TABLE 1 Baseline Characteristics, Predisposing Factors, Management, and Outcomes of Patients Presenting With SCAD, With or Without Cardiac Arrest

	Patients With Cardiac Arrest (n = 14)	Patients Without Cardiac Arrest (n = 88)	p Value
Baseline characteristics			
Age, yrs	44 ± 11	48 ± 10	0.19
Female	13 (93)	75 (85)	0.45
Hypertension	6 (43)	25 (28)	0.28
Hyperlipidemia	1 (7)	27 (31)	0.07
Obesity	1 (7)	15 (17)	0.35
Tobacco Use	7 (50)	17 (19)	**0.01**
Presentation with STEMI	11 (79)	32 (36)	**<0.001**
EF, %	44 ± 12	53 ± 13	0.13
Management			
Conservative management	7 (50)	56 (64)	0.33
PCI	7 (50)	30 (34)	0.25
CABG	0 (0)	3 (3)	0.49
ICD placed	5 (36)	2 (2)	**<0.001**
Outcomes			
30-day readmission	4 (29)	10 (12)	0.11
Repeat SCAD occurrence	3 (21)	5 (6)	**0.04**
Mortality	0 (0)	0 (0)	NA

Values are mean ± SD or n (%). The p values in **bold** are statistically significant. Peripartum state and history of gestational hypertension/pre-eclampsia/eclampsia was relevant for female patients only (n = 13 for patients with cardiac arrest and n = 75 for patients without cardiac arrest). Thirty-day readmission was only available for 83 patients without cardiac arrest.

CABG = coronary artery bypass grafting; EF = ejection fraction; ICD = implantable cardioverter-defibrillator; PCI = percutaneous coronary intervention; SCAD = spontaneous coronary artery dissection; STEMI = ST-segment elevation myocardial infarction.

therapy in SCAD, it is sometimes employed in practice. Although ICD therapy may intuitively make sense, the risk-benefit ratio in SCAD is unclear. Most importantly, nationwide collaborative efforts are imperative to consolidate existing data from SCAD and SCD registries to guide ICD placement in SCAD patients.

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REFERENCES

1. Saw J, Aymong E, Sedlak T, et al. Spontaneous coronary artery dissection: association with predisposing arteriopathies and precipitating stressors and cardiovascular outcomes. Circ Cardiovasc Interv 2014;7:645–55.

2. Wood MJ, Drachman DE. Angiographic and interventional management of spontaneous coronary artery dissections: when less is more, or "when 'better' flow is the enemy of good flow". Catheter Cardiovasc Interv 2017;89:68–70.

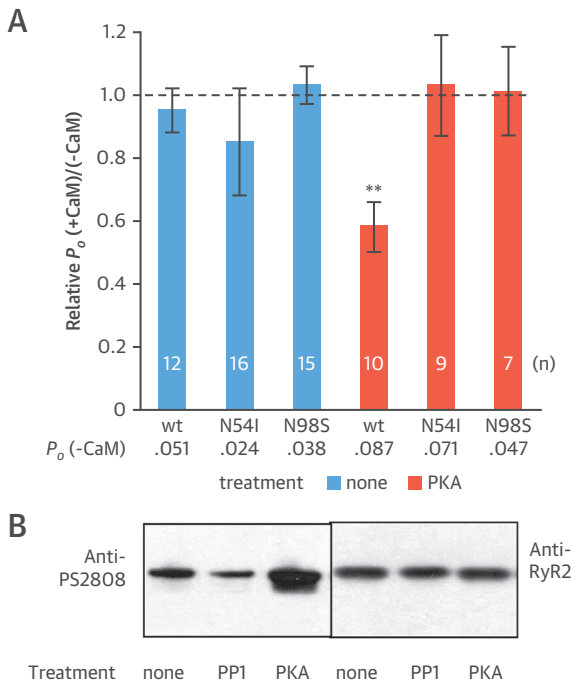
3. Tweet MS, Hayes SN, Pitta SR, et al. Clinical features, management, and prognosis of spontaneous coronary artery dissection. Circulation 2012;126:579–88.

