Author/s:
Soh, MS;Bagnall, RD;Semsarian, C;Scheffer, IE;Berkovic, SF;Reid, CA

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DR. MING SHIUAN SOH (Orcid ID : 0000-0002-5689-2082)

PROF. INGRID E SCHEFFER (Orcid ID : 0000-0002-2311-2174)

PROF. SAMUEL F. BERKOVIC (Orcid ID : 0000-0003-4580-841X)

PROF. CHRISTOPHER ALAN REID (Orcid ID : 0000-0002-1457-8028)

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Title: Rare SUDEP *SCN5A* variants cause changes in channel function implicating cardiac arrhythmia as a cause of death

Ming S. Soh[1], Richard D. Bagnall[2,3], Christopher Semsarian[2,3], Ingrid E. Scheffer[1,4,5,6], Samuel F. Berkovic[4] and Christopher A. Reid[1,4*]

[1]Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, VIC, Australia

[2]Agnes Ginges Centre for Molecular Cardiology at Centenary Institute, The University of Sydney, Sydney, NSW, Australia

[3]Faculty of Medicine and Health, The University of Sydney, NSW, Australia

[4]Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC, Australia

[5]Murdoch Children's Research Institute, The Royal Children's Hospital, Parkville, VIC, Australia

[6]Department of Paediatrics, University of Melbourne, Royal Children's Hospital, VIC, Australia

Correspondence:
Christopher A. Reid
christopher.reid@florey.edu.au
+61 3 9035 6372
ORCID: 0000-0002-1457-8028

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Summary

Sudden Unexpected Death in Epilepsy (SUDEP) is a leading cause of premature death in epilepsy. The underlying pathological mechanisms are likely to be multi-factorial. Cardiac arrhythmia has been suggested as a cause of death in some patients with SUDEP. *SCN5A* encodes the cardiac $Na_V1.5$ sodium channel. *SCN5A* variants that result in either loss or gain of channel function cause cardiac arrhythmias. Rare *SCN5A* variants have been reported in SUDEP cases but the impact of these variants on channel function is unknown. Here we use whole-cell voltage clamp recordings to perform functional analyses of rare *SCN5A* SUDEP variants, p.V223G, p.I397V and p.R523C. Expression and biophysical properties including activation, inactivation and recovery from inactivation were probed. Each *SCN5A* variant significantly impacted human $Na_V1.5$ channel function indicating that they could cause cardiac arrhythmias. The patient carrying the p.R523C variant was on lamotrigine, an antiseizure medication implicated in SUDEP. Therapeutic concentration of lamotrigine caused a slowing of the rate of recovery from inactivation and a hyperpolarizing shift in the voltage of inactivation of human $Na_V1.5$ wild-type, but not p.R523C channels, implicating a gene-by-drug interaction. These data suggest that *SCN5A* arrhythmogenic variants may confer increased risk of sudden death in individuals with epilepsy.

Keywords: SUDEP, epilepsy, cardiac arrhythmia, genetics, ion channels

Introduction

Sudden Unexpected Death in Epilepsy (SUDEP) is a leading cause of premature death for patients with epilepsy [1]. Well-established seizure-related risk factors include frequent tonic-clonic seizures, epilepsy duration, frequent changes in doses of anti-seizure medication and polytherapy [2-4]. However, how seizures lead to death is not well-established and the underlying pathological mechanisms are likely to differ between individuals. The retrospective MORTEMUS study revealed that seizure-mediated terminal apnea preceded terminal asystole suggesting that death was caused by respiratory failure triggered by a tonic-clonic seizure [5]. Another factor widely implicated in SUDEP has been the role of abnormal cardiac rhythms [6]. Variants in genes associated with cardiac arrhythmias occur in up to 15% of SUDEP cases [7]. Recently, we reported that loss-of-function *KCNH2* variants are enriched in a SUDEP population compared to an aged patient population with epilepsy who served as controls as they have effectively 'escaped' SUDEP [8]. Loss-of-function *KCNH2* is a well-established risk factor for long-QT syndrome (LQTS) that increases the risk of 'torsades de pointes', a type of ventricular tachycardia that triggers cardiac arrest [9]. This suggests a model in which genetic risk factors that predispose to cardiac arrhythmia may contribute to SUDEP risk in certain epilepsy patients.

