an alanine repeat in the homeobox gene *PHOX2B*, is the predominant cause of congenital central hypoventilation syndrome, with frameshift, nonsense, and missense mutations in *PHOX2B* accounting for a small proportion of cases. Subjects with smaller *PHOX2B* expansions can present in later life with nocturnal hypoventilation and some have coexistent epilepsy. The involvement of variants in *PHOX2B* in SUDEP is, therefore, an attractive hypothesis; however, no polyalanine repeat expansions or point mutations were identified in a large SUDEP cohort (76). Furthermore, no rare variants were identified in and additional five genes with plausible roles in central control of ventilation (*ASCL1*, *BDNF*, *EDN3*, *GDNF*, and *RET*) among 61 SUDEP cases (51).

CONCLUSION

Sudden unexpected death in epilepsy is a tragic event and failure to identify a cause of death has major clinical, emotional, and

psychological effects on the surviving family. The discovery, validation, and pathophysiological role of genetic variants in SUDEP is important for further understanding of risk factors and prediction in families of people with SUDEP. Exome or genome sequencing of SUDEP enables a search for variants in multiple genes related to epilepsy, cardiac arrhythmia, and respiratory function, with plausible roles in the underlying pathophysiology of SUDEP. Molecular autopsy has revealed a surprising number of SUDEP cases with variants in cardiac arrhythmia genes that may heighten risk of unexplained sudden death and SUDEP in people with LQTS mutations may be predictable and preventable. There are also a number of *de novo* mutations reported in cardiac arrhythmia and epilepsy genes, which has important implications for the surviving family members as the risk of sudden death will

be low, and may partly explain why SUDEP is largely non-familial. The ultimate goal will be to use genetic screening of people with epilepsy to identify gene variants that could increase the risk of sudden unexpected death.

AUTHOR CONTRIBUTIONS

RB, DC, and CS wrote the manuscript and approved the final version.

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Impaired Baroreflex Sensitivity after Bilateral Convulsive Seizures in Patients with Focal Epilepsy

Kevin G. Hampel[1,2], Christian E. Elger[1] and Rainer Surges[1]*

[1] Department of Epileptology, University Hospital Bonn, Bonn, Germany, [2] Multidisciplinary Epilepsy Unit, Neurology Service, University Hospital La Fe, Valencia, Spain

Background: Sudden unexpected death in epilepsy (SUDEP) is probably due to an autonomic failure in the early postictal phase after bilateral convulsive seizures (BCS) in the majority of cases. The baroreflex sensitivity (BRS) is an established and reliable biomarker of autonomic function and sudden cardiac death.

Objective: To investigate whether postictal BRS depends on seizure type.

Methods: Beat-to-beat systemic blood pressure and heart rate were continuously and non-invasively recorded with the ccNexfin® device in patients with focal epilepsy undergoing video-EEG monitoring. BRS was calculated using the sequence as well as the spectral method. A random mixed linear model was applied to analyze the influence of seizure type on BRS during three different time periods of 15-min length each (interictal, preictal, and postictal). In addition, the possible effects of other factors (hypertension, hemispheric lateralization of ictal activity, epilepsy type, body position, vigilance state) were explored. Data are given as median with interquartile range.

Results: A total of 26 seizures of 26 patients were analyzed. In BCS ($n = 7$), BRS significantly dropped from a preictal value of 15.0 ms/mm Hg (13.0–19.4) and an interictal value of 15.6 ms/mm Hg (12.0–20.4) to 3.1 ms/mm Hg (2.7–10.5) during the postictal period ($p < 0.0001$) according to the sequence method. This finding was replicated with the spectral method. In contrast, focal seizures ($n = 19$) did not lead to significant alterations of BRS in the postictal phase.

Conclusion: Postictal BRS depends on the seizure type and is markedly impaired after BCS. The present study provides further evidence for a disturbed autonomic function following BCS. These findings might be related to cardiovascular failure in the context of SUDEP.

Keywords: epileptic seizures, autonomic nervous system, systemic blood pressure, heart rate, mortality, sudden unexpected death in epilepsy

INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) is the most frequent directly epilepsy-related cause of premature death in people with epilepsy (1). Except for stroke, no other neurologic disorder leads to more loss of potential patient years than SUDEP (2). The current knowledge points toward an early postictal autonomic failure after bilateral convulsive seizures (BCS) as the cause of death in most SUDEP cases (3). Recently, we found that heart rate (HR) and systemic blood pressure (BP) are differentially modulated in the early postictal phase after BCS, suggesting

possible autonomic imbalance (4). While HR was elevated by 75% 2 min after BCS-cessation, the mean arterial BP was only increased by 15% and even dropped slightly below the preictal values 5 min after seizure termination. Because systemic BP is a critical hemodynamic factor, it is tightly regulated to maintain adequate perfusion of vital organs like the heart and brain (5). The baroreflex regulates short- and long-term BP modulation and represents one of the most reliable indicators for autonomic function (6). Baroreflex dysfunction was previously found in patients with temporal lobe epilepsy in the interictal period as compared to healthy controls (7). However, seizure-related changes of the baroreflex function have not been reported yet. Here, we investigated whether postictal baroreflex sensitivity (BRS) depends on the seizure type.

PATIENTS AND METHODS

Patients

Adult patients aged 18 years or older who were evaluated by video-EEG-monitoring for epilepsy surgery or differential diagnosis were prospectively enrolled from September 2013 to December 2015 in the Department of Epileptology, University Hospital Bonn (Germany). Data on seizure-related HR and systemic BP of included patients were previously published (4). Epilepsies and seizures were classified according to the revised International League Against Epilepsy terminology (8).

Data Recording and Processing

Patients underwent conventional scalp EEG recordings according to the 10–20 system or invasive presurgical monitoring with intracranial electrodes according to the results of prior non-invasive video-EEG telemetry (Micromed S.p.A., Mogliano Veneto, Italy). In addition, pulse rate, oxygen saturation (LNCS DC-I® reusable sensor, Masimo, Irvine, CA, USA), and arterial BP were continuously and non-invasively recorded from beat-to-beat using the ccNexfin® device (BMEYE, Amsterdam, the Netherlands) for up to 8 h a day (maximum approved time span for the device applied at a single finger). The exact methodology for the data recording was described previously (4). For each seizure, consecutive values of systolic BP and HR obtained by the ccNexfin® device at three different time periods (interictal, i.e., starting 5 min after the beginning of the recordings or starting 20 min before the end of the recording; preictal, i.e., up to the last 2 min before the seizure start; postictal, i.e., starting 2 min after seizure end) of 15 min duration each were considered. The period length of 15 min was chosen, because the sequence method for BRS requires a time interval of at least 15 min to obtain reliable results (9). Seizures with (i) overlapping time intervals of interictal and preictal or interictal and postictal intervals or (ii) seizures with periictal time intervals below 15 min were excluded from the analysis. We assessed BRS with two different methods, the sequence and the spectral method. The modified sequence method was applied with the following settings: the threshold for change in R wave-to-R wave interval (R-R interval) 4 ms; a zero time shift between the systolic PB pulse and R-R interval; and a correlation coefficient threshold of 0.8 between systolic PB and R-R interval sequences and the whole average of negative and positive slopes (10, 11).

The modified spectral method was applied using the average of the whole low frequency band (12–14).

Statistical Analysis

A linear random mixed effect model was applied with restricted maximum likelihood to estimate the effect of time interval (interictal, preictal, and postictal) and seizure type (BCS, FS) on BRS (15). Since the BRS calculated with the sequence and the spectral method was right skewed, we used the log-transformed BRS as the outcome variable for the analysis. As a fixed effect we entered time interval and seizure type, as well as possible influencing factors including hemispheric lateralization of ictal activity (left, right, or bilateral), epilepsy type (temporal lobe epilepsy or extra-temporal lobe epilepsy), preictal vigilance state (awake or asleep), preictal body position (laying or sitting), circumstances (spontaneous or triggered), hypertension (taking antihypertensive drugs or not), and postictal generalized EEG suppression (PGES present or not). As random effects, we included random intercepts for patients to account for non-independence in the data. We used backward selection to find the best model (16). Normality of residuals and random effects of the final model were validated by visual inspection and the Shapiro–Wilk test. Data are given as median with the interquartile range (IQR) or as geometric mean with corresponding 95% confidence interval. p-values ≤0.05 were considered significant. We used simultaneous inference procedures to adjust the p-values with the Holm–Bonferroni method to further test the comparison between the different time intervals of FS and BCS (17). The statistical analysis and the graphs were performed using R version 3.3.0 with the packages dplyr version 0.4.3, multcomp version 1.4.5, and ggplot2 version 2.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Forty-five seizures were considered for the analysis, but 19 seizures had to be excluded because time intervals were shorter than the required time length of 15 min. Finally, a total of 26 seizures of 26 patients with drug-resistant focal epilepsy met the inclusion criteria and were analyzed. **Table 1** displays the clinical summary data of these patients (further details on patient and seizure characteristics are provided in **Tables 2** and **3**). Several factors and potential confounders were tested for possible associations and interactions with BSR. Since preictal vigilance state, preictal body position, circumstances, hypertension, epilepsy type, and hemispheric lateralization appeared to have no significant effect on BSR, the final model for both methods (sequence and spectral method) included time interval, seizure type, and the interaction between time interval and seizure type. **Tables 4** and **5** display the summary statistics of the two models.

BSR According to the Sequence Method

In BCS (n = 7), BRS significantly dropped from a preictal value of 15.0 ms/mm Hg (IQR 13.0−19.4, adjusted p-value < 0.0001) and interictal value of 15.6 ms/mm Hg (IQR 12.0−20.4, adjusted p-value < 0.0001) to a postictal value of 3.1 ms/mm Hg (IQR 2.7−10.5). Although not statistically significant, BCS with PGES (n = 3) tended to be associated with a lower postictal BRS as compared to BCS without PGES (n = 4, adjusted p-value = 0.10).

In FS ($n = 19$), no significant difference was found for the pre-ictal BRS of 10.5 ms/mm Hg (IQR 7.6−13.2) as compared to the interictal BRS of 8.6 ms/mm Hg (IQR 7.1−12.6) or the postictal BRS of 11.4 ms/mm Hg (IQR 6.7−13.9). **Figure 1A** (left panel) summarizes the BRS values calculated with the sequence method in patients with BCS and FS. **Figure 2A** (left panel) shows the individual profiles of the patients.

BSR According to the Spectral Method

In BCS ($n = 7$), BRS significantly decreased from 10.2 ms/mm Hg (IQR 8.8−11.6, adjusted p-value = 0.014) preictally and 10.0 ms/mm Hg (IQR 7.4−14.0, adjusted p-value = 0.014) interictally to 5.3 ms/mm Hg (IQR 3.6−7.6) postictally. BRS in BCS with or without PGES did not differ significantly during the postictal time interval (adjusted p-value = 0.24). In FS ($n = 19$), postictal BRS (7.8 ms/mm Hg, IQR 6.8−9.4) was not different from preictal (8.4 ms/mm Hg,

IRQ 5.8−10.8) or interictal (7.8 ms/mm Hg, IQR 5.5−11.1) values. **Figure 1B** (right panel) summarizes the BRS values calculated with the spectral method in patients with BCS and FS. **Figure 2B** (right panel) shows the individual profiles of the patients.

DISCUSSION

In summary, we found that in contrast to FS, BRS is markedly impaired in the early postictal period following BCS, suggesting that BCS lead to substantial postictal autonomic dysfunction.

Potential Mechanisms of Seizure-Related Modulation of BP and BRS

The mechanisms of how epileptic seizures affect systemic BP and BRS are not fully understood [1]. The anatomic basis consists of the sympathetic and parasympathetic branches of the autonomic nervous system, which regulates BP *via* their effects on HR, cardiac output, and total peripheral resistance. The central pathway of the baroreflex involves several brain structures [6]. The nucleus tractus solitarius receives the afferent input of baroreceptors. From there the parasympathetic branch is processed through the nucleus ambiguous and finally inhibits the sinus node to reduce HR and cardiac output. The sympathetic pathway is processed through the caudal ventrolateral medulla, rostral ventrolateral medulla and preganglionic sympathetic neurons [5]. From there the sympathetic outflow mainly increases total peripheral resistance through vasoconstriction of muscle, renal, and mesenteric blood vessels. In addition, subordinate centers continuously modulate the baroreflex arc.

TABLE 1 | Summary of clinical characteristics.

Characteristics	BCS ($n = 7$)	FS ($n = 19$)
Age (median, IQR)	33.4 (27.7–44.6)	39.5 (32–43.5)
Female (no.)	5	7
Age at disease onset (median, IQR)	20 (10–23)	19 (9.5–29)
Localization of seizure onset (no.)		
Temporal	5	13
Extra-temporal	2	6

IQR, interquartile range; BCS, focal seizure evolving to bilateral convulsive seizure; FS, focal seizure.

TABLE 2 | Clinical characteristics of patients with BCS and FS.

Patient no.	Sex	Age (years)	Age at onset (years)	Type of recorded seizure	Epilepsy type (hemispheric lateralization)	EEG (interictal)	Cerebral MRI
1	F	31.9	13	FS	FLE (U)	No epileptic activity	Normal
2	F	42.8	37	FS	TLE (L)	Normal	Gliosis temporal (L)
3	F	51.6	6	BCS	TLE (R)	Temporal-occipital (R)	Hippocampal sclerosis (B)
4	F	44.2	34	FS	FLE (R)	Frontal (B)	No epileptic lesion
5	F	42.1	20	BCS	TLE (R)	Temporal (B)	Hippocampal sclerosis (R)
6	M	23.7	19	FS	TLE (R)	Temporal (L)	Gliosis occipital (R)
7	M	32.1	11	FS	TLE (L)	No epileptic activity	Normal
8	M	47.0	31	BCS	TLE (R)	Temporal-occipital (R)	Hippocampal sclerosis (R)
9	M	32.7	22	FS	TLE (R)	Generalized	Tumor temporal (R)
10	F	29.1	25	BCS	TLE (R)	Temporal (B)	No epileptic lesion
11	M	48.2	17	FS	TLE (L)	Temporal (L)	Tumor occipital (R)
12	F	33.4	21	BCS	Temporal-parietal (L)[a]	Frontal-temporal-central (L)	No epileptic lesion
13	F	26.2	14	BCS	TLE (R)	Temporal (L)	Heterotopia lateral ventricle (R)
14	M	25.8	1	FS	TLE (R)	Temporal (R)	Hippocampal sclerosis (R)
15	M	38.8	31	FS	FLE (L)	Normal	Normal
16	F	39.5	16	FS	TLE (L)	Temporal (B)	No epileptic lesion
17	F	19.3	7	FS	FLE (L)	Normal	Focal cortical dysplasia cingulate cortex (L)
18	M	40.4	27	FS	TLE (L)	Temporal (L)	Normal
19	M	39.6	30	FS	TLE (L)	Normal	Multiple lesions
20	M	18.0	4	BCS	FLE (L)	Frontal (R)	Focal cortical dysplasia frontal (R)
21	M	61.9	22	FS	TLE (R)	Normal	No epileptic lesion
22	M	20.9	7	FS	FLE (L)	Frontal (L)	Normal
23	F	55.3	2	FS	TLE (R)	Temporal (R)	Hippocampal sclerosis (R)
24	M	40.3	31	FS	TLE (R)	Temporal (R)	Hippocampal sclerosis (R)
25	F	46.9	8	FS	FLE (L)	Generalized	Extensive focal cortical dysplasia (L)
26	M	38.5	28	FS	TLE (L)	Temporal (L)	Hippocampal sclerosis (L)

B, bilateral; BCS, bilateral convulsive seizures; FLE, frontal lobe epilepsy; F, female; FSs, focal seizures; L, left; M, male; R, right; TLE, temporal lobe epilepsy; U, undefined.
[a]According to intracranial EEG monitoring extended seizure onset zone left temporal-parietal.

TABLE 3 | Basic event characteristics of BCS and FS.

Patient no.	Vigilance	Body position	Circumstances	Impaired consciousness	Seizure duration (s)	EEG onset (ictal)	PGES	AHD	AED
1	Asleep	Laying	Spontaneous	Yes	32	Fronto-temporal (R)	No	No	LEV, LTG, CLB
2	Awake	Sitting	Triggered by music	Yes	50	Temporal (L)	No	No	LTG
3	Awake	Laying	Spontaneous	Yes	102	Generalized	No	No	LTG
4	Awake	Laying	Spontaneous	No	12	No focal onset	No	No	LTG, OXC, TPM, RFM, DZP
5	Awake	Laying	Spontaneous	Yes	94	Temporal (B)	Yes	No	OXC, LTG
6	Asleep	Laying	Spontaneous	Yes	123	Temporal-occipital (R)	No	No	No
7	Awake	Laying	Spontaneous	No	11	Temporal-occipital (L)	No	No	LTG, CLB, PER, LCM, PGB
8	Awake	Laying	Spontaneous	Yes	84	Temporal-occipital (R)	Yes	MP	LEV, LCM
9	Awake	Laying	Spontaneous	Yes	16	Temporal (R)	No	No	LEV, LCM, LTG
10	Awake	Sitting	Spontaneous	Yes	138	Generalized	Yes	No	OXC
11	Awake	Laying	Triggered by stimulation[a]	No	203	Temporal (L)	No	No	LEV, LCM
12	Awake	Laying	Spontaneous	Yes	98	Temporal-parietal (L)	No	No	LTG
13	Awake	Sitting	Spontaneous	Yes	146	Temporal-occipital (R)	No	No	No
14	Awake	Laying	Spontaneous	Yes	71	Temporal-occipital (R)	No	No	TPM
15	Awake	Laying	Spontaneous	No	13	No ictal rhythm	No	No	CBZ, OXC, VPA, CLB
16	Asleep	Laying	Spontaneous	Yes	102	Temporal (L)	No	No	No
17	Awake	Laying	Spontaneous	480	41	No focal onset	No	No	LEV, OXC
18	Awake	Laying	Spontaneous	Yes	77	Temporal (L)	No	No	No
19	Awake	Sitting	Spontaneous	No	36	No focal onset	No	No	No
20	Awake	Laying	Triggered by stimulation[a]	Yes	678	Frontal (R)	No	No	LTG, TPM
21	Asleep	Laying	Spontaneous	Yes	96	Temporal (R)	No	CAN, HCT	CBZ
22	Asleep	Laying	Spontaneous	No	32	No focal onset	No	No	LEV, ZNS, OXC
23	Awake	Laying	Spontaneous	Yes	62	No focal onset	No	No	LEV, LCM, PER
24	Awake	Laying	Spontaneous	No	40	Temporal (R)	No	No	LTG, PER
25	Awake	Laying	Spontaneous	Yes	19	No focal onset	No	No	PHB, LEV, LCM, PER
26	Awake	Laying	Spontaneous	No	27	No focal onset	No	No	No

AED, anti-epileptic drug on the day of the seizure; AHD, antihypertensive drug on the day of the seizure; BCS, bilateral convulsive seizures; CAN, candesartan; CBZ, carbamazepine; CLB, clobazam; DZP, diazepam; ESL, eslicarbazepine; FSs, focal seizures; HCT, hydrochlorothiazide; L, left; MP, metoprolol; LCM, lacosamide, LTG, lamotrigine; LEV, levetiracetam; OXC, oxcarbazepine; PER, perampanel; PHB, phenobarbital; PGB, pregabalin; PGES, postictal generalized EEG suppression; R right; RFM, rufinamide; TPM, topiramate; ZNS, zonisamide.
[a]Stimulation refers to electrical stimulation of an implanted depth electrode.

TABLE 4 | Summary of the final model (sequence method).

Variable	Coefficient β	95% confidence interval	*T*-value	*p*-Value
Intercept[a]	10.1[b] ms/mm Hg	7.8–13[b] ms/mm Hg	18.2	<0.001
Interictal time interval	99.6%	79.4–124.91%	−0.036	0.97
Postictal time interval	89.8%	67.4–119.6%	−0.755	0.45
Seizure type (BCS)	158%	95.6–261.1%	1.88	0.07
Interaction (interictal and BCS)	98.6%	63.7–152.7%	−0.06	0.95
Interaction (postictal and BCS)	33%	19–57.4%	−4.035	<0.001

[a]Intercept refers to the reference class of the model focal seizure (FS) and preictal time interval.
[b]Geometric mean of baroreflex sensitivity (BRS).
BCS, bilateral convulsive seizures.

