

DR MARIA THOM (Orcid ID : 0000-0001-7712-2629)

Article type : Annual Review Issue - Review

**The past, present and future challenges in epilepsy related and sudden deaths and biobanking.**

Maria Thom<sup>1</sup>, Maura Boldrini<sup>2</sup>, Elizabeth Bundock<sup>3</sup>, Mary N. Sheppard<sup>4</sup>, Orrin Devinsky<sup>5</sup>

<sup>1</sup>Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London WC1N, 3BG,

<sup>2</sup>Department of Psychiatry, Columbia University Medical Centre, Divisions of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New York, NY, 10032 USA

<sup>3</sup>Office of the Chief Medical Examiner, Burlington, VT 05401, USA,

<sup>4</sup> Department of Pathology, St George's University Hospitals NHS Foundation Trust, Blackshaw Road, London SW17 0QT

<sup>5</sup> Department of Neurology, NYU Epilepsy Center, 223 E 34th Street, NY, NY 10021.

Corresponding Author:

Maria Thom  
Dept of Neuropathology  
UCL Institute of Neurology  
London WC1N 3BG  
M.Thom@ucl.ac.uk  
Tel : 020 3448 4233

Key words: SUDEP, Epilepsy-related death, post-mortem, biobanking

Short Running Title: Post-mortem practices in SUDEP

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nan.12453

This article is protected by copyright. All rights reserved.

## Abstract

Awareness and research on epilepsy-related deaths (ERD), in particular Sudden Unexpected Death in Epilepsy (SUDEP), have exponentially increased over the last two decades. Most publications have focused on guidelines that inform clinicians dealing with these deaths, educating patients, potential risk factors and mechanisms. There is a relative paucity of information available for pathologists who conduct these autopsies regarding appropriate post-mortem practice and investigations. As we move from recognizing SUDEP as the most common form of ERD toward in-depth investigations into its causes and prevention, health professionals involved with these autopsies and post-mortem procedure must remain fully informed. Systematizing a more comprehensive and consistent practice of examining these cases will facilitate 1) more precise determination of cause of death, 2) identification of SUDEP for improved epidemiological surveillance (the first step for an intervention study), and 3) bio-banking and cell-based research. This article reviews how pathologists and healthcare professionals have approached ERD, current practices, logistical problems and areas to improve and harmonize. The main neuropathology, cardiac and genetic findings in SUDEP are outlined, providing a framework for best practices, integration of clinical, pathologic and molecular genetic investigations in SUDEP, and ultimately prevention.

## List of abbreviations

ERD, Epilepsy-Related Death; FCD, Focal Cortical Dysplasia; GTCS, Generalised tonic-clonic seizure; HS, Hippocampal Sclerosis; MCD, Mild malformation of cortical development; SCD, Sudden Cardiac Death; SUDEP, Sudden Unexpected Death in Epilepsy; TLE, temporal lobe epilepsy.

## Introduction

Pathologists have long recognised that patients with epilepsy can die suddenly and unexpectedly ('mors subita') and that following post-mortem we can be 'none the wiser' as to the cause of death [1]. Indeed, in Sommer's 1880 seminal post-mortem study of the hippocampus in epilepsy, several patients had died suddenly, in some cases associated with a seizure [2]. In the 1980s, Leestma commented that although forensic pathologists were well acquainted with seizure-associated sudden death, clinical practitioners seemed less aware [3]. Sudden death is now widely recognised

by neurologists as a global healthcare concern [4] affecting all age groups with epilepsy [5]. Recent estimates suggest the incidence of sudden unexpected death in epilepsy (SUDEP) is around 1 to 1.2 per 1000 per year in people with epilepsy [6, 7] and there is no indication of a decline. Indeed, Terence *et al* commented in 1981, that although SUDEP was recognized since 1910 despite all the medical advances in treating epilepsy through the last century, as best they could determine the frequency had remained unchanged [8].

Although still a relatively rare event (representing around 0.095% of all deaths), SUDEP accounts for approximately 500 of all 1,200 epilepsy related deaths (ERD) per year in the UK [9, 10], often affecting young and often otherwise healthy adults. A recent study in San Francisco County found that more than half of SUDEP cases may go unrecognized even after retrieval of medical records, thorough autopsy and toxicological evaluation, and review by an expert panel of forensic pathologists as well as a cardiologist and neurologist [11]. The exact mechanisms of sudden death remain unproven in human studies, with hypotheses and theories mainly derived from animal experimental studies and anecdotal clinical evidence. SUDEP can occur in many epilepsy syndromes, with different seizure types, underlying neuropathology and genetic conditions. It primarily associates with generalised tonic-clonic seizures (GTCS) and most often occurs in bed during sleep hours with the person found in a prone position (reviewed in [5]). When witnessed and recorded in an epilepsy monitoring unit, SUDEP typically followed a GTCS when the obtunded patient experiences depressed arousal and respiration, non-tachyarrhythmic cardiac dysfunction, and profound depression of cortical EEG activity (postictal generalized EEG suppression, PGES) [12]. For such witnessed SUDEP cases an arbitrary cut-off of death within 1 h from acute collapse was proposed [13].

Epilepsy can cause death or contribute to the circumstances of death in numerous ways. Deaths due to status epilepticus, complications following seizure such as aspiration pneumonia, injury or drowning sustained during a seizure, complications of treatment, or suicide [14, 15] are classified for epidemiologic and research purposes as epilepsy-related deaths (ERD). SUDEP is defined as the sudden, unexpected death, witnessed or unwitnessed, of a person with epilepsy, where the above ERD are excluded and complete post-mortem does not reveal another toxicological or anatomic cause of death [13]. Sudden deaths can also occur with symptomatic seizures in patients without an epilepsy diagnosis, for example in alcohol withdrawal [11] (Table 1). Because the diagnosis of SUDEP is made by exclusion of other causes of death this therefore requires a clinical history of epilepsy,

witness statements and details of the scene and circumstances of death, and complete post-mortem examination including toxicology.

### **Past and current post-mortem practice in epilepsy related deaths**

In suspected ERD and SUDEP cases, a 'complete' post-mortem examination is generally considered to be external and full internal examination, including examination of the whole brain by a neuropathologist or personnel with neuropathology training, and toxicology, including anticonvulsant levels [16, 17]. National audits of ERD highlighted inadequacy of post-mortem investigations in 87% in the UK in 2002 [17] and 41% in a Melbourne study in 2016 [16]. Insufficient or absent neuropathological investigations were common and in some regions access to neuropathology services is limited.

Recognizing that in some cases autopsies were either not done at all or incomplete (e.g., head only) or the cause of death was inconclusive, categories of **definite, probable and possible SUDEP** were devised [18] and subsequently revised [13] to facilitate classification of deaths for purposes of epidemiology and research. In '**probable SUDEP**' cases, circumstances are most consistent with SUDEP but post-mortem examinations are lacking or incomplete. In '**possible SUDEP**' cases, a potential competing cause of death (other than epilepsy) is identified, meaning that epilepsy and another unrelated cause are both plausible. Cardiac, pulmonary diseases and drug intoxication are the most common competing causes in the 'possible SUDEP' deaths as well as death in water without evidence of submersion or drowning [13]. In instances where the cause of death is considered to be the combined effect of epilepsy plus a concomitant condition, the term '**definite SUDEP plus**' has been proposed [13] (Table 1).

### **Medicolegal aspects and death certification**

International and regional autopsy practice in ERD vary and may be conducted by general pathologists or forensic pathologists, typically under jurisdiction of a Coroner or Medical Examiner. Determining cause of death in ERD cases is a challenge for the Medical Examiner or Coroner since they are often confronted with incomplete data (e.g. unwitnessed death, insufficient medical history, decomposed body), competing causes of death, or compelling circumstances without confirmatory anatomic or toxicological findings.

The cause of death is the aetiologically specific disease or injury that initiated the sequence of physiologic events leading directly to death. Examples of aetiologically specific causes of death include idiopathic epilepsy, atherosclerotic cardiovascular disease, drowning, and blunt force trauma. Mechanisms of death, the intervening steps that link the cause to the death, are the altered physiology or biochemistry through which the cause exerts its lethal effect and are not aetiologically specific (i.e. they can be due to many underlying aetiologies). Because the physiology of organ systems is complex and intertwined, there is never just one mechanism. Pulmonary oedema, cerebral hypoxia-ischaemia, arrhythmia and seizure are mechanisms of death. Each of these mechanisms may be happening during the death of a person with epilepsy. Pulmonary oedema is a finding that should be documented when present in ERD but is non-specific and it is recommended that this is not used in isolation as the cause of death in a patient with epilepsy.

Because there is often little evidence at post-mortem examination to determine when a seizure has occurred, it is often impossible to be certain that epilepsy was the cause of death in an unwitnessed case. When a person with epilepsy dies suddenly and is found to have another potentially lethal disease (e.g. Coronary artery disease), the Medical Examiner must weigh which aetiology was the more likely cause of death using details from the investigation and post-mortem examination.

Pulmonary oedema and tongue bites, particularly lateral tongue bites, are considered suggestive but not pathognomonic for seizure since they may be due to other aetiologies [19, 20]. Even a description of terminal seizure-like activity is not specific since persons dying of other diseases may have such activity without established epilepsy. Therefore, medical history and circumstances of death are critical to the final evaluation in any case. Absolute certainty of the cause is not needed for competent death certification. Once the Medical Examiner or Coroner has explored and excluded potential causes unrelated to epilepsy, through detailed investigation and complete post-mortem examination, the sudden death of a person with epilepsy is most likely caused by epilepsy.

The choice of wording for the death certificate varies widely between jurisdictions and between individuals within jurisdictions. Inconsistencies and lack of standardisation of wording remain widespread [6, 21] limiting our ability to track SUDEP rates over time. Despite the ubiquitous reference to SUDEP as a cause of death in the literature, it is an acronym that describes the circumstances of death; sudden, in a person with epilepsy, not due to an unnatural cause (e.g. not trauma or drowning), not the result of status epilepticus, and in the setting of a negative post-mortem examination (i.e. no obvious toxicological or other anatomic cause identified) (Nashef, 1997). Some Medical Examiners and Coroners are resistant to using SUDEP as a cause of death statement and favour use of the specific aetiology (epilepsy) instead. Unfortunately, the sudden

nature of the death is therefore often omitted from the cause statement, making identification and ascertainment of SUDEP cases for epidemiology and research difficult. Nevertheless although rarely used in previous decades [22], 'SUDEP' is now more commonly recognized as an entity and listed on death certificates [23] and accepted as a valid diagnosis [11, 22].

### **Neuropathology in SUDEP: Why it is needed**

The primary objectives of the neuropathological examination in an ERD are to (i) exclude any unexpected cause of death, (ii) detect any underlying lesion causing the epilepsy and (iii) explore any neuropathological consequences of chronic or recent seizure activity. No neuropathological feature can establish a seizure as the mechanism of death. Furthermore, epilepsy is a clinical diagnosis supported by EEG and there are no specific neuropathological alterations that can categorically confirm epilepsy without confirmatory investigations undertaken during life. Neuropathological lesions that cause sudden death are often dramatic, macroscopically straightforward diagnoses; these include acute traumatic brain injury or spontaneous intracerebral haemorrhages [24]. More focal or subtle neuropathologies, such as fatal colloid cysts [25] or acute encephalitis [26, 27], require a systematic, careful approach to brain examination with confirmatory immunohistochemistry or molecular studies, for example for viral markers, if necessary.

