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#### Heart Rate Variability in Epilepsy: A Potential Biomarker of SUDEP Risk

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#### Summary

<u>Objective</u>: Sudden unexpected death in epilepsy (SUDEP) is a tragic and devastating event for which the underlying pathophysiology remains poorly understood; this study investigated whether abnormalities in heart rate variability (HRV) are linked to SUDEP in patients with epilepsy due to mutations in sodium channel (SCN) genes.

<u>Methods</u>: We retrospectively evaluated HRV in epilepsy patients using EEG studies, in order to study the potential contribution of autonomic dysregulation to SUDEP risk. We extracted HRV data, in wakefulness and sleep, from 80 patients with drug-resistant epilepsy; 40 patients with mutations in SCN genes, and 40 control patients with non-SCN drug-resistant epilepsy. From the SCN group, 10 patients had died of SUDEP. We compared HRV between SUDEP and non-SUDEP groups; specifically studying awake HRV and sleep:awake HRV ratios. <u>Results</u>: The SUDEP patients had the most severe autonomic dysregulation, showing lower awake HRV and either extremely high or low ratios of sleep-to-awake HRV in a subgroup analysis. A secondary analysis comparing the SCN and non-SCN groups indicated that

autonomic dysfunction was slightly worse in the SCN epilepsy group.

*Signficance*: These findings suggest that autonomic dysfunction is associated with SUDEP risk in patients with epilepsy due to sodium channel mutations. The relationship of HRV to SUDEP merits further study; HRV may eventually have potential as a biomarker of SUDEP risk, which would allow for more informed counseling of patients and families, and also serve as a useful outcome measure for research aimed at developing therapies and interventions to reduce SUDEP risk.

Key Words: Sudden death, SCN1A, Dravet syndrome, autonomic, sodium channel, sleep

#### Introduction

Sudden unexpected death in epilepsy (SUDEP) is a rare but tragic phenomenon, defined as "... death in people with epilepsy occurring in the absence of a known structural cause of death ..."<sup>1,2</sup> The frequency of SUDEP is 1.2 per 1000 patient-years in people with epilepsy overall; those with drug-resistant epilepsy have increased risk, estimated at 4.2 per 1000 patient-years.<sup>3-5</sup> Patients at particularly high risk are those with developmental and epileptic encephalopathies due to sodium channel (SCN) gene mutations. The most common of these is Dravet syndrome, occurring with *SCN1A* mutations in > 80% of cases,<sup>6</sup> with a SUDEP rate of 9.32 per 1000 patient-years.<sup>7</sup> Variants in other SCN genes, including *SCN2A*, *SCN4A*, *SCN5A*, *SCN8A*, *SCN10A* and *SCN11A*, have also been associated with SUDEP in human and animal models.<sup>8-11</sup>

SUDEP risk factors have been identified, including a major association with uncontrolled tonicclonic seizures, as well as medication noncompliance, male sex, epilepsy onset before 16 years, and duration of epilepsy greater than 15 years.<sup>5</sup> Despite recognition of these risk factors, counseling patients precisely regarding SUDEP risk is challenging, with a recent review by Devinsky et al stating "Seizures and SUDEP are probabilistic events that can not be accurately predicted."<sup>5</sup> There is a desperate need for biomarkers to identify patients at greatest risk so that prevention strategies can be devised and put in place. Currently no quantitative biomarkers of SUDEP risk exist, though one attractive candidate is heart rate variability (HRV).

HRV is an index of autonomic activity, with increased HRV reflecting dominance of parasympathetic over sympathetic activity, whereas, decreased HRV suggests a shift towards sympathetic dominance.<sup>12</sup> HRV increases in sleep and decreases in wakefulness. Lower HRV was originally shown to be predictive of mortality in cardiac and elderly populations,<sup>13,14</sup> and has more recently become a topic of interest in epilepsy. Interictal HRV is lower in individuals with epilepsy, including those who are newly diagnosed and drug naïve.<sup>15,16</sup> However, the pattern of dysregulation is not as simple as a uniform decrease across sleep and wakefulness. A recent study found that people with generalized epilepsy had enhanced increase in HRV, reflecting parasympathetic tone, in sleep compared to healthy controls.<sup>17</sup> Unfortunately, the data on sleep:wake HRV differences are limited, primarily because many studies have evaluated HRV using cardiac monitoring devices such as Holter monitors which do not allow for a distinction between sleep and awake states.