**TABLE 5 | Summary of the final model (spectral method).**

Variable	Coefficient β	95% confidence interval	*T*-value	*p*-Value
Intercept[a]	8.1[b] ms/mm Hg	6.8–9.6[b] ms/mm Hg	24.3	<0.001
Interictal time interval	95.5%	78.6–116.2%	−0.47	0.64
Postictal time interval	96.9%	80.4–116.8%	−0.35	0.73
Seizure type (BCS)	122.3%	86.9–172%	1.21	0.24
Interaction (interictal and BCS)	108.9%	74.7–158.8%	0.45	0.65
Interaction (postictal and BCS)	55.7%	38.9–79.7%	−3.28	0.002

[a]Intercept refers to the reference class of the model focal seizure (FS) and preictal time interval.
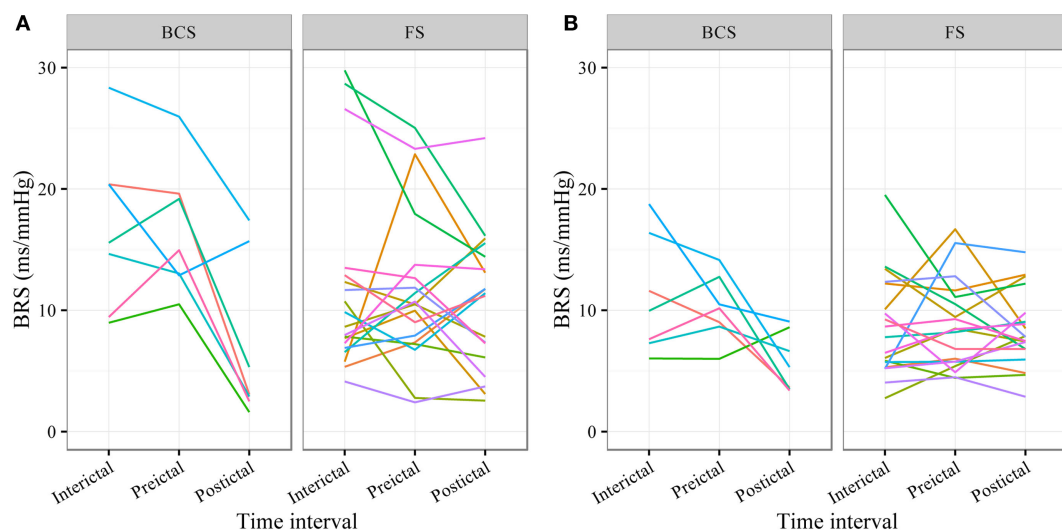*[b]Geometric mean of baroreflex sensitivity (BRS).*
BCS, bilateral convulsive seizures.
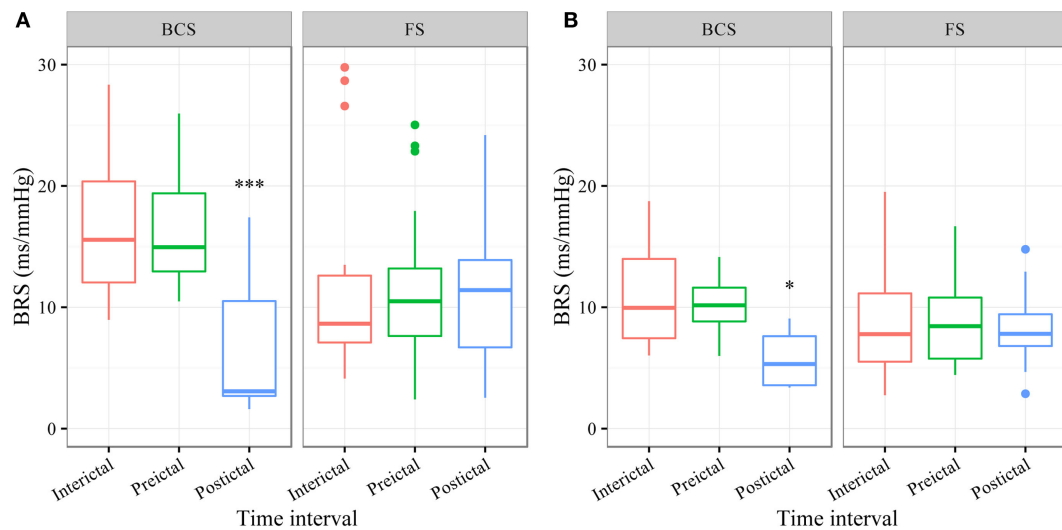
FIGURE 1 | The baroreflex sensitivity (BRS) is markedly decreased after bilateral convulsive seizures (BCS), but not after focal seizures (FSs). Box plots of BRS during the different time intervals for BCS and FS calculated with the sequence **(A)** and spectral method **(B)**. ***BRS calculated with the sequence method is markedly reduced during the postictal time interval compared to preictal (adjusted p-value < 0.0001) and interictal time interval (adjusted p-value < 0.0001). *BRS calculated with the spectral method is markedly reduced during the postictal time interval compared to preictal (adjusted p-value = 0.014) and interictal time interval (adjusted p-value = 0.014).

FIGURE 2 | Individual plots of baroreflex sensitivity (BRS) for each patient during the different time intervals for bilateral convulsive seizures (BCS) and focal seizures (FSs) calculated with the sequence (A) and spectral method (B).

In FS, BP, and HR both display a transient increase with a similar time course, probably due to an increase of the sympathetic activity through stimulation of central autonomic centers by epileptic activity (4). In BCS, systemic BP appears to display at least two different patterns. The first pattern is characterized by a simultaneous increase of systemic BP and HR during the seizure (4, 18, 19). The second pattern consists of a biphasic behavior of BP with an initial increase followed by a drop (4, 19). The HR is commonly accelerated during BCS (4, 20). Importantly, the time course of seizure-related HR and BP alterations are different.

While the HR remains elevated up to 30 min after seizure termination, systemic BP is only slightly elevated by about 15% in the early postictal phase and returns to baseline levels or even drops slightly below preictal values within 5 min after seizures cessation (4, 20). This opposed behavior of HR and systemic BP in spite of a considerable seizure-related release of catecholamines is somewhat surprising, but most likely caused by an immediate muscular hyperemia that commonly follows exercise of skeletal muscles which, in turn, leads to a decreased systemic vascular resistance and ultimately to a drop in systemic BP (21). To

counteract the drop in systemic vascular resistance and systemic BP, HR may be increased *via* the arterial baroreflex, which could partially explain the increase of HR following BCS (4, 20). The abovementioned seizure-related local and systemic metabolic effects, however, may also impair BRS to some extent.

An alternative explanation for impaired BRS after BCS is an inhibitory effect on central autonomic centers due to exhausted or suppressed brain activity (18). For example, BCS are frequently associated with a PGES (22, 23). In one case report, postictal hypotension and PGES were associated (18). However, in a larger study, no association between postictal BP changes and PGES was found (4). In the present study, BCS (assessed with the sequence method) with PGES tended to have lower BRS during the postictal period than BCS without PGES. This result has to be considered with caution because of the small sample size and because this finding could not be reproduced by the BRS analysis according to the spectral method.

Potential Clinical Implications of Impaired BRS following BCS

The supply of metabolites and oxygen depends on the blood perfusion, which is directly linked to the systemic BP and tightly regulated in most organs (5). The baroreflex continuously stabilizes systemic BP and prevents excessive BP rises or falls (24). A *permanently decreased* BRS has been observed in many chronic diseases including diabetes mellitus, obesity, hypertension, and coronary artery disease and predicts a poor prognostic outcome (5). For instance, patients with myocardial infarction or congestive heart failure are at higher risk of sudden cardiac death (13). *Acute non-selective* baroreflex failure usually leads to hypertensive crisis or fluctuating hypertension (25). In particular subjects with selective baroreflex failure in whom the efferent parasympathetic pathway to the heart remains intact, however, can also suffer from hypotensive episodes (26).

Our study indicates a marked *acute* autonomic dysfunction after BCS. Most SUDEP cases are probably caused by a cardiorespiratory failure in the early postictal phase following BCS (3). Importantly, a recent case report described a significant drop of systemic BP in the aftermath of a BCS (18). If severe postictal hypotension in conjunction with an impaired BRS were one of the mechanisms facilitating SUDEP, these events may be prevented by an early cardiopulmonary resuscitation. Indeed, in seven out of nine near-SUDEP cases, cardiopulmonary resuscitation was successfully carried out within the first 3 min of seizure cessation (3). In contrast, in 8 of 12 SUDEP cases, cardiopulmonary resuscitation was initiated later than 10 min after seizure termination. Therefore, it seems advisable that systemic BP, HR, and breathing rate should be monitored in the early postictal period if possible, e.g., in video-EEG telemetry units during assessment for epilepsy surgery.

Study Limitations

Our study comes with some limitations. As we retrospectively selected patients, this study has an observational character and may be subject to various confounders. Firstly, because of the relatively small sample size, we may have overlooked smaller effects of an influencing factor in our patient sample. For example, because only three BCS were followed by PGES, the model may have failed

to detect a significant effect on BRS. Second, patients with lesions in areas which affect autonomic regulatory sites may have additional or stronger postictal alterations of BRS. Due to the small sample size of patients with these lesions and epilepsy-type matched patients without such lesions, this question could not be directly addressed in our study. Third, we cannot rule out possible effects of anticonvulsant drugs or the withdrawal of anticonvulsant drugs on BRS. These putative effects, however, should be the same for both FS and BCS, strengthening the finding of differential effects on BSR depending on the seizure type. Fourth, confounders such as variable body position, hypertension, or vigilance state may have an impact on the BRS. However, we assessed this issue by including these variables into the model without apparent significant effect on BRS. In addition, we verified our findings comparing the BRS of postictal interval not only with the BRS of the preictal but also with the BRS of the interictal time interval. Fifth, spontaneous BRS methods have some limitations compared to the gold standard, the phenylephrine method (27). For example, the measures do not always correlate well with pharmacological methods (28). However, the phenylephrine method has also several drawbacks. For instance, it demonstrates a large intra-individual variability of response if it is repeated in the same individual (29). In addition, phenylephrine induces changes in venous compliance and venous return and may activate the afferent branch of baroreflex pathways regardless of the increase in systemic BP (29). Furthermore, the pharmacological methods involve patients to risks, because they are invasive procedures with all the potential harmful effects. We therefore chose a spontaneous baroreflex method, whose baroreflex nature has been demonstrated on an animal model (30). This method was also validated against the phenylephrine method in healthy subjects (31). In addition, we reproduced our results using a different technique, the spectral method, thereby strengthening our findings and conclusions (12). Finally, our interictal and preictal BRS values were in a similar range than that of healthy subjects reported in previous studies, which underlines the reliability of our estimates (12, 14, 32).

CONCLUSION

We found that postictal BRS is markedly reduced after BCS in patients with focal epilepsy, providing additional evidence for severe autonomic dysfunction related to BCS. Our findings might be linked to cardiovascular failure facilitating SUDEP. Further studies with larger sample sizes are needed to verify our results and to deepen our understanding of periictal modulation of systematic BP and BRS.

ETHICS STATEMENT

The study was approved by the local medical ethical committee of the University of Bonn. Each patient had signed an informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

KH has conducted the recordings, contributed to the study design, the data analysis, and writing of the manuscript. CE has

contributed to interpretation of the data and has revised the manuscript critically for important intellectual content. RS has contributed to the study design, the data analysis, and writing of the manuscript.

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Antiepileptic Drugs Impair Shortening of Isolated Cardiomyocytes

Johanna Hulbert[1], Christian E. Elger[2], Rainer Meyer[1]* and Rainer Surges[2]*

[1] Institute of Physiology II, University Hospital Bonn, Bonn, Germany, [2] Department of Epileptology, University Hospital Bonn, Bonn, Germany

Background: Most antiepileptic drugs (AEDs) inhibit seizure generation by acting on voltage-dependent ion channels. Voltage-dependent sodium and calcium channels are commonly expressed in brain and heart, suggesting that AEDs may have considerable cardiodepressive effects, thereby facilitating sudden cardiac death as a potential cause of sudden unexpected death in epilepsy. Here, we investigated the effects of carbamazepine (CBZ), lamotrigine (LTG), and levetiracetam (LEV) alone and in combination on the shortening properties of isolated ventricular cardiomyocytes of wild-type mice.

Methods: Properties of murine cardiomyocytes were determined by recording the sarcomere shortening with a video imaging system before, during, and after administration of AEDs in different concentrations and combinations. We assessed (i) the number of successful shortenings during continuous electrical stimulation (electromechanical coupling) and (ii) the shortening amplitude as well as other shortening-related properties upon repetitive electrical stimulation at 4 Hz. Data are given as mean ± SEM.

Results: At 100 μM, CBZ (10 cells), LTG (11 cells), and LEV (11 cells) alone had no effect on the electromechanical coupling but reversibly reduced shortening amplitudes by 15 ± 4, 24 ± 3, and 11 ± 3%, respectively. Increasing the LTG concentration to 250 (21 cells) and 500 μM (4 cells) reversibly inhibited the electromechanical coupling in 62 and 100% of the experiments. Importantly, simultaneous application of CBZ, LTG, and LEV at 100 μM also impaired the electromechanical coupling in 8 of 19 cardiomyocytes (42%) and reduced the shortening amplitude by 21 ± 4%.

Conclusion: Our data show that AEDs reversibly impair cardiac excitation and contraction. Importantly, the blocking effect on electromechanical coupling appears to be additive when different AEDs are simultaneously applied. The translational value of these experimental findings into clinical practice is limited. Our results, however, suggest that rationale AED therapy may be important with respect to cardiac side effects and potential facilitation of serious cardiac dysfunction especially when AEDs are used in combination or at very high doses.

Keywords: antiepileptic drugs, cardiac block, cardiomyocytes, contraction, epilepsy, side effects, sudden unexpected death in epilepsy

INTRODUCTION

In people with epilepsy, the risk of dying prematurely is increased 2.5-fold as compared to the general population (1), about one fifth of these cases are due to sudden unexpected death in epilepsy (SUDEP) (2). The MORTEMUS study has shown that the fatal event is probably due to a severe suppression of cardiorespiratory function shortly after a generalized tonic–clonic seizure in the majority of SUDEP cases (3). Given the large spectrum of epilepsy-related cardiorespiratory alterations, however, SUDEP is likely to be a heterogeneous phenomenon and to have multiple causes (4). For instance, SUDEP may also occur in the absence of epileptic seizures with a similar pattern as described in the MORTEMUS study (5). It is also plausible that, in some cases, SUDEP is due to fatal cardiac arrhythmias such as bradyarrhythmias or ventricular tachyarrhythmias that are not directly related to epileptic seizures. This assumption is supported by several facts: (i) people with epilepsy frequently display abnormal cardiac and autonomic features in the absence of epileptic seizures (6), (ii) epilepsy is a risk factor for sudden cardiac death in the general population (7), (iii) sudden cardiac death occurred in people with epilepsy without evidence of precedent epileptic seizures (8), and (iv) about 10% of witnessed SUDEP cases were reported without apparent seizure activity (9).

The apparent propensity to cardiac mortality and sudden cardiac death in people with epilepsy may have genetic or acquired, epilepsy-related causes. Furthermore, antiepileptic drugs (AEDs) appear to be an independent risk factor for sudden cardiac death, especially in those patients taking AEDs that target voltage-gated sodium channels (VGSC) (10, 11). Numerous experimental studies have revealed that many AED predominantly act on excitatory VGSC and voltage-gated calcium channels (VGCC) or interact with ligand-gated chloride- and mixed-cation channels. A single AED, however, can bind to multiple targets (12, 13). For instance, the drugs that are usually considered as "sodium channel blockers" such as carbamazepine (CBZ), lamotrigine (LTG), and phenytoin also inhibit high-threshold VGCC, whereas levetiracetam (LEV) appears to mediate its antiepileptic effects *via* binding to the presynaptic synaptic vesicle protein 2A but also acts on high-threshold VGCC (12, 13).

Voltage-gated sodium channels and VGCC are expressed both in the brain and heart. The different subtypes of these channels, however, display predominant expression patterns, e.g., within cerebral or cardiac tissue. For instance, the VGSC subtypes NaV1.1, 1.2, 1.3, and 1.6 are primarily responsible for neuronal depolarization in the brain, whereas NaV1.4 and 1.5 are predominantly found in skeletal and cardiac muscle (14). The VGCC subtypes CaV1.2 and 1.3 as well as CaV3.1 and 3.2 are expressed in neurons and cardiomyocytes (15).

In our study, we wanted to examine the effects on cardiac tissue of three selected AEDs alone and in combination. We have chosen CBZ as it is considered the prototype of sodium channel blockers used in the treatment of people with epilepsy. LEV and LTG were selected, because they are nowadays the most widely used AEDs in the developed countries (16). Furthermore, CBZ and LTG were discussed as potential risk factors for SUDEP in previous studies (17). As a single AED can modulate both VGSC

and VGCC, we have not investigated effects on a selected molecular target (e.g., by performing patch-clamp recordings to measure specific ion currents) but have recorded the shortening of isolated cardiomyocytes upon electrical stimulation. This experimental approach does not allow the attribution of the observed effects to a specific molecular target but represents the sum of all pathways that are involved in the excitation and shortening of cardiomyocytes. We have assessed electromechanical coupling and the shortening properties upon application of CBZ, LTG, and LEV alone and in combination.
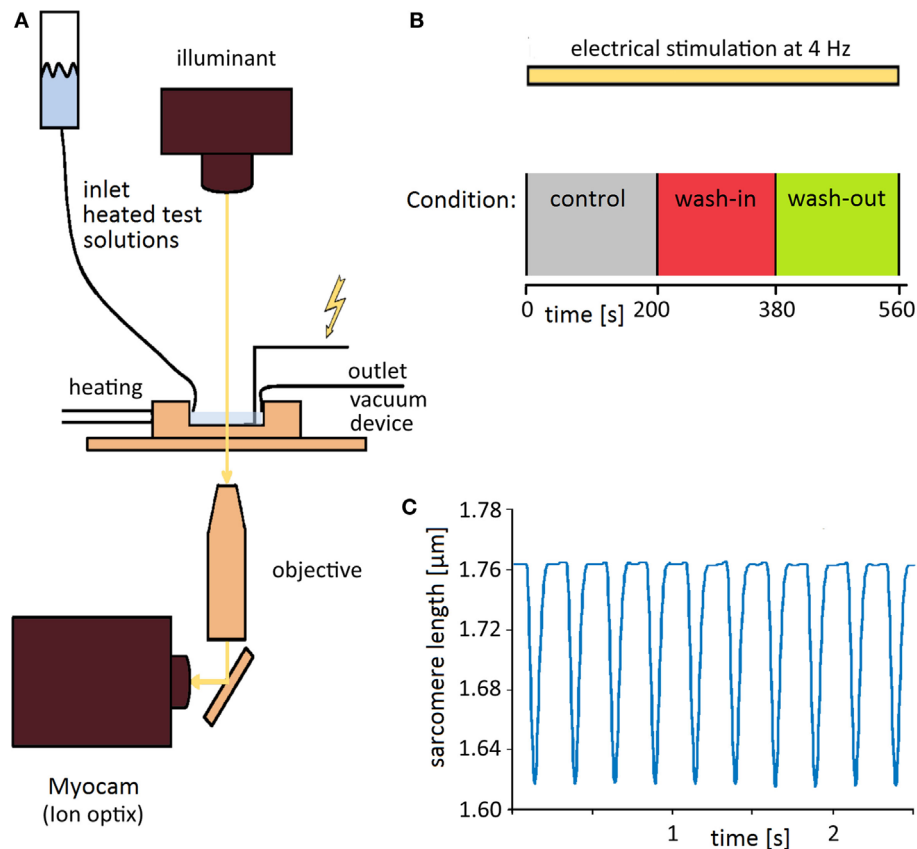
MATERIALS AND METHODS

Animals

C57BL/6 mice of male sex (Charles River/Breeding in the Department of Anatomy at the University of Bonn) were housed at 22°C with 12-h light–dark cycle and fed with water and food *ad libitum*. Twelve- to sixteen-week-old mice were used for the experiments. Animals were anesthetized using isoflurane in room air and euthanized by cervical dislocation.

Murine Cardiomyocyte Isolation

Myocytes were isolated as previously described (18). The hearts were removed and transferred to a cold cardioplegic EGTA-Tyrode solution (NaCl 135 mM, KCl 4 mM, MgCl$_2$ 1 mM, HEPES 2 mM, and EGTA 0.1%, pH 7.4). The removed hearts were rapidly attached to a modified Langendorff apparatus and perfused with oxygenated and heated solutions (35–36°C). Hearts were firstly perfused with EGTA-Tyrode, then with a high-potassium solution (NaCl 4 mM, KCl 10 mM, MgCl$_2$ 1 mM, CaCl$_2$ 0.025 mM, K-glutamate 130 mM, HEPES 4 mM, glucose monohydrate 9 mM), followed by high-potassium solution supplemented with trypsin (150 U/ml Type I, Sigma-Aldrich) and finally by high-potassium solution supplemented with collagenase (16 U/ml—caseinase activity—Sigma blend Type L, Sigma-Aldrich). Afterward, the ventricles were cutoff the Langendorff apparatus, finely minced and completely dismantled by stirring with a glass bar inside experimental Tyrode [NaCl 135 mM, KCl 4 mM, MgCl$_2$ 1 mM, CaCl$_2$ 1.8 mM, HEPES 2 mM, glucose monohydrate 10.1 mM, bovine serum albumin 0.1%, and trypsin inhibitor (Type II-S Soybean 1.67 mg/100 ml, pH 7.4)]. The solution was filtered through a 125-μm nylon mesh and centrifuged slowly for about 10 s. The cells were resuspended in fresh solution, allowed to settle for 7 min (heating cabinet 36°C) and resuspended again. Isolated cells were kept in oxygenated experimental Tyrode solution at room temperature for up to 6 h.