Among lesions that cause epilepsy, published series do not suggest that any single type is over-represented in SUDEP (Table 2, Figure 2). The range of pathologies encompass those commonly encountered in surgical epilepsy series, including hippocampal sclerosis, cortical malformations such as focal cortical dysplasia (FCD) and low grade tumours [28]. Focal neuropathological abnormalities occur in ~50% of SUDEP autopsies ranging from 34 to 89% between larger SUDEP series (Table 2). Leestma, identified brain lesions in 60% of SUDEP cases compared to 11% in non-epilepsy autopsies [3], but not all SUDEP studies support this [23, 29] and there are few post-mortem non-SUDEP epilepsy studies for comparison (Figure 2). The variability in lesion detection may reflect the population demographics of the study as well as methodological differences between studies (e.g., macroscopically visible versus additional histological pathologies being reported). Identification of brain pathology pertaining to epilepsy is highly dependent on (i) the brain being examined in detail following a period of fixation [3, 30, 31], (ii) the number of histological regional samples taken [31], (iii) the level of scrutiny, for example if routine immunohistochemistry was carried out, as recommended in epilepsy surgical diagnostic protocols, [32], and (iv) the experience and training of

the pathologist. In the forensic setting, the depth and detail of neuropathological investigation may be negatively affected by limited resources, including cost and access to special histologic techniques, neuropathology services and reporting time restrictions. Indeed, many epilepsy related pathologies are focal and visible only microscopically, some requiring specific immunohistochemistry to confirm diagnosis. Examples include milder or atypical forms of hippocampal sclerosis (ILAE type 3), granule cell dispersion [33] and hippocampal mossy fibre sprouting, considered to be a relatively specific secondary process in epilepsy (reviewed in [34]) (Figure 1c,d). Mild malformations of cortical development (MCD), in particular mild MCD type II (heterotopic neurones in the white matter), have been reported in SUDEP [35] but are recognised to require quantitative immunohistochemistry confirmation with comparison to validated control ranges [36]. Currently we lack international agreement on precise diagnostic criteria for Mild MCD type II in surgical TLE or other brain regions [37] and because of this its recognition and reporting in ERD may widely vary. Even well-defined epileptogenic pathologies likely go undetected at post-mortem examination due to under-sampling. For example, symptomatic FCD are not infrequently MRI 'occult' even with modern neuroimaging [38] (Figure 1e,f); such lesions are typically macroscopically less visible with absent hallmark features, as regions of cortical thickening or blurring of the grey-white matter interface [37]. Access to clinical notes and any localising investigations (e.g. EEG, seizure semiology, MRI, PET findings) is good practice and may help to focus the pathologist on the epileptogenic zone and anecdotally can aid in the identification of underlying pathology, although data is lacking to prove this increases overall lesion detection rate. Given these methodological approaches that may influence the sensitivity of the post-mortem, there is lack of clarity from the available neuropathological series as to whether structural brain lesions are more frequent in SUDEP than epilepsy without SUDEP and therefore if symptomatic epilepsy compared to idiopathic/genetic causes represents a higher risk.

### **Regional Neuropathology - insights into mechanisms in SUDEP**

Repetitive seizures can initiate molecular signals and cellular processes that ultimately alter the brain structure in terms of neuronal loss, gliosis [39], microgliosis and inflammation [40], blood brain barrier breakdown [41], vascular changes and axonal reorganisation [42, 43]. These can occur in the area of seizure onset in focal epilepsies but widespread brain alterations in the cortex [44], thalamus and cerebellum can be observed at post-mortem [45]. Many of these sequelae represent a 'pathological' repair process that can further alter the seizure threshold, as exemplified by astrogliosis [39]. One key question is whether specific or strategic patterns of epilepsy-related brain

injury occur in SUDEP which could increase vulnerability or mechanistically be relevant to the cause of death. Central autonomic networks and cardiorespiratory centres are obvious candidate areas to investigate [12, 46]. These include the insular cortex, prefrontal cortex, hippocampus and amygdala through their connections to the hypothalamus, pons and medulla [47]. Ictal autonomic phenomena, as urinary urge, piloerection, and heart rate changes arise due to excitation or inhibition of these brain regions and are particularly associated with limbic or TLE [48]. Neuropathology studies have focused on chronic structural alteration as well as acute pathology. Inherent caveats to SUDEP research include imprecise times of death as most are unwitnessed, the unanticipated nature of death that incurs logistical delays in brain removal, and lack of suitable controls groups such as non-epilepsy sudden deaths and non-SUDEP epilepsy cases of similar age and severity of epilepsy.

**Brainstem:** Central control of respiration is regulated by interconnected medullary nuclear groups forming the ventral respiratory column [46] including the Bötzing and pre-Bötzing complex [49] and modulated by other brainstem networks including the medullary raphe [50]. Notably, in experimental models of SUDEP, cortical seizures initiated spreading depolarisations or ictal activity in these brainstem regions which resulted in cardiorespiratory arrest [51, 52]. In neurodegenerative conditions with accompanying central respiratory deficits, selective reduction of specific respiratory (somatostatin/neurokinin 1Receptor<sup>+</sup>) nuclear groups occurs [53], and in Sudden Infant Death Syndrome (SIDS), alterations to the medullary serotonergic neurones is observed [54]. In MRI studies, medullary volume loss, proposed to be secondary to sustained seizure propagation, is of potential relevance to ictal autonomic disturbances in SUDEP [55] but there are limited neuropathology studies investigating specific cellular alterations [56, 57].

**Amygdala:** In TLE amygdala neuronal loss or sclerosis is variably present [58, 59]. Seizure spread or stimulation of the basolateral and lateral nucleus amygdala results in transient apnoea [60, 61], the central nucleus of the amygdala being connected to brainstem cardio-respiratory and other autonomic nuclei; therefore, underlying amygdala pathology could be mechanistically relevant in SUDEP. A stereological study of the lateral nucleus of the amygdala confirmed significantly reduced neuronal density and higher astrocytic densities in SUDEP compared to non-epilepsy controls (Figure 1i,j) [62]; this was more prominent in the left side and independent of hippocampal sclerosis but no significant alteration in the central nucleus was found. MRI studies in SUDEP show higher amygdala volumes on the right side compared to control [63]. A more recent pathology study, however, failed to confirm significant differences in density of GFAP<sup>+</sup> astroglia in the lateral, basal and accessory basal nucleus of the amygdala in SUDEP compared to controls [56].



**Hippocampus:** There is no evidence that hippocampal sclerosis is more prevalent in SUDEP. HS is reported in up to 21% of 145 SUDEP cases [31] (Table 2, Figure 2), compared to 30.5% of 650 [64] and 45% of 235 epilepsy post mortems [65]. Some unexplained deaths in infancy and childhood may be seizure-related and unexpected deaths in young patients with febrile seizures may share similar pathogenetic mechanisms to SUDEP [66, 67]. Developmental lesions of the dentate gyrus of the hippocampus have been reported in SIDS including varying degrees of bi-lamination of the dentate gyrus, noted in 41% of 153 cases [68]. Similarly, hippocampal abnormalities, including excessive convolutions of the dentate gyrus, granule cell bilamination, folding of the subiculum, and hippocampal malrotation were associated with sudden death in children (>1 year old) with a history of febrile seizures [69, 70]. Hippocampal malrotation is a developmental anatomical variant, identified in 23% of the general population on MRI in one series and more commonly left sided [71]. Malrotation of the hippocampus was found on MRI in 8.8% of patients with febrile status in the FEBSTAT study [72] with predilection for the left side, compared to 2.1% in their control groups [73]. Hippocampal malrotation may represent a substrate to febrile seizure and epilepsy [74] and granule cell dispersion is postulated as a morphological biomarker in SIDS that could indicate impaired connectivity between the hippocampus and brainstem [68] or a manifestation of previous unrecognised ictal activity [75]. Granule cell dispersion, a common and often prominent feature in approximately 40% of adult TLE patients with hippocampal sclerosis [34], was reported in only 4% of 145 SUDEP cases without sclerosis [31]. However, as no study has systematically evaluated SUDEP cases comparable to SIDS [68-70] or surgical studies [76], this feature may be under-recognised. Similarly, hippocampal malrotation of varying degrees occur in 9.7% of SUDEP cases independently of sclerosis, which was more common on the left side [31]. In a quantitative MRI study, increased hippocampal volumes in SUDEP were noted on the right side [63]. More systematic analysis of hippocampal pathology and any lateralisation is warranted in SUDEP to explore structural/developmental alterations predisposing to SUDEP as a surrogate biomarker.

**Thalamus:** A reduction of grey matter volume in the posterior thalamus was noted in SUDEP on quantitative MRI which is of potential significance as this region has roles in breathing inhibition following episodes of hypoxia [63]. Involvement of the posterior thalamus in seizure initiation and cardiovascular modulation [77] and altered connectivity between thalamus, brainstem and frontal cortex may be relevant in some patients at high risk for SUDEP [78]. In post-mortem studies, the severity of thalamic pathology, based on reduced synaptophysin immunolabelling and gliosis, correlates with duration of seizures [79] but regional patterns of thalamic vulnerability in SUDEP, including any predilection for pulvinar or posterior lobe, remains to be addressed.

**Cerebellum:** Macroscopic atrophy or selective Purkinje cell loss were reported in 29% and 12% of SUDEP subjects respectively [31] but in up to 57% of other SUDEP series [80]. By comparison *In vivo* MRI findings showed cerebellar atrophy in 22% to 36% of non-SUDEP epilepsy groups on MRI measurements [81]. The fastigial nucleus of the cerebellum in functional imaging studies has roles in normal autonomic regulation of baroreflexes [82]. Cerebellar damage has been implicated in SUDEP [83] but requires more rigorous pathology and molecular assessment in order to identify its role in the pathogenesis of SUDEP.

**Cerebral cortex :** Left insular cortex damage has been reported in SUDEP and associated with autonomic instability [84] and is a candidate region for further study[85], but the mechanisms by which these anatomical abnormalities relate to sudden death are unclear. Apart from documentation of acute neuronal changes (see below) in the insula, there are no neuropathological correlative studies.

#### **SUDEP – evidence for acute brain injury**

SUDEP may be the consequence of extensive, irreversible neuronal damage during a seizure, strategic injury in critical autonomic regions or the cumulative effects of recent poor seizure control [86]. Such cellular injury or neurochemical exhaustion could decompensate normal homeostatic, auto-resuscitative mechanisms following a fatal seizure. Acute eosinophilic neuronal change has been reported in up to half of SUDEP cases [31], although with notable differences between series (Table 2) which may reflect to its varying extent and hence detection (Figure 2). It primarily involves the hippocampus (CA1/subiculum) although widespread involvement including cortical laminar patterns can occur [35]. This non-specific cytopathic change likely reflects hypoxic and/or excitotoxic cellular stresses occurring acutely (rather than immediately) in the last six hours. Hypoxia in epilepsy reflects ictal hypoperfusion [87], impaired central respiration/transient apnoea, external airway compromise during seizure or a synergistic interaction of these. Acute neuronal injury in SDUEP was significantly more frequent when seizure episodes occurred in the 24 h prior to death, the body was found in a prone position, external airways were obstructed, or brain swelling was present [31]. In sudden unexplained death in childhood, hyper-eosinophilic acute ischaemic neurones were identified at similar rates to controls groups with known cause of death [69].

Investigations in SUDEP of markers of acute neuronal death (necrosis, apoptosis) or reversible injury, including cell-stress signals promoting neuronal survival (VEGF, JNK1-3 , BDNF) [88], enable

more objective data regarding the type, severity and distribution of brain injury, and could aid in localising the epileptogenic zone and explore secondary effects of ictal discharges involving central autonomic networks [48]. Heat Shock Protein (HSP-70)-positive neurones in all hippocampal subfields were more frequent in SUDEP compared to non-epilepsy and other epilepsy sudden deaths; similar differences were not noted for c-JUN neuronal expression [57]. Labelling of insular cortex and brainstem nuclei with HSP-70 and c-JUN was also observed [57]. Hippocampal neuronal hypoxia-inducible factor 1-alpha (HIF1- $\alpha$ ) and vascular endothelial growth factor (VEGF) expression, both upregulated in response to hypoxia, occur in SUDEP although did not significantly differ from control groups [89]. A more recent study identified fewer HIF1- $\alpha$  neurones in the parahippocampal gyrus in SUDEP compared to non-epilepsy sudden death controls but no differences in the hippocampus, amygdala or brainstem, where neurones in the pre-Böttinger complex were labelled strongly [56]. Further investigations correlating evidence of neuronal injury with clinical and genetic variables and circumstantial evidence of death will be important to inform on mechanisms in SUDEP.

Seizure induced neuroinflammation [40] and acute blood brain barrier dysfunction [41] pose obvious candidate mechanisms in SUDEP. Increased, but statistically non-significant, brain weights and brain swelling, likely due to acute cerebral oedema, occur in SUDEP (Table 2) [31]. However, studies as yet do not indicate significant neuroinflammation in SUDEP; less lymphocytic inflammation was noted than controls [80] and quantitative analysis of HLA-DR activated microglia in the hippocampus, amygdala and medulla did not differ between SUDEP, and non-epilepsy sudden deaths [56] (Figure 1g, h). Albumin and IgG, tissue markers of acute blood brain barrier breakdown, showed less labelling in SUDEP in the parahippocampal gyrus, and fewer CD163-positive peripheral macrophages (indicative of increased vascular leakage) were found in the ventrolateral medulla [56]. In summary is unclear if neuroinflammation, or its cumulative effects in the presence of repeated seizures, is related to the pathogenesis of SUDEP.