Interrogating sleep-wake differences in HRV may be crucial as autonomic dysregulation has been proposed as a factor in SUDEP.<sup>18</sup> Although decreased awake HRV has been shown to correlate with other SUDEP risk factors,<sup>19</sup> a direct link between HRV and SUDEP has not yet been demonstrated.<sup>20</sup> Epilepsy patients rarely have Holter studies or other prolonged cardiac monitoring, so retrospective heart rate data are not usually available for individuals suffering SUDEP. We hypothesized that awake HRV would be lower, and sleep:awake HRV ratios higher, in patients with SCN mutations who go on to suffer SUDEP. We also hypothesized that awake HRV higher in patients with SCN-related epilepsy when compared to those with drug-resistant epilepsy due to other causes, since a prior study had suggested HRV derangements were more severe in Dravet syndrome than in other epilepsy

phenotypes.<sup>21</sup> To test these hypotheses, we extracted heart rate data from EEG recordings of patients with drug-resistant epilepsy, allowing us to investigate whether lower HRV in wakefulness, and sleep-wake HRV differences, could be predictive of SUDEP risk in certain patients with epilepsy.

#### Methods

We analysed routine and prolonged telemetry EEG studies of patients with drug-resistant epilepsy and SCN mutations, and a control group of age-matched patients with non-SCN drug-resistant epilepsy. Inclusion criteria for the SCN epilepsy group were: mutation in a sodium channel gene associated with epilepsy and an EEG with at least 5 minutes of recording in wakefulness and/or sleep without seizures and with an interpretable ECG lead derivation. The non-SCN group had the same inclusion criteria, excepting the SCN mutation, and included patients with either a clear non-genetic cause for epilepsy (e.g. perinatal brain injury), a non-SCN genetic cause (e.g. *DEPDC5* mutation), and/or negative screening for a gene panel including major SCN genes (*SCN1A*, *SCN1B*, *SCN2A*, *SCN8A*, *SCN9A*). Classification of SUDEP followed the Nashef et al (2012) criteria; and was based on available clinical data and the post-mortem report, if performed. We excluded any patients known to have cardiac disease/arrhythmias or who were known to be receiving beta-blockers or other anti-arrhythmic agents.

Consecutive R-R intervals were manually measured using EEG software caliper tools and recorded for a five-minute period in the awake state, as well as a five-minute period in stage 1/2 sleep if available. Whenever possible, a time period in wakefulness was chosen when the patient was resting quietly and not eating, with no seizure in the previous 10 minutes, with corroborative video being available in most cases. If a patient had a major convulsive event (i.e. a tonic-clonic or tonic seizure) during the EEG, we endeavoured to find a 5-minute period at least 1-2 hours removed for HRV evaluation. Time period selection and data collection was performed by the same pediatric neurologist (KAM) for all cases.

HRV measures were calculated using Microsoft Excel. The primary outcome measure was root mean square of successive differences (RMSSD), a time domain HRV measure calculated as the square root of the average of the squared differences between successive R-R intervals over a period of time  $(\sqrt{\frac{1}{N-1}}(\sum_{i=1}^{N-1} {\{(R-R)_{i+1} - (R-R)_i\}}^2})$  where *N* is the number of R-R intervals in the period of analysis). RMSSD was chosen as the primary outcome measure because this variable gives a good measure of beat-to-beat variability and should be sensitive to even subtle variations in autonomic balance. Secondary HRV outcome measures were the standard deviation of R-R intervals over the period of time (SDNN), and the percentage of consecutive R-R intervals differing by more than 50 milliseconds (pNN50). For patients for whom a fiveminute sleep interval was available, the ratio of sleep to wakefulness for each HRV variable was calculated.