Sarcomere Shortening

For sarcomere length measurement cells were placed in a Laminin coated, heated chamber (35–36°C) on an inverted microscope (ZeissAxiovert 100 TV, Jena, Germany, lens: Neofluar 40/0.75) and continuously perfused with the experimental solutions. Sarcomere shortening of isolated ventricular myocytes was recorded with a video imaging system (Myocam) and SarcLen software (IonOptix; Milton, MA, USA) as previously described (**Figure 1A**) (19). The regular striation pattern of the sarcomeres

FIGURE 1 | **Setup and time course of experiments**. **(A)** Experimental setup allowing optical measurement of sarcomere lengthening (see Materials and Methods for details). **(B)** A scheme of the time course of experiments. Shortenings were elicited by regular stimulations at 4 Hz before, during, and after wash-out of the test solutions. **(C)** Example of recording traces. The sarcomere length shortens upon extracellular electrical stimulation from about 1.76 to 1.62 μm.

is analyzed by fast Fourier transformation. Sarcomere shortening shifts the power spectrum peak, which corresponds to the absolute sarcomere length (**Figure 1C**) (20). Shortenings were induced by bipolar external stimuli *via* two gold electrodes (0.4 ms, 40 V, SD9, Grass; Quincy, MA, USA). During the recordings, the isolated cardiomyocytes were continuously stimulated at a frequency of 4 Hz. After a "run-in period" of about 200 s to establish steady-state shortenings with continuous bathing with experimental Tyrode, the solution was switched to a drug-containing Tyrode for 3 min. Afterward, the solution was switched back to Tyrode solution again for 3 min to show wash-out of the drug (**Figure 1B**).

Drugs

Stock solutions containing CBZ and LTG were dissolved in dimethyl sulfoxide (DMSO). LEV was prepared in distilled water. Stock solutions were stored at −20°C or prepared freshly and added to experimental Tyrode just before use. The final experimental concentrations used in our experiments were 100 μM CBZ, 100, 250, and 500 μM LTG, and 100 μM LEV. The concentration of DMSO did not exceed 1:1,000 (1:500 for LTG 500 μM). The Tyrode solution used before and after drug wash-in (control condition) contained DMSO in corresponding

concentrations. Drugs and all other chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Data Analysis

The evaluation of the cardiomyocyte shortening was carried out using IonWizard 6 (IonOptix). Only rod shaped faultless cells with a uniform striation pattern were selected for recordings. Recordings were included in the final analysis when cardiomyocytes displayed a stable steady state at the point of data acquisition and when they survived the recurrent stimulation for the whole recording period. To exclude a toxic effect or gradual death of the recorded cardiomyocytes, we only included those recordings in which the drug effect was reversible. We assessed (i) the number of successful shortenings following individual electrical stimulations (electromechanical coupling) and (ii) the shortening properties (sarcomere length, shortening amplitude, maximal shortening velocity, maximal relengthening velocity, and the time to return to 90% of the baseline) upon repetitive electrical stimulation at 4 Hz before, during, and after application of the AEDs.

The shortening velocity (in micrometers per second) characterizes the maximal speed with which the cell shortens. The relengthening velocity (in micrometers per second) is the

maximal speed in the return phase of the transient. The time to return to 90% of the baseline (in milliseconds) is a measure of cellular relaxation. To quantify the shortening properties, we have averaged the last 10 shortening signals before wash-in and wash-out, respectively. For statistical analysis, a repeated-measures ANOVA followed by Newman–Keuls corrected *post hoc* analysis was used when appropriate. ANOVA tests were performed using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). *p*-Value <0.05 was considered as significant (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). All data are given as mean ± SEM.

RESULTS

External Ca²⁺ Reversibly Modulates Shortening Amplitudes of Isolated Cardiomyocytes

To explore the recording conditions and the validity of our experimental approach, we measured sarcomere shortening at three different external calcium concentrations, namely, 1.2, 1.8 (defined as control condition), and 3.6 mM. As expected, the switch from 1.8 to 1.2 mM external Ca²⁺ reversibly decreased the shortening amplitude of the isolated cardiomyocytes by $46 \pm 5\%$ (from 0.073 ± 0.012 μm under control condition to 0.041 ± 0.009 μm, $p < 0.001$, **Figure 2A**). Conversely, the increase of the external Ca²⁺ concentration from 1.8 to 3.6 μM led to a reversible enhancement of the shortening amplitude by $44 \pm 5\%$ (from 0.093 ± 0.008 to 0.13 ± 0.01 μm, $p < 0.0001$, **Figure 2B**).

AEDs Reversibly Impair Shortening Properties of Isolated Cardiomyocytes

First, the effects on the shortening properties of cardiomyocytes were tested for each AED separately. All three drugs at a concentration of 100 μM did not affect electromechanical coupling (i.e., each electrical stimulation induced a shortening) but led to a reversible reduction in shortening amplitudes. Original recordings of sarcomere lengths and shortenings before, during, and after application of LEV are shown in **Figure 3A**. While the depressive effects of CBZ (10 cells) and LEV (11 cells) ranged between 11 and 15% (**Figures 3A,B**; **Table 1**), LTG (11 cells) led to a reduction of the shortening amplitude by about 24% (**Figure 3C**; **Table 1**). Furthermore, LTG also reduced the shortening velocity by $23 \pm 4\%$ and the relengthening velocity by $23 \pm 5\%$ (**Table 1**), whereas CBZ and LEV did not affect the shortening and relengthening velocities at the given concentration. The time of return to 90% of the baseline was not affected by any of the tested drugs.

As 100 μM LTG appeared to have the greatest impact on cardiomyocytes, we have also explored the effects of LTG at higher concentrations (250 and 500 μM). Interestingly, during application of LTG at 250 (21 cells) and 500 μM (4 cells), a variable proportion of the cardiomyocytes failed to shorten in response to every single electrical stimulation (for original traces see **Figure 4A**). The number of the failed shortenings increased with increasing time of application. At 250 and 500 μM LTG, the electromechanical coupling was irregular in 62 and 100% of the cells, respectively (**Figure 4B**). The shortening amplitudes after a failed excitation

in 500 μM LTG were higher than those under control conditions, because murine cardiomyocytes develop an increased post-rest shortening after a stimulation pause compared to the steady state.

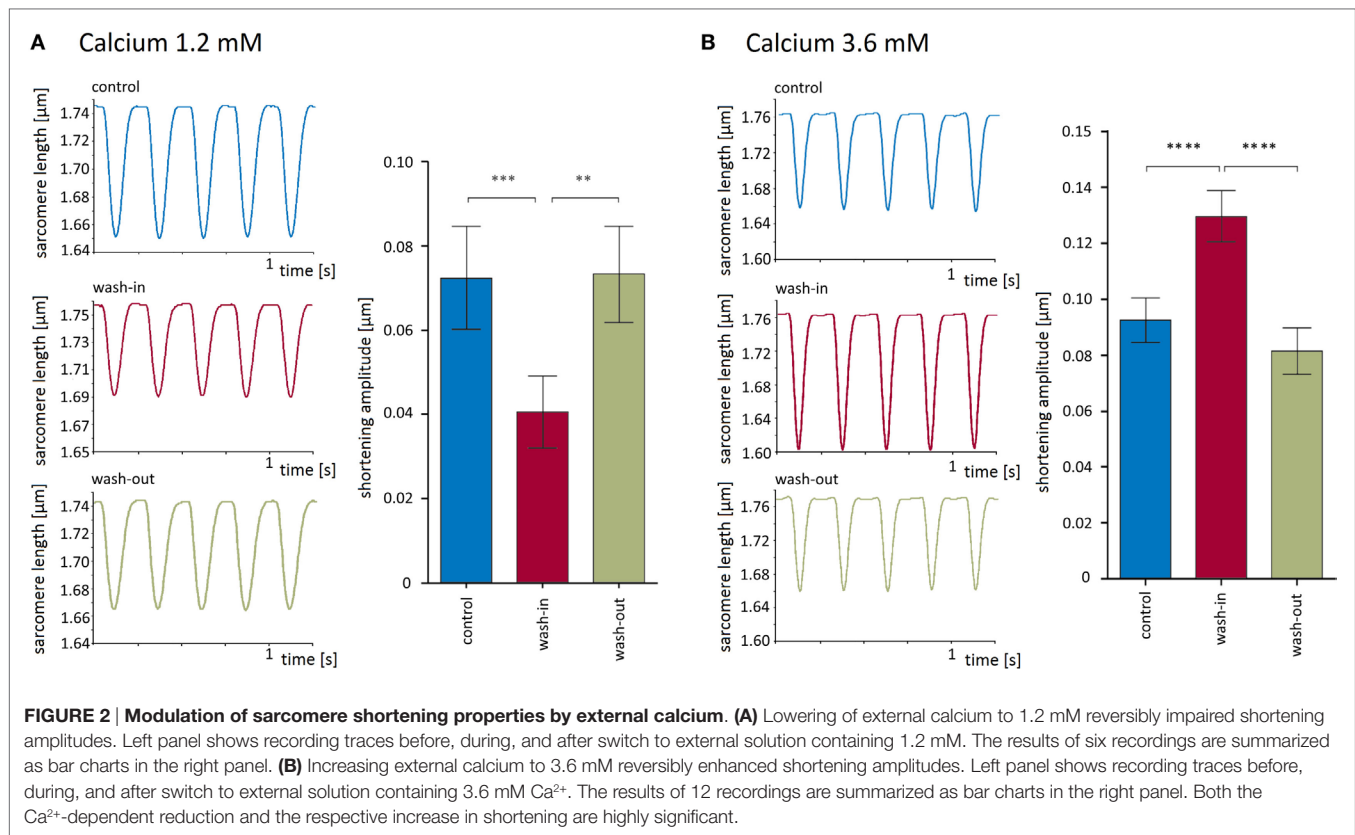## Effect of Simultaneously Applied AEDs on Electromechanical Coupling

So far, we have applied the AEDs separately. In clinical practice, however, people with epilepsy often take more than one AED to achieve better seizure control. Therefore, CBZ, LTG, and LEV were simultaneously applied at a concentration of 100 μM each, i.e., at the same concentration as compared to the abovementioned experiments when each AED was applied alone. The combination of all three drugs led to electromechanical coupling failure in eight cells (42% of all recordings, **Figure 4C**). In the 11 cells with undisturbed coupling, effects on the shortening properties of the simultaneous drug application could be properly determined. The shortening amplitude was inhibited by $21 \pm 4\%$ (**Figure 4C**), the shortening and relengthening velocities were reduced by 23 ± 3 and $18 \pm 5\%$, respectively (**Table 1**).

To further characterize the blocking effects on the electromechanical coupling, we have quantified the number of failed stimulations (i.e., no shortening) with respect to the total number of electrical stimulations for those cells that showed a disturbed electromechanical coupling. The failure rate amounted to $49 \pm 11\%$ upon simultaneous application of CBZ, LEV, and LTG (i.e., every second stimulation failed to evoke a shortening) and was even higher during application of very high LTG concentrations (**Figure 5C**).

DISCUSSION

Depressed Cardiac Shortening: Mechanisms of Action and Possible Clinical Implications

All three AEDs alone reversibly reduced the shortening amplitudes of isolated cardiomyocytes (**Figure 5A**). Our study was not designed to investigate the mechanisms that are involved in the modulation of shortening properties of isolated cardiomyocytes. In the case of CBZ and LTG, however, the well-described inhibitory effects on VGSC and VGCC most likely underlie the observed depressive action of CBZ and LTG (12, 13). CBZ and LTG at 100 μM reduced the shortening amplitudes by about 15 and 24%, respectively. It is difficult to translate the extent and importance of these *in vitro* findings into clinical practice for at least two reasons: first, we have applied relatively high concentrations of CBZ and LTG as compared to the typical therapeutic serum levels in epilepsy patients (LTG 12–58 μM, CBZ 17–51 μM) (21). Second, the inhibitory effects of CBZ and LTG on VCSC are use dependent, i.e., the inhibition gets stronger with increasing stimulation frequencies (22, 23). In our experiments, the cardiomyocytes were stimulated at 4 Hz only, as the recordings were stable for at least 10–15 min at this particular frequency. This stimulation frequency equals a heart rate of 240 beats per minute, which is low as compared to the habitual resting heart rate between 500 and 600 beats per minute in mice, but still much higher than the resting heart rate in adult humans. Thus, the effect size of CBZ or

FIGURE 2 | Modulation of sarcomere shortening properties by external calcium. (A) Lowering of external calcium to 1.2 mM reversibly impaired shortening amplitudes. Left panel shows recording traces before, during, and after switch to external solution containing 1.2 mM. The results of six recordings are summarized as bar charts in the right panel. **(B)** Increasing external calcium to 3.6 mM reversibly enhanced shortening amplitudes. Left panel shows recording traces before, during, and after switch to external solution containing 3.6 mM Ca^{2+}. The results of 12 recordings are summarized as bar charts in the right panel. Both the Ca^{2+}-dependent reduction and the respective increase in shortening are highly significant.

LTG on cardiac contraction at typical therapeutic concentrations is likely to be lower in epilepsy patients. In the instance of a CBZ or LTG overdose, however, the inhibitory effects on contraction properties as well as on electromechanical coupling (as shown at 250 and 500 µM LTG) appear to be of clinical importance, as illustrated by previous case reports (24, 25).

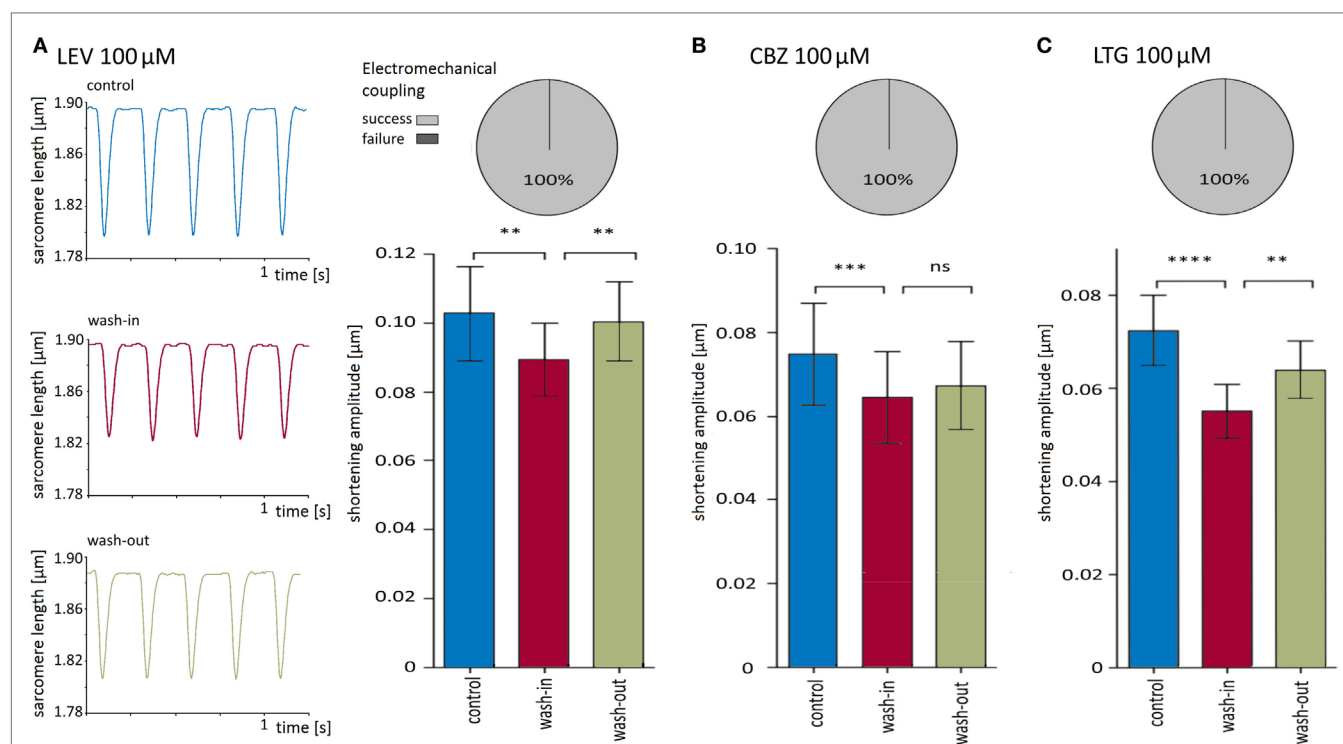Levetiracetam at 100 µM reversibly reduced the shortening amplitude by about 11%. This depressive LEV effect was rather unexpected but can be explained by its inhibitory action on voltage-gated L-type calcium channels (26, 27). Although the concentration of LEV was well within the typical serum levels of epilepsy patients (70–270 µM) (21), the cardiodepressive effect is small and unlikely to be clinically significant, given the fact that rapid intravenous infusion of LEV had no apparent cardiovascular effects in healthy controls and epilepsy patients (28, 29).

Faulty Electromechanical Coupling: Mechanisms of Action and Possible Clinical Implications

Electromechanical coupling was reversibly blocked only at very high concentrations of LTG or when all three AEDs at 100 µM were simultaneously applied (**Figures 5B,C**). In our experimental constellation, this phenomenon may be due to an inhibition of cellular excitation, of the excitation–shortening coupling, the shortening itself, or a combination of these elements, probably *via* binding to VGSC and VGCC.

In our point of view, the effect of the simultaneous application of the AEDS is particularly important in the clinical context. About one-third of the epilepsy patients are difficult to treat, i.e., they commonly need more than one AED to achieve better or full seizure control (30). Polypharmacy, in turn, was suspected to increase the risk of SUDEP (31). Subsequent studies have finally revealed that polypharmacy is not a risk factor itself, but rather a surrogate marker of severe epilepsy and insufficient seizure control (32) and that efficacious adjunctive AED treatment reduces the SUDEP risk (33). It appears, however, plausible that simultaneous administration of AEDs that bind to the same cellular targets increases the risk of target-related side effects. Therefore, it is not surprising that cardiac side effects such as atrioventricular block, sinus node dysfunction, or arrhythmogenic ST–T abnormalities were reported in the context of simultaneous intake of AED that inhibits VGSC (34–36). We think that cardiac side effects can indeed, if long lasting and severe enough, facilitate sudden cardiac death as a rare cause of SUDEP in some epilepsy patients. This view is also supported by the finding that AED intake is an independent risk factor for sudden cardiac death (10, 11). In this context, it is of note that CBZ and LTG were discussed as potential risk factors for SUDEP in previous studies (17). However, these assumptions could not be replicated in subsequent meta-analyses or when studies were adjusted for the frequency of generalized tonic–clonic seizures (17, 32, 37). To date, no single AED has unequivocally been proven to increase the SUDEP risk.

FIGURE 3 | Carbamazepine (CBZ), lamotrigine (LTG), and levetiracetam (LEV) reversibly reduced shortening amplitudes. **(A)** Left panel shows original recording traces of one cell before, during and after wash-in of 100 μM LEV. The bar chart summarizes the significant and reversible reduction of shortening amplitudes of 11 cells, and the pie chart visualizes the efficacy of electromechanical coupling. **(B)** 100 μM CBZ (10 cells) also reduced significantly the shortening amplitude (bar chart) but did not influence excitation–shortening coupling (pie chart). **(C)** 100 μM LTG (11 cells) developed comparable effects as LEV and CBZ.

TABLE 1 | Absolute effects of antiepileptic drugs on shortening properties.