Calmodulin Mutants Linked to Catecholaminergic Polymorphic Ventricular Tachycardia Fail to Inhibit Human RyR2 Channels

Calmodulin (CaM) is a calcium-binding protein that can directly inhibit cardiac ryanodine receptor calcium release channels (ryanodine receptor 2 [RyR2]) (1). CaM mutations can cause an autosomal-dominant form of catecholaminergic polymorphic ventricular tachycardia (CPVT), a syndrome characterized by exercise- and/or emotional stress-induced ventricular arrhythmia and sudden death (2). We previously reported that CPVT-linked mutant CaMs (N54I and N98S) had either no or a slight stimulating effect on the activity of sheep RyR2, whereas wild-type (wt)-CaM inhibited sheep RyR2 by approximately 40% and inhibited sarcoplasmic reticulum (SR) calcium release in permeabilized mouse cardiomyocytes (3). Although the mutant CaMs failed to inhibit mouse and sheep RyR2, they bound even more tightly to RyR2 than wild-type (wt)-CaM (3), providing an explanation for why CPVT mutant CaM could have a dominant effect on RyR2 in the presence of excess wt-CaM. To date, data are lacking on CaM regulation of human RyR2. To address this question, we investigated the action of wt and CPVT mutant CaMs on the single channel activity of RyR2 isolated from hearts of healthy human donors.

Human left ventricular tissues were obtained from the Human Heart Tissue Repository at the University of Sydney with approval from the human research ethics committees of the University of Newcastle (approval number H-2009-0369) and the University of Sydney (#09-2009-12146). SR membranes containing RyR2 were isolated from these tissues and incorporated into artificial lipid bilayers (4). RyR2 channel gating was measured by single-channel recording (4) in the presence and absence of physiological CaM

FIGURE 1 The Effect of wt-CaM and CPVT CaM Mutants on Activity of Human RyR2



RyR2 is in the presence of 100 nmol/l cytoplasmic Ca^{2+} and 2 mmol/l ATP and 0.1 mmol/l luminal [Ca^{2+}] with membrane potential −40 mV. **(A)** Relative effect of wt-CaM on RyR2 open probability (P_o) compared with that of N54I and N98S (mean ± SEM with replicate numbers in each **bar**). P_o (-CaM) denotes mean absolute P_o before wt or mutant CaM application. **Significant difference to 1 ($p < 0.01$). **(B)** Western blots of RyR2 subjected to incubations in PP1 (15 min) or PKA (5 min) at 30°C probed with antibodies for PS2808 **(left)**, stripped and reprobed with antibodies for RyR2 **(right)**. Ca^{2+} = calcium ion; CaM = calmodulin; CPVT = catecholaminergic polymorphic ventricular tachycardia; PKA = protein kinase A; PP1 = protein phosphatase 1; RyR2 = ryanodine receptor 2; wt = wild-type.

concentrations (0.1 nmol/l). CaM was added and removed from the RyR2 complex by using continuous local perfusion via a tube placed close to the bilayer, enabling solution changes within 1 s. Because CaM readily dissociates from RyR2 in 25 s (1,3), channels that are incorporated into artificial lipid bilayers are devoid of CaM unless exogenous CaM is applied. The effect of CaM was measured by repeatedly applying CaM for 1 min interleaved by 1-min periods of washout. Surprisingly, neither wt-CaM nor CPVT mutant CaMs (N54I and N98S) had any effect on the activity of human RyR2 (Figure 1A). The lack of inhibition of human RyR2 by wt-CaM was not anticipated because CaM is inhibitory in all animal models investigated so far (sheep, dog, and mouse [1,3]). This common lack of efficacy of wt and mutant CaMs on human RyR2 activity necessitates a re-examination of our hypothesis for the role of CaM regulation of RyR2 in CPVT.