We used one-tailed Wilcoxon ranked sums test for comparisons evaluating whether awake HRV was lower in those who had SUDEP when compared to those who were alive at last review or died of other causes, as well as for comparisons of awake HRV measures between the SCN and non-SCN groups (including SUDEP cases). Two-tailed Wilcoxon tests were used for comparisons of sleep:wake HRV ratios, as there was less confidence in the hypothesis that this would be higher in SUDEP versus non-SUDEP and SCN versus non-SCN comparisons. Two-tailed tests were also used for sleep HRV measures, age and NN. We did not remove outliers in our primary analysis, but did incorporate Tukey's test<sup>22</sup> to assess the effect of removing outliers in a secondary analysis.

Since sleep:awake HRV ratios appeared to be either extremely high or extremely low, we performed a post hoc analysis comparing deviation from the mean  $(\Delta x_a = |x_a - \bar{x}|)$  between the SUDEP and non-SUDEP groups.

The study was approved by the University of Calgary Conjoint Health Research Ethics Board (ID REB16-0218) and the Human Research Ethics Committee of Austin Health (Project No. H2007/02961).

#### Results

#### Characteristics of Epilepsy Patients Studied

40 patients (27 females) with SCN mutations participated, aged 15 months to 38 years (median age 7.3 y) at the time of their combined EEG-ECG recording. This included 38 with *SCN1A* mutations and Dravet syndrome, and one each with *SCN2A* and *SCN8A* mutations (both with early infantile developmental and epileptic encephalopathy). We also recruited 40 age-matched control patients with non-SCN drug-resistant epilepsy; this group was comprised of patients who either had a clear non-genetic cause for epilepsy (e.g. perinatal brain injury), a non-SCN genetic cause (e.g. *DEPDC5* mutation), and/or negative screening for a gene panel including major SCN genes (*SCN1A*, *SCN1B*, *SCN2A*, *SCN8A*, *SCN9A*). Based on the most up-to-date information in the research file, none of the non-SCN patients had died of SUDEP.

From the SCN epilepsy group, ten patients were classified as SUDEP (Table 1). Based on the 2012 consensus definitions,<sup>1</sup> four patients had definite SUDEP, five probable SUDEP and another possible SUDEP. The interval between the EEG studied and the patient's death ranged from three weeks to 10 years (median 2.5 y, standard deviation 4.0 y). The "possible SUDEP" case was a 21-month-old girl who slept between her parents; she appeared well at 1 am and was dead at 3 am. Her post-mortem showed pneumonia which was considered a competing cause of death; however, the index of suspicion for SUDEP was very high.

#### Comparing HRV between SUDEP and non-SUDEP Epilepsy Patients

Awake RMSSD was lower in the group of SCN epilepsy patients who suffered SUDEP when compared to the non-SUDEP patients, with median RMSSD of SUDEP patients being half of that of the non-SUDEP group (12.0 ms versus 24.6 ms, p = 0.039; Table 2). One of the secondary HRV measures, pNN50, was also lower in the SUDEP group (p = 0.018), while the other, SDNN, trended lower but did not meet statistical significance (p = 0.07).

When examined on a patient-by-patient basis, however, we noted that while eight SUDEP patients had very low awake HRV values, one 11-year-old boy had unusually high values, and a 38-year-old woman (the oldest patient) intermediate values. If the latter two patients are removed from the analysis as outliers, based on being more than 1.5 interquartile ranges from the mean,<sup>22</sup> the difference in awake RMSSD between SUDEP and non-SUDEP becomes even more dramatic (median 9.1 ms versus 24.6 ms,  $p = 1.8 \times 10^{-3}$ ). With outliers removed, awake pNN50 and SDNN are also both clearly lower in the SUDEP group ( $p = 8.3 \times 10^{-4}$  and 6.5 x  $10^{-3}$ , respectively).