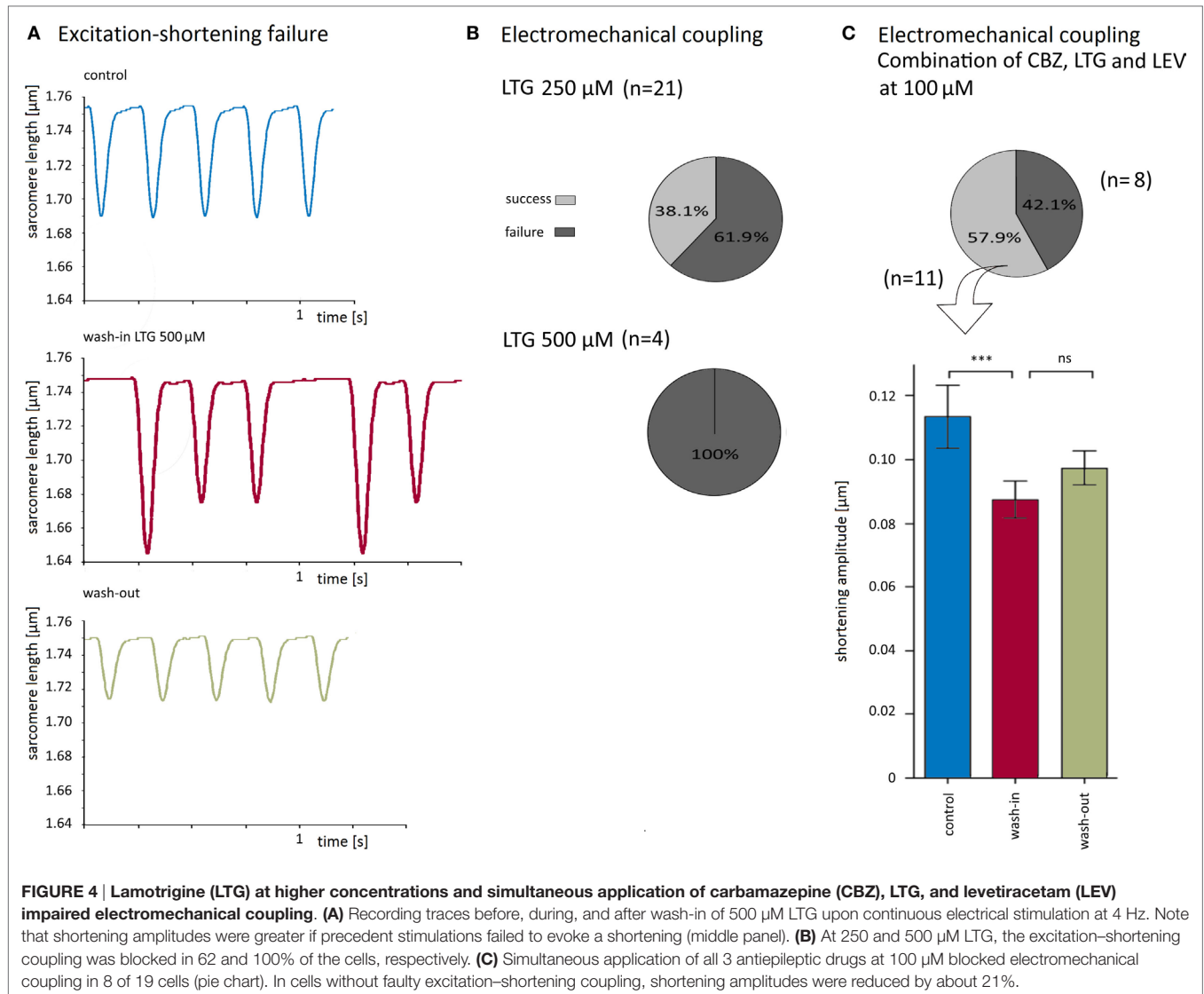|	Lamotrigine 100 μM ($n = 11$)	Carbamazepine 100 μM ($n = 10$)	Levetiracetam 100 μM ($n = 11$)	Combination of all 3 drugs ($n = 11$)
Shortening amplitude (μm)	-0.0174 ± 0.003 **$p < 0.0001$**	-0.0103 ± 0.0022 **$p < 0.001$**	-0.0134 ± 0.0038 **$p < 0.01$**	-0.0264 ± 0.0051 **$p < 0.001$**
Shortening velocity (μm/s)	-0.778 ± 0.149 **$p < 0.001$**	-0.3775 ± 0.2089 $p > 0.05$	-0.6415 ± 0.277 $p > 0.05$	-1.442 ± 0.269 **$p < 0.0001$**
Relengthening velocity (μm/s)	0.4733 ± 0.1363 **$p < 0.01$**	0.1414 ± 0.1183 $p > 0.05$	0.4669 ± 0.204 $p > 0.05$	0.9769 ± 0.248 **$p < 0.01$**
Time to return to baseline 90% (ms)	-0.727 ± 4.433 $p > 0.05$	2.80 ± 4.08 $p > 0.05$	2.727 ± 3.14 $p > 0.05$	6.455 ± 2.695 $p > 0.05$

p-Values are compared to the control condition (before application of the drug).
Significant p-values are highlighted in bold.

Study Limitations and Translational Value of Our Findings for SUDEP in Humans

People with epilepsy often display acquired or genetic alterations of voltage-gated ionic channels that are expressed in both heart and brain (6, 38). In the present study, however, we have tested the effects of three AEDs on the shortening properties of isolated cardiomyocytes of wild-type mice only, but not on cardiomyocytes of mice with genetic or acquired epilepsy. In this context, it is of note that significant disturbances of electrical properties were found in cardiomyocytes of rodents with acquired or genetic epilepsies. For instance, the expression of NaV1.1 was increased in ventricular cardiomyocytes of a rat model of acquired epilepsy

(kainic acid-induced status epilepticus model), leading to a prolonged duration of cardiac action potential and most likely to a prolongation of the QT interval (39). In a mouse model of Dravet syndrome, a severe form of childhood epilepsy with high mortality which is commonly caused by heterozygous mutations in NaV1.1, ventricular myocytes surprisingly exhibited an increase in voltage-dependent sodium currents, probably due to an enhanced expression of NaV1.5 (40). These alterations were associated with an increased excitability, a prolongation of action potential duration, and an increase in triggered activity of cardiomyocytes in mice carrying the Dravet mutation. These two examples nicely illustrate possible modifications of cardiac

FIGURE 4 | Lamotrigine (LTG) at higher concentrations and simultaneous application of carbamazepine (CBZ), LTG, and levetiracetam (LEV) impaired electromechanical coupling. **(A)** Recording traces before, during, and after wash-in of 500 µM LTG upon continuous electrical stimulation at 4 Hz. Note that shortening amplitudes were greater if precedent stimulations failed to evoke a shortening (middle panel). **(B)** At 250 and 500 µM LTG, the excitation–shortening coupling was blocked in 62 and 100% of the cells, respectively. **(C)** Simultaneous application of all 3 antiepileptic drugs at 100 µM blocked electromechanical coupling in 8 of 19 cells (pie chart). In cells without faulty excitation–shortening coupling, shortening amplitudes were reduced by about 21%.
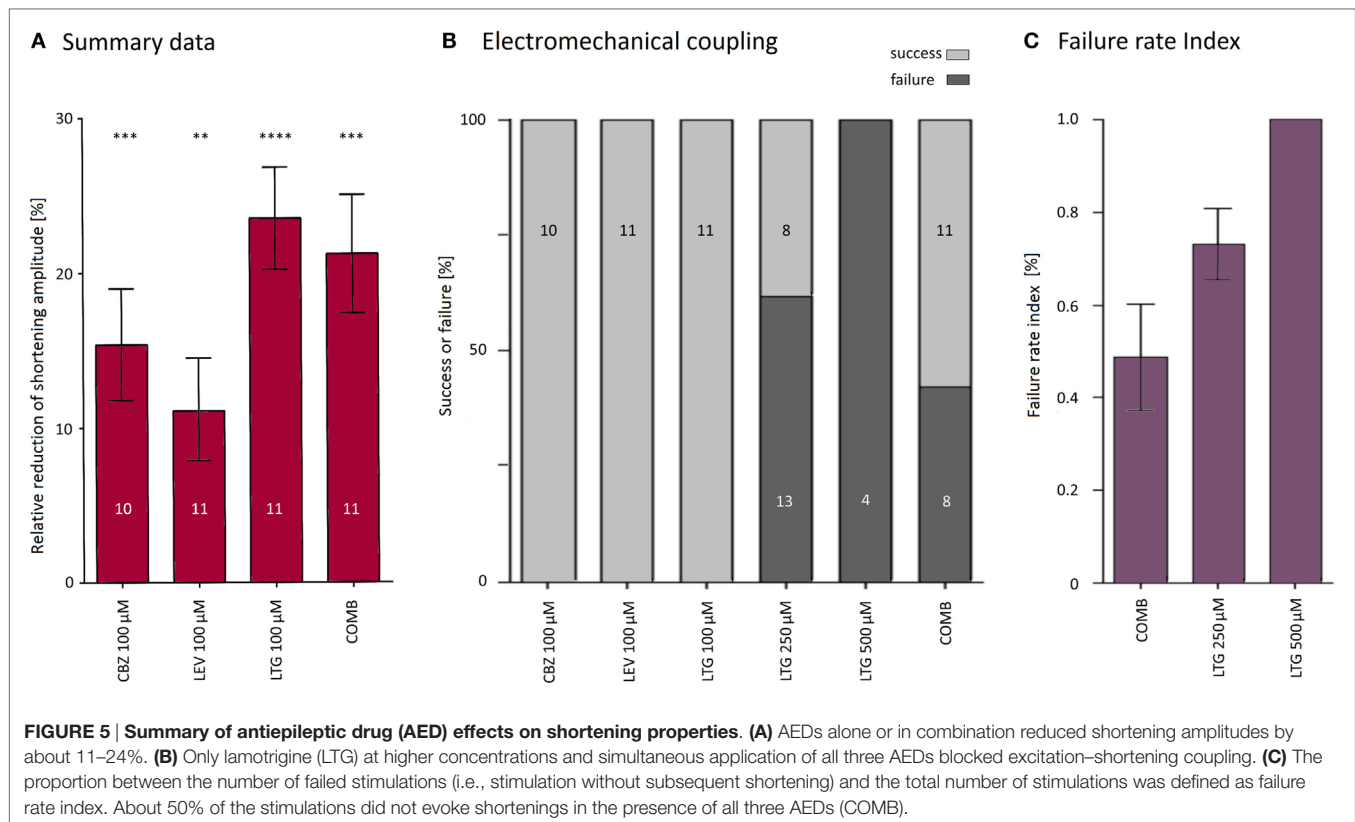
properties at the level of gene expression in animal models of epilepsy but also demonstrate the difficulties in predicting potential consequences of such alterations for AED effects. A measurable effect on modulation of voltage-gated sodium currents by AED in cardiomyocytes of humans or animals with epilepsy, however, seems likely, given the differential sensitivities of splice variants and types of VGSC to AEDs (23, 41, 42). Thus, future studies are required to compare the effects of AEDs at different concentrations (including other AEDs than CBZ, LEV, and LTG) on cardiomyocytes of wild-type mice to those of mice with an epileptic phenotype.

Given these considerations, the translational value of our study for SUDEP in humans is rather limited. In view of the large spectrum of epilepsy-related cardiorespiratory alterations, SUDEP is likely to be a heterogeneous phenomenon and to have multiple etiologies (4, 5). Therefore, it appears plausible that in some cases, SUDEP is caused by fatal cardiac dysfunction due to myocardial

failure, bradyarrhythmias, or ventricular tachyarrhythmias. Here, we have shown that CBZ, LEV, and LTG reversibly reduced the shortening of isolated wild-type cardiomyocytes and that LTG at higher concentrations or simultaneous application of all three AEDs blocked electromechanical coupling. Our findings may at least partially explain some of the cardiac disturbances, which were reported in epilepsy patients treated with AEDs in mono- or polytherapy. For instance, myocardial failure was reported in the context of CBZ intoxication (24, 43, 44), a complete atrioventricular block upon an overdose of LTG (25) and occurrence of sinus node dysfunction, atrial flutter/fibrillation, atrioventricular block, or ventricular tachycardia in association with the combined use of sodium channel-blocking AEDs (34, 35, 45, 46).

In summary, our results suggest that rationale AED therapy may be important with respect to cardiac side effects and potential facilitation of serious cardiac dysfunction especially when AEDs are used in combination or at very high doses.

FIGURE 5 | Summary of antiepileptic drug (AED) effects on shortening properties. (A) AEDs alone or in combination reduced shortening amplitudes by about 11–24%. **(B)** Only lamotrigine (LTG) at higher concentrations and simultaneous application of all three AEDs blocked excitation–shortening coupling. **(C)** The proportion between the number of failed stimulations (i.e., stimulation without subsequent shortening) and the total number of stimulations was defined as failure rate index. About 50% of the stimulations did not evoke shortenings in the presence of all three AEDs (COMB).

ETHICS STATEMENT

The animal handling and care conformed to the German federal laws and to NIH Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

AUTHOR CONTRIBUTIONS

JH has performed the experiments and the primary data analysis and contributed to the writing of the manuscript. CE has contributed to interpretation of the data and revised the manuscript critically for important intellectual content. RM has supervised the experiments and contributed to the study design, data analysis, and writing of the manuscript. RS has contributed to the study design, data analysis, and writing of the manuscript.

FUNDING

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Unexpected Death of a Child with Complex Febrile Seizures— Pathophysiology Similar to Sudden Unexpected Death in Epilepsy?

Brian J. Dlouhy[1,2]*, Michael A. Ciliberto[3], Christina L. Cifra[3], Patricia A. Kirby[4], Devin L. Shrock[4], Marcus Nashelsky[4]*[†] and George B. Richerson[2,5]*[†]

[1] Department of Neurosurgery, University of Iowa Hospitals and Clinics, Iowa City, IA, USA, [2] Pappajohn Biomedical Institute, University of Iowa Carver College of Medicine, Iowa City, IA, USA, [3] Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA, USA, [4] Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA, USA, [5] Department of Neurology, University of Iowa Hospitals and Clinics, Iowa City, IA, USA

Febrile seizures are usually considered relatively benign. Although some cases of sudden unexplained death in childhood have a history of febrile seizures, no documented case of febrile seizure-induced death has been reported. Here, we describe a child with complex febrile seizures who died suddenly and unexpectedly after a suspected seizure while in bed at night during the beginning phases of sleep. She was resuscitated and pronounced brain dead 2 days later at our regional medical center. Autopsy revealed multiorgan effects of hypoperfusion and did not reveal an underlying (precipitating) disease, injury, or toxicological cause of death. Although a seizure was not witnessed, it was suspected as the underlying cause of death based on the medical examiner and forensic pathologist (author Marcus Nashelsky) investigation, the post-resuscitation clinical findings, and multiple aspects of the clinical history. The child had a history of complex febrile seizures that had previously caused apnea and oxygen desaturation. She had two febrile seizures earlier on the same day of the fatal event. Interestingly, her mother also experienced a febrile seizure as a child, which led to respiratory arrest requiring cardiorespiratory resuscitation. This case suggests that in a child with complex febrile seizures, a seizure can induce death in a manner that is consistent with the majority of cases of sudden unexpected death in epilepsy (SUDEP). Further work is needed to better understand how and why certain individuals, with a history of epilepsy or not, die suddenly and unexpectedly from seizures. This will only occur through better understanding of the pathophysiologic mechanisms underlying epileptic and febrile seizures and death from seizures including SUDEP.

Keywords: febrile seizures, epilepsy, sudden unexplained death in childhood, sudden unexpected death in epilepsy, sudden infant death syndrome, sudden death in the young, sudden unexpected infant death

INTRODUCTION

Febrile seizures are usually considered relatively benign, and no reported cases of febrile seizure-induced death have been documented (1, 2). By contrast, sudden unexpected death in epilepsy (SUDEP) is a well-established phenomenon but excludes patients without a diagnosis of epilepsy (3). However, in susceptible individuals, the pathophysiologic mechanisms underlying SUDEP (4)

could potentially be induced by a first unprovoked seizure, or even by a provoked seizure (5). Increasing evidence suggests that some cases of sudden unexplained death in childhood (SUDC) are associated with a history of febrile seizures (6, 7).

Here, we describe a case of a child with complex febrile seizures who died suddenly and unexpectedly after a suspected seizure while in bed at night, with her face partially turned and covered, and during the beginning phases of sleep. We propose that this death may have resulted from the same pathophysiologic mechanisms as those believed to occur in the majority of SUDEP cases as detailed in MORTEMUS (8), outlined in multiple reviews (4, 5, 9) and discussed in recent studies examining the pathophysiology surrounding SUDEP (10)—a generalized tonic–clonic seizure (GTCS) leads to ictal and postictal respiratory dysfunction and hypoxemia, which is exacerbated by being facedown in bed, during sleep, and which ultimately leads to bradycardia and asystole.

METHODS

All inpatient and outpatient records were reviewed, and patient demographics, clinical history, clinical presentation, radiographic findings, medical examiner autopsy findings, and neuropathology were recorded and analyzed. Written and informed consent was obtained.

CASE REPORT

A 21-month-old girl with normal healthy development and without significant past medical history initially presented twice on the same day with two witnessed complex febrile GTCSs 12 months prior to her death. One seizure led to loss of breathing and oxygen desaturation with cyanosis of the lips and cyanosis and mottling of the skin. The child was febrile to 39.4°C in our emergency department following the second seizure. She was postictal, sleepy, and ataxic, with an otherwise normal examination and was admitted for observation. Her hospital course was unremarkable, and she was discharged the following day.

One month later, she had a simple febrile seizure in the setting of croup and, 7 months later, had two more GTCSs in the setting of another upper respiratory illness. One of these seizures occurred at night while in bed with her mother, who awakened to the GTCS. The child again became cyanotic with the seizure, requiring stimulation by her mother, and ultimately began breathing again in the postictal period. In all of these episodes, she quickly returned to baseline after the seizures and the febrile illnesses resolved without complication.

She continued to develop normally and, at 33 months of age, she had witnessed another GTCS. At that time, she was seen in the emergency department, where she was postictal and sleepy with a temperature of 38.2°C. She was otherwise normal on neurological examination. A few hours later, she had witnessed another GTCS with postictal drowsiness. She recovered but did not return to her baseline level of activity that day. That night, she was placed in her parents' bed to sleep between her mother and her sister. When last seen alive by her father, she was on her left side facing her sister. Approximately 30 min later, her father saw that she was still on her left side closely adjacent to her sister, who was also on her left

side. Her face was close to a pillow and her sister's back. The father found that she was unresponsive, pulseless, and apneic.

Her mother, a certified registered nurse anesthetist, and paramedics performed cardiorespiratory resuscitation. There was eventual return of spontaneous circulation after approximately 30 min after being found unresponsive. She was transported to our hospital. Neurological examination revealed no brainstem reflexes and no movement of the extremities. A CT scan of the brain showed diffuse swelling of the cerebral hemispheres, consistent with hypoperfusion brain injury. An electroencephalogram showed severe generalized voltage suppression without clear cerebral activity. An echocardiogram revealed adequate cardiac function with a normal heart rate and blood pressure. A chest radiograph showed airspace changes in the upper lobes of the lungs. Clinical evaluation revealed no evidence of traumatic injury. She was pronounced brain dead 2 days after admission. Her parents consented to organ donation, which was followed by autopsy performed by a medical examiner (author Marcus Nashelsky).

The cause of death was determined by the medical examiner and forensic pathologist (author Marcus Nashelsky) to be "global hypoxic/ischemic encephalopathy due to prolonged, resuscitated cardiopulmonary arrest due to complications of recurrent complex febrile seizures or epilepsy." At autopsy, no toxicological or structural underlying cause of death was identified. Neuropathological examination of the brain revealed global cerebral edema and marked softening of the brain with central, uncal, and tonsillar herniation—expected findings days after an anoxic brain injury. No masses were identified. Microscopy revealed diffuse hypoxic–ischemic neuronal injury in all areas sampled. There was no evidence of mesial temporal sclerosis, gliosis, neuronal loss, or cortical dysplasia. No histologic findings that have been associated with epilepsy were found.

Interestingly, and as a possible forewarning, when the child's mother was a child, she had a febrile seizure that led to respiratory arrest, thereby requiring cardiorespiratory resuscitation and a weeklong hospitalization.

DISCUSSION

Febrile seizures are usually considered relatively benign, occur most commonly between the ages of 6 months and 6 years, have a peak incidence at 18 months, and occur in 2–5% of children before the age of 5 years (1, 2). In the case presented here, a 2.75-year-old child with a history of complex febrile GTCSs had a suspected seizure in bed at night. She was found unresponsive, pulseless, and apneic approximately 30 min after she was last checked by her father. Although a seizure was not witnessed, it was suspected based on the circumstances, clinical findings surrounding death, and aspects of the clinical history. First, the child had a history of multiple GTCSs during febrile episodes and had two febrile GTCSs on the same day she was later found unresponsive in bed. Second, the child was found with her face turned slightly downward, near her sister's shoulder and the pillow, a position that may have limited airflow, a common finding during seizures that lead to death. Remarkably, the scenario surrounding this death is commonly found in epilepsy patients who die from SUDEP after

a GTCS. However, this child did not have a diagnosis of epilepsy and therefore, by definition, cannot be called SUDEP.