### **Cardiopulmonary pathology and investigations in SUDEP**

Pulmonary pathology is a common autopsy finding in SUDEP (Table 2) reported in 72% of cases, with the most frequent findings being pulmonary congestion or oedema [90]. Although the incidence of post-ictal pulmonary oedema has been debated [91], radiographic evidence was reported in 29% of patients following GTCS [92]. The pathogenic mechanisms of oedema following GTCS are not understood, and include increased sympathetic drive, mediated by the medulla and hypothalamus, altered hydrostatic pressures and increased pulmonary capillary permeability ('neurogenic

pulmonary oedema') [91]. In SUDEP, additional exacerbating factors include central respiratory inhibition and cardiogenic and haemodynamic dysfunction.

Cardiac examination in ERD and SUDEP is essential to exclude cardiac causes of death such as undiagnosed myocardial disease, valve disease, coronary artery disease or structural abnormalities of the conduction system. When the clinical diagnosis of epilepsy is less certain and the cause of death is considered likely cardiac, but no lesion is identified, sampling for histology is required with mapped blocks of anterior, lateral and posterior right and left ventricle and septum from a representative mid-ventricular transverse slice and right ventricular outflow tract. In addition blocks from conduction tissue is taken if history of conduction defect is recorded clinically. In some situations, fixation of the heart and referral to a specialist cardiac pathologist is recommended as best practice and the organ can be returned following examination. There should be a low threshold for sending the whole heart to a specialist centre for expert opinion, at the discretion of the pathologist and Coroner or Medical examiner and discussion with the family (for UK guidance see <https://www.rcpath.org/profession/clinical-effectiveness/clinical-guidelines/autopsy-guidelines.html>). Following status epilepticus or a convulsive seizure, stress-induced cardiac changes or *Takotsubo* cardiomyopathy can occur [90] (Figure 3). When mild to moderate cardiac pathology is found in a case with circumstances and clinical features suggestive of SUDEP, the classification should be 'definite SUDEP plus' if the cardiac condition could be a contributing cause, or 'possible SUDEP' if the cardiac condition is a competing cause of death. This nuanced distinction, often challenging to apply in specific cases, was introduced in the more recent SUDEP classification [13]. For example, if a 30-year-old man with treatment resistant nocturnal convulsions is found dead in bed in the morning in the prone position with a tongue laceration, and post-mortem shows a 40% occlusion of the left anterior coronary artery or mild cardiomegaly, the cause of death is likely epilepsy, although the cardiac pathology may have contributed (definite SUDEP Plus). By contrast, a 55-year-old woman with infrequent seizures who dies alone in her flat during the daytime with no evidence of a seizure and who is found to have moderate cardiomegaly with left ventricular hypertrophy could have died from the cardiac disease or epilepsy (possible SUDEP).

Subtle cardiac pathologies reported in SUDEP series include myocyte hypertrophy and mild interstitial fibrosis (Figure 3) (Table 2 and reviewed in [90]). It remains unclear whether these changes represent a sequel of seizures or antiepileptic drugs, or may be similar to baseline rates of cardiac pathology in other non-cardiac sudden deaths; their relevance to the mechanism of SUDEP is unknown. Nevertheless, this again suggests that routine systematic histological examination of the myocardium, even in what appear to be typical SUDEP cases [93], is warranted in to monitor the

significance of these unexpected findings. Ictal disturbance in cardiac electrophysiology that induces dysrhythmias has long been considered a candidate mechanism of death in SUDEP [94]. Further, several ion channels have shared expression in brain and heart and cardiac genes associated with long QT syndrome (LQT), bradycardia, and SCD (*KCNQ1*, *KCNH2*, *SCN5A*, *RYR2*, *HCN4*) can cause both epilepsy and arrhythmias or increase the risk of seizure-induced arrhythmias and have been linked to SUDEP (reviewed in [5] and see section below) (Table 3).

### **Genetics of SUDEP and the molecular autopsy**

In cases where the scene investigation, autopsy and toxicological studies do not reveal a probable or definite cause of death, post-mortem genetic testing can reveal a variant or mutation that could cause sudden death. These 'molecular autopsies' emerged from studies in which sudden cardiac death was suspected but only confirmed when post-mortem DNA analysis revealed genetic findings that established the likely mechanism of death. De novo or known pathogenic mutations in cardiac channelopathy or cardiomyopathy genes were found in 27% of children and young adults with unexplained sudden cardiac death [95]. While molecular autopsy is well established in evaluating cases of suspected sudden cardiac death, its role in defining the mechanism of death in epilepsy patients remains less well defined. Unlike sudden cardiac death, where pathogenic variants in genes that modulate the cardiac rhythm and myocardium are associated with sudden death and have a well-characterized lethal mechanism, defining a SUDEP gene is more challenging. In the largest study to date, 61 SUDEP cases underwent post-mortem whole exome sequencing. The investigators identified mutations in cardiac arrhythmia (all long QT) genes (7%), candidate pathogenic variants in dominant cardiac arrhythmia genes (15%) and mutations or candidate pathogenic variants in dominant epilepsy genes in 25% [96]. The unanswered question is the potential pathogenic relationship and role of these mutations in SUDEP.

Some genetic mutations are associated with an increased risk of SUDEP in humans and in some animal models of SUDEP. However, most of these mutations are established causes of epilepsy that are often treatment-resistant and associated with frequent GTCS (e.g., *SCN1A*, *SCN8A*, *DEPDC5*, dup15q; Table 3). The epileptic encephalopathies are associated with high rates of sudden death and status epilepticus [97]. In Dravet syndrome [98], the mortality rate/1000-person-years was 15.8 and SUDEP rate was 9.3/1000-person-years. These rates are more than 10-fold higher than other paediatric epilepsies but similar to cohorts of adults with refractory epilepsy being evaluated for surgery (6-9/1000-person-years) [99].

The association of these genes with SUDEP could result solely from their role in causing severe epilepsy with frequent convulsive seizures and status epilepticus (e.g., *SCN1A*, *SCN8A*, dup15q) or epilepsy of variable severity (e.g., *DEPDC5*). However, specific genetic mutations may also independently contribute to the mechanism of death by facilitating autonomic instability, cardiac or respiratory dysfunction, or more prolonged postictal depression of arousal following a seizure or interfering with post-ictal autonomic recovery. Experimental models of Dravet syndrome reveal parasympathetic hyperactivity accompanies SUDEP and death can be prevented by parasympathetic blockade [100]. The relevance of this to human Dravet patients is uncertain as we lack autonomic data related to SUDEP, Dravet syndrome, and the broader epilepsy population. Marked sympathetic hyperactivity (elevated electrodermal activity) was documented post-ictally in a 20-year-old SUDEP case (Picard et al 2017 Neurology In press). Sympathetic hyperactivity is well documented after most GTCs in animals and humans. The mechanism of death in SUDEP may be related to postictal abnormalities of both parasympathetic [101] (e.g., bradycardia, hypotension) and sympathetic systems [5].

The challenges in linking an epilepsy gene to SUDEP are highlighted by *DEPDC5*. This gene codes for a member of the IML1 protein family involved in G-protein signalling networks. The protein coded by *DEPDC5* is a component of the GATOR1 complex that inhibits the mTORC1 pathway and can cause autosomal dominant familial focal epilepsy of variable severity. Pathogenic variants of *DEPDC5* were enriched in the largest genetic study of a SUDEP cohort [96]. In a series of 61 SUDEP cases, five cases had *DEPDC5* variants; three were nonsense mutations that were very likely pathogenic while two were missense variants, one of which is highly conserved in evolution. Yet *DEPDC5* is the most common gene associated with familial focal epilepsy and is a common gene for sporadic focal epilepsy [102]. The challenges of differentiating genetic from other factors in the mechanism of death is exemplified by the two brothers with *DEPDC5* mutations who both died from SUDEP but both had histories of AED nonadherence [103]. Since *DEPDC5* patients often have frontal lobe foci and frontal lobe deficits, this can potentially impair planning and judgement, and this gene may increase SUDEP risk by causing treatment-resistant epilepsy as well as increasing the risk of life-style factors such as sleep deprivation, medication nonadherence and excess alcohol use.

SUDEP is not limited to treatment resistant epilepsies. SUDEP rarely complicates benign epilepsy of childhood with centrotemporal spikes and a history of convulsions not treated with antiepileptic drugs [104]. Further, one third of young children who die suddenly and have unrevealing

comprehensive death investigation, have a history of febrile seizures (ten-fold greater than the general population), suggesting seizure as a mechanism of death [67]. Thus, any patient with seizures is likely at increased risk for SUDEP post-ictally. Larger genetic studies are needed comparing SUDEP cases and control epilepsy populations matched for epilepsy severity, to determine if epilepsy genes increase SUDEP risk beyond the increased risk related to epilepsy severity.

As detailed above SUDEP is also associated with variants and mutations of neuro-cardiac channelopathy genes. The presence of LQT gene mutations in SUDEP suggests that a seizure could provoke a lethal cardiac arrhythmia, or sudden cardiac death could occur independently of a seizure. Both possibilities may occur in some cases, although the available literature on deaths recorded in epilepsy monitoring units do not identify a single case where a seizure led to lethal arrhythmia or where a lethal arrhythmia spontaneously occurred [12]. However, several near-SUDEP cases had arrhythmias and it is possible that patients with LQT gene mutations and epilepsy have treatment-responsive epilepsy and are rarely admitted to epilepsy monitoring units. Further, the extensive literature on cardiac changes during and after seizures recorded in epilepsy monitoring units very rarely identifies potentially lethal seizure-induced cardiac arrhythmias [94, 105, 106]. The most serious cardiac rhythm changes associated with seizures are ictal asystole, atrial fibrillation, and ST-wave depression. These are less often associated with mutations in cardiac genes than torsade des pointes ventricular tachyarrhythmias. However, abnormalities in cardiac repolarization on ECG are more frequent in SUDEP than control epilepsy cases [107], suggesting a potential role of cardiac gene variants in SUDEP. As noted above, pathogenic and rare variants of cardiac channel genes are found in SUDEP cases [108]. Moreover, all channelopathy genes are expressed to some extent in the brain, including the brainstem. Thus, if these cardiac channelopathy genetic variants increase SUDEP risk, they could do so by alterations in the heart or brain, or both organs. The extremely rare occurrence of ventricular tachyarrhythmias in recorded SUDEP cases, or during seizures recorded in epilepsy monitoring units, suggest that if cardiac channel genes contribute to SUDEP pathogenesis, it may be via a different mechanism than their role in sudden cardiac death. Finally, some or many variants in cardiac genes may be unrelated to SUDEP pathogenesis.

#### **The future and biobanking in SUDEP**

There is current momentum from professional groups, as well as patient support groups, to move from mere recognition and documentation of SUDEP to identifying its causes and preventing its



occurrence [109]. Despite an initial 'head start' by pathologists in recognising SUDEP, human tissue and cell based research programmes in ERD have somewhat lagged behind. The reasons are complex and manifold: (i) lack of clinical recognition and importance of the problem to allocate proportionate research funding in this area, (ii) establishing research collections in forensic setting where priorities and human tissue legislation is primarily geared toward establishing cause of death, with limited or restricted capacity to interact with research programmes, (iii) problems obtaining consent from immediately bereaved families, (iv) lack of easy access to a local or referral neuropathology services in some regions [16], (v) problems with post-mortem decomposition of autopsy tissues [80] with many epilepsy research programmes focussing resources on optimally preserved surgical tissues, (vi) lack of sufficient numbers of suitable control cases and tissues, (vii) lack of standardised guidance documents for conducting SUDEP autopsies, the core practice of which can vary between regions and jurisdictions. This last point is pertinent, as despite a flurry of clinical practice documents on SUDEP aimed at neurologists and health care professionals (14 from the first half of 2017 alone [6, 7, 9, 110-120] there are very few directed at the pathologist regarding the elements and scope of the post-mortem examination. There are no consensus international best practice guidelines (in contrast to surgical epilepsy neuropathology practice [32, 33, 37] and informal enquiry indicates that very few countries have any form of autopsy guidelines for investigations in ERD. In the UK guidance documents for the approach to post-mortem examination, tissue sampling protocols (Figure 1 ; Table 4) and classification of cause of death in suspected epilepsy and sudden cardiac cases are issued by the Royal College of Pathologists series (<https://www.rcpath.org/profession/clinical-effectiveness/clinical-guidelines/autopsy-guidelines.html>) ; these provide guidelines and do not mandate practice and are based on current evidence available in ERD and SUDEP.