The sleep:awake ratio of HRV could be evaluated in five individuals from the SUDEP group who had at least five minutes of sleep recorded. Three had very high sleep:awake RMSSD ratios, with two having ratios far higher than any in the non-SUDEP group. The other two patients had very low sleep:awake HRV ratios, actually showing slight decreases in HRV in sleep, and equivalent to the lower end of the non-SUDEP patients (Figure 1A). In order to study this apparent tendency towards extremes, we compared difference from the mean ( $\Delta x_a = |x_a - \bar{x}|$ ) and found that the SUDEP group tended to deviate by a much greater degree (p = 5.3 x 10<sup>-4</sup>; Figure 1B).

For all these data, it should be noted that the living SCN patients may still have SUDEP at a later date.

#### SCN versus Non-SCN Epilepsy

Next, we compared all patients with SCN mutations (including those suffering SUDEP) with non-SCN drug-resistant epilepsy patients. Awake HRV measures were lower in the SCN group compared with the non-SCN epilepsy group (p < 0.05, Table 3); the primary outcome measure, (RMSSD), was 19% lower in the SCN epilepsy group. The secondary awake HRV measures, (SDNN) and (pNN50), were 26% and 40% lower in the SCN group, respectively. In addition, non-significant trends were also observed for lower sleep HRV and greater sleep:awake ratios in the SCN group; however, larger cohort sizes are needed to determine if these are meaningful trends.

We plotted age against awake RMSSD for both groups to investigate whether awake HRV varies with age; there was no significant correlation between age and HRV in either group, with  $r^2$  values of 0.01 and 0.06 for SCN and non-SCN epilepsy, respectively (Supplementary Figure).

#### Discussion

We present evidence that individuals who have died of SUDEP associated with SCN mutations have more severe autonomic dysregulation than individuals with drug-resistant epilepsy without SUDEP. Analysing HRV we found that awake HRV was lower, and sleep:awake HRV ratio outside of the normal range, in those who later suffered SUDEP. Our findings emphasise the importance of considering arousal state when interpreting HRV data, an issue that has only been looked at previously in a cohort with generalized epilepsy.<sup>17</sup> This has important implications for our understanding of the pathophysiology of SUDEP, as well as the clinical management of patients with drug-resistant epilepsy. We note, however, that in a retrospective study such as this, certain biases are inevitable, and these should be taken into account when evaluating the data; due to study design, complete observer blinding was not possible.

#### SUDEP Pathophysiology

The mechanism by which autonomic dysfunction increases SUDEP risk remains unclear. Our data show a direct association between interictal HRV and SUDEP; however, a second autonomic pathological "hit" may be necessary for death to occur. There have been two published cases of sudden death in patients with seizures in which HRV analysis in the acute premorbid period was undertaken in the setting of video-EEG monitoring. An adult male with focal cortical dysplasia showed progressive increase in HRV in the hours and days before SUDEP, following a focal seizure evolving to generalized convulsion.<sup>23</sup> The second case was a girl with a chromosomal disorder and febrile seizures who suffered sudden death without a clear inciting ictal event.<sup>24</sup> Her baseline HRV was abnormally low (RMSSD 6-8 ms) but rose suddenly eight minutes prior to death, immediately following onset of diffuse EEG suppression. In both, the patients demonstrated altered HRV leading up to death, with a sudden centrally-mediated event

associated with the terminal deterioration. There is also a report of a patient who showed progressive decrease in HRV in the months leading up to SUDEP, though only three data points were available so interpretation was difficult.<sup>25</sup>

If a second hit is needed to trigger SUDEP in vulnerable individuals, seizures are a logical culprit, since most cases of SUDEP occur post-ictally.<sup>26,27</sup> Peri-ictal changes in HRV have been extensively studied and partially depend on seizure type. In general, HRV decreases during the ictal phase, gradually returning to baseline in the post-ictal period.<sup>28-31</sup> However, a patient who aspirates during a seizure might also have a sudden increase in HRV when vagal tone increases during the act of coughing or choking. One possibility is that people with epilepsy and severe autonomic dysfunction are closer to intolerable extremes of both parasympathetic and sympathetic tone (Figure 2). An inciting event such as a seizure or vagal event, could push such individuals into a fatal autonomic deterioration.