Despite semantic and classification differences, the commonalities between this case and the majority of SUDEP (**Table 1**) cases suggest a common pathophysiologic mechanism. The MORTEMUS study (8) revealed that SUDEP cases are often induced by a GTCS while the individual is in bed, at night, often during sleep and facedown. A GTCS can lead to activation/inactivation of brain regions that are functionally connected to the brainstem respiratory network (4), which can result in ictal and postictal respiratory dysfunction and oxygen desaturation. Recent work by Dlouhy et al. (10) found that seizure spread to the amygdala as well as electrical stimulation of the amygdala resulted in apnea and oxygen desaturation in humans. Being prone, facedown in bed, and asleep would likely exacerbate this oxygen desaturation. This hypoxemia may ultimately lead to bradycardia, asystole, and death. The fact that many SUDEP cases are found prone in bed suggests that this position plays a role in the pathophysiology. The prone position where the face may be covered by pillows or blankets likely predisposes to impaired ventilation and oxygenation during inhibition of respiratory motor output. This position also suggests a loss of arousal by patients, as they do not sense the alarm of rising CO_2 concentrations (4). Mechanistically, this loss of arousal during the peri-ictal period may be explained by an especially striking observation in the study by Dlouhy et al. (10) in which apnea evoked by amygdala stimulation was not accompanied by dyspnea. In fact, the patients were completely unaware that they had stopped breathing. Therefore, a loss of arousal to CO_2 may occur with amygdala seizures.

The child discussed here was known to have apnea (witnessed and described by the patient's mother who is an experienced health-care provider) during her febrile seizures with oxygen desaturation causing cyanosis, further supporting the hypothesis that a seizure in this case may have led to a cascade of events similar to what is seen in SUDEP cases. Strikingly, and supporting the likelihood that a seizure led to death in this case, the mother of the child also experienced a febrile seizure in childhood, which led to respiratory arrest requiring cardiorespiratory resuscitation and prolonged hospitalization.

TABLE 1 | Shared and different characteristics between SUDC, SUDEP, and case presented here.

Clinical feature	SUDC	SUDEP	Case
Occurrence at night	Yes	Yes	Yes
Occurrence during sleep	Yes	+/−	Yes
Prone positioning	Yes	Yes	Yes
Facedown	Yes	Yes	Yes
History of febrile seizures	Yes	+/−	Yes
History of GTCS	Yes	Yes	Yes
Suspected respiratory dysfunction at death	Unknown	Yes	Suspected
History of apneic seizures	Unknown	Unknown	Yes
Apparent lack of struggle at death	Yes	Yes	Yes
History of epilepsy	No	Yes	No
Age at death	1–5 years	Any age	2.75 years

SUDC, sudden unexplained death in childhood; SUDEP, sudden unexpected death in epilepsy; GTCS, generalized tonic–clonic seizure.

Increasing evidence, as discussed above, suggests peri-ictal respiratory dysfunction as the primary cause for SUDEP. However, death still may have occurred through other mechanisms in this child. Although evidence suggests a seizure occurred immediately prior to death, it is possible that a seizure immediately proceeding death did not occur. Prolonged autonomic effects from the two complex febrile seizures occurring earlier in the day could have resulted in cardiac changes or arrhythmia at night resulting in death.

We acknowledge that this is a rare case. However, in a large population-based cohort of children, Vestergaard et al. (11) found that mortality is increased in children during the 2 years after having a complex febrile seizure. Additionally, retrospective analysis of 121 cases of SUDC as part of the San Diego SUDC research project found that 48.8% of SUDC cases had a personal and/or family history of febrile seizures and therefore was a significant risk factor for SUDC (7). In another retrospective review of 123 consecutive children with SUDC reported to the SUDC program of the SUDC Foundation, 31.7% of SUDC cases had a personal history of febrile seizures (6). An ongoing prospective SUDC registry may help support the hypothesis that febrile seizures can lead to death with similar pathophysiology to SUDEP and how commonly this occurs.

The cause of febrile seizures is unclear and likely multifactorial. No single susceptibility gene has been found to cause febrile seizures (12). However, some genetic mutations that result in epilepsy syndromes have a component of recurrent febrile seizures (2). Mutations in SCN1A, a sodium ion channel, can result in both generalized epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome (DS) (2, 12). The risk of SUDEP is high in children with DS, probably more than other infantile epilepsies (13). It is unclear why patients with DS have a high rate of early mortality but it may be related to the severity and frequency of the seizures (5). Mutations in SCN1B are linked to GEFS+, temporal lobe epilepsy, and DS (14–16). Further work in linking SCN1B and other genes encoding ion channels in the brain to SUDEP are needed. The child in this case did not fit the clinical picture of having an epilepsy syndrome with febrile seizures.

As further progress is made in defining the mechanisms involved in SUDEP and SUDC, more of these cases will be "explained" with respect to pathophysiologic causes. Mechanistic overlap likely exists, and further evidence may support that some cases of SUDC are due to unrecognized seizures, and some cases of sudden death are induced by provoked seizures. As that happens, these categorical terms will begin to lose meaning and hinder our ability to accurately describe the cause of death—therefore, as we learn more, it will be more rational to classify these deaths based on pathophysiologic mechanism.

CONCLUSION

This case suggests that febrile seizures can lead to sudden unexpected death in children through mechanisms similar to those involved in SUDEP. Further study is needed to better understand how seizures of any kind, including provoked and epileptic, cause death. As we learn more, it will become possible to divide SUDC into subgroups based on pathophysiologic mechanism.

ETHICS STATEMENT

The parents of the child described here consented to this study and report.

AUTHOR CONTRIBUTIONS

Author contributions to the study and manuscript preparation include the following. Conception and design: BD, MN, and GR. Acquisition of data, and analysis and interpretation of data: all the authors. Draft of the article: BD. Critical revision of the article and review of submitted version of the manuscript: all the authors. Approval of the final version of the manuscript on behalf of all the authors, administrative/technical/material support, and study supervision: BD.

FUNDING

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Risk Assessment for Sudden Death in Epilepsy: The SUDEP-7 Inventory

Jennifer L. Novak[1], Patrick R. Miller[1], Daniela Markovic[2], Sheba K. Meymandi[3,4] and Christopher M. DeGiorgio[1,4]*

[1] *Department of Neurology, University of California Los Angeles School of Medicine, Sylmar, CA, USA,* [2] *Department of Biomathematics, University of California Los Angeles School of Medicine, Sylmar, CA, USA,* [3] *Department of Cardiology, University of California Los Angeles School of Medicine, Sylmar, CA, USA,* [4] *Olive View-UCLA Medical Center, Sylmar, CA, USA*

Background: Sudden unexpected death in epilepsy (SUDEP) is a major cause of death in those with drug-resistant epilepsy (DRE). There is a need for inventories and biomarkers associated with the risk for SUDEP.

Objective: To explore the revised SUDEP Risk Inventory (SUDEP-7) in a cohort with DRE and determine the association with Heart Rate and other covariates.

Methods: Twenty-five subjects with severe DRE were enrolled in a clinical trial for epilepsy. Baseline demographics, duration of epilepsy, seizure types, seizure frequency, seizure severity, AEDs, and vital signs were collected. Heart rate variability (HRV) was calculated from 1-h recordings of ECG. A SUDEP Risk Inventory (SUDEP-7) was administered, which included seven validated and weighted risk factors initially identified by Walczak et al. as factors associated with SUDEP risk.

Results: The total score on the revised SUDEP-7 ranged from 1 to 7, mean = 3.4 (SD 1.8). The SUDEP Risk Inventory score was inversely correlated with RMSSD (Pearson $r = -0.45$, $p = 0.027$). The following variables were significantly associated with RMSSD: epilepsy duration ($p = 0.02$), age ($p = 0.03$), and developmental intellectual disability ($p < 0.001$). The correlation between RMSSD and SUDEP-7 tended to persist also after the adjustment for patient age ($r = -0.40$, $p = 0.05$). Two subjects died of SUDEP: their SUDEP-7 scores were above average and in the upper twenty-fifth and fiftieth percentiles, respectively (6 and 4, mean = 3.4).

Conclusion: RMSSD, a measure of low frequency HRV, was significantly associated with SUDEP Risk Inventory (SUDEP-7) scores. Using a multivariate model, the covariates of developmental intellectual disability, age, and duration of epilepsy were also significantly associated with decreased HRV. The correlation between decreased HRV and a higher SUDEP-7 score remained unchanged even after the adjustment for patient age. The results suggest that older age, greater duration of epilepsy, and the presence of developmental intellectual disability may increase the risk of SUDEP through their direct influence on decreasing the vagus nerve-mediated HRV. Further validation of the SUDEP-7 inventory is indicated.

Trial Registration: ClinicalTrials.gov, NCT00871377.

Keywords: SUDEP, SUDEP-7, heart rate variability, epilepsy, mortality, sudden death in epilepsy

INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) accounts for 16–36% of deaths in people with epilepsy and has an annual incidence of three to nine per 1,000 in the general epilepsy population (1). In drug-resistant epilepsy (DRE), the annual incidence of SUDEP is about one in 150 (2). Clinical risk factors have been prospectively identified in multiple studies. These risk factors include frequent generalized tonic–clonic seizures and long duration of epilepsy among others (2, 3).

There is a critical need for biomarkers and a screening inventory so patients at risk may be identified prior to death. This would create an opportunity to educate, monitor, and intervene to reduce the risk of SUDEP. In 2011, our group reported the first SUDEP Risk Inventory (SUDEP-7), which includes seven validated risk factors for SUDEP (4). The SUDEP-7 has been associated with two biomarkers of SUDEP risk: RMSSD and postictal generalized EEG suppression (PGES) (4, 5). RMSSD, the root mean square differences of successive R–R intervals, is a measure of vagus-mediated heart rate variability (HRV) and autonomic regulation of the heart. Low HRV, as measured by RMSSD, has been associated with atrial fibrillation, cardiovascular disease, and poor outcomes in patients with heart failure (6). In this report, we evaluate a revised SUDEP-7 Risk Inventory in a complete cohort of 25 patients with severe DRE. We correlate the revised SUDEP-7 with HRV, age, duration of epilepsy, and other key covariates and evaluate the associations of HRV with the individual risk factors of SUDEP-7 in bivariate fashion and in a multivariable model.

METHODS

Subjects were enrolled in a prospective double-blind crossover trial of a study of fish oil for epilepsy (clinicaltrials.gov # NCT00871377). Institutional Research Committee approval was secured prior to initiating the study, and informed consent was obtained from each subject or their guardian at enrollment. Inclusion criteria were as follows: ages 18–70; a history of localized, partial epilepsy; a history of generalizes tonic–clonic or tonic seizures with loss of consciousness; DRE with three or more simple partial, complex partial, or tonic–clonic seizures per month (1981 ILAE classification, partial onset seizures with or without loss of consciousness); prior exposure to at least one or more antiepileptic drugs at therapeutic doses alone or in combination; an EEG and/or an MRI consistent with a localization related epilepsy; and at least three seizures per month for at least 2 months prior to the study. Exclusion criteria were as follows: progressive medical, cardiac, or other illness; allergy to fish products or fish oil; history of coagulation disorder; history of non-epileptic seizures; consumption of fish oil 30 days or less prior to enrollment; any change in antiepileptic drugs 30 days or less prior to enrollment; warfarin treatment 30 days or less prior to enrollment; history of poor compliance with therapy; drug or alcohol abuse; uncountable seizures as a result of seizure clustering; and pregnancy.

At entry, subjects underwent a history and a physical exam, and their seizure calendars were reviewed and validated. A baseline seizure frequency was calculated from the validated seizure calendars. A National Hospital/Chalfont seizure severity scale was administered, and a score was calculated at baseline. Vital signs (heart rate, blood pressure, and weight) were determined and recorded. Subjects underwent a 1-h electrocardiogram in the resting, awake state in the sitting position, using a Philips Digitrak-plus 24 digital Holter monitoring system with a frequency range of 0.5–60 Hz and a sampling rate of 175 Hz (Philips Digitrak-Plus 24). Time-dependent measures of HRV were calculated as defined by Stein et al., and they included SDNN, SDANN, and RMSSD. SDNN is defined as the mean of the SDs for all R–R intervals; SDANN is defined as the SDs of all R–R intervals in successive 5-min epochs; and RMSSD is defined as the root mean square difference of successive R–R intervals (7).

For this study, a revised SUDEP-7 Risk Inventory was adapted from the original SUDEP-7 Risk Inventory. The SUDEP-7 Risk Inventory (Version 2.0) includes seven risk factors validated by Walczak et al. and weighted by multiplying the reported odds ratios by the natural log ($\log_e$) (3). The revised SUDEP Risk Inventory uses a modified scoring methodology to prevent overinflating subjects' SUDEP score. Subjects with risk factor 1 (three or more tonic–clonic seizures in the last year) scored a 0 for risk factor 2 (one tonic–clonic seizures in the last year). Similarly, subjects with risk factor 4 (50 or more seizures per month) scored a 0 for risk factor 3 (one seizure in the last year).

All subjects were assessed with the SUDEP-7 Risk Inventory (SUDEP-7), and 24/25 completed HRV testing.

Statistical Analysis

We evaluated the relationship between SUDEP-7 versus RMSSD using the Pearson correlation after confirming linearity. We reported the summary statistics for RMSSD including the mean (SD) and median by quartiles of the SUDEP-7 score and evaluate the association of RMSSD versus the SUDEP-7 quartile using the Spearman correlation (test of trend).

We compared the baseline RMSSD measure by level of each individual risk factor of SUDEP-7 using the Wilcoxon rank sum test or the *t*-test as appropriate. We evaluated the relationship between RMSSD versus the subset of the most important risk factors of SUDEP simultaneously using the non-parametric linear regression model with bootstrapping.

To evaluate the relationship between SUDEP-7 versus RMSSD while controlling for the covariates, such as age and sex, we used the linear regression model after confirming the normality and constant variance assumptions. Linearity was confirmed by fitting splines.

RESULTS

Twenty-five subjects with severe DRE were enrolled. Mean age was 33.0 years (SD 10.3), with 15 females and 10 males. Average duration of epilepsy was 21.5 years (SD 11.2). Patients in this cohort were highly drug resistant, with a mean seizure frequency of 25.1 seizures per month (SD 59.4).

Table 1 summarizes the clinical data for the cohort. **Table 1** is divided into four quartiles depending on SUDEP-7 score. The total score on the SUDEP-7 ranged from 1 to 7, mean = 3.4 (SD 1.8) out of a maximum possible score of 10. **Table 2** summarizes the core components of the SUDEP-7 inventory, and the number of subjects with each factor.

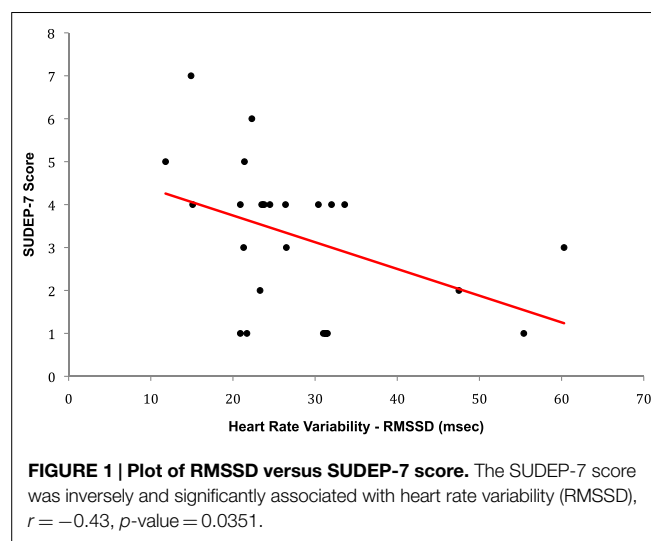**TABLE 1 | Summary of data for the cohort of patients, stratified by SUDEP Risk Inventory score (SUDEP-7).**

Quartile by SUDEP-7 score	SUDEP-7 score	Age	Sex	Duration of epilepsy (years)	Seizures per month	RMSSD (ms)	Mean RMSSD by quartile
76–100%	7	45	M	45	6	14.9	17.6 ms SD 5.1
	6	21	M	16	60	22.3	
	6	18	F	17	24	N/A	
	5	46	F	31	3	21.4	
	5	28	F	28	20	11.8	
51–75%	4	56	F	44	5	15.1	25.6 ms SD 5.8
	4	44	M	33	6	32.0	
	4	32	M	31	3	23.8	
	4	40	F	34	3	20.9	
	4	42	F	30	30	26.4	
	4	32	M	14	3	30.4	
	4	30	M	22	5	33.6	
	4	34	F	29	5	23.5	
	4	30	M	26	15	24.5	
25–50%	3	28	F	20	8	60.3	35.8 ms SD 17.2
	3	19	M	5	60	26.5	
	3	37	M	15	300	21.3	
	2	32	F	10	10	23.3	
	2	22	F	12	4	47.5	
0–25%	1	28	F	7	7	20.9	32.0 ms SD 12.5
	1	24	M	10	12	31.3	
	1	37	F	7	5	31.5	
	1	26	F	21	10	31.0	
	1	22	F	17	10	55.4	
	1	53	F	13	13	21.7	
Mean	2.96	33.04		21.48	25.08	27.9	27.9
SD	1.59	10.3		11.20	59.35	11.8	11.8

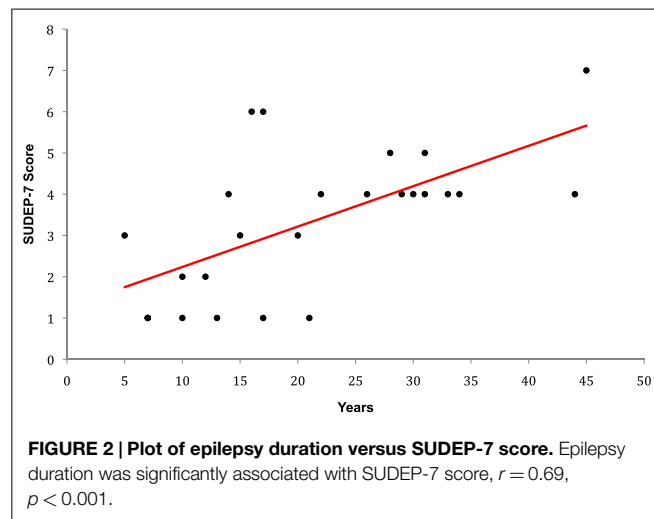*The mean RMSSD decreased with each increasing quartile of SUDEP-7, p = 0.032, trend test.*

TABLE 2 | The SUDEP Risk Inventory (SUDEP-7, version 2.0) with each risk factor, weighting, and scoring convention.

SUDEP Risk Inventory (version 2.0)	Odds ratio	Weighting $\log_e \times$ odds ratio	Number of subjects with each risk factor
1. More than three tonic–clonic seizures in last year	8.1	0 or 2	6
2. One or more tonic–clonic seizures in last year (if factor 1 present, score as 0)	2.4	0 or 1	9
3. One or more seizures of any type over the last 12 months (if factor 4 present, score as 0)	2.2, 3.8, 4.6	0 or 1	24
4. >50 seizures of any type per month over the last 12 months	11.5	0 or 2	3
5. Duration of epilepsy >30 years	13.9	0 or 3	7
6. Use of three or more AEDs	4.0	0 or 1	9
7. Developmental disability, I.Q. <70 or too impaired to test	5.0	0 or 2	3

FIGURE 1 | Plot of RMSSD versus SUDEP-7 score. The SUDEP-7 score was inversely and significantly associated with heart rate variability (RMSSD), $r = -0.43$, p-value = 0.0351.

The SUDEP-7 score was inversely correlated with RMSSD, one of the three core measures of HRV (Pearson $r = 0.43$, p-value = 0.035, **Figure 1**). Subjects in the highest SUDEP-7 quartile (scores = 5–7) had significantly lower RMSSD values than subjects in the lowest SUDEP-7 quartile (scores = 1). The mean RMSSD for the highest SUDEP-7 quartile was 17.6 ms (SD = 5.1). The mean RMSSD for the lowest SUDEP-7 quartile was 32.0 ms (SD = 12.5). RMSSD decreased significantly with each increasing quartile of SUDEP-7, $p = 0.032$, trend test, see **Table 1**. The SUDEP-7 score was also correlated with duration of epilepsy ($r = 0.69$, $p < 0.001$, Pearson moment correlation, **Figure 2**).

FIGURE 2 | Plot of epilepsy duration versus SUDEP-7 score. Epilepsy duration was significantly associated with SUDEP-7 score, $r = 0.69$, $p < 0.001$.

TABLE 3 | SUDEP-7 scores of two subjects who died of autopsy-confirmed SUDEP.

SUDEP-7 Risk Inventory (SUDEP-7)	Subject 1	Subject 13
1. More than three tonic–clonic seizures in last year	2	2
2. One or more tonic–clonic seizures in last year	0	0
3. One or more seizures of any type over the last 12 months	1	1
4. >50 seizures of any type per month over the last 12 months	0	0
5. Duration of epilepsy ≥30 years	0	0
6. Concurrent use of >3 antiepileptic drugs	1	1
7. Developmental intellectual disability, I.Q. <70, or too impaired to test	0	2
Total score	4	6

Subjects with developmental intellectual disabilities (mental retardation) had significantly lower baseline RMSSD values compared to subjects without developmental intellectual disabilities: mean RMSSD values for patients with developmental intellectual disabilities were 16.3 versus 29.6 ms in those without developmental intellectual disabilities ($p = 0.026$, Wilcoxon rank sum test). Age was inversely correlated with RMSSD values, with lower RMSSD values in older subjects ($r = 0.45$, $p = 0.028$). In addition, greater duration of epilepsy was correlated with decreased RMSSD ($r = 0.65$, $p = 0.02$, for persons with >20 years duration). The covariate of epilepsy duration (adjusted mean rate of change = 0.71 ms/year, $p = 0.008$, for patients with >20 years duration) remained significantly associated with decreased RMSSD even when controlled for in the same multivariable model. Older age tended to be associated with greater SUDEP-7 score, although this association did not reach statistical significance ($r = 0.24$, $p = 0.25$). The correlation between decreased RMSSD and greater SUDEP-7 score trended even after the adjustment for age ($r = 0.40$, $p = 0.05$), but any effect of age on SUDEP-7 entirely disappeared once RMSSD was accounted for in the multivariable model.