In establishing the Centres for SUDEP research [121], the extent of the logistical problems and existing barriers to biobanking in ERD and SUDEP became apparent. These obstacles need to be addressed in the future and invested in to accelerate research into this condition. All biobanking should be conducted in full agreement with Coroners and Medical Examiners and following discussion and informed consent of the families (Figure 4). Local practical and economical strategies to more accurately determine the cause of death in ERD should utilise regional expertise in neuro and cardiac pathology to augment the investigation with timely and detailed reports (Figure 4). Post-mortem imaging, including MRI, CT and angiography is likely to play an increasing role in sudden death examination [122, 123], where it may supplement standard autopsy procedures by revealing lesions difficult to assess by macroscopic examination. There is no published study applying post-mortem imaging in ERD, however, high resolution MRI of whole brains or regions provides more



Accepted Article

detailed information of structural brain abnormalities enabling correlation with histological findings as ongoing areas of research [124, 125]. Prospective collection of DNA from brain, heart and lungs could also expand the range and scope of investigations. Techniques such as single cell RNAseq, proteomics usually requiring fresh frozen tissue, will provide complementary data on gene expression ; however, with newer technologies these methods are feasible with even formalin fixed, paraffin-embedded (FFPE) [126]. Greater availability of bio-specimens could facilitate numerous studies, including 1) systematic comparison of DNA exomes derived from blood and brain to examine the role of possible somatic mutations, 2) brainstem RNAseq in areas of interest (e.g., medullary raphe and pre-Bötzinger nuclei), 3) detailed examination of the cardiac conduction system. Such systematic tissue biobanking may enable better stratification of cause of death in SUDEP through integrated clinical, imaging, pathological and molecular diagnosis (Figure 4).

In summary, the evidence indicates that SUDEP is a heterogeneous condition in terms of both the underlying epilepsy neuropathology, genetics as well as mechanism of death. SUDEP at present provides a convenient 'umbrella' term under which to collect, stratify and further investigate these cases to shed light on any divergent or convergent pathogenetic mechanisms. Hopefully, in the future, epilepsy centres, neuropathologists, cardiac pathologists and medical examiners, coroner offices, and government funding agencies will collaborate in establishing international biorepositories to advance SUDEP research.

#### Acknowledgements

MT, OD MB are part of the Centres for SUDEP research and supported by funding from NIH/NINDS (Grant number neuropathology of SUDEP: 5U01NS090415 and SUDEP admin core grant: U01-NS090405). OD is the principal investigator of the North American SUDEP registry. This work was undertaken at UCLH/UCL, which received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. Epilepsy Society supports the UCL Epilepsy Brain and Tissue Bank. We are grateful to Zuzanna Michalak for her help with the figures. We are very grateful to the helpful comments from and international colleagues in Neuropathology who provided detailed feedback on the availability of SUDEP guidelines in their countries, particularly Eleonora Aronica, Beatrice Paradiso, Homa Adle-Biassette, Joanne Sy, Hajime Miyata and Ingmar Blümcke.

#### Author contributions

The authors have no conflicts of interest to declare other than grants as stated in the acknowledgements. All authors contributed to the preparation of this review article with particular focus on the sections relevant to their areas of professional expertise: OD for the section on the clinical aspects, molecular autopsy and biobanking, MNS to the section on cardiac pathology, EB to the medicolegal aspects and MB and MT to the neuropathology sections and biobanking.

## Figure legends

### Figure 1. Neuropathology in SUDEP and sampling protocols

**A and B.** Neuropathology block sampling protocols and findings in SUDEP. Recommended sampling strategy includes the frontal watershed regions (1), temporal cortex (2), amygdala (3), thalamus (4) hippocampus from both sides and at several levels if indicated (5) and insular cortex with basal ganglia (6); see Table 4 for details of coronal levels and other recommended or essential samples as part of the routine neuropathology examination. **C:** Mild granule cell dispersion in SUDEP case. **E.** Mossy fibre sprouting in the hippocampus with ZnT3 immunostaining in SUDEP **E:** Temporal lobe cortex from a patient with a SUDEP. Macroscopic examination of the brain was unremarkable but samples from the left hemisphere showed regions of focal cortical dysplasia type IIA. This was confirmed with immunohistochemistry for neurofilament (shown in F). **G, H. Study of neuroinflammation in SUDEP** [56] ; No significant increase in HLADR expressing microglia in hippocampal subfields in SUDEP (G) compared to non-epilepsy sudden deaths (H). **I, J. Studies of chronic alterations in SUDEP:** The lateral nucleus of the amygdala showing increased gliosis in SUDEP (I) compared to non-epilepsy control (J), shown in one study on stereology [57] , which could be relevant to altered function of this nucleus. Bar in G equivalent to 75 microns for G – J; C, D & F taken with x 20 objective and E with x 20 objective.

### Figure 2. Scatter graph of relative frequency of neuropathology lesions reported in SUDEP series.

Red triangles represent data from reported series (as detailed in Table 2) and the black line is the average for all SUDEP series. 'Inflammatory' in this context refers to inflammatory diagnosis at autopsy as meningoencephalitis. This is compared to data from epilepsy PM series (\*these also include some SUDEP cases) (Green triangle) [65, 127] and to surgical series (Blue triangle; data from the European Epilepsy Brain Bank of over 3,000 cases).

### Figure 3. Cardiac pathology and sampling in ERD and SUDEP

**A.** Mild myocardial hypertrophy in a sudden death case. **B.** Takotsubu cardiomyopathic changes with several myocytes showed an increased band of eosinophil staining and early contraction band formation (arrows). Bar = 50 microns.

### Figure 4. Future approaches in ERD.

Vision of future post-mortem examinations in epilepsy related deaths with intergrated clinical-pathology-genetics classifications and stratification of cause of death and intergration with research biobanks. \*A SUDEP gene could be defined as a gene mutation of pathogenic variant that causes epilepsy and increases the SUDEP risk via the central or peripheral nervous system or end-organ effects on respiratory, cardiac or other autonomic functions [5]. ~ Some post-mortem investigations would be carried out as relevant on a case by case basis. COD= cause of death, SCD = sudden cardiac death

Category of sudden death	Definition / criteria	Post mortem findings pertaining to cause of death
<b>DEFINITE SUDEP</b>	Sudden, unexpected, witnessed or unwitnessed. Exclude traumatic causes and drowning (death in 'benign' circumstances). Clinical diagnosis of epilepsy*. Status epilepticus cases excluded (seizure $\geq$ 30 min or serial seizures without recovery between).	No cause of death identified (including toxicology, histology and neuropathology examination).
<b>DEFINITE SUDEP PLUS</b>	Criteria as for Definite SUDEP.	Concomitant condition identified: not proven to be the cause of death but may have contributed.
<b>PROBABLE SUDEP</b>	Criteria as for Definite SUDEP.	Not conducted or Elements of the examination incomplete (e.g. no neuropathology or toxicology screen).
<b>POSSIBLE SUDEP</b>	Criteria as for Definite SUDEP.	A competing cause of death is identified at post-mortem examination.
<b>SUDDEN DEATH WITH SYMPTOMATIC SEIZURE</b>	Clinical diagnosis of epilepsy lacking* (e.g. alcohol withdrawal seizures).	Pathology relating to underlying condition may be found.

Table 1. Categories of Sudden Death in Epilepsy (adapted from [11, 13]). \* Epilepsy defined as  $\geq$  2 unprovoked seizures at least 24 hours apart or a single unprovoked seizure with an enduring risk for further seizures.

AUTHOR	PERIOD OF STUDY	REGION, COUNTRY	NUMBER SUDEP (AGE RANGES)	TYPE OF STUDY	NEUROPATHOLOGY (number (%) of cases with structural lesions/pathology)	PULMONARY PATHOLOGY	CARDIAC PATHOLOGY
KROHN[1]	10 years circa 1963	Oslo, Norway	14 (10-70 ys)	Retrospective	<b>5/14 (36%)</b> : atrophic/senile atrophy, slight meningoencephalitis, seizure-related change, NOS	No detail	No detail
TERRENCE[8]	1978-1979	Pittsburgh, USA	8 (9-31ys)	Retrospective	<b>3/8 (38%)</b> ; cerebral oedema 2/8 (25%), old TBI 1/8 (12%). Acute neuronal injury in hippocampus and cerebellum also described.	<b>(100%)</b> Pulmonary oedema	No detail
LEESTMA[3]	1976-1979	Cook County, USA	66 (10 months -60 ys)	Retrospective	<b>46/66 (60%)</b> : Old TBI/contusions (26%), tumours (4.5%), HS (6%), MCD (6%), scars (9%), old CVA (3%) atrophy/hemiatrophy (7.5%), TS (1.5%) cerebral oedema (12%)	<b>19/66 (29%)</b> Pulmonary oedema/congestion	No detail
LEESTMA[128]	1983	Cook County, USA	60 (8 month - 83 ys)	Prospective	<b>24/60 (40%)</b> : contusions (11%), scars (20%), old CVA (17%), HS (12%), cerebellar atrophy (7%), MCD (7%), VM (2%) tumour (2%)	<b>(42/52) 80%</b> Pulmonary oedema	<b>(9/60) 15%</b> Mild to moderate atherosclerosis <b>2/60 (3%)</b> myocardial fibrosis
EARNEST[129]	1982-1987	Denver County, USA	44 (3-58 ys)	Retrospective Review, PM Findings	<b>15/44 (34%)</b> : 14/44 (31.8%) cerebral oedema but no herniation	<b>38/44 (86.4%)</b> Pulmonary congestion/oedema	<b>5/44 (11%)</b> focal interstitial fibrosis
NATELSON[130]	1998	Morris County, USA	7 (12-44 ys)	Case Control Study	<b>1/7 (14%)</b> : communicating hydrocephalus	no detail	<b>5/6 (83%)</b> myocyte vacuolisation, <b>4/8 (50%)</b> interstitial fibrosis
THOM[62]	1999	UCL London UK	15 (14-69)	Case Control/ Quantitative Evaluation	Amygdala: Lower neuronal densities and increased astrocytic densities in lateral nucleus of amygdala compared to controls	no details	No detail
ANTONIUK[131]	1990-1999	Curitiba, Brazil	20 (11-50 ys)	Retrospective Review, PM Findings	Cerebral oedema 11/20 (55%)	<b>12/20 (60%)</b> pulmonary oedema Pulmonary haemorrhage (1/20)	No detail
BLACK & GRAHAM[30]	1991-1996	Glasgow, UK	131 (adults)	Retrospective Review, PM Findings	<b>66%</b> : old TBI, HS, hypoxic-ischaemic brain injury.	No detail	No detail
SHIELDS[80]	1996-2000	Louisville, USA	70 (16-71 ys)	Retrospective	<b>32/60 (53%)</b> : old TBI (59%), hippocampal/cortical atrophy (26%), cerebellar atrophy (31%), VM (6%), Tumour (3%), cerebellar damage (57%), inflammation (38%)	<b>(81%)</b> Pulmonary oedema/congestion	No detail
DAVIS & MCGWIN[132]	1986-2000	Jefferson County, USA	57 (14-77 ys)	Retrospective, Case Control	No detail	No difference in lung weights compared to control group	No contraction band necrosis in SUDEP group ; no differences in heart weights
P-CODREA[133]	1998-2000	Northern Jutland, Denmark	15 (17-56 ys)	Prospective study, PM Findings/ Quantitative Evaluation	<b>8/15 (53%)</b> : old TBI, old ICH, FCD, Microcephaly, heterotopia	No detail	Significant foci of myocardial fibrosis compared to age matched controls (p=0.03)
THOM[57]	2003	UCL, London UK	18 (17-69 ys)	Case Control / Quantitative Evaluation	Increased HSP-70 <sup>+</sup> neurones in hippocampal in SUDEP compared to control groups. Also noted in insular cortex.	<b>13/18 (72%)</b> Pulmonary oedema	No detail