Our data should be interpreted with caution, however. Given the data were collected retrospectively, we could not control for confounding factors which might also influence HRV. Such factors include anti-epileptic medications, seizure frequency, and co-morbid medical conditions. Additionally, not all of the non-SCN group were sequenced for all SCN genes, so it is theoretically possible that a patient with post-traumatic epilepsy could also have a *SCN1A* variant. We also note that the time intervals between HRV measurement and SUDEP varied considerably; for some individuals, HRV might have changed by the time of their death.

#### Differences Between SCN and Non-SCN Epilepsy

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We studied patients with epilepsy due to SCN mutations because high rates of SUDEP have been reported in these populations.<sup>7,32</sup> While SUDEP has not been studied, one study found that HRV, measured on 24-hour Holter monitors, was lower in patients with Dravet syndrome compared to people with epilepsy and healthy children.<sup>21</sup> Because of the methodology used, this study was not able to evaluate sleep:awake differences.

In our study, the SCN group had lower awake HRV values. For sleep HRV and sleep:awake HRV ratio, we did not find significant differences between the SCN and non-SCN drug-resistant epilepsy groups. The differences between the SCN and non-SCN groups are important in considering how broadly these findings can be extrapolated; for example, we do not know if all genetic etiologies carry a similar SUDEP risk. The same patterns of altered HRV may be present in non-SCN drug-resistant epilepsy patients who suffer SUDEP; however, further studies are required to clarify this. A smaller scale study of adults with various forms of epilepsy, did not find a difference in HRV when comparing SUDEP and non-SUDEP cases, suggesting our results may not extrapolate well to adult, non-SCN populations.<sup>20</sup> When autonomic dysfunction is present in non-SCN patients who suffer SUDEP, other factors, such as insular damage, may contribute in less predictable ways.<sup>33</sup>

#### Future Directions

There may be clinical management implications from the correlation of altered HRV with SUDEP, though further study is required. Following establishment of normal and abnormal ranges, HRV measurement could potentially be incorporated into standard EEG protocols and used as a SUDEP risk index. This would greatly enhance clinicians' abilities to counsel patients and families regarding the possibility of SUDEP and highlight specific interventions or precautions (e.g. internal or external defibrillators) that could be undertaken in high risk cases.

There are also treatment implications if HRV proves a reliable SUDEP biomarker, as certain therapies may have a beneficial effect on HRV. Children with infantile spasms have low HRV at the time of diagnosis, but this improves following treatment.<sup>34,35</sup> As well, therapeutic application of vagal nerve stimulation (VNS) is associated with a reduction in T wave alternans, a measure of cardiac instability, making VNS an attractive candidate to reduce SUDEP risk; however, the effects of VNS on HRV have not yet been thoroughly studied, and a clear reduction in SUDEP incidence post-VNS implantation has not been shown.<sup>36,37</sup> In the future, clinicians may use sequential HRV measurements to assess whether a patient's SUDEP risk had changed on a given

treatment. Changes in HRV could be an additional clinically relevant outcome measure when assessing the efficacy of new therapies.

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#### **Disclosure of Potential Conflicts of Interest**

Dr. Myers received a travel grant from Zynerba, and has received research grant funding from Citizens United for Research in Epilepsy and Supporting Families with Koolen-de Vries Syndrome. Dr. Zuberi receives research funding from Epilepsy Research UK, Dravet Syndrome UK, Glasgow Children's Hospital Charity, and UCB Pharma. He has attended advisory boards and received speaker's honoraria paid to his institution's charitable fund from Zogenix, GW Pharma, and Biocodex. Dr. Sadleir serves on the epilepsy advisory board for Nutricia and receives funding from Health Research Council of New Zealand grant 15/070, Cure Kids, and the Ted and Mollie Carr Endowment Trust. Dr. Scheffer serves on the editorial boards of *Neurology*® and *Epileptic Disorders* and has served on the editorial board of *Annals of Neurology Epilepsy Currents*; has received revenue from Medvet Science and Bionomics for patents; serves on the epilepsy advisory board for Nutricia; may accrue future revenue on a pending patent on therapeutic compound; has received speaker honoraria from Athena Diagnostics, UCB, GSK, Eisai, and Transgenomics; has received funding for travel from Athena Diagnostics, UCB, and GSK; and receives/has received research support from the National Health and Medical Research Council, Australian Research Council, NIH, Health Research Council of New Zealand, March of Dimes, Weizmann Institute, Citizens United for Research in Epilepsy, US Department of Defense, and Perpetual Charitable Trustees. None of the remaining authors has any conflicts of interest to disclose.