Two patients died from autopsy-confirmed SUDEP. One died during the study, and one died after exiting the study. The SUDEP-7 score for subject one was 4, and the SUDEP-7 score for subject 13 was 6. **Table 3** summarizes the SUDEP-7 for the two subjects who died of SUDEP. Note, both reported three or more GTC seizures, and both were taking three or more AEDs. Subject 13 had a developmental intellectual disability (see **Table 3**).

DISCUSSION

We report the revised SUDEP-7 Risk Inventory in a cohort with severe DRE. Scores in the top SUDEP-7 quartile ranged from 5 to 7. All scores in the lowest SUDEP-7 quartile were 1. Two subjects (8%) died of SUDEP. One was in the highest quartile (SUDEP-7 score = 7), and the other was in the second highest quartile (SUDEP-7 score = 4). Both subjects had above-average SUDEP-7 scores when compared with the mean score of 3.4. The

high incidence of SUDEP is likely due to the high severity and longstanding duration of epilepsy in this cohort.

The revised SUDEP-7 Risk Inventory was associated with the biomarker RMSSD, a measure of vagus-mediated HRV. High SUDEP-7 scores (SUDEP-7 scores of >5) were significantly associated with low values of RMSSD. Low SUDEP-7 scores, indicating lower risk for SUDEP, were associated with higher values of vagus-mediated HRV (RMSSD). Age, duration of epilepsy, and developmental intellectual disability were all associated with reduced vagus-mediated HRV (RMSSD). The correlation of RMSSD and SUDEP-7 persisted even after the adjustment for patient age, but any effect of age on SUDEP-7 completely disappeared once RMSSD was accounted for in the multivariable model. The results suggest that older age, greater duration of epilepsy, and presence of developmental disability may increase SUDEP risk through their direct influence on vagus nerve mediated HRV (8).

This article adds to data from the initial cohort of 19 patients published in 2010 and now includes the entire study cohort of 25 subjects, with stratification of SUDEP-7 scores into quartiles, and needed modifications to the original methodology for calculating the SUDEP-7 score. This study adds to evidence that patients with developmental disability may have higher risk for SUDEP. This expanded study also provides new evidence that patients with SUDEP-7 scores in the highest quartile have significantly greater autonomic dysfunction of the heart than patients with low SUDEP-7 scores (6, 7, 9, 10). The values of RMSSD for the highest SUDEP-7 quartile averaged 17.6 ms and are similar to values in patients with heart disease at high risk for heart failure. In a recent study of 4,652 patients with cardiac risk factors who underwent HRV testing, subjects with RMSSD values similar to those in the highest SUDEP-7 quartile had significantly higher risk for heart failure. In that study, hazard ratios (HR) for heart failure ranged from a HR of 4.7:1 for RMSSD values <16 to 2.8:1 for RMSSD values of 16–26.9 ms. This indicates that patients at highest risk of SUDEP have autonomic dysfunction similar to that found in patients at high risk for heart failure.

The SUDEP-7 Risk Inventory now requires further validation in larger populations. Moseley et al. have correlated the SUDEP-7 with another biomarker, PGES. PGES was found to occur following seizures in 32.4% of 37 children monitored in a pediatric epilepsy unit (5). PGES was highly correlated with the SUDEP-7 inventory (5). Children with PGES had significantly higher SUDEP-7 scores than those without PGES SUDEP-7 score = 4.2 for those with PGES, versus 2.8 for those without PGES (5). The strong association between the SUDEP-7 inventory with a second biomarker is an important discovery and provides further independent validation.

The question of whether to include the risk factor of three or more ongoing AEDs is an ongoing question (8). Hesdorffer et al. found that the number of AEDs was not an independent predictor of SUDEP risk, after adjusting for the presence/absence of generalized tonic–clonic seizures (GTCS), age at death, and gender (11). The number of AEDs may simply reflect the severity

of epilepsy and not SUDEP risk. Walczak et al. did report that three or more AEDs were an independent risk factor for SUDEP, and both subjects who died of SUDEP scored positive for the risk factor of greater than three AEDs. Further exploration regarding inclusion of three or more AEDs as a risk factor is indicated.

In summary, we report the revised SUDEP-7 Risk Inventory (SUDEP-7) in a cohort with severe DRE. The SUDEP-7 was associated with a biomarker of vagus-mediated HRV. The highest SUDEP-7 scores of 5–7 were associated with levels of autonomic function found in patients at high risk for heart failure, which reflects severely deranged autonomic control of the heart. We believe further investigation of the SUDEP-7 Risk Inventory is indicated.

FUNDING

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Ranking the Leading Risk Factors for Sudden Unexpected Death in Epilepsy

Christopher M. DeGiorgio[1,2,3]*, Daniela Markovic[2], Rajarshi Mazumder[1,2] and Brian D. Moseley[4]

[1] UCLA Department of Neurology, Los Angeles, CA, United States, [2] David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [3] Olive View-UCLA Medical Center, Sylmar, CA, United States, [4] Department of Neurology and Rehabilitation Medicine, University of Cincinnati, Cincinnati, OH, United Statesio

Background: Sudden unexpected death in epilepsy (SUDEP) is rare in well-controlled epilepsy. However, SUDEP is a common cause of death in drug-resistant epilepsy. Over the last 30 years, multiple cohort and population studies have identified clinical risk factors associated with an increased risk for SUDEP.

Objective: To identify and rank the leading SUDEP risk factors from major cohort and population-based studies. The incidence of SUDEP is also evaluated in special clinical situations, including antiepileptic drug treatment, epilepsy surgery, devices, and assignment to placebo in clinical trials.

Methods: A PubMed search for English language human cohort studies for the terms Sudden, Death, and Epilepsy was performed for the years 1987–2017. Risk factors for SUDEP were identified and ranked by the weighted log adjusted odds ratio (OR)/relative risk ratio (RR).

Findings: The top 10 leading risk factors ranked from highest to lowest log adjusted OR/RR are the following: ≥3 GTC seizures per year; ≥13 seizures in the last year; No Antiepileptic Drug (AED) treatment; ≥3 AEDs; ≥3 GTCs in the past year; 11–20 GTC seizures in the last 3 months; age of onset 0–15 years old; IQ < 70; 3–5 AED changes in the last year; ≥3 AEDs. Two risk factors from separate sources (≥3 GTC seizures and ≥3 AEDs) occur twice in the top 10 risk factors.

Conclusion: The top 10 risk factors for SUDEP are identified and ranked. A ranking of the top risk factors could help clinicians identify patients at highest risk for SUDEP.

Keywords: epilepsy, seizures, sudden death, sudden unexpected death in epilepsy, mortality, odds ratio

INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) is an important cause of death in people with epilepsy (1, 2). Average incidence is 0.2 per 1,000 persons/year in children and 1.0 per 1,000 persons/year in adults (1, 3, 4). The recent American Academy of Neurology guideline reassures patients and families that SUDEP is rare in people with well-controlled epilepsy (1). However, SUDEP is relatively common in persons with drug-resistant epilepsy, accounting for 14.7–17.4% of deaths with an incidence of 2.46–5.94 per 1,000 persons/year (5–8). Risk factors associated with increased

risk for SUDEP risk are frequent GTC seizures, polytherapy, early onset, long duration of epilepsy, frequent antiepileptic drug (AED) changes, and low IQ (9–13).

The purpose of this report is to review and rank the leading SUDEP risk factors from studies published in core clinical and epilepsy journals from 1987 to 2017. We also review the incidence of SUDEP in specific clinical situations. The authors hope this report will provide an accessible reference for clinicians to help identify persons at highest risk for SUDEP.

METHODS

A PubMed search for all English Language Human publications from 1987 to 2017 was performed for the terms "Sudden," "Death," and "Epilepsy" in journal titles. This search yielded 310 publications. An identical similar search including the term "Population" or "Cohort" yielded 64 and 30 publications, respectively. A search of core clinical journals for these terms yielded 20 and 18 publications, respectively. Both prospective and retrospective studies were included and duplicate studies were eliminated. Publications from core clinical journals and core epilepsy journals indexed in PubMed were included.

Risk factors from cohort studies using matched control subjects and analyzed using multivariate or univariate analysis were included. The crude and adjusted odds ratios or relative risk ratios (ORs or RRs) from these studies were analyzed. For ranking of risk factors for inclusion in **Table 1**, the adjusted log OR/RR was used. The weighted log of the adjusted OR was calculated by multiplying the adjusted log OR × 1/SE to adjust for the size and variability of the point estimates of the source studies. The top 10 SUDEP risk factors were then ranked by the weighted log OR or RR. Variables accounting for the adjusted OR are listed in **Table 1** for each individual study, and include: age, gender, seizure frequency, geographic region, epilepsy duration, and data source.

RESULTS

Table 1 summarizes the 10 leading clinical risk factors ranked by the weighted log of the adjusted OR or RR. **Figure 1** graphically displays the adjusted OR/RR for these 10 risk factors with their corresponding 95% confidence intervals (CI). The top 10 leading risk factors by weighted log OR/RR are the following: #1 ≥3 GTC seizures per year (versus 0, Hesdorffer et al.) (13); #2 ≥13 seizures (of any type) in the last year (versus 0–2); #3 No AED treatment (versus 1–2 AEDs); #4 ≥3 AEDs (versus 1); #5 ≥3 GTCs in the past year (versus 0, Walczak et al.) (10); #6 11–20 GTC seizures in the last 3-months (versus 0–5); #7 age of onset 0–15 years old (versus > age 45); #8 IQ < 70; #9 3–5 AED changes/year in the last year; #10 ≥3 AEDs (versus 0–2) (8–11, 13).

Specific Clinical Scenarios
GTC Seizures and Overall Seizure Frequency
GTC and frequent GTC seizures are consistently identified as the leading risk factors for SUDEP with the highest OR (10–13). GTC seizures account for 3 of the 10 leading risk factors reported in **Table 1**. Three or more GTC seizures in the last year (reported

by Hesdorffer et al.) ranks as the leading risk factor overall and are repeated again as #5 (also reported by Walczak et al.) (10, 13). High seizure frequency of any type is also an important risk factor for SUDEP (9, 10). In their study of 6,880 subjects, Nilsson et al. reported that 13–50 seizures/year and >50 seizures/year were associated with SUDEP (crude OR of 8.64 and 10.16, respectively) (9). Similarly, Walczak et al. reported that >50 seizures of any type per month were associated with risk of SUDEP with an OR of 11.5 (CI 1.3–99.3) (10).

AED Therapy
Antiepileptic drug therapy and polytherapy have been extensively studied in relation to SUDEP risk (14–16). Hesdorffer et al. evaluated four large cohort studies of SUDEP, and found the risk of SUDEP in patients treated with monotherapy versus no treatment was reduced for phenytoin, carbamazepine, and valproic acid (OR ranged from 0.5 to 0.7, CI 0.2–1.6) (16).

Since CI were wide and statistical significance was not reached, these results do not provide compelling evidence of a protective effect of AED therapy against SUDEP (14–16). However, no AED treatment in patients with epilepsy has been identified as a risk factor for SUDEP and is listed in **Table 1** (11).

Polytherapy accounts for 2 of the top 10 leading risk factors for SUDEP. Nilsson et al. reported that three or more AEDs were associated with an increased risk of SUDEP (9). In their series, 12 of 57 (21%) subjects who died of SUDEP were taking at least 3 AEDs, versus 7 of 171 controls (4%). Walczak found a moderate association between SUDEP and two or more AEDs (10). However, Hesdorffer et al. in their 2012 meta-analysis of published case–control studies, when adjusting for the number of GTC seizures, found a reduced association between SUDEP risk and polytherapy (16). It is likely that the risk of polytherapy is more a reflection of severe drug resistance and high seizure frequency, rather than the risk of AEDs *per se* (16).

Frequent changes in AED dose are also a risk factor. Nilsson et al. found that frequent AED dose changes were associated with the risk of SUDEP (9). The RR associated with three or more changes in AED dose in the last year was 6.08 (CI 1.99–18.56) (9).

Randomization to placebo clinical AED trials is associated with a very high risk of SUDEP. Ryvlin et al. reported an analysis of 20,101 patients enrolled in 112 randomized controlled AED trials (17). Fourteen of 7,678 patients randomized to placebo died of SUDEP, versus only 3 of 3,297 patients randomized to effective doses of investigational AEDs (17). The incidence of SUDEP was 6.9 per 1,000 patient years for those randomized to placebo, versus only 0.9 per 1,000 patient years assigned to effective doses of AEDs. Overall, the relative risk of SUDEP was 7.5 times higher in those assigned to placebo than those randomized to active treatment arms (17).

Resective Epilepsy Surgery
Resective epilepsy surgery is associated with reductions in mortality, especially in those who become seizure free. In 1999, Sperling et al. reported a cohort of 393 drug-resistant patients who underwent various epilepsy surgical procedures over the period 1986 to 1996 (18). The mortality of those who were not seizure free was 5.7% (CI 2.9–9.9%) for a mortality rate of 13.7
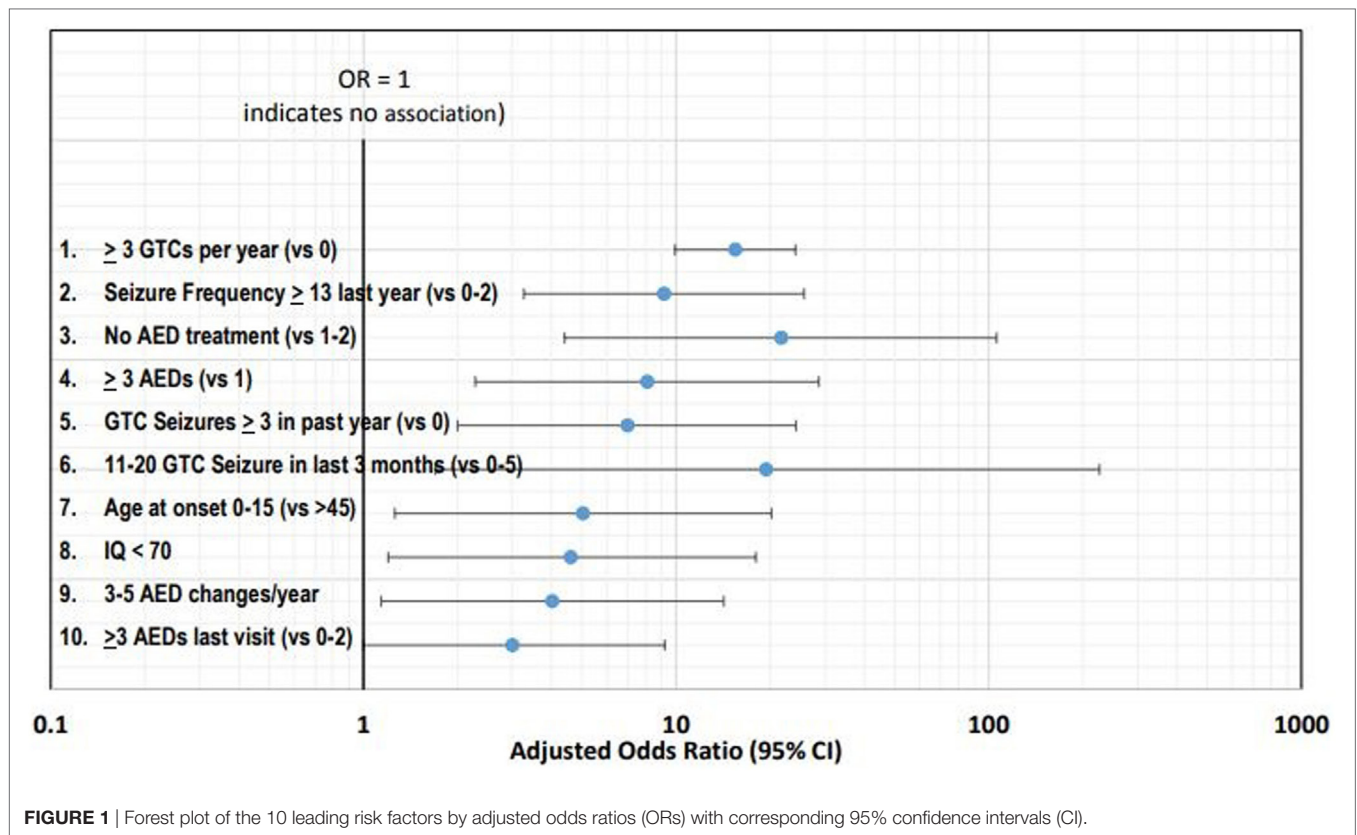
TABLE 1 | Top 10 risk factors for SUDEP sorted by the weighted log OR estimate.

Ranking by weighted log OR	Risk factor	Adjusted OR	Lower CI	Upper CI	Weighted log OR [*adjusted log OR × 1/SE*]	Reference	Study methodology/OR adjustments
1	Three or more GTC seizures per year (versus 0 seizures)	15.46	9.92	24.10	12.10	Hesdorffer et al. (13)	Pooled analysis from four cohort studies. Risk factors were examined using logistic regression. ORs were adjusted for data source, gender, age at death, and epilepsy duration
2	≥ 13 seizures of any type in the last year (versus 0–2 seizures)	9.15	3.26	25.68	4.20	Nilsson et al. (9)	Cohort Study: relative risk ratios (RRs) were calculated using multiple logistic regression. Each index case of SUDEP was compared with three living controls matched for year of birth, sex, and assessment period randomly selected from the study. ORs were adjusted for population age, gender, and seizure frequency
3	No AED treatment (versus one or two AEDs)	21.70	4.40	106.00	3.78	Langan et al. (11)	Cohort Study: ORs were calculated using index SUDEP case compared with 4 randomly selected controls from patients with epilepsy enrolled in the MRC General Practice Research Framework. ORs were adjusted for age, geographic region, and seizure frequency
4	Three AEDs (versus 1)	8.09	2.28	28.62	3.24	Nilsson et al. (9)	Cohort Study: relative RRs were calculated using multiple logistic regression. Each index case of SUDEP was compared with three living controls matched for year of birth, sex, and assessment period randomly selected from the study. ORs were adjusted for population age, gender, and seizure frequency
5	Three or more GTC Seizures in past year (versus 0)	7.00	2.00	24.20	3.04	Walczak et al. (10)	Cohort Study: ORs were calculated using logistic regression. Index case of SUDEP compared with 4 randomly selected controls also enrolled in prospective study at 3 Midwest US epilepsy centers. ORs were adjusted for seizure frequency
6	11–20 GTC Seizures in the last 3 months (versus 0–5)	19.40	1.70	226.00	2.39	Langan et al. (11)	Cohort Study: ORs were calculated using index SUDEP case compared with 4 randomly selected controls from patients with epilepsy enrolled in the MRC General Practice Research Framework. ORs were adjusted for age, geographic region
7	Age at onset 0–15 (versus >45)	5.04	1.26	20.19	2.29	Nilsson et al. (9)	Cohort Study: relative RRs were calculated using multiple logistic regression. Each index case of SUDEP was compared with three living controls matched for year of birth, sex, and assessment period randomly selected from the study. ORs were adjusted for population age, gender, and seizure frequency
8	IQ < 70	4.60	1.20	18.00	2.23	Walczak et al. (10)	Cohort Study: ORs were calculated using logistic regression. Index case of SUDEP compared with 4 randomly selected controls also enrolled in prospective study at 3 Midwest US epilepsy centers. ORs were adjusted for seizure frequency
9	3–5 changes in AEDs per year (versus 0)	4.02	1.14	14.21	2.16	Nilsson et al. (9)	Cohort Study: relative RRs were calculated using multiple logistic regression. Each index case of SUDEP was compared with three living controls matched for year of birth, sex, and assessment period randomly selected from the study. ORs were adjusted for population age, gender, and seizure frequency
10	≥3 AEDs last visit (versus 0–2)	3.00	1.00	9.20	1.96	Walczak et al. (10)	Cohort Study: ORs were calculated using logistic regression. Index case of SUDEP compared with 4 randomly selected controls also enrolled in prospective study at 3 Midwest US epilepsy centers. ORs were adjusted for seizure frequency

The weighted log OR is defined as the log adjusted OR × 1/SE. CI are reported.
OR, odds ratio; CI, confidence intervals; SUDEP, sudden unexpected death in epilepsy; AED, antiepileptic drug.