ZHOU[23]	2007-2009	Maryland, USA	74 (14-63 ys)	Retrospective	<b>43/74 (58.1%)</b> ; old TBI (13.5%), MCD (6.8%), cerebellar atrophy (5.4%), VM (4.1%), Hippocampal gliosis (2.7%), cortical/hippocampal atrophy (2.7%), CVA (2.7%). Tumours (2.7%), TS (1.4%), acute neuronal injury (1.4%)	<b>(100%)</b> Pulmonary congestion/oedema	<b>(9.5%)</b> moderate LVH <b>(4.1%)</b> mild coronary atherosclerosis <b>(21.2%)</b> myocyte hypertrophy <b>(42.3%)</b> myocardial fibrosis
FEAST[89]	2012	UCL London UK	7 (adults)	Case Control / Quantitative Evaluation	Hippocampal HIF1 $\alpha$ expression in SUDEP compared to controls	No detail	No details
RYVLIN[12]	1968-2007	Multi-Centre, Inter-National	8 (with PM)	Survey	<b>5/8 (62%)</b> : Hippocampal atrophy (2/8), probable encephalitis (2/8), Ganglioglioma (1/8): These were ante-mortem diagnosis and not detailed or confirmed in autopsy findings available	<b>3/8 (37%)</b> Pulmonary oedema	1/8 (12.5%) subendocardial fibrosis, 1/8 (12.5%) coronary atherosclerosis
THOM[31]	2016	Multi-centre, UK	145 (2-82 ys)	Retrospective	Neuropathology Macroscopic <b>(52%)</b> , Microscopic <b>(89%)</b> : Mild brain swelling (28%), MCD (15%), Tumour (6.8%), HS (21%), Old TBI (17%), CVA (6.9%), Cerebellar atrophy (41%), acute neuronal injury (55%)	<b>(68%)</b> Pulmonary oedema/congestion	No detail
MICHALAK[56]	2017	UCL London, UK	28 (18-69 ys)	Case Control Study / Quantitative Evaluation	Neuroinflammation in SUDEP: Brainstem reduced CD163-positive macrophages in SUDEP & lower IgG in CA1 and parahippocampal gyrus than controls	No detail	No detail

Table 2. Pathology studies in SUDEP based on series with more than one case and the positive findings are detailed. CVA = Cerebro-vascular accident, FCD= focal cortical dysplasia, HS= hippocampal sclerosis, NOS= not otherwise specified, TBI = traumatic brain injury, TS = Tuberos Sclerosis, VM= vascular malformation, PM=post-mortem, ICH = intra cranial haemorrhage, ys = years. Studies with details of macro and/or histological findings are included.

Likely SUDEP Gene	Protein/Cellular Role	Clinical Disorder(s)	Expression Site(s)
<b>Primary cardiac genes</b>			
<i>KCNQ1</i> [134]	Potassium channel KvLQT1/Kv7.1; ventricular repolarization	Long QT	Brain, heart, lung
<i>KCNH2</i> [134, 135]	Potassium channel hERG1/Kv11.1; repolarization of cardiac action potential	Long QT Epilepsy	Brain, heart, lung
<i>SCN5a</i> [134, 135]	Sodium channel, Nav1.5; rapid depolarizing sodium current underlying cardiac action potential upstroke	Long QT Brugada	Brain, heart, lung
<i>NOS1AP</i> [134]	Cytosolic protein that binds to neuronal nitric oxide synthase	Long QT	Brain, heart, lung
<i>RYR2</i> [134, 135]	Cardiac ryanodine receptor 2, acts as intracellular Ca <sup>2+</sup> release channel, coupling excitation–contraction	Sudden cardiac death	Brain, heart, lung
<i>HCN4</i> [134, 135]	Hyperpolarization activated cyclic nucleotide gated potassium channel 4; slow kinetics of activation and inactivation, cardiac pacemaker role	Brugada, Sick sinus rhythm	Brain, heart, lung
<i>LBD3</i> [136]	Involved in protein kinase-C mediated signalling in striated muscle	Dilated cardiomyopathy, arrhythmogenic ventricular dysplasia	Heart, brain, lung
<i>DSC2</i> [136]	Component of intercellular desmosome junctions	Arrhythmogenic ventricular dysplasia, cardiomyopathy	Heart, brain, lung
<i>KCNE1</i> [136]		Long QT	Heart, brain, lung
<b>Primary Epilepsy or Brain Gene</b>			
<i>SCN1A</i> [99]	Sodium channel Nav1.1	Dravet/EE, GEFS+	Brain, heart, lung
<i>SCN2A</i> [137]	Sodium channel Nav 1.2	EE	Brain, heart, lung
<i>SCN8A</i> [137]	Sodium channel Nav 1.6	EE	Brain, heart, lung
<i>PRRT2</i> [135]	Proline-rich transmembrane protein 2	Benign familial infantile seizures	Brain
<i>DEPDC5</i> [103]	G-protein signalling pathway. Component of the GATOR1 complex which inhibits mTORC1 pathway	Focal epilepsy (broad spectrum of phenotypes)	Brain, heart, lung
<i>CSTB</i>	A stefin that inhibits intracellular thiol protease; may prevent protease leakage from lysosomes	Unverricht-Lundborg disease	Brain, heart, lung
<i>TSC2, TSC1</i>	Hamartin (TSC1), Tuberin (TSC2); Downregulate the mTORC1 pathway	Tuberous sclerosis complex	Brain, heart, lung

<i>HCN2</i> [135]	Hyperpolarization activated cyclic nucleotide gated potassium channel 2; Contributes to spontaneous rhythmic activity in heart (sinoatrial node) and brain	Generalized epilepsy	Brain, heart, lung
<i>KCNT1</i> [138]	Outwardly rectifying potassium channel subunit	Focal epilepsy, EE	Brain, lung, heart
<b>Chromosomal disorders*</b>			
Dup15q11 [99]	supernumerary isodicentric chromosome 15; extra copies of UBE3A (Angelman syndrome) and GABRB3 (GABA receptor)	EE; variable epilepsy phenotype	UBE3A - brain, heart, lung  GABRB3 - brain, heart, lung
5q14.3 deletion [139]	Haploinsufficiency of MEF2C (role in myogenesis) and EFNA5 - receptor protein-tyrosine kinases involved in neurodevelopment	Variable severity of epilepsy and neurodevelopmental disability	MEF2C - brain, heart, lung  EFNA5 - brain, heart, lung

**Table 3. Genes implicated in SUDEP.** ES – epilepsy severity; EE – epileptic encephalopathy; \*No animal models exist for these genetic disorders and with most genes, it is uncertain if the risk of SUDEP is solely accounted for by the severity of epilepsy and developmental delays

Brain region	Approximate coronal level* / side for sampling	Rationale for sampling in routine POST-MORTEM examination in ERDs	Evidence implicating relevance to SUDEP : Clinical / neuroimaging / pathological studies
Frontal watershed regions (F1/2)	Anterior level/ Both hemispheres	<ul style="list-style-type: none"> <li>Assessment of recent hypoxic-ischaemic brain damage in watershed regions</li> <li>Exclude unsuspected meningitis/encephalitis</li> </ul>	
Fronto-basal cortex	Anterior level/ Both hemispheres	<ul style="list-style-type: none"> <li>Investigation of old or subtle TBI</li> </ul>	<ul style="list-style-type: none"> <li>Old TBI, particularly cortical contusions, frequent in SUDEP series (see Table 1)</li> </ul>
Insular cortex with basal ganglia	Several levels/ Both hemispheres	<ul style="list-style-type: none"> <li>'Screening' block to exclude unsuspected meningitis/encephalitis, small vessel disease, metabolic or neurodegenerative process</li> </ul>	<ul style="list-style-type: none"> <li>Insular cortex has cardiac autonomic roles</li> <li>Clinical/pathology evidence linking this region to SUDEP [84]</li> <li>HSP-70 protein expression shown in SUDEP [57]</li> </ul>
Amygdala	Both Hemispheres	<ul style="list-style-type: none"> <li>Frequent evidence for amygdala MRI volume alterations and gliosis/sclerosis, mainly linked with TLE (recently reviewed in [58]) as sequel of seizures</li> </ul>	<ul style="list-style-type: none"> <li>Stimulation of amygdala induces apnoea [60, 61]</li> <li>Loss of neurones in lateral nucleus in SUDEP [62]</li> <li>Altered volume in MRI studies of SUDEP [63]</li> </ul>
Thalamus	Mid Coronal level	<ul style="list-style-type: none"> <li>Evidence for thalamic pathology in epilepsy as possible sequel of seizures (reviewed in [79])</li> </ul>	<ul style="list-style-type: none"> <li>Role in seizure initiation and modulation</li> <li>Altered volume in MRI studies in SUDEP including pulvinar nucleus [63]</li> <li>Cardioregulatory roles</li> </ul>
Temporal cortex Superior and middle temporal gyrus	Posterior level/ Both hemispheres	<ul style="list-style-type: none"> <li>To exclude unsuspected meningitis/encephalitis or hypoxic/ischaemic brain damage</li> <li>Exclude epilepsy related pathology include temporal lobe sclerosis, cortical dysplasia, mild malformations etc.</li> </ul>	
Hippocampus	Posterior or several levels (or where it appears abnormal)/ Both sides	<ul style="list-style-type: none"> <li>HS frequent in TLE and other epilepsy syndromes</li> <li>HS may be unilateral or bilateral</li> <li>Specific cellular alterations support epilepsy mediated damage over other causes (e.g. ischaemic, neurodegenerative) (reviewed in [34])</li> </ul>	<ul style="list-style-type: none"> <li>Links with cardiorespiratory regulatory brain regions and stimulation induces apnoea [60]</li> <li>Pathology associated with SUDEP (see Table1) and reported in SIDS</li> <li>Altered volume in MRI studies in SUDEP [63]</li> </ul>
Cerebellum cortex	Watershed region (or region of atrophy)	<ul style="list-style-type: none"> <li>Frequent atrophy in TLE and other epilepsies</li> </ul>	<ul style="list-style-type: none"> <li>Atrophy variably reported in SUDEP PM series (see Table 1)</li> <li>Autonomic roles, including blood pressure regulation [140]</li> </ul>
Medulla	Mid/high obex level	<ul style="list-style-type: none"> <li>Exclude unsuspected inflammatory/vascular/neoplastic disease</li> </ul>	<ul style="list-style-type: none"> <li>Cardio-regulatory nuclei</li> <li>Evidence from SUDEP models [51]</li> <li>Altered volume in MRI studies of SUDEP [55]</li> </ul>
Macroscopic abnormality/ brain lesion		<ul style="list-style-type: none"> <li>Could pertain to cause of death or underlying epilepsy</li> <li>Correlate block sampling with any localising investigations during life to maximise detection (e.g.EEG, MRI findings, seizure semiology)</li> </ul>	

**Table 4. Suggested neuropathological sampling protocols for histology in the investigation of suspected SUDEP and the rationale.** Based on recommendations from the Royal college of Pathologists autopsy series: deaths in epilepsy, issued in 2006 (currently withdrawn for revision). The sampling strategy is a minimal approach of a set of blocks which aim to exclude other unsuspected pathology (not visualised macroscopically) as cause of death or epilepsy as well as disclose common, secondary, epilepsy-related pathologies. Such a systematic sampling protocol could facilitate research work between centres in SUDEP. Other desirable blocks that could be considered/bio-banked for future research include regions



with cardio-regulatory function including midbrain, pons (parabrachial pontine nuclei involved in respiratory rhythmogenesis, raphe nuclei (RN) comprising serotonergic neurons, retrotrapezoid nucleus and parafacial respiratory group, deep cerebellar nuclei, pulvinar, cortical blocks from cingulate, anterior frontal and more extensive blocks of insular, thalamus and amygdala nuclei. \*Anterior coronal levels correspond to the nucleus accumbens, mid-level to the mammillary bodies and posterior level to the lateral geniculate nucleus.