SCN5A encodes the $Na_v1.5$ cardiac voltage-gated sodium channel subunit; variation in this gene is a well-established cause of arrhythmias [9]. Variants in *SCN5A* that give rise to loss- and gain- of channel function lead to Brugada syndrome and LQTS type 3 (LQTS3) respectively [10]. Both these arrhythmia types can trigger sudden cardiac death. Rare *SCN5A* missense variants, including one confirmed *de-novo* variant, have been reported in SUDEP cases implicating them in the cause of death [6, 7, 11]. However, no analysis of the effect of these variants on channel function has been performed. Here, we use whole-cell electrophysiology to determine the impact of the p.V223G, p.I397V and p.R523C *SCN5A* SUDEP variants on channel function. Each *SCN5A* variant caused changes in the biophysical properties of the channel suggesting that they could contribute to arrhythmia risk and, as a consequence, an increased risk of SUDEP.

Methods

SCN5A mutagenesis and cell culture

CHO-K1 cells were transiently transfected with wild-type or variant cDNA using Lipofectamine 3000 (Thermo Fisher Scientific). Cells were also co-transfected with EGFP for selection during recording. Cells were maintained in DMEM F-12 supplemented with

GlutaMAX™, 10% foetal bovine serum, and 1% penicillin-streptomycin (Thermo Fisher Scientific) at 37°C in 5% CO_2 incubator. Experiments were performed 3-4 days after transfection.

Whole-cell voltage clamp electrophysiology

Whole-cell recordings and data acquisition were made using Multiclamp 700B amplifier and pCLAMP 9/DigiData 1440 (Molecular Devices) software (details in Supporting document).

Electrophysiology analysis

Current amplitudes were analyzed offline using AxoGraph v1.7.4 (AxoGraph Scientific) and curves were generated using the Prism v8.1.0 software (GraphPad) (details in Supporting document).

Statistical analysis

One-way ANOVA with Dunnett's post-hoc correction was used for statistical comparison to wild-type values with significance set at $p < 0.05$. Paired *t*-test was used for before-after lamotrigine comparison. All data points are shown as mean ± S.E.M.

Results

Three rare *SCN5A* missense variants have been described in patients who died of SUDEP [6, 7, 11]. p.V223G and p.I397V variants were identified in our cohort study [7] while p.R523C was described in a case report [11]. To determine the functional consequence of the SUDEP *SCN5A* variants, voltage-dependent sodium currents were recorded from transiently-expressing CHO-K1 cells using whole-cell voltage clamp. Voltage protocols to probe activation, inactivation and recovery from inactivation were delivered to cells. All *SCN5A* SUDEP variants generated voltage-dependent sodium currents (Figure 1A-E, Supplementary Figure 1). Current density was significantly smaller for p.V223G, while p.R523C and p.I397V expressed at similar levels to the wild-type channel (Figure 1A-B, Table 1). The impact on the voltage-dependence of activation was mixed with a significant depolarizing shift observed for p.R523C and p.V223G, while a hyperpolarizing shift was noted for p.I397V (Figure 1C-E, Table 1). The impact on the voltage dependence of inactivation was more uniform with significant depolarizing shifts seen in all variants relative to wild-type (Figure 1C-E, Table 1). Recovery from inactivation was significantly faster for p.R532C, but no change was noted for p.V223G and p.1397V (Figure 1F-G, Table 1). These data demonstrate that each rare *SCN5A* SUDEP variant caused significant changes in the biophysical properties of the channel (Table 1).