FIGURE 1 | Forest plot of the 10 leading risk factors by adjusted odds ratios (ORs) with corresponding 95% confidence intervals (CI).

per 1,000 persons/year (18). This is substantially higher than the mortality rate of 0 per 1,000 person/year for those patients who were seizure free during the follow-up period (CI 0.0–1.8%) (18). Six of 11 subjects who died during follow-up died of probable or definite SUDEP (54.5%) (18). Of the 6 subjects who died of SUDEP, 5/6 (83%) were not seizure free, indicating that seizure freedom after epilepsy surgery is a factor in the risk for SUDEP (18). Later in 2016, Sperling et al. reported the mortality in a larger cohort of 1,110 drug-resistant patients evaluated for epilepsy surgery (19). The cohort consisted of 1,006 patients who underwent epilepsy surgery, and 104 patients who underwent pre-surgical evaluation but were treated with medical therapy only. In the 104 patients treated with medical therapy only, the mortality rate was 25.3 per 1,000 persons/year versus 8.6 per 1,000 persons/year for surgically treated patients (19). Overall, the SUDEP rate for surgically treated patients was low: 15 died of SUDEP in 8,126 persons/year (incidence = 0.6 per 1,000 persons/year) (19).

Devices: Vagus Nerve Stimulation (VNS) and Responsive Neurostimulation (RNS)

Annegers et al. first reported SUDEP rates with VNS therapy (20). The incidence of SUDEP in their initial cohort of 1,891 patients with drug-resistant epilepsy was 4.1 per 1,000 persons/year (20). Later, in a follow-up study, Annegers reported that the incidence of SUDEP dropped from 5.5 per 1,000 persons/year to 1.7 per 1,000 persons/year after two years of VNS therapy (21). More recently, Granbichler et al., in a series from

Kings College, reported SUDEP rates in persons with drug-resistant epilepsy treated with VNS over a 15-year period (1995 through 2010). They reported SUDEP rate of 3.7 per 1,000 persons/year (22). It is currently unknown how long-term VNS therapy exerts its potential protective effect on SUDEP (22). In RNS, Heck et al. and Bergey et al. reported four probable or definite SUDEP cases, representing an incidence of 3.5 per 1,000 persons/year (23, 24). This rate is lower than the 25.3 per 1,000 persons/year reported by Sperling et al. in epilepsy surgical candidates treated with medical therapy only (19, 23, 24).

DISCUSSION

In this review, we identify the leading risk factors for SUDEP from published cohort studies of SUDEP and rank them by the weighted log OR. The 10 leading risk factors for SUDEP ranked by weighted log OR are the following: #1 ≥3 GTC seizures per year (versus 0); #2 ≥13 seizures of any type in the last year (versus 0–2); #3 No AED treatment (versus 1–2 AEDs); #4 ≥3 AEDs (versus 1); #5 ≥3 GTCs in the past year (versus 0); #6 11–20 GTC seizures in the last 3 months (versus 0–5); #7 age of onset 0–15 years old (versus > age 45); #8 IQ < 70 (mental retardation or developmental delay); #9 3–5 AED changes/year in the last year; #10 ≥3 AEDs (versus 0–2) (8–11, 13).

We also review the incidence and risk of SUDEP in specific clinical scenarios. For the drug-resistant patient, resective epilepsy surgery imparts a protective effect versus medical therapy alone (18, 19). This is especially true when patients are seizure

free after surgery (18, 19). VNS may also be associated with long-term reductions in SUDEP risk. The data on RNS are still early. However, VNS and RNS may be associated with lower mortality rates than surgical candidates treated with medical therapy only (18–24).

Polytherapy (defined as ≥3 AEDs), frequent AED changes (3–5 per year), and assignment to placebo during clinical trials are associated with increases in SUDEP risk (9, 17). However, the role of polytherapy in SUDEP risk is controversial (16). Hesdorffer found that when the risk of polytherapy, when adjusted for the frequency of GTC seizures is diminished (16). The OR for polytherapy (>3 AEDs) when adjusted for the frequency of GTC seizures is reduced from 2.8 to 1.4 (16). Supporting a diminished role for polytherapy in SUDEP risk is the Ryvlin et al.'s study of the risk of investigational AEDs versus placebo (17). Patients on effective investigational AEDs were on multiple AEDs, yet their risk was lower than those on placebo (17).

A better understanding of the leading risk factors for SUDEP could help clinicians identify patients at highest risk. For these patients, protective measures such as listening devices, nocturnal supervision, or seizure detection devices could be considered (25, 26). Langan et al. in their large UK study reported that listening devices and nocturnal supervision reduce the risk for SUDEP up to 80–90% (11, 25). Strategies have been published that could help physicians intervene to reduce the risk of SUDEP. However, their efficacy is unproven (25, 26). http://Dannydid.org is a non-profit organization committed to reducing the risk of SUDEP, and is a good resource for patients, families, and clinicians.

Clinicians should work diligently to aggressively control GTC seizures and make patients seizure free whenever possible. Since epilepsy surgery imparts a significant reduction in mortality compared with medical therapy in potential surgical candidates, efforts to explore surgical interventions should be pursued when feasible.

Community education can be expanded to inform people with epilepsy about the risks associated with SUDEP (25). Pharmaceutical companies and regulatory bodies could change the structure of clinical trials to employ novel designs that reduce exposure to placebo. Such designs could include the use of historic controls or include robust escape criteria to allow exit for placebo patients when seizures worsen.

Risk inventories should be updated to include risk factors with the highest OR. The top 10 risk factors could be included in new SUDEP risk factor inventories, such as the SUDEP-7 (27). The original SUDEP-7 was sourced from a single prospective study reported by Walczak et al. and does not include many of the risk factors identified in **Table 1** (10, 27, 28)[1]. A revision to the SUDEP risk inventory is under consideration (27, 28).

This analysis has limitations. Risk factors were derived from different studies of variable sizes, ages, and cohorts. There were variations in statistical analysis and matching techniques (four matched controls or three matched controls). The incidence of SUDEP and numbers of cases of SUDEP varied significantly from study to study. For example, there were 20 cases of SUDEP in the Walczak et al.'s study, yet 154 cases in the Langan et al.'s study (10, 11). CI were wide for many ORs and RRs, which likely reflected the relatively rare nature of SUDEP.

In conclusion, the recent AAN guideline appropriately reassures persons with well-controlled epilepsy and their families that SUDEP is rare (1). However, those with one or more risk factors listed in **Table 1** should be considered at higher risk for SUDEP. Recognition of those at high risk could lead to education and individualized interventions to reduce the risk of SUDEP.

AUTHOR CONTRIBUTIONS

CD served as the lead author. RM helped in the concept of ranking and odds ratios, BM made several additions and contributions to the manuscript, and DM analyzed the statistics from the references cited in **Table 1**.

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[1] Available from: http://Dannydid.org

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Vitamin D3 for the Treatment of Epilepsy: Basic Mechanisms, Animal Models, and Clinical Trials

Kevin Pendo[1] and Christopher M. DeGiorgio[2]*

[1] Princeton University, Princeton, NJ, USA, [2] Department of Neurology, University of California Los Angeles, Los Angeles, CA, USA

There is increasing evidence supporting dietary and alternative therapies for epilepsy, including the ketogenic diet, modified Atkins diet, and omega-3 fatty acids. Vitamin D3 is actively under investigation as a potential intervention for epilepsy. Vitamin D3 is fat-soluble steroid, which shows promise in animal models of epilepsy. Basic research has shed light on the possible mechanisms by which Vitamin D3 may reduce seizures, and animal data support the efficacy of Vitamin D3 in rat and mouse models of epilepsy. Very little clinical data exist to support the treatment of human epilepsy with Vitamin D3, but positive findings from preliminary clinical trials warrant larger Phase I and II clinical trials in order to more rigorously determine the potential therapeutic value of Vitamin D3 as a treatment for human epilepsy.

Keywords: cholecalciferol, vitamin D3, epilepsy, SUDEP, animal models, clinical trials

INTRODUCTION

Epilepsy affects approximately two million Americans and 65 million people worldwide (1). Among those with epilepsy, 22–30% have drug-resistant epilepsy (DRE) (1, 2). DRE causes cognitive and mood impairment, injuries, and increased risk of death including sudden death in epilepsy (SUDEP) (1–3). Antiepileptic drugs (AEDs) are the primary medical treatment for epilepsy. However, even for those whose seizures are well controlled by AEDs, allergies, neurological and systemic toxicity, depression, memory loss, and osteoporosis are common problems (4, 5). Because of the limitations and potential toxicity of existing AEDs, there is significant clinical interest in finding alternative therapies for epilepsy.

In the search for alternative epilepsy treatments, Vitamin D3 is an intriguing candidate (6). As early as 1974, Christiansen postulated that supplementation of Vitamin D might improve calcium and magnesium levels and may decrease hyperexcitability in patients with epilepsy. In the four decades since, progress has been made in understanding the biochemical and cellular mechanisms of Vitamin D3's anticonvulsant properties. Animal data have supported the anticonvulsant effects of Vitamin D3 in mice and rats (7–11). Existing evidence for the use of Vitamin D3 in treating human epilepsy is very limited (6, 12). There is a critical need for larger clinical trials to establish the safety and efficacy of vitamin D3 in epilepsy. In this review, we will critically analyze the animal and human evidence to date supporting the use of Vitamin D3 as a treatment for epilepsy.

VITAMIN D3 OVERVIEW: BIOCHEMISTRY AND ROLE IN HUMAN HEALTH

The most biologically active form of Vitamin D in humans is Vitamin D3 (cholecalciferol), which is a fat-soluble steroid hormone (13). Dietary sources of Vitamin D3 include dairy, meat, fish, and mushrooms (14). The primary source of Vitamin D3 is exposure of the skin to ultraviolet sunlight (14). The metabolic pathway of Vitamin D3 is summarized in **Figure 1**. 7-dehydrocholesterol is converted to Vitamin D3 in the skin after exposure to sunlight. Vitamin D3 is converted to 25-hydroxy-cholecalciferol (25-OH Vitamin D3) in the liver. 25-OH Vitamin D3 is the major circulating form of Vitamin D, but it itself is biologically inactive and must be converted to the active form 1,25-dihydroxy-Vitamin D3 (1,25 Vitamin D3) in the kidneys (13–15). Vitamin D3 is important for calcium metabolism, bone health, cardiac function, and blood pressure maintenance, among other health benefits (14, 16, 17). Vitamin D3 deficiency is a marker of poor health and overall mortality (16). However, 40–50% of Americans have insufficient Vitamin D3 levels, and insufficiency is even more prevalent in underserved populations, including Hispanics (69%) and African Americans (82%) (18).

VITAMIN D3 IN THE BRAIN AND NERVOUS SYSTEM

Among its variety of health benefits, Vitamin D3 plays an important role in the human brain and nervous system, as indicated by increasing evidence gathered over the past several decades. Researchers have explored the role of Vitamin D3 in Alzheimer's disease and dementias (19, 20), Parkinson's disease (19, 21), multiple sclerosis (22–24), schizophrenia (25), affective disorders (13, 26), cognitive decline (13, 27), and epilepsy (6, 12). Vitamin D3 is also involved in neuroprotection (15, 28, 29), brain cell proliferation and differentiation (30, 31), and brain development (30, 32, 33). A neurological role of Vitamin D3 is further

supported by the presence of Vitamin D3-specific receptors and enzymes in neurons and glial cells throughout the brain, in the spinal cord, and in the peripheral nervous system (34–37). The broad role of Vitamin D3 in the nervous system has engendered research into Vitamin D3's anticonvulsant action in the brain, and the proposed mechanisms of action can generally be categorized as either genomic or non-genomic.
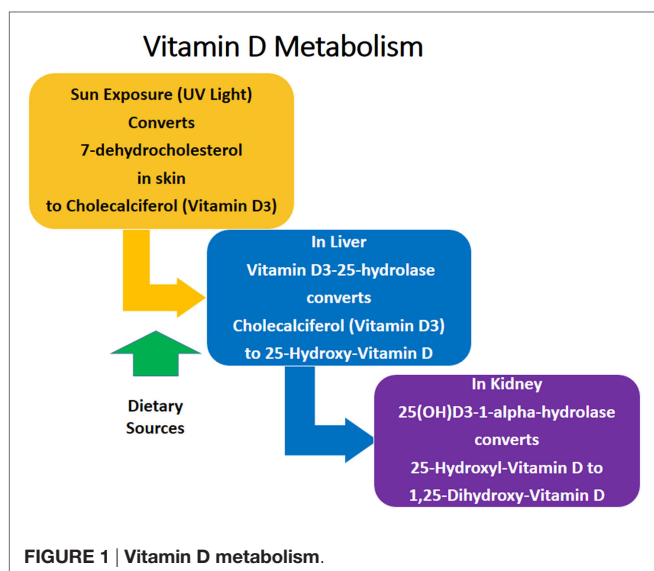
Genomic Mechanisms of Action

Genomic mechanisms behind Vitamin D3's anticonvulsant effect are based on Vitamin D3's ability to regulate the expression of genes, a process that is mediated by a nuclear Vitamin D3 receptor (VDR) (38). VDR is a ligand-specific transcription factor, which is activated by Vitamin D3 and subsequently alters gene expression (28, 29). Through this mechanism, Vitamin D3 lowers the expression of certain proconvulsant cytokines, such as IL-1β and TNF-α. These cytokines can increase seizure susceptibility in several ways. IL-1β is involved in a pathway that results in phosphorylation of the NR2B subunit of the NMDA receptor, which is a glutamate receptor that is important in the generation of seizures (39). The phosphorylation of this NMDA receptor subunit increases Ca^{2+} influx into neurons (40) and stabilizes the receptor in the membrane (41), leading to the hyperexcitability of neurons that can cause seizures (39, 42). IL-1β can also cause neuronal hyperexcitability by increasing the release probability of glutamate (43), an excitatory neurotransmitter, and inhibiting its reuptake (39, 44). In addition, IL-1β can reduce inhibitory GABA-ergic Cl^- flux (45), furthering the proconvulsant effect of this cytokine (39). The TNF-α cytokine acts as a proconvulsant because it initiates both the recruitment of AMPA receptors to the neuronal membrane and the endocytosis of GABA$_A$ receptors away from the membrane (46, 47). The TNF-α-induced overexpression of AMPA receptors and under-expression of GABA$_A$ receptors on the neuronal membranes results in more excitatory synaptic transmission and less inhibitory signaling, which increases the likelihood of epileptic activity.

Through its nuclear VDR, Vitamin D3 can also increase the expression of anticonvulsant growth factors GDNF and NT3 (15, 29, 48–50). NT3 leads to an anticonvulsant effect by downregulating TrkA and TrkC receptors, which are receptors that regulate synaptic strength (50). The mechanism behind GDNF's anticonvulsant action remains largely unknown, but it is speculated that, similar to that of NT3, it involves some modulation of synaptic transmission (51). Vitamin D3-activated VDR also promotes expression of the calcium-binding proteins parvalbumin and calbindins, which inhibit epileptic episodes (15, 29, 52). By binding to Ca^{2+} in the presynaptic terminal, these calcium-binding proteins prevent excessive Ca^{2+}-induced neurotransmitter release and thus protect against epileptic activity (52, 53).

Non-Genomic Mechanisms of Action

Faster, non-genomic mechanisms of Vitamin D3's anticonvulsant effect have been proposed as well. Vitamin D3's ability to increase calcium uptake from the intestine can alter plasma and brain Ca^{2+} concentrations, which may decrease neuronal excitability and prevent seizures. However, evidence suggests that Vitamin

FIGURE 1 | Vitamin D metabolism.

Vitamin D Metabolism

Sun Exposure (UV Light) Converts 7-dehydrocholesterol in skin to Cholecalciferol (Vitamin D3)

Dietary Sources

In Liver
Vitamin D3-25-hydrolase converts Cholecalciferol (Vitamin D3) to 25-Hydroxy-Vitamin D

In Kidney
25(OH)D3-1-alpha-hydrolase converts 25-Hydroxyl-Vitamin D to 1,25-Dihydroxy-Vitamin D

D3's anticonvulsant effect is not wholly attributable to its role in altering calcium levels (6, 8, 9). Rather, it is more likely that Vitamin D3's rapid, anticonvulsant effect results from its ability to fine-tune Ca^{2+} and Cl^- currents across neuronal membranes (54, 55). Vitamin D3 initiates non-genomic signal transduction pathways that ultimately alter the conductance of L-type calcium channels and chloride channels, therefore affecting neuronal excitability and seizure susceptibility at the threshold level (55–57). The details of these non-genomic signal transduction pathways are debated, and although it used to be thought that they were mediated by a distinct membrane Vitamin D3 receptor (VDR_{mem}) (58), more recent evidence suggests that these rapid, non-genomic anticonvulsant pathways are actually mediated by the same protein – VDR – that mediates Vitamin D3's genomic actions (54, 57, 59–61), with different domains of VDR being involved in the genomic and non-genomic pathways that lead to Vitamin D3's anticonvulsant effects.

VITAMIN D3 IN ANIMAL MODELS OF SEIZURES

Rat Models

In 1984, Siegel et al. published a seminal paper describing the effect of Vitamin D3 on seizure thresholds in rat hippocampi (7). Using artificial electrical stimulation to model seizures, they found that stereotactic injection of 50 or 100 µg of 1,25 Vitamin D3 into the hippocampus of rats significantly elevated the seizure threshold in all rats treated. This elevation in threshold was noticeable 5–10 min after the injection of 1,25 Vitamin D3, and the effect lasted at least 120–180 min. Intravenous injection of 1,25 Vitamin D3 also significantly elevated seizure threshold, but the effect was transient, lasting only 30 min, perhaps due to limited uptake of 1,25 Vitamin D3 in the brain. Most rats were Vitamin D3-sufficient, but they found that in one Vitamin D3-deficient rat, a lower dose of 1,25 Vitamin D3 was required to raise the seizure threshold to a similar extent.

Mouse Models

Over two decades after Siegel et al.'s rat study, Kalueff et al. explored the anticonvulsant effects of Vitamin D3 in a mouse model of seizures (8). Subcutaneous injection of 33 µg of 1,25 Vitamin D3 incurred an anticonvulsant effect in a chemically induced model of seizures. Compared to controls, mice injected with 1,25 Vitamin D3 40 min prior to the injection of pentylenetetrazol (PTZ), a seizure-inducing chemical, exhibited longer mean latency to seizure onset (77 vs. 55 s), shorter mean duration of tonic–clonic seizures (10 vs. 32 s), and lower mortality (18 vs. 55%). However, the anticonvulsant effects of 1,25 Vitamin D3 were nearly gone if Vitamin D3 injection occurred 3, 6, 12, or 24 h before PTZ injection. The acute efficacy of 1,25 Vitamin D3 suggests that the anticonvulsant effect in this model was due to non-genomic actions of the steroid. In addition, differences in Ca^{2+} levels between control and experimental mice were non-significant, suggesting that 1,25 Vitamin D3 exerted an anticonvulsant effect independent of its role in calcium metabolism (8).

In a separate study, Kalueff et al. found that the partial deletion of the VDR gene in mice led to increased seizure severity in the model of PTZ-induced seizures (9). Compared to wild-type mice, VDR-knockout mice demonstrated significantly shorter latencies to seizure onset (50.4 vs. 66.9 s), higher Racine scores of seizure severity (5.9 vs. 4.9), and increased mortality (90 vs. 40%). Of note, none of the mice in either the control or experimental condition showed spontaneous seizure activity, suggesting that the VDR gene acts at the threshold level of seizures. Both wild-type and VDR-knockout mice had normal calcium levels, suggesting that the partial deletion of the VDR gene increases seizure intensity *via* a non-calcium mechanism and providing further evidence of an anticonvulsant effect of Vitamin D3 that is independent from its role in calcium metabolism (9).

In two studies, Borowicz et al. have shown that certain doses of Vitamin D3 enhance the efficacy of several AEDs in a mouse electroshock model of epilepsy without altering the concentrations of the drugs, suggesting a synergistic pharmacological interaction (10, 11). The authors also reported some anticonvulsant action of Vitamin D3 in its own right (10), and they found that treatment with Vitamin D3 led to no deleterious changes in motor coordination, long-term memory, or anxiety (10, 11).

Overall, existing evidence from rat and mouse studies supports an acute anticonvulsant effect of Vitamin D3 in electric shock and chemically induced models of seizure. Further research is needed to explore the longer-term effects of Vitamin D3 therapy in diverse animal models of epilepsy.

VITAMIN D3 IN HUMAN EPILEPSY

People with epilepsy are often Vitamin D3-deficient, along with having decreased bone density and higher rates of osteoporosis (62). Furthermore, certain AEDs, such as carbamazepine and phenytoin, are known to decrease Vitamin D3 levels in people who are taking them due to increased metabolic clearance of Vitamin D3 and conversion to inactive forms (63, 64). People with epilepsy face a sixfold risk for bone fracture compared to the normal population, likely an interplay between frequent falls, reduced bone density, and low levels of Vitamin D3 (62). Maternal Vitamin D3 deficiency during pregnancy has also been associated with hypocalcemia-induced seizures in neonates, which have been successfully treated with calcium and Vitamin D3 supplementation in several case studies (65–68).