## References

- 1 Krohn W. Causes of Death among Epileptics. *Epilepsia* 1963; 4: 315-22
- 2 Sommer W. Erkrankung des Ammonshorns als aetiologisches Moment der Epilepsie. *Archiv für Psychiatrie und Nervenkrankheiten* 1880; 10: 631-75
- 3 Leestma JE, Kalelkar MB, Teas SS, Jay GW, Hughes JR. Sudden unexpected death associated with seizures: analysis of 66 cases. *Epilepsia* 1984; 25: 84-8
- 4 Levira F, Thurman DJ, Sander JW, Hauser WA, Hesdorffer DC, Masanja H, Odermatt P, Logroscino G, Newton CR, Epidemiology Commission of the International League Against E. Premature mortality of epilepsy in low- and middle-income countries: A systematic review from the Mortality Task Force of the International League Against Epilepsy. *Epilepsia* 2017; 58: 6-16
- 5 Devinsky O, Hesdorffer DC, Thurman DJ, Lhatoo S, Richerson G. Sudden unexpected death in epilepsy: epidemiology, mechanisms, and prevention. *Lancet neurology* 2016; 15: 1075-88
- 6 Sveinsson O, Andersson T, Carlsson S, Tomson T. The incidence of SUDEP: A nationwide population-based cohort study. *Neurology* 2017:
- 7 Harden C, Tomson T, Gloss D, Buchhalter J, Cross JH, Donner E, French JA, Gil-Nagel A, Hesdorffer DC, Smithson WH, Spitz MC, Walczak TS, Sander JW, Ryvlin P. Practice guideline summary: Sudden unexpected death in epilepsy incidence rates and risk factors: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology and the American Epilepsy Society. *Neurology* 2017; 88: 1674-80
- 8 Terrence CF, Rao GR, Perper JA. Neurogenic pulmonary edema in unexpected, unexplained death of epileptic patients. *Annals of neurology* 1981; 9: 458-64
- 9 Shankar R, Donner EJ, McLean B, Nashef L, Tomson T. Sudden unexpected death in epilepsy (SUDEP): what every neurologist should know. *Epileptic disorders : international epilepsy journal with videotape* 2017; 19: 1-9
- 10 Shankar R, Jalihal V, Walker M, Laugharne R, McLean B, Carlyon E, Hanna J, Brown S, Jory C, Tripp M, Pace A, Cox D, Brown S. A community study in Cornwall UK of sudden unexpected death in epilepsy (SUDEP) in a 9-year population sample. *Seizure* 2014; 23: 382-5
- 11 Devinsky O, Friedman D, Cheng JY, Moffatt E, Kim A, Tseng ZH. Underestimation of sudden deaths among patients with seizures and epilepsy. *Neurology* 2017; 89: 886-92
- 12 Ryvlin P, Nashef L, Lhatoo SD, Bateman LM, Bird J, Bleasel A, Boon P, Crespel A, Dworetzky BA, Hogenhaven H, Lerche H, Maillard L, Malter MP, Marchal C, Murthy JM, Nitsche M, Patariaia E, Rabben T, Rheims S, Sadzot B, Schulze-Bonhage A, Seyal M, So EL, Spitz M, Szucs A, Tan M, Tao JX, Tomson T. Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): a retrospective study. *The Lancet Neurology* 2013; 12: 966-77
- 13 Nashef L, So EL, Ryvlin P, Tomson T. Unifying the definitions of sudden unexpected death in epilepsy. *Epilepsia* 2012; 53: 227-33
- 14 Garcia Espinosa A, Andrade Machado R, Borges Gonzalez S, Garcia Gonzalez ME, Perez Montoto A, Toledo Sotomayor G. Wisconsin Card Sorting Test performance and impulsivity in patients with temporal lobe epilepsy: suicidal risk and suicide attempts. *Epilepsy & behavior : E&B* 2010; 17: 39-45
- 15 Baker GA. Depression and suicide in adolescents with epilepsy. *Neurology* 2006; 66: S5-12

- 16 Clark D, Riney K. A population-based post mortem study of sudden unexpected death in epilepsy. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia* 2016; 23: 58-62
- 17 Hanna NJ. National sentinel clinical audit of epilepsy-related death : report 2002. Norwich: Stationery Office. 2002
- 18 Annegers JF. United States perspective on definitions and classifications. *Epilepsia* 1997; 38: S9-12
- 19 Brigo F, Nardone R, Bongiovanni LG. Value of tongue biting in the differential diagnosis between epileptic seizures and syncope. *Seizure* 2012; 21: 568-72
- 20 Brigo F, Storti M, Lochner P, Tezzon F, Fiaschi A, Bongiovanni LG, Nardone R. Tongue biting in epileptic seizures and psychogenic events: an evidence-based perspective. *Epilepsy & behavior : E&B* 2012; 25: 251-5
- 21 Atherton DS, Davis GG, Wright C, Devinsky O, Hesdorffer D. A survey of medical examiner death certification of vignettes on death in epilepsy: Gaps in identifying SUDEP. *Epilepsy research* 2017; 133: 71-5
- 22 Schraeder PL, Delin K, McClelland RL, So EL. Coroner and medical examiner documentation of sudden unexplained deaths in epilepsy. *Epilepsy research* 2006; 68: 137-43
- 23 Zhuo L, Zhang Y, Zielke HR, Levine B, Zhang X, Chang L, Fowler D, Li L. Sudden unexpected death in epilepsy: Evaluation of forensic autopsy cases. *Forensic science international* 2012; 223: 171-5
- 24 Agesen FN, Risgaard B, Zachariasardottir S, Jabbari R, Lyng TH, Ingemann-Hansen O, Ottesen GL, Thomsen JL, Haunso S, Krieger DW, Winkel BG, Tfelt-Hansen J. Sudden unexpected death caused by stroke: A nationwide study among children and young adults in Denmark. *International journal of stroke : official journal of the International Stroke Society* 2017: 1747493017724625
- 25 Lagman C, Rai K, Chung LK, Nagasawa DT, Beckett JS, Tucker AM, Yang I. Fatal Colloid Cysts: A Systematic Review. *World neurosurgery* 2017:
- 26 Kawamura Y, Ohashi M, Ihira M, Hashimoto S, Taniguchi K, Yoshikawa T. Nationwide survey of rotavirus-associated encephalopathy and sudden unexpected death in Japan. *Brain & development* 2014; 36: 601-7
- 27 Ramanathan S, Bleasel A, Parratt J, Orr C, Dale RC, Vincent A, Fung VS. Characterisation of a syndrome of autoimmune adult onset focal epilepsy and encephalitis. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia* 2014; 21: 1169-75
- 28 Blumcke I. Neuropathology of focal epilepsies: a critical review. *Epilepsy & behavior : E&B* 2009; 15: 34-9
- 29 Opeskin K, Berkovic SF. Risk factors for sudden unexpected death in epilepsy: a controlled prospective study based on coroners cases. *Seizure* 2003; 12: 456-64
- 30 Black M, Graham DI. Sudden unexplained death in adults caused by intracranial pathology. *Journal of clinical pathology* 2002; 55: 44-50
- 31 Thom M, Michalak Z, Wright G, Dawson T, Hilton D, Joshi A, Diehl B, Koeppe M, Lhatoo S, Sander JW, Sisodiya SM. Audit of practice in sudden unexpected death in epilepsy (SUDEP) post mortems and neuropathological findings. *Neuropathology and applied neurobiology* 2016; 42: 463-76
- 32 Blumcke I, Aronica E, Miyata H, Sarnat HB, Thom M, Roessler K, Rydenhag B, Jehi L, Krsek P, Wiebe S, Spreafico R. International recommendation for a comprehensive neuropathologic workup of epilepsy surgery brain tissue: A consensus Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia* 2016; 57: 348-58
- 33 Blumcke I, Thom M, Aronica E, Armstrong DD, Bartolomei F, Bernasconi A, Bernasconi N, Bien CG, Cendes F, Coras R, Cross JH, Jacques TS, Kahane P, Mathern GW, Miyata H, Moshe SL, Oz B, Ozkara C, Perucca E, Sisodiya S, Wiebe S, Spreafico R. International consensus classification of

hippocampal sclerosis in temporal lobe epilepsy: a Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia* 2013; 54: 1315-29

34 Thom M. Review: Hippocampal sclerosis in epilepsy: a neuropathology review. *Neuropathology and applied neurobiology* 2014; 40: 520-43

35 Meencke HJ, Janz D. Neuropathological findings in primary generalized epilepsy: a study of eight cases. *Epilepsia* 1984; 25: 8-21

36 Liu JY, Ellis M, Brooke-Ball H, de Tisi J, Eriksson SH, Brandner S, Sisodiya SM, Thom M. High-throughput, automated quantification of white matter neurons in mild malformation of cortical development in epilepsy. *Acta neuropathologica communications* 2014; 2: 72

37 Blumcke I, Thom M, Aronica E, Armstrong DD, Vinters HV, Palmieri A, Jacques TS, Avanzini G, Barkovich AJ, Battaglia G, Becker A, Cepeda C, Cendes F, Colombo N, Crino P, Cross JH, Delalande O, Dubeau F, Duncan J, Guerrini R, Kahane P, Mathern G, Najm I, Ozkara C, Raybaud C, Represa A, Roper SN, Salamon N, Schulze-Bonhage A, Tassi L, Vezzani A, Spreafico R. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia* 2011; 52: 158-74

38 Shi J, Lacuey N, Lhatoo S. Surgical outcome of MRI-negative refractory extratemporal lobe epilepsy. *Epilepsy research* 2017; 133: 103-8

39 Robel S. Astroglial Scarring and Seizures: A Cell Biological Perspective on Epilepsy. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 2016:

40 de Vries EE, van den Munckhof B, Braun KP, van Royen-Kerkhof A, de Jager W, Jansen FE. Inflammatory mediators in human epilepsy: A systematic review and meta-analysis. *Neuroscience and biobehavioral reviews* 2016; 63: 177-90

41 Han H, Mann A, Ekstein D, Eyal S. Breaking Bad: the Structure and Function of the Blood-Brain Barrier in Epilepsy. *The AAPS journal* 2017; 19: 973-88

42 Walker LE, Janigro D, Heinemann U, Riikonen R, Bernard C, Patel M. WONOEP appraisal: Molecular and cellular biomarkers for epilepsy. *Epilepsia* 2016; 57: 1354-62

43 Farrell JS, Wolff MD, Teskey GC. Neurodegeneration and Pathology in Epilepsy: Clinical and Basic Perspectives. *Advances in neurobiology* 2017; 15: 317-34

44 Blanc F, Martinian L, Liagkouras I, Catarino C, Sisodiya SM, Thom M. Investigation of widespread neocortical pathology associated with hippocampal sclerosis in epilepsy: a postmortem study. *Epilepsia* 2011; 52: 10-21

45 Corsellis JA. The neuropathology of temporal lobe epilepsy. *Modern trends in neurology* 1970; 5: 254-70

46 Smith JC, Abdala AP, Borgmann A, Rybak IA, Paton JF. Brainstem respiratory networks: building blocks and microcircuits. *Trends in neurosciences* 2013; 36: 152-62

47 Edlow BL, McNab JA, Witzel T, Kinney HC. The Structural Connectome of the Human Central Homeostatic Network. *Brain connectivity* 2016; 6: 187-200

48 Moseley B, Bateman L, Millichap JJ, Wirrell E, Panayiotopoulos CP. Autonomic epileptic seizures, autonomic effects of seizures, and SUDEP. *Epilepsy & behavior : E&B* 2013; 26: 375-85

49 Koshiya N, Oku Y, Yokota S, Oyamada Y, Yasui Y, Okada Y. Anatomical and functional pathways of rhythmogenic inspiratory premotor information flow originating in the pre-Botzinger complex in the rat medulla. *Neuroscience* 2014; 268: 194-211

50 Benarroch EE. Medullary serotonergic system: organization, effects, and clinical correlations. *Neurology* 2014; 83: 1104-11

51 Aiba I, Noebels JL. Spreading depolarization in the brainstem mediates sudden cardiorespiratory arrest in mouse SUDEP models. *Science translational medicine* 2015; 7: 282ra46

52 Salam MT, Montandon G, Genov R, Devinsky O, Del Campo M, Carlen PL. Mortality with brainstem seizures from focal 4-aminopyridine-induced recurrent hippocampal seizures. *Epilepsia* 2017:

- 53 Schwarzacher SW, Rub U, Deller T. Neuroanatomical characteristics of the human pre-Botzinger complex and its involvement in neurodegenerative brainstem diseases. *Brain* 2011; 134: 24-35
- 54 Kinney HC, Richerson GB, Dymecki SM, Darnall RA, Nattie EE. The brainstem and serotonin in the sudden infant death syndrome. *Annual review of pathology* 2009; 4: 517-50
- 55 Mueller SG, Bateman LM, Laxer KD. Evidence for brainstem network disruption in temporal lobe epilepsy and sudden unexplained death in epilepsy. *NeuroImage Clinical* 2014; 5: 208-16
- 56 Michalak Z, Obari D, Ellis M, Thom M, Sisodiya SM. Neuropathology of SUDEP: Role of inflammation, blood-brain barrier impairment, and hypoxia. *Neurology* 2017; 88: 551-61
- 57 Thom M, Seetah S, Sisodiya S, Koepp M, Scaravilli F. Sudden and unexpected death in epilepsy (SUDEP): evidence of acute neuronal injury using HSP-70 and c-Jun immunohistochemistry. *Neuropathology and applied neurobiology* 2003; 29: 132-43
- 58 Yilmazer-Hanke D, O'Loughlin E, McDermott K. Contribution of amygdala pathology to comorbid emotional disturbances in temporal lobe epilepsy. *Journal of neuroscience research* 2016; 94: 486-503
- 59 Nakayama Y, Masuda H, Shirozu H, Ito Y, Higashijima T, Kitaura H, Fujii Y, Kakita A, Fukuda M. Features of amygdala in patients with mesial temporal lobe epilepsy and hippocampal sclerosis: An MRI volumetric and histopathological study. *Epilepsy research* 2017; 135: 50-5
- 60 Lacuey N, Zonjy B, Londono L, Lhatoo SD. Amygdala and hippocampus are symptomatogenic zones for central apneic seizures. *Neurology* 2017; 88: 701-5
- 61 Dlouhy BJ, Gehlbach BK, Kreple CJ, Kawasaki H, Oya H, Buzza C, Graner MA, Welsh MJ, Howard MA, Wemmie JA, Richerson GB. Breathing Inhibited When Seizures Spread to the Amygdala and upon Amygdala Stimulation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2015; 35: 10281-9
- 62 Thom M, Griffin B, Sander JW, Scaravilli F. Amygdala sclerosis in sudden and unexpected death in epilepsy. *Epilepsy research* 1999; 37: 53-62
- 63 Wandschneider B, Koepp M, Scott C, Micallef C, Balestrini S, Sisodiya SM, Thom M, Harper RM, Sander JW, Vos SB, Duncan JS, Lhatoo S, Diehl B. Structural imaging biomarkers of sudden unexpected death in epilepsy. *Brain* 2015; 138: 2907-19
- 64 Meencke HJ, Veith G, Lund S. Bilateral hippocampal sclerosis and secondary epileptogenesis. *Epilepsy research Supplement* 1996; 12: 335-42
- 65 Novy J, Belluzzo M, Caboclo LO, Catarino CB, Yogarajah M, Martinian L, Peacock JL, Bell GS, Koepp MJ, Thom M, Sander JW, Sisodiya SM. The lifelong course of chronic epilepsy: the Chalfont experience. *Brain* 2013; 136: 3187-99
- 66 Dlouhy BJ, Ciliberto MA, Cifra CL, Kirby PA, Shrock DL, Nashelsky M, Richerson GB. Unexpected Death of a Child with Complex Febrile Seizures-Pathophysiology Similar to Sudden Unexpected Death in Epilepsy? *Frontiers in neurology* 2017; 8: 21
- 67 Hesdorffer DC, Crandall LA, Friedman D, Devinsky O. Sudden unexplained death in childhood: A comparison of cases with and without a febrile seizure history. *Epilepsia* 2015; 56: 1294-300
- 68 Kinney HC, Cryan JB, Haynes RL, Paterson DS, Haas EA, Mena OJ, Minter M, Journey KW, Trachtenberg FL, Goldstein RD, Armstrong DD. Dentate gyrus abnormalities in sudden unexplained death in infants: morphological marker of underlying brain vulnerability. *Acta Neuropathol* 2015; 129: 65-80
- 69 Hefti MM, Cryan JB, Haas EA, Chadwick AE, Crandall LA, Trachtenberg FL, Armstrong DD, Grafe M, Krous HF, Kinney HC. Hippocampal malformation associated with sudden death in early childhood: a neuropathologic study: Part 2 of the investigations of The San Diego SUDC Research Project. *Forensic science, medicine, and pathology* 2016; 12: 14-25
- 70 Kinney HC, Poduri AH, Cryan JB, Haynes RL, Teot L, Sleeper LA, Holm IA, Berry GT, Prabhu SP, Warfield SK, Brownstein C, Abram HS, Kruer M, Kemp WL, Hargitai B, Gastrang J, Mena OJ, Haas EA, Dastjerdi R, Armstrong DD, Goldstein RD. Hippocampal Formation Maldevelopment and Sudden

Unexpected Death across the Pediatric Age Spectrum. *Journal of neuropathology and experimental neurology* 2016; 75: 981-97

71 Cury C, Toro R, Cohen F, Fischer C, Mhaya A, Samper-Gonzalez J, Hasboun D, Mangin JF, Banaschewski T, Bokde AL, Bromberg U, Buechel C, Cattrell A, Conrod P, Flor H, Gallinat J, Garavan H, Gowland P, Heinz A, Ittermann B, Lemaitre H, Martinot JL, Nees F, Paillere Martinot ML, Orfanos DP, Paus T, Poustka L, Smolka MN, Walter H, Whelan R, Frouin V, Schumann G, Glaunes JA, Colliot O, Consortium I. Incomplete Hippocampal Inversion: A Comprehensive MRI Study of Over 2000 Subjects. *Frontiers in neuroanatomy* 2015; 9: 160

72 McClelland AC, Gomes WA, Shinnar S, Hesdorffer DC, Bagiella E, Lewis DV, Bello JA, Chan S, MacFall J, Chen M, Pellock JM, Nordli DR, Jr., Frank LM, Moshe SL, Shinnar RC, Sun S, Team FS. Quantitative Evaluation of Medial Temporal Lobe Morphology in Children with Febrile Status Epilepticus: Results of the FEBSTAT Study. *AJNR American journal of neuroradiology* 2016; 37: 2356-62

73 Chan S, Bello JA, Shinnar S, Hesdorffer DC, Lewis DV, MacFall J, Shinnar RC, Gomes W, Litherland C, Xu Y, Nordli DR, Pellock JM, Frank LM, Moshe SL, Sun S, Team FS. Hippocampal Malrotation Is Associated With Prolonged Febrile Seizures: Results of the FEBSTAT Study. *AJR American journal of roentgenology* 2015; 205: 1068-74

74 Gamss RP, Slasky SE, Bello JA, Miller TS, Shinnar S. Prevalence of hippocampal malrotation in a population without seizures. *AJNR American journal of neuroradiology* 2009; 30: 1571-3

75 Ackerman MJ, Andrew TA, Baker AM, Devinsky O, Downs JC, Keens T, Kuntz J, Lin P, Lear-Kaul KC, Reichard R, Robinson DA. An Association of Hippocampal Malformations and Sudden Death? We Need More Data. *Forensic science, medicine, and pathology* 2016; 12: 229-31

76 Blumcke I, Kistner I, Clusmann H, Schramm J, Becker AJ, Elger CE, Bien CG, Merschhemke M, Meencke HJ, Lehmann T, Buchfelder M, Weigel D, Buslei R, Stefan H, Pauli E, Hildebrandt M. Towards a clinico-pathological classification of granule cell dispersion in human mesial temporal lobe epilepsies. *Acta neuropathologica* 2009; 117: 535-44

77 Scorza FA. SUDEP on the table: Some thoughts about posterior thalamus. *Epilepsy & behavior* : E&B 2016; 54: 148-9

78 Tang Y, Chen Q, Yu X, Xia W, Luo C, Huang X, Tang H, Gong Q, Zhou D. A resting-state functional connectivity study in patients at high risk for sudden unexpected death in epilepsy. *Epilepsy & behavior* : E&B 2014; 41: 33-8

79 Sinjab B, Martinian L, Sisodiya SM, Thom M. Regional thalamic neuropathology in patients with hippocampal sclerosis and epilepsy: a postmortem study. *Epilepsia* 2013; 54: 2125-33

80 Shields LB, Hunsaker DM, Hunsaker JC, 3rd, Parker JC, Jr. Sudden unexpected death in epilepsy: neuropathologic findings. *The American journal of forensic medicine and pathology* 2002; 23: 307-14

81 Hermann BP, Bayless K, Hansen R, Parrish J, Seidenberg M. Cerebellar atrophy in temporal lobe epilepsy. *Epilepsy & behavior* : E&B 2005; 7: 279-87

82 Kimmerly DS. A review of human neuroimaging investigations involved with central autonomic regulation of baroreflex-mediated cardiovascular control. *Autonomic neuroscience : basic & clinical* 2017:

83 Scorza FA, Terra VC, Arida RM, Sakamoto AC, Harper RM. Sudden death in a child with epilepsy: potential cerebellar mechanisms? *Arquivos de neuro-psiquiatria* 2011; 69: 707-10

84 Lacuey N, Zonjy B, Theerannaew W, Loparo KA, Tatsuoka C, Sahadevan J, Lhatoo SD. Left-insular damage, autonomic instability, and sudden unexpected death in epilepsy. *Epilepsy & behavior* : E&B 2016; 55: 170-3

85 Li J, Ming Q, Lin W. The insula lobe and sudden unexpected death in epilepsy: a hypothesis. *Epileptic disorders : international epilepsy journal with videotape* 2017; 19: 10-4

86 Chen D, Si Y, He J, Deng Y, Chen T, He YJ, Liu L, Zhou D. Terminal seizure frequency and its relation to SUDEP. *Epilepsy & behavior* : E&B 2017; 70: 173-6