#### **Ethical Publication Statement**

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

#### Key Points

- Heart rate variability was studied in in 40 patients with epilepsy due to sodium channel mutations and 40 with other drug-resistant epilepsy.

- Awake heart rate variability was lower in patients with sodium channel mutations who suffered SUDEP.

- Awake heart rate variability was lower in epilepsy patients SCN mutations when compared to those with non-SCN drug-resistant epilepsy.

- Autonomic dysfunction is a likely contributing factor in SUDEP in patients with epilepsy due to SCN mutations.

- Heart rate variability has potential as a biomarker of SUDEP risk.

# Script

#### **Figure Legends**

**Figure 1.** Subgroup analysis of sleep:awake ratios of HRV. (A) Ratio of RMSSD from sleep to wakefulness is shown for SUDEP and non-SUDEP drug-resistant epilepsy patients. (B) The mean deviation from the average sleep:awake RMSSD ratio ( $Dev_i = |x_i - \bar{x}|$ ).

**Figure 2.** Hypothetical model of autonomic "second hit" SUDEP mechanism. Diurnal variation in HRV is based on data from this study, as well as previous studies <sup>15,17,38</sup>. HRV is lower in patients with epilepsy (blue line) than healthy controls (orange line) during the waking hours and then increases well above the healthy control level at night time. This same pattern is seen in patients who go on to have SUDEP (red line), but is more extreme. With a seizure in wakefulness, there is a further decrease in HRV, which may cause patients with already low awake HRV to enter a pathologic zone of sympathetic hyperactivity. Conversely, if an aspiration event occurs in sleep, the vagal response may elicit pathologically increased parasympathetic tone in a patient with baseline elevated sleep HRV and elevate them above the SUDEP threshold.

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#### Tables

#	Sex	Gene	Age at	Age at	Autopsy	SUDEP
	=	Mutated	EEG (y)	Death (y)		Classification <sup>1</sup>
1	F	SCN2A	1.75	1.8	Pneumonia	Possible SUDEP
2	М	SCNIA	13	16	Not performed	Probable SUDEP
3	F	SCNIA	1.7	11.5	No other cause	Definite SUDEP
4	F	SCN1A	1.5	10.5	No other cause	Definite SUDEP
5	М	SCNIA	7	9	No other cause	Definite SUDEP
6	М	SCN1A	20	28	Not performed	Probable SUDEP
7	F	SCNIA	1.25	3.3	Not performed	Probable SUDEP
8	М	SCNIA	11	14	Not performed	Probable SUDEP
9	F	SCN1A	3	3	Not performed	Probable SUDEP
10	F	SCN1A	38	38	No other cause	Definite SUDEP

Table 1. Sudden unexpected death in epilepsy (SUDEP) cases.

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**Table 2.** HRV in SUDEP versus non-SUDEP patients. Comparison of HRV in 10 SCN epilepsy patients suffering SUDEP versus 70 non-SUDEP patients who were alive at last review or had died of other causes. Sleep data are from 5 SUDEP patients and 45 non-SUDEP who had at least 5 minutes of sleep recorded during EEG.