Because CPVT is an arrhythmia brought on by β-adrenergic-induced RyR2 phosphorylation, we simulated adrenergic stress by incubating SR vesicles containing RyR2 with protein kinase A (PKA) before incorporation into bilayers. Western blots using phospho-specific antibodies (Figure 1B) confirmed an increased phosphorylation at RyR2 residue S2808, an accepted PKA consensus site. We found that wt and CPVT-mutant CaMs had clearly divergent effects on RyR2 when S2808 phosphorylation levels were increased by PKA (Figure 1A). wt-CaM caused approximately 40% inhibition of RyR2, whereas N54I and N98S had no effect. Taken together, our results support the hypothesis that wt-CaM inhibition blunts RyR2 activity after β-adrenergic stimulation, whereas CPVT mutant CaMs fail to do so. Hence, mutant CaMs facilitate pathological overactivation of RyR2 during periods of stress and exercise. These results indicate that, in humans, CaM functions to protect against aberrant SR Ca^{2+} release during periods of adrenergic stress where RyR2 is highly phosphorylated. The failure to inhibit phosphorylated RyR2 likely explains the pathogenic role of mutant CaM in CPVT.

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REFERENCES

1. Xu L, Meissner G. Mechanism of calmodulin inhibition of cardiac sarcoplasmic reticulum Ca2+ release channel (ryanodine receptor). Biophys J 2004;86:797–804.

2. Gomez-Hurtado N, Boczek NJ, Kryshtal DO, et al. Novel CPVT-associated calmodulin mutation in CALM3 (CALM3-A103V) activates arrhythmogenic Ca waves and sparks. Circ Arrhythm Electrophysiol 2016;9:e004161.

3. Hwang HS, Nitu FR, Yang Y, et al. Divergent regulation of ryanodine receptor 2 calcium release channels by arrhythmogenic human calmodulin missense mutants. Circ Res 2014;114:1114–24.

4. Walweel K, Li J, Molenaar P, et al. Differences in the regulation of RyR2 from human, sheep, and rat by Ca2+ and Mg2+ in the cytoplasm and in the lumen of the sarcoplasmic reticulum. J Gen Physiol 2014;144:263–71.

Differences Between Conditional and Marginal Propensity Score Estimates

A Real-World Application

The paper by Elze et al. (1) is an important investigation analyzing the application of different propensity score (PS) methods compared with covariate adjustment to estimate treatment effect in 4 cardiovascular studies. Although the article aimed to provide scientists with the necessary information to decide on the most suitable PS approach, necessary distinctions that would allow for a meaningful interpretation of the results have not been drawn.

Although mentioned as a limitation, marginal and conditional estimates produced by the different PS approaches were compared in the paper. Marginal methods estimate the population average and conditional methods the covariate-stratum-specific effect (2). The intrinsic differences between the model structures employed by these methods result in hazard ratios (HRs) that cannot be compared. The only exceptions are linear models and situations in which the true HR is 1. The phenomenon of incompatibility of conditional and marginal effect estimates is known as the noncollapsibility property and applies to various measures including HRs and odds ratios (2). For example, in the CHARM (Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity) study, marginal models compare the risk of all-cause mortality in beta-blocker users and nonusers, whereas conditional models compare mortality risk in users and nonusers with similar covariates (1).

Further clarification may also be necessary for the authors' assertion that "doubly robust methods all estimate conditional HR" as some doubly robust methods can produce marginal effect estimates (1,3).

Finally, and possibly most importantly, it is crucial to note that when using real-world data, the true effect remains unknown (4). Definitive statements on the actual performance of different inference methods can therefore not be made. For instance, in the presence of residual confounding, a larger standard error for a biased effect estimate may be desirable as the related confidence interval will be more likely to cover the true underlying effect, despite the bias. Confounding is a causal concept and cannot be adequately assessed with observational data alone (4).

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REFERENCES

1. Elze MC, Gregson J, Baber U, et al. Comparison of propensity score methods and covariate adjustment: evaluation in 4 cardiovascular studies. J Am Coll Cardiol 2017;69:345–57.

2. Pang M, Kaufman JS, Platt RW. Studying noncollapsibility of the odds ratio with marginal structural and logistic regression models. Stat Methods Med Res 2016;25:1925–37.

3. Funk MJ, Westreich D, Wiesen C, Sturmer T, Brookhart MA, Davidian M. Doubly robust estimation of causal effects. Am J Epidemiol 2011;173:761–7.

4. Pearl J. Causality: Models, Reasoning, and Inference. 2nd Edition. New York, NY: Cambridge University Press, 2009.