The patient carrying the p.R523C variant was on lamotrigine, a sodium channel blocker implicated in increased risk of SUDEP, at the time of death (Supplementary Table 1). Therapeutic concentration of lamotrigine (50 μM) shifted inactivation $V_{1/2}$ to more hyperpolarized potentials and slowed the recovery from inactivation in wild-type channels. Lamotrigine did not significantly alter p.R523C biophysical properties (p = 0.06; see Supplementary Figure 2-3 and Supplementary Table 2). The blunted impact of lamotrigine on the rate of recovery from inactivation suggests a gene-by-drug interaction (Supplementary Figure 2A, J, K). Vehicle control has no significant impact on any biophysical properties. These data demonstrate the activity of lamotrigine against human cardiac $Na_V1.5$ channels. A variant-specific impact was noted but the implication for SUDEP risk requires further investigations.

Discussion

In this study we have performed functional analysis of rare *SCN5A* variants associated with SUDEP. Importantly, all three variants changed channel function. The magnitude of the changes in the biophysical properties noted here are in line with those reported previously for variants associated with cardiac arrhythmia and sudden cardiac death [12]. The overall impact of each variant on channel function is complex with multiple different biophysical properties altered. It is therefore challenging to conclude whether the overall impact of these biophysical changes results in a gain- or loss-of-function. However, both loss- and gain-of-function *SCN5A* variants are associated with arrhythmogenic syndromes [10]. It should be noted that our recordings were completed at room temperature, as is usual in the field, and that different characteristics may emerge at higher temperatures. Nevertheless, our data convincingly demonstrates that the three rare *SCN5A* SUDEP variants characterised alter the function of $Na_V1.5$ channels.

Both p.R523C and p.I397V have been described in patients with arrhythmias. p.R523C was described in a proband with long-QT syndrome [13], while p.I397V was reported in an individual with Brugada syndrome [14]. Moreover, other missense mutations at position I397, p.I397T and the pathogenic gain-of-function variant p.I397F, were identified in LQTS3 patients [15-17]. While p.V223G has not been reported previously, p.V223L causes a partial loss of function and has been proposed as a likely cause of Brugada syndrome [18]. Functional analysis, presented here,

further supports the role that these rare *SCN5A* variants predispose to arrhythmias. These findings support a model in which epilepsy patients harbouring arrhythmogenic variants are at greater risk of SUDEP.

The patient carrying the p.R523C variant was on lamotrigine at the time of SUDEP. Lamotrigine is a sodium channel blocker that has been implicated in increasing the risk of SUDEP. Here, we show that therapeutic concentration of lamotrigine significantly altered the biophysical properties of the wild-type $Na_V1.5$ channel. These data suggest that lamotrigine has an impact on human cardiac $Na_V1.5$ channels and are postioned to independently increase the risk of arrhythmia and consequent death. While the impact of lamotrigine on the p.R523C variant was not significant, the general pattern of effect was largely similar to that of wild-type. The exception was the recovery from inactivation which was blunted for the p.R523C variant suggesting a gene-by-drug interaction. It is therefore plausible that there may be additive risk for patients carrying pathogenic variants in arrhythmogenic genes and taking lamotrigine (or other pro-arrhythmic drugs). Further preclinical and clinical studies are warranted to test this idea.

Variants in genes associated with cardiac arrhythmias occur in up to 15% of SUDEP cases [6, 7]. Recently, we reported that *KCNH2* variants that cause loss of function are ~3-fold enriched in patients with SUDEP when compared to an aged epilepsy population who have 'escaped' SUDEP [8]. This raises the possibility that arrhythmogenic variants could act as genetic biomarkers for SUDEP risk in patients with epilepsy. Genetic screening in epilepsy is becoming more common, providing an opportunity to identify such variants which may carry management implications, such as increased surveillance of patients in sleep. Several tools, including functional analysis, can be used to predict the likely clinical impact of a given variant. These should be used in conjunction with standard clinical investigations to assess the likely risk of arrhythmia. Prolonged EEG with cardiac monitoring might be warranted to specifically explore ictal and interictal changes in cardiac rhythm. This would allow the detection of additional biomarkers of risk, including arrhythmogenic markers such as prolonged QT intervals, especially during seizures. Clinical strategies that have been successfully used to reduce the risk of sudden cardiac death could be employed, although clinical trials specific to epilepsy are needed.