	SUDEP	Non-SUDEP	p value*
Awake – Median Age (S.D.)	5.3 y (11.8)	8.5 y (10.7)	0.33
Awake – Median NN (S.D.)	480 ms (206)	586 ms (145)	0.19
Awake – Median RMSSD (S.D.)	12.0 ms (31.6)	24.6 ms (22.4)	0.039
Awake – Median SDNN (S.D.)	24.9 ms (24.9)	39.7 ms (23.3)	0.070
Awake – Median pNN50 (S.D.)	0 (18.6)	4.3 (14.1)	0.018
Sleep – Median Age (S.D.)	3.0 y (15.7)	8.7 y (10.9)	0.53
Sleep – Median NN (S.D.)	573 ms (217)	717 ms (200)	0.21
Sleep – Median RMSSD (S.D.)	86.7 ms (82.8)	43.0 ms (61.2)	0.97
Sleep – Median SDNN (S.D.)	74.8 ms (67.1)	39.5 ms (44.4)	0.97
Sleep – Median pNN50 (S.D.)	32.4 (23.1)	22.0 (26.0)	0.98
Sleep:Wake – Median NN (S.D.)	1.26 (0.22)	1.21 (0.17)	0.51
Sleep:Wake – Median RMSSD (S.D.)	4.5 (9.3)	1.6 (1.5)	0.49
Sleep:Wake – Median SDNN (S.D.)	3.2 (2.9)	1.1 (0.9)	0.57

\* p value based on Wilcoxon ranked sum test (1-sided for Awake RMSSD, SDNN and pNN50, 2-sided for all other outcome measures). The primary outcome measure was awake RMSSD; all other variables are secondary outcomes and corrections for multiple comparisons have not been made. Abbreviations: NN = R-R interval; RMSSD = root mean square of successive differences; SDNN = standard deviation of R-R intervals; pNN50 = percentage of consecutive R-R intervals differing by more than 50 milliseconds; S.D. = standard deviation.

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**Table 3**. HRV in SCN versus non-SCN patients. Comparison of patients with SCN epileptic encephalopathies to other drug-resistant epilepsy cases. Awake RMSSD, as well as the secondary awake HRV measures, SDNN and pNN50, were lower in the SCN epilepsy group.

	SCN Epilepsy	Other Drug-resistant	р
		Epilepsy	value*
Median Age (S.D.)	7.3 y (11.4 y)	8.6 y (10.2 y)	0.60
Awake – Median NN (S.D.)	569 ms (137 ms)	586 ms (167 ms)	0.35
Sleep – Median NN (S.D.)	670 ms (166 ms)	729 ms (228 ms)	0.21
Sleep:Awake Median NN (S.D)	1.23 (0.20)	1.22 (0.15)	0.82
Awake – Median RMSSD (S.D.)	15.5 ms (25.1 ms)	31.0 ms (22.0 ms)	0.048
Sleep – Median RMSSD (S.D.)	31.0 ms (65.1 ms)	62.4 ms (61.6 ms)	0.18
Sleep:Awake Median RMSSD (S.D.)	1.6 (4.6)	1.6 (1.4)	0.74
Awake – Median SDNN (S.D.)	32.8 ms (21.4 ms)	43.9 ms (24.0 ms)	0.0083
Sleep – Median SDNN (S.D.)	35.0 ms (49.1 ms)	49.1 ms (44.4 ms)	0.30
Sleep:Awake Median SDNN (S.D.)	1.4 (1.6)	1.1 (0.8)	0.42
Awake – Median pNN50 (S.D.)	0.55 (14.1)	6.2 (14.9)	0.013
Sleep – Median pNN50 (S.D.)	8.9 (25.8)	35.1 (24.5)	0.12

\* p value based on 2-tailed Wilcoxon ranked sum test, with the exception of awake RMSSD, SDNN and pNN50, where 1-tailed Wilcoxon ranked sum tests were used based on hypothesis that SCN epilepsy patients would have lower awake HRV than drug-resistant epilepsy controls. The primary outcome measure was awake RMSSD; p values shown for other variables do not include correction for multiple tests. Abbreviations: NN = R-R interval; RMSSD = root mean square of successive differences; SDNN = standard deviation of R-R intervals; pNN50 = percentage of R-R intervals > 50 milliseconds; S.D. = standard deviation.

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