In humans, little clinical data exist about the effect of Vitamin D3 supplementation on seizures. In 1974, Christiansen et al. conducted a pilot study in which they treated 23 epilepsy patients with Vitamin D3 (6). Subjects were divided into two groups (A and B) for the duration of the 12-week study, which was divided into a 4-week observation phase (T1) followed by two 4-week treatment periods (T2 and T3). Group A ($n = 9$) received 4,000 IU/day of Vitamin D3 during T2, followed by 16,000 IU/day during T3. Group B ($n = 14$) received placebo during T2, followed by 8,000 IU/day of Vitamin D3 during T3. During T2, Group A (treated with 4,000 IU/day of Vitamin D3) experienced a mean reduction in seizure frequency of 32% from baseline, while Group B (placebo) experienced an 8% reduction in mean seizure frequency from baseline. During T3, Group A (treated

with 16,000 IU/day of Vitamin D3) experienced a 29% reduction in mean seizure frequency from baseline, while Group B (being treated with 8,000 IU/day of Vitamin D3) experienced a similar. In both groups, high dose vitamin D3 (8000 to 16000 IU/day) was associated with reductions in seizure frequency 33% reduction in mean seizure frequency from baseline. The authors concluded that high dose Vitamin D3 significantly reduced the number of seizures in patients with poorly controlled epilepsy, and, contrary to the authors' hypothesis, it did so independently of calcium or magnesium levels (6).

Nearly 40 years after Christiansen et al.'s findings, Holló et al. conducted the most recent clinical study of Vitamin D3 therapy in human epilepsy (12). Their subjects consisted of 13 patients with DRE. At baseline, low 25-OH-Vitamin D3 levels <30 ng/ml were present in 12/13 patients and deficient levels (<12 ng/ml) were present in 8/13 patients; 1/13 patients had a normal Vitamin D3 level at baseline. Treatment consisted of Vitamin D3 supplementation aimed at normalizing the serum Vitamin D3 levels of all the subjects. To the 12 patients with low or deficient Vitamin D3 levels at baseline, an oral dose of 40,000–200,000 IU bolus of Vitamin D3 was administered, and treatment was continued with a daily maintenance dose of 2,000–2,600 IU/day of Vitamin D3. The one subject with normal baseline Vitamin D3 level only received the daily maintenance doses. Vitamin D3 levels were rechecked 3 months after treatment onset to determine successful normalization of Vitamin D3 levels and rule out potential Vitamin D3 toxicity. Vitamin D3 supplementation was determined to be safe, as no subjects showed toxic levels of Vitamin D3 at the 3-month follow-up (12). Median Vitamin D3 level rose from 11.8 ng/ml at baseline (range: <4–34.2 ng/ml) to 38.0 ng/ml at 3-month follow-up (range: 23.3–45.0 ng/ml). This elevation in Vitamin D3 levels was significant ($p = 0.001$, sign test), and the posttreatment Vitamin D3 levels of all subjects were within or close to the normal range (12). The efficacy of the Vitamin D3 treatment in reducing seizures was determined by comparing the number of seizures experienced during the 90 days prior to treatment onset to the number of seizures experienced in the 90 days after treatment onset. Among all subjects, 10/13 experienced fewer seizures after initialization of Vitamin D3 treatment, 2/13 had more seizures, and 1/13 had the same number of seizures. The median reduction in seizure number following treatment onset was 40% and was significant ($p = 0.04$). In addition, 5/13 patients experienced a ≥50% reduction in number of seizures. The existing clinical evidence suggests a therapeutic effect of Vitamin D3 in human epilepsy, but there is a need for larger Phase I trials and Phase II randomized, placebo-controlled trials to investigate optimal dosing and short-term and long-term efficacy.

DOES VITAMIN D3 HAVE A POTENTIAL ROLE IN REDUCING SUDEP RISK?

Vitamin D3 status is strongly associated with risk of sudden cardiac death in heart disease and patients with severe kidney disease on hemodialysis. In a large prospective study of 2,300 patients in the Cardiovascular Health Study, the risk of sudden cardiac death was 2-times higher in those with Vitamin D3 levels <20 ng/ml (4 events/1,000) than in those with Vitamin D3 levels >20 ng/ml (2 events/1,000) (69). Similarly, in a study of 1,108 patients with chronic kidney disease, very low levels of Vitamin D3 (25-hydroxy-Vitamin D3 levels <25 nmol/l) were 3-times more likely to sustain sudden cardiac death than those with high levels >75 nmol/l (hazard ratio = 2.99) (70).

Common to severe heart and kidney disease is impaired heart rate variability (HRV), particularly vagus-mediated high-frequency HRV (69–72). Patients with DRE, who are at high risk for SUDEP, have impaired vagus-mediated HRV, similar in magnitude to patients with heart failure (69, 70, 73, 74). Recently, subjects with DRE, at high risk of SUDEP, as measured by the SUDEP-7 inventory, were found to have severe impairment in RMSSD, a measure of vagus-mediated HRV (73, 74). In a recent study linking SUDEP risk in patients with DRE, those with the highest SUDEP-7 inventory risk scores in the highest quartile had RMSSD values of 17.6 ms, vs. 32.0 ms for those with the lowest SUDEP-7 inventory scores ($p = 0.03$, trend test) (74). This finding is relevant since Vitamin D3 supplementation improves vagus-mediated HRV (71, 72, 75). Recently, Vitamin D3 supplementation ranging from 5,000 to 10,000 IUs in normal controls resulted in significant improvements in high-frequency HRV, as measured by the low-frequency/high-frequency HRV ratio (75). A similar result was recently found in patients with IGA nephropathy, where high-frequency HRV, as measured by the LF/HF HRV ratio, also increased after Vitamin D3 supplementation (71).

CONCLUSION AND FUTURE DIRECTIONS

The weight of evidence from basic research and animal models over the past several decades supports an anticonvulsant effect of Vitamin D3. Vitamin D3's anticonvulsant action may be *via* genomic and non-genomic mechanisms. Epidemiological data as well as a variety of case studies also point to a connection between Vitamin D3 and epilepsy and support the use of Vitamin D3 as a potential therapy for human epilepsy, both in its own right and in conjunction with existing AEDs. However, the clinical data that exist are limited by small sample size and/or lack of randomization and double-blind placebo control. Despite these limitations, existing clinical data have, in the opinion of this review, been positive enough to warrant larger Phase I and Phase II clinical trials in order to more rigorously determine the potential therapeutic value of Vitamin D3 as a treatment for human epilepsy. Recently, our group has received an IND for a Phase I study of Vitamin D3 in DRE to study the safety, preliminary efficacy, and potential cardiac benefits of Vitamin D3 5,000 IU/day in DRE.

AUTHOR CONTRIBUTIONS

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Fish Oil Supplementation Reduces Heart Levels of Interleukin-6 in Rats with Chronic Inflammation due to Epilepsy

Mariana Bocca Nejm[1], André Abou Haidar[2], Aparecida Emiko Hirata[2], Lila Missae Oyama[2], Antonio-Carlos Guimarães de Almeida[3], Roberta Monterazzo Cysneiros[4], Esper Abrão Cavalheiro[1], Carla Alessandra Scorza[1] and Fulvio Alexandre Scorza[1]*

[1] *Disciplina de Neurociência, Escola Paulista de Medicina/Universidade Federal de São Paulo (EPM/UNIFESP), São Paulo, Brazil,* [2] *Departamento de Fisiologia, Escola Paulista de Medicina/Universidade Federal de São Paulo (EPM/UNIFESP), São Paulo, Brazil,* [3] *Departamento de Engenharia de Biossistemas, Universidade Federal de São João Del Rei (UFSJ), São João Del Rei, Brazil,* [4] *Programa de Pós-Graduação em Distúrbios do Desenvolvimento do Centro de Ciências Biológicas e da Saúde da Universidade Presbiteriana Mackenzie, São Paulo, Brazil*

Sudden unexpected death in epilepsy (SUDEP) is a major cause of premature death related to epilepsy. The causes of SUDEP remain unknown, but cardiac arrhythmias and asphyxia have been suggested as a major mechanism of this event. Inflammation has been implicated in the pathogenesis of both epilepsy and ventricular arrhythmia, with interleukin-6 (IL-6) being recognized as a crucial orchestrator of inflammatory states. Our group previously reported that levels of IL-6 were increased in the hearts of epileptic rats. In this scenario, anti-inflammatory actions are among the beneficial effects of fish oil dietary supplementation. This investigation revealed that elevated levels of IL-6 in the heart were markedly reduced in epileptic rats that were treated in the long-term with fish oil, suggesting protective anti-inflammatory actions against dangerously high levels of IL-6. Based on these findings, our results suggest beneficial effects of long-term intake of fish oil in reducing the inflammation associated with chronic epilepsy.

Keywords: epilepsy, sudden unexpected death in epilepsy, inflammation, heart, interleukin-6, fish oil

INTRODUCTION

The mortality rate in people with epilepsy is substantially higher than that observed in the general population, which is a matter of concern among specialists (1). In this scenario, sudden unexpected death in epilepsy (SUDEP) is a leading cause of premature mortality directly related to epilepsy (2).

Sudden unexpected death in epilepsy is defined as "sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death in patients with epilepsy, with or without evidence for a seizure, and excludes documented *Status epilepticus* (SE), in which postmortem examination does not reveal a toxicological or anatomic cause for death" (3). The fatal event is most likely provoked by functional disturbance, and researchers have proposed cardiac arrhythmia as a potential cause of SUDEP (2). Recently, clinically relevant genetic variants in cardiac arrhythmia and epilepsy have been found in a considerable number of SUDEP cases (4). Inflammation has been implicated in the pathophysiological events of both epilepsy and life-threatening ventricular arrhythmia (5, 6), in which the central role of the interleukin-6 (IL-6) is well recognized.

Our group was the first to describe increased levels of IL-6 in the hearts of rats with chronic epilepsy (7). Omega-3 polyunsaturated fatty acids, which are abundant in fish oil, have been described as potent anti-inflammatory agents (8). Thus, in the search for complementary therapy for epilepsy, this study was conducted to examine the potential anti-inflammatory effects of fish oil supplementation on the levels of the pro-inflammatory cytokine IL-6 in the heart of rats with chronic epilepsy.

ANIMALS AND METHODS

Animals

Adult male Wistar rats (220–280 g) were housed under standard controlled conditions (7:00 a.m./7:00 p.m. light/dark cycle; 20–22°C; 45–55% humidity) with food and water *ad libitum*. All animal experiments were carried out in accordance with the National Institutes of Health guide for care and use of laboratory animals and approved by the Animal Care Committee of UNIFESP (CEUA 188439).

Induction of Epilepsy

Epilepsy was induced according to the procedure described previously (9). In brief, 30 min after methyl scopolamine injection (1 mg/kg, s.c.—Sigma, MO, USA), pilocarpine was administered (350 mg/kg, i.p.—Sigma, MO, USA). Animals developed SE. To terminate SE, diazepam (10 mg/kg—Cristalia, Compaz) was administered subcutaneously 3 h after SE onset. Rats evolved through the latent period to the chronic phase of the pilocarpine model. To confirm the presence of epilepsy, animals were continuously video monitored for 60 days, and spontaneous recurrent seizures observed. At the end, nine rats with epilepsy ($n = 9$) were used in the experiments.

Fish Oil Treatment

Animals were randomly divided into the following groups: (1) animals treated daily with vehicle (0.009% cremophor) (control vehicle); (2) animals treated daily with 85 mg/kg fish oil (control fish oil); (3) animals with epilepsy treated daily with vehicle (epilepsy vehicle); and (4) animals with epilepsy treated with 85 mg/kg fish oil (epilepsy fish oil). During the 90-day treatment, animals received vehicle (cremophor 0.009%) or fish oil (PROEPA®, 85 mg/kg). A single daily dose was given *via* oral gavage between 11:00 and 12:00 a.m. Volume was adjusted according to animal weight, which was verified three times a week during the 90-day treatment period. Each fish oil capsule contained eicosapentaenoic acid (EPA), 180 mg, and docosahexaenoic acid (DHA), 120 mg. Capsule contents were dissolved in 0.009% cremophor, yielding a final concentration of 21.25 mg/ml fish oil, corresponding to 3.82 mg/ml EPA and 2.55 mg/ml DHA, and the final composition of fish oil was administered as 1 ml/250 g of body weight. Animals were killed by decapitation. Hearts were removed, and ventricles were separated and split in half (for Western blot and ELISA procedures) and then stored at −80°C until use.

Measurements of IL-6 Protein Levels

One half of the ventricle was used for the Western blotting procedure, and the other half was used for the ELISA assay. For Western blot analysis, samples were prepared as previously described by Nejm et al. (10). The primary antibodies used were anti-IL-6 (Millipore-Chemicon, USA) and anti-β-actin (Sigma Aldrich, USA). ELISA assays (DuoSet ELISA, R&D Systems, Minneapolis, MN, USA) were performed following the recommendations of the manufacturer. All samples were run in duplicate, and the mean value was reported.
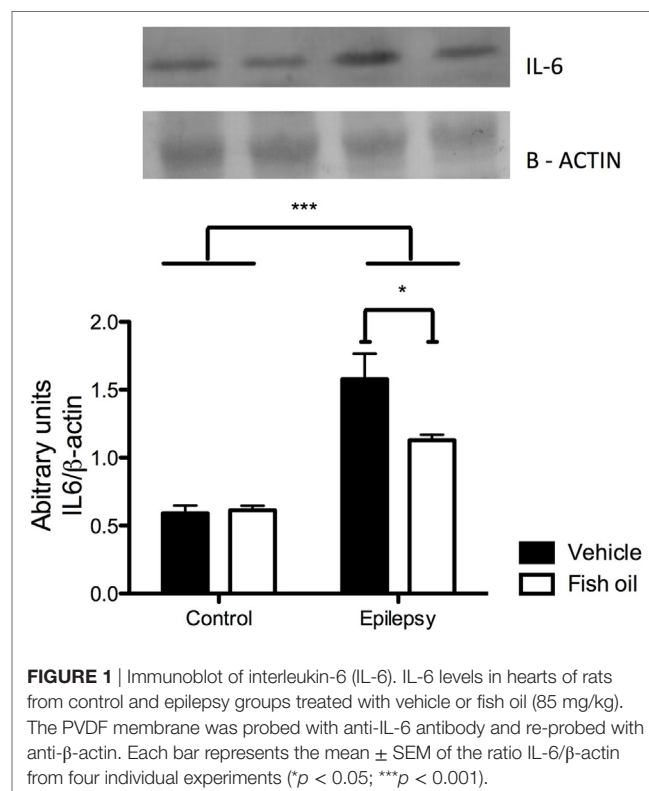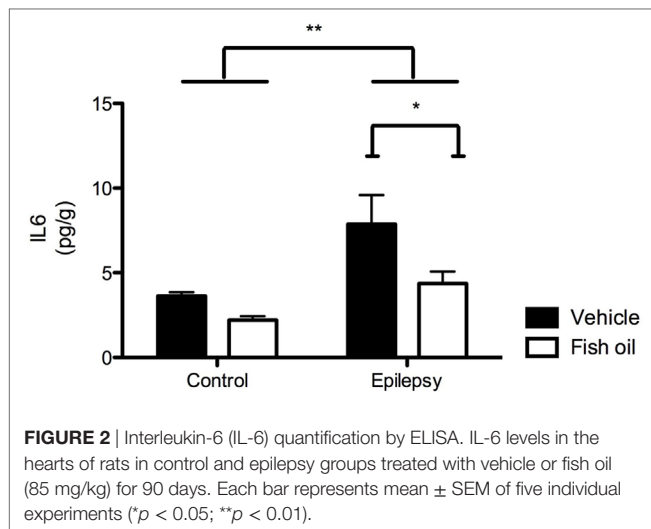
Statistical Analysis

Data were expressed as the mean ± standard error. Statistical analysis was performed using two-way ANOVA [grouped as control versus experimental and by treatment (vehicle versus fish oil)] followed by Bonferroni posttest. *p* Values of 0.05 or less were considered significant.

RESULTS

The results of Western blot analysis showed that IL-6 levels in the heart of rats with epilepsy were substantially increased when compared with control rats [$F(1;12) = 54.78$; $p < 0.0001$]. Animals with epilepsy that were treated with fish oil for an extended time exhibited a marked reduction in IL-6 levels, since an interaction effect was observed between epilepsy and fish oil treatment [$F(1;12) = 5.34$; $p = 0.039$], as shown in **Figure 1**.

In **Figure 2**, the ELISA results corroborated the results obtained with immunoblotting analysis as increased levels of IL-6 were found in the hearts of animals with epilepsy compared with those in control rats [$F(1;16) = 10.26$; $p = 0.0059$]. Furthermore,

FIGURE 1 | Immunoblot of interleukin-6 (IL-6). IL-6 levels in hearts of rats from control and epilepsy groups treated with vehicle or fish oil (85 mg/kg). The PVDF membrane was probed with anti-IL-6 antibody and re-probed with anti-β-actin. Each bar represents the mean ± SEM of the ratio IL-6/β-actin from four individual experiments (*$p < 0.05$; ***$p < 0.001$).

FIGURE 2 | Interleukin-6 (IL-6) quantification by ELISA. IL-6 levels in the hearts of rats in control and epilepsy groups treated with vehicle or fish oil (85 mg/kg) for 90 days. Each bar represents mean ± SEM of five individual experiments (*$p < 0.05$; **$p < 0.01$).

the ELISA results show the effectiveness of the fish oil treatment, which reduced IL-6 levels in both control and epileptic rats [$F(1;16) = 6.05$; $p = 0.0264$]. There was no interaction effect between epilepsy and fish oil treatment [$F(1;16) = 1.06$; $p = 0.3178$].

DISCUSSION

In this rodent pilocarpine model of seizures, fish oil supplementation reduced cardiac levels of IL-6, indicating an anti-inflammatory effect of fish oil.

Consistent preclinical and clinical findings support the presence of a persistent inflammatory condition in the brain and plasma of subjects with epilepsy (4, 6, 11). Epileptic seizures often affect heart rate and rhythm, and poor seizure control is by far the major clinical risk factor for SUDEP (2). However, despite the available therapeutic arsenal, 30–40% of individuals with epilepsy have uncontrolled seizures. Studies focused on the pathological basis of epilepsy have focused on the role of inflammatory events (6). Basic and clinical studies has been described that chronic inflammatory state contribute to the pathogenesis of seizures and maintance of epilepsy (6, 11, 12). Previously, we demonstrated chronically elevated levels of the pro-inflammatory cytokine IL-6 in the ventricles of rats with seizures (7). Here, our findings support the existence of persistent inflammation in the ventricles of rats with epilepsy, which exhibited elevated levels of IL-6. Elevated circulating levels of IL-6 have been described in patients with heart failure, and these higher levels are positively correlated with the presence and duration of cardiac arrhythmias and with the severity of disease and mortality risk (5, 13). Taken

together, there is compelling evidence that increased levels of IL-6 are deeply involved in the pathophysiological mechanisms underlying both epilepsy and cardiac diseases.

Using the pilocarpine model of epilepsy, Ferrari and colleagues in our group demonstrated that chronic treatment with fish oil promoted neuroprotection and positive plastic changes in the brain of rats with epilepsy (14). The beneficial anti-inflammatory effects of omega-3 fatty acids in chronic inflammatory diseases have been consistently documented (8). Fish oil dietary consumption is suggested as a good source of omega-3 polyunsaturated fatty acids for consumers. This study suggests that chronic oil fish supplementation has a protective anti-inflammatory effect against elevated levels of IL-6 in the heart of rats with epilepsy, but the underlying mechanisms of these effects remain unknown.

CONCLUSION

Our findings report the beneficial anti-inflammatory effects of long-term oil fish intake in the hearts of rats with chronic inflammation associated with epilepsy. Our results suggest the potential therapeutic value of dietary supplementation with oil fish in patients with epilepsy.

ETHICS STATEMENT

All animals were treated according to protocols for animal care, and this study was carried out in accordance with the recommendations established by the Federal University of Sao Paulo. All efforts were made to minimize animal suffering (Comissão de Ética no Uso de Animais—CEUA).

AUTHOR CONTRIBUTIONS

MN: conducted the experiments, acquired data, analyzed data, critically discussed the manuscript, and wrote the manuscript. AAH: conducted the experiments, acquired data, analyzed data, and critically discussed the manuscript. AEH, LO, A-CA, and RC: analyzed data and critically discussed the manuscript. EC: conceived the work and wrote the manuscript. CS: critically discussed the manuscript and wrote the manuscript. FS: conceived the work, critically discussed the manuscript, and wrote the manuscript.

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