In summary, we demonstrate that three *SCN5A* variants alter channel function suggesting that they could contribute to arrhythmias during a seizure and cause sudden death. These data support the premise that variants in arrhythmia genes can act as genetic biomarkers of SUDEP risk in some epilepsy patients.

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Disclosure of conflicts of interest

SFB declares unrestricted educational grants from UCB Pharma, SciGen and Eisai and consultancy fees from Praxis Precision Medicines. IES has served on scientific advisory boards for UCB, Eisai, GlaxoSmithKline, BioMarin, Nutricia, Rogcon, Encoded Therapeutics, Knopp Biosciences, Xenon Pharmaceuticals; received speaker honoraria from GlaxoSmithKline, UCB, BioMarin, Biocodex, Chiesi, Liva Nova and Eisai; received funding for travel from UCB, Biocodex, GlaxoSmithKline, Biomarin and Eisai; served as an investigator for Zogenix, Zynerba, Ultragenyx, GW Pharma, UCB, Eisai, Anavex Life Sciences, Ovid Therapeutics, Epigenyx, Encoded Therapeutics, Marinus, Xenon Pharmaceuticals; consulted for Zynerba Pharmaceuticals, Atheneum Partners, Ovid Therapeutics, Care Beyond Diagnosis, Epilepsy Consortium and UCB; is a Non-Executive Director of Bellberry Ltd. IES may accrue future revenue on pending patent WO61/010176 (filed: 2008): Therapeutic Compound; has a patent for *SCN1A* testing held by Bionomics Inc. and licensed to various diagnostic companies; has a patent molecular diagnostic/theranostic target for benign familial infantile epilepsy (BFIE) [PRRT2] 2011904493 & 2012900190 and PCT/AU2012/001321 (TECH ID:2012-009). The remaining authors have no conflicts of interest.

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.

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Figure 1: Human *SCN5A* SUDEP variants significantly affect channel function. (A) Representative whole-cell current trace recordings of $Na_v1.5$ wild-type (WT) and SUDEP variants V223G, I397V and R523C, elicited from the activation protocol shown under WT trace current. (B) Current density-voltage relationship. (C-E) Voltage-dependence of normalized peak conductance (G/G_{max}) and steady-state inactivation (I/I_{max}) for (C) V223G, (D) I397V, and (E) R523C. (F) Representative recovery from inactivation-elicited current trace recordings using the voltage protocol with variable time duration (Δt) shown below the traces. (G) Time- course of recovery from inactivation curves.

Table 1: Summary of whole-cell voltage clamp electrophysiology parameters of wild-type hSCN5A and SUDEP variants

	Number of cells	pA/pF_{max}	Activation $V_{1/2}$ (mV)	Activation slope	Inactivation $V_{1/2}$ (mV)	Inactivation slope	Recovery $t_{1/2}$ (ms)	Recovery τ (ms)
Wild-type	20	-58.7 ± 6.3	-21.8 ± 0.5	10.9 ± 0.4	-52.9 ± 0.5	-11.6 ± 0.4	2.3 ± 0.1	3.9 ± 0.8
V223G	12	-29.4 ± 7.6*	-17.1 ± 0.6****	10.5 ± 0.5	-48.8 ± 0.5****	-12.8 ± 0.5*	2.2 ± 0.1	3.1 ± 0.3
I397V	14	-52.9 ± 9.0	-26.0 ± 0.4****	8.1 ± 0.4***	-49.2 ± 0.4****	-10.1 ± 0.4*	2.4 ± 0.1	3.3 ± 0.4
R523C	15	-56.5 ± 7.6	-19.0 ± 0.4***	9.0 ± 0.3**	-45.0 ± 0.4****	-10.0 ± 0.3*	1.3 ± 0.1****	2.1 ± 0.2****
GFP control	11	-7.3 ± 1.1****	-	-	-	-	-	-