- 87 Leal-Campanario R, Alarcon-Martinez L, Rieiro H, Martinez-Conde S, Alarcon-Martinez T, Zhao X, LaMee J, Popp PJ, Calhoun ME, Arribas JI, Schlegel AA, Stasi LL, Rho JM, Inge L, Otero-Millan J, Treiman DM, Macknik SL. Abnormal Capillary Vasodynamics Contribute to Ictal Neurodegeneration in Epilepsy. *Scientific reports* 2017; 7: 43276
- 88 Tovar-y-Romo LB, Penagos-Puig A, Ramirez-Jarquín JO. Endogenous recovery after brain damage: molecular mechanisms that balance neuronal life/death fate. *Journal of neurochemistry* 2016; 136: 13-27
- 89 Feast A, Martinian L, Liu J, Catarino CB, Thom M, Sisodiya SM. Investigation of hypoxia-inducible factor-1alpha in hippocampal sclerosis: a postmortem study. *Epilepsia* 2012; 53: 1349-59
- 90 Nascimento FA, Tseng ZH, Palmiere C, Maleszewski JJ, Shiomi T, McCrillis A, Devinsky O. Pulmonary and cardiac pathology in sudden unexpected death in epilepsy (SUDEP). *Epilepsy & behavior : E&B* 2017; 73: 119-25
- 91 Balofsky A, George J, Papadakos P. Neuropulmonology. *Handbook of clinical neurology* 2017; 140: 33-48
- 92 Kennedy JD, Hardin KA, Parikh P, Li CS, Seyal M. Pulmonary edema following generalized tonic clonic seizures is directly associated with seizure duration. *Seizure* 2015; 27: 19-24
- 93 Neff JL, Lin PT. An Unexpected Finding in SUDEP Involving a Child: Focal Myocardial Infarct Adjacent to Bundle of His. *Journal of forensic sciences* 2017:
- 94 Ravindran K, Powell KL, Todaro M, O'Brien TJ. The pathophysiology of cardiac dysfunction in epilepsy. *Epilepsy Res* 2016; 127: 19-29
- 95 Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, Davis AM, Thompson T, Connell V, Wallace J, Naylor C, Crawford J, Love DR, Hallam L, White J, Lawrence C, Lynch M, Morgan N, James P, du Sart D, Puranik R, Langlois N, Vohra J, Winship I, Atherton J, McGaughan J, Skinner JR, Semsarian C. A Prospective Study of Sudden Cardiac Death among Children and Young Adults. *The New England journal of medicine* 2016; 374: 2441-52
- 96 Bagnall RD, Crompton DE, Petrovski S, Lam L, Cutmore C, Garry SI, Sadleir LG, Dibbens LM, Cairns A, Kivity S, Afawi Z, Regan BM, Duflou J, Berkovic SF, Scheffer IE, Semsarian C. Exome-based analysis of cardiac arrhythmia, respiratory control, and epilepsy genes in sudden unexpected death in epilepsy. *Annals of neurology* 2016; 79: 522-34
- 97 Shmueli S, Sisodiya SM, Gunning WB, Sander JW, Thijs RD. Mortality in Dravet syndrome: A review. *Epilepsy & behavior : E&B* 2016; 64: 69-74
- 98 Cooper MS, McIntosh A, Crompton DE, McMahon JM, Schneider A, Farrell K, Ganesan V, Gill D, Kivity S, Lerman-Sagie T, McLellan A, Pelekanos J, Ramesh V, Sadleir L, Wirrell E, Scheffer IE. Mortality in Dravet syndrome. *Epilepsy research* 2016; 128: 43-7
- 99 Devinsky O. Sudden, unexpected death in epilepsy. *The New England journal of medicine* 2011; 365: 1801-11
- 100 Kalume F, Westenbroek RE, Cheah CS, Yu FH, Oakley JC, Scheuer T, Catterall WA. Sudden unexpected death in a mouse model of Dravet syndrome. *The Journal of clinical investigation* 2013; 123: 1798-808
- 101 Szurhaj W, Troussiere AC, Logier R, Derambure P, Tyvaert L, Semah F, Ryvlin P, De Jonckheere J. Ictal changes in parasympathetic tone: Prediction of postictal oxygen desaturation. *Neurology* 2015; 85: 1233-9
- 102 Tsai MH, Chan CK, Chang YC, Yu YT, Chuang ST, Fan WL, Li SC, Fu TY, Chang WN, Liou CW, Chuang YC, Ng CC, Hwang DY, Lim KS. DEPDC5 mutations in familial and sporadic focal epilepsy. *Clinical genetics* 2017:
- 103 Nascimento FA, Borlot F, Cossette P, Minassian BA, Andrade DM. Two definite cases of sudden unexpected death in epilepsy in a family with a DEPDC5 mutation. *Neurology Genetics* 2015; 1: e28
- 104 Doumlele K, Friedman D, Buchhalter J, Donner EJ, Louik J, Devinsky O. Sudden Unexpected Death in Epilepsy Among Patients With Benign Childhood Epilepsy With Centrotemporal Spikes. *JAMA neurology* 2017; 74: 645-9

- 105 Nei M, Sperling MR, Mintzer S, Ho RT. Long-term cardiac rhythm and repolarization abnormalities in refractory focal and generalized epilepsy. *Epilepsia* 2012; 53: e137-40
- 106 Schuele SU. Effects of seizures on cardiac function. *Journal of clinical neurophysiology* : official publication of the American Electroencephalographic Society 2009; 26: 302-8
- 107 Chyou JY, Friedman D, Cerrone M, Slater W, Guo Y, Taupin D, O'Rourke S, Priori SG, Devinsky O. Electrocardiographic features of sudden unexpected death in epilepsy. *Epilepsia* 2016; 57: e135-9
- 108 Coll M, Allegue C, Partemi S, Mates J, Del Olmo B, Campuzano O, Pascali V, Iglesias A, Striano P, Oliva A, Brugada R. Genetic investigation of sudden unexpected death in epilepsy cohort by panel target resequencing. *International journal of legal medicine* 2016; 130: 331-9
- 109 Hirsch LJ, Donner EJ, So EL, Jacobs M, Nashef L, Noebels JL, Buchhalter JR. Abbreviated report of the NIH/NINDS workshop on sudden unexpected death in epilepsy. *Neurology* 2011; 76: 1932-8
- 110 Galli F, Vignoli A, Canevini MP, Cerioli G, Vegni E. Sudden unexpected death in epilepsy (SUDEP) disclosure in pediatric epilepsy: An Italian survey on "to tell or not to tell". *Epilepsy & behavior* : E&B 2017; 67: 33-8
- 111 Donner EJ, Camfield P, Brooks L, Buchhalter J, Camfield C, Loddenkemper T, Wirrell E. Understanding Death in Children With Epilepsy. *Pediatric neurology* 2017; 70: 7-15
- 112 Jones LA, Thomas RH. Sudden death in epilepsy: Insights from the last 25 years. *Seizure* 2017; 44: 232-6
- 113 Louik J, Doumlele K, Hussain F, Crandall L, Buchhalter J, Hesdorffer D, Donner E, Devinsky O, Friedman D. Experiences with premorbid SUDEP discussion among participants in the North American SUDEP Registry (NASR). *Epilepsy & behavior* : E&B 2017; 70: 131-4
- 114 McLean B, Shankar R, Hanna J, Jory C, Newman C. Sudden unexpected death in epilepsy: measures to reduce risk. *Practical neurology* 2017; 17: 13-20
- 115 Lapham G, Gaillard WD, Sexter J, Berl MM. Increasing Awareness of Sudden Death in Pediatric Epilepsy Together. *Pediatrics* 2017; 139:
- 116 Hampel KG, Rocamora Zuniga R, Quesada CM. Unravelling the mysteries of sudden unexpected death in epilepsy. *Neurologia* 2017:
- 117 Friedman D. Epilepsy: A new guideline on sudden unexpected death in epilepsy. *Nature reviews Neurology* 2017; 13: 388-9
- 118 Berl MM, Goodkin HP, Kroner BL, Bumbut A, Lapham G, Gaillard WD. Sudden Death in Epilepsy: Knowledge among Pediatric Providers. *The Journal of pediatrics* 2017:
- 119 Nisbet T, Turbull S, Mulhern S, Razvi S. 'Breaking Good News': Neurologists' experiences of discussing SUDEP with patients in Scotland. *Epilepsy & behavior* : E&B 2017; 70: 72-9
- 120 Thurman DJ, Logroscino G, Beghi E, Hauser WA, Hesdorffer DC, Newton CR, Scorza FA, Sander JW, Tomson T, Epidemiology Commission of the International League Against E. The burden of premature mortality of epilepsy in high-income countries: A systematic review from the Mortality Task Force of the International League Against Epilepsy. *Epilepsia* 2017; 58: 17-26
- 121 Lhatoo S, Noebels J, Whittemore V, Research NCFs. Sudden unexpected death in epilepsy: Identifying risk and preventing mortality. *Epilepsia* 2015; 56: 1700-6
- 122 Ahmad MU, Sharif KA, Qayyum H, Ehsanullah B, Balyasnikova S, Wale A, Shanmuganandan A, Siddiqui MRS, Athanasiou T, Kemp GJ. Assessing the use of magnetic resonance imaging virtopsy as an alternative to autopsy: a systematic review and meta-analysis. *Postgraduate medical journal* 2017:
- 123 van Rijn RR, Beek EJ, van de Putte EM, Teeuw AH, Nikkels PGJ, Duijst W, Nievelstein RA, Dutch NG. The value of postmortem computed tomography in paediatric natural cause of death: a Dutch observational study. *Pediatric radiology* 2017:
- 124 Reeves C, Tachrount M, Thomas D, Michalak Z, Liu J, Ellis M, Diehl B, Misericocchi A, McEvoy AW, Eriksson S, Yousry T, Thom M. Combined Ex Vivo 9.4T MRI and Quantitative Histopathological Study in Normal and Pathological Neocortical Resections in Focal Epilepsy. *Brain pathology* 2016; 26: 319-33

- 125 Zucca I, Milesi G, Medici V, Tassi L, Didato G, Cardinale F, Tringali G, Colombo N, Bramerio M, D'Incerti L, Freri E, Morbin M, Fugnanesi V, Figini M, Spreafico R, Garbelli R. Type II focal cortical dysplasia: Ex vivo 7T magnetic resonance imaging abnormalities and histopathological comparisons. *Annals of neurology* 2016; 79: 42-58
- 126 Drummond ES, Nayak S, Ueberheide B, Wisniewski T. Proteomic analysis of neurons microdissected from formalin-fixed, paraffin-embedded Alzheimer's disease brain tissue. *Scientific reports* 2015; 5: 15456
- 127 Thom M, Liu JY, Thompson P, Phadke R, Narkiewicz M, Martinian L, Marsdon D, Koeppe M, Caboclo L, Catarino CB, Sisodiya SM. Neurofibrillary tangle pathology and Braak staging in chronic epilepsy in relation to traumatic brain injury and hippocampal sclerosis: a post-mortem study. *Brain : a journal of neurology* 2011; 134: 2969-81
- 128 Leestma JE, Walczak T, Hughes JR, Kalelkar MB, Teas SS. A prospective study on sudden unexpected death in epilepsy. *Annals of neurology* 1989; 26: 195-203
- 129 Earnest MP, Thomas GE, Eden RA, Hossack KF. The sudden unexplained death syndrome in epilepsy: demographic, clinical, and postmortem features. *Epilepsia* 1992; 33: 310-6
- 130 Natelson BH, Suarez RV, Terrence CF, Turizo R. Patients with epilepsy who die suddenly have cardiac disease. *Archives of neurology* 1998; 55: 857-60
- 131 Antoniuk SA, Oliva LV, Bruck I, Malucelli M, Yabumoto S, Castellano JL. Sudden unexpected, unexplained death in epilepsy autopsied patients. *Arquivos de neuro-psiquiatria* 2001; 59: 40-5
- 132 Davis GG, McGwin G, Jr. Comparison of heart mass in seizure patients dying of sudden unexplained death in epilepsy to sudden death due to some other cause. *The American journal of forensic medicine and pathology* 2004; 25: 23-8
- 133 S PCT, Dalager-Pedersen S, Baandrup U, Dam M, Vesterby-Charles A. Sudden unexpected death in epilepsy: is death by seizures a cardiac disease? *The American journal of forensic medicine and pathology* 2005; 26: 99-105
- 134 Glasscock E. Genomic biomarkers of SUDEP in brain and heart. *Epilepsy & behavior : E&B* 2014; 38: 172-9
- 135 Goldman AM, Behr ER, Semsarian C, Bagnall RD, Sisodiya S, Cooper PN. Sudden unexpected death in epilepsy genetics: Molecular diagnostics and prevention. *Epilepsia* 2016; 57 Suppl 1: 17-25
- 136 Hata Y, Yoshida K, Kinoshita K, Nishida N. Epilepsy-related sudden unexpected death: targeted molecular analysis of inherited heart disease genes using next-generation DNA sequencing. *Brain Pathol* 2017; 27: 292-304
- 137 Wegiel J, Schanen NC, Cook EH, Sigman M, Brown WT, Kuchna I, Nowicki K, Imaki H, Ma SY, Marchi E, Wierzbica-Bobrowicz T, Chauhan A, Chauhan V, Cohen IL, London E, Flory M, Lach B, Wisniewski T. Differences between the pattern of developmental abnormalities in autism associated with duplications 15q11.2-q13 and idiopathic autism. *Journal of neuropathology and experimental neurology* 2012; 71: 382-97
- 138 Moller RS, Heron SE, Larsen LH, Lim CX, Ricos MG, Bayly MA, van Kempen MJ, Klinkenberg S, Andrews I, Kelley K, Ronen GM, Callen D, McMahon JM, Yendle SC, Carvill GL, Mefford HC, Nabbout R, Poduri A, Striano P, Baglietto MG, Zara F, Smith NJ, Pridmore C, Gardella E, Nikanorova M, Dahl HA, Gellert P, Scheffer IE, Gunning B, Kragh-Olsen B, Dibbens LM. Mutations in KCNT1 cause a spectrum of focal epilepsies. *Epilepsia* 2015; 56: e114-20
- 139 Veeramah KR, O'Brien JE, Meisler MH, Cheng X, Dib-Hajj SD, Waxman SG, Talwar D, Girirajan S, Eichler EE, Restifo LL, Erickson RP, Hammer MF. De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. *American journal of human genetics* 2012; 90: 502-10
- 140 Macey PM, Ogren JA, Kumar R, Harper RM. Functional Imaging of Autonomic Regulation: Methods and Key Findings. *Frontiers in neuroscience* 2015; 9: 513





