



## ORIGINAL ARTICLE

# Transcriptional profiles of genes related to electrophysiological function in *Scn5a*<sup>+/-</sup> murine hearts

Michael Takla<sup>1,2</sup> | Charlotte E. Edling<sup>1</sup> | Kevin Zhang<sup>1,3</sup> | Khalil Saadeh<sup>1,4</sup> | Gary Tse<sup>1,5</sup> | Samantha C. Salvage<sup>6</sup> | Christopher L.-H. Huang<sup>1,6</sup>  | Kamalan Jeevaratnam<sup>1</sup> 

<sup>1</sup>Faculty of Health and Medical Science, University of Surrey, Guildford, UK

<sup>2</sup>Christ's College, University of Cambridge, Cambridge, UK

<sup>3</sup>School of Medicine, Imperial College London, London, UK

<sup>4</sup>Clinical School, University of Cambridge, Cambridge, UK

<sup>5</sup>Second Hospital of Tianjin Medical University, Tianjin, China

<sup>6</sup>Department of Biochemistry, University of Cambridge, Cambridge, UK

## Correspondence

Kamalan Jeevaratnam, Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7AL, UK.  
Email: drkamalanjeeva@gmail.com

## Funding information

The work described here was funded by grants from the University of Surrey and Medical Research Council Medical Research Council (MR/M001288/1).

## Abstract

The *Scn5a* gene encodes the major pore-forming Na<sub>v</sub>1.5 (α) subunit, of the voltage-gated Na<sup>+</sup> channel in cardiomyocytes. The key role of Na<sub>v</sub>1.5 in action potential initiation and propagation in both atria and ventricles predisposes organisms lacking *Scn5a* or carrying *Scn5a* mutations to cardiac arrhythmogenesis. Loss-of-function Na<sub>v</sub>1.5 genetic abnormalities account for many cases of the human arrhythmic disorder Brugada syndrome (BrS) and related conduction disorders. A murine model with a heterozygous *Scn5a* deletion recapitulates many electrophysiological phenotypes of BrS. This study examines the relationships between its *Scn5a*<sup>+/-</sup> genotype, resulting transcriptional changes, and the consequent phenotypic presentations of BrS. Of 62 selected protein-coding genes related to cardiomyocyte electrophysiological or homeostatic function, concentrations of mRNA transcribed from 15 differed significantly from wild type (WT). Despite halving apparent ventricular *Scn5a* transcription heterozygous deletion did not significantly downregulate its atrial expression, raising possibilities of atria-specific feedback mechanisms. Most of the remaining 14 genes whose expression differed significantly between WT and *Scn5a*<sup>+/-</sup> animals involved Ca<sup>2+</sup> homeostasis specifically in atrial tissue, with no overlap with any ventricular changes. All statistically significant changes in expression were upregulations in the atria and downregulations in the ventricles. This investigation demonstrates the value of future experiments exploring for and clarifying links between transcriptional control of *Scn5a* and of genes whose protein products coordinate Ca<sup>2+</sup> regulation and examining their possible roles in BrS.

## KEYWORDS

arrhythmia, Brugada syndrome, mechanisms, sodium channel, transcription

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Physiological Reports* published by Wiley Periodicals LLC on behalf of The Physiological Society and the American Physiological Society

## 1 | INTRODUCTION

The Brugada syndrome (BrS) poses a major worldwide public health problem, accounting for one in five sudden cardiac deaths among patients without reported structural cardiac defects (Antzelevitch et al., 2005; Matsuo et al., 2001). It is inherited as an incompletely penetrant autosomal dominant trait. Of clinical BrS cases, 10 to 30% have an identifiable causal mutation (Chen et al., 1998). Of these, the most frequent involve loss-of-function in the *Scn5a* gene (Chockalingam et al., 2012).

Clinical Type I BrS is characterized by electrocardiographic right precordial coved-type ST elevations with a transient or stable lead V1–V3 T-wave inversion (Gussak et al., 1999; Kurita et al., 2002), and significantly elevated risks of polymorphic ventricular tachycardia, atrial fibrillation, and ventricular fibrillation (Amin et al., 2010; Kusano et al., 2008). The nature of the relationship between ST elevation and tachyarrhythmia is uncertain. Early experiments in canine hearts had suggested a repolarization hypothesis, invoking acute decreases in inward  $\text{Na}^+$  current ( $I_{\text{Na}}$ ), during phase 1 of the right ventricular epicardial action potential resulting in regional differences in transmural repolarization (Yan & Antzelevitch, 1999). In contrast, subsequent experimental and some clinical studies suggested a depolarization hypothesis (Meregalli et al., 2005) in which a compromised  $I_{\text{Na}}$  slows the conduction velocity of the epicardial action potential, doing so to a greater extent in the right ventricular