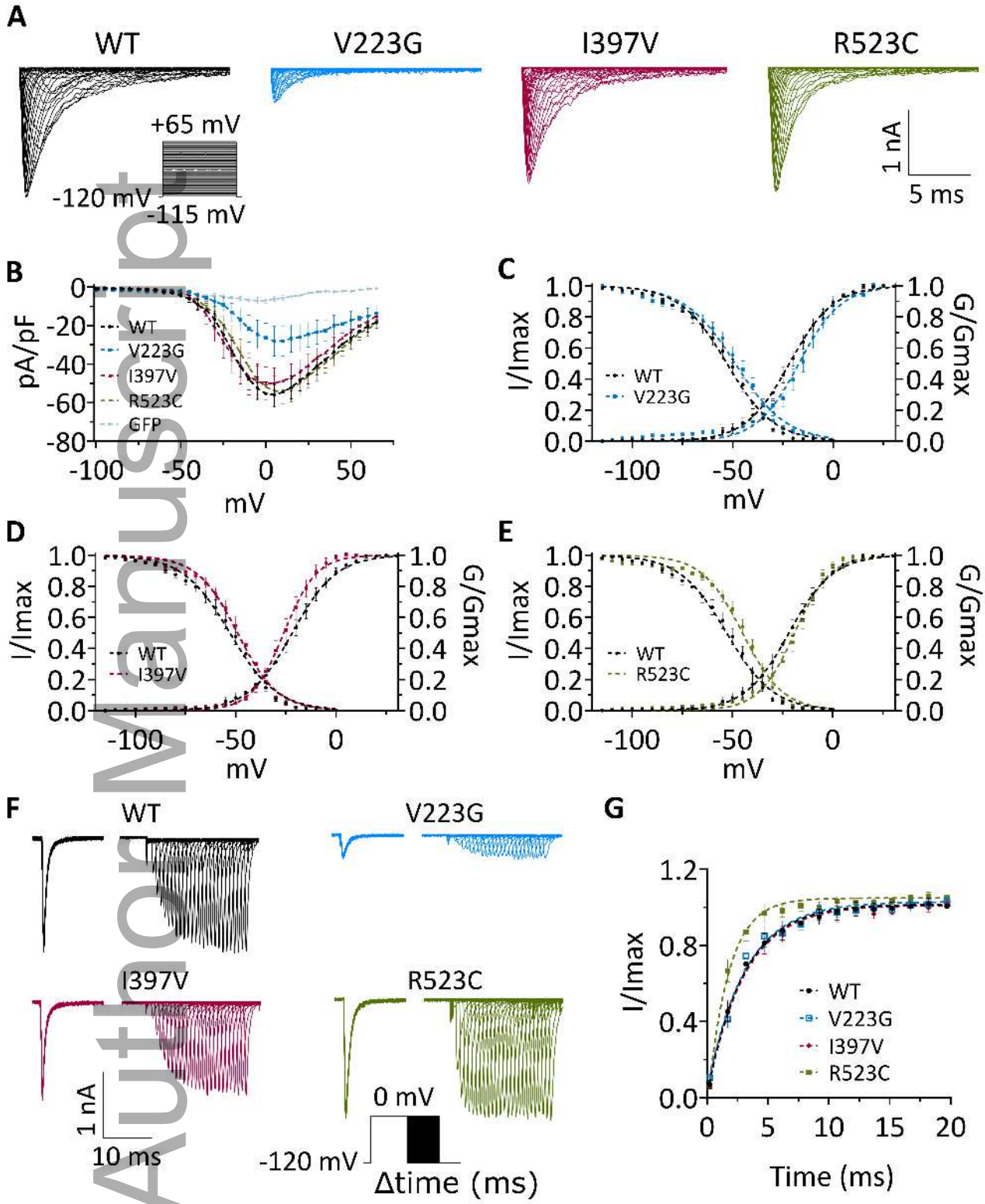
Compared to WT (one-way ANOVA with Dunnett's post-hoc):

* P < 0.05

** P < 0.01

*** P < 0.001

**** P < 0.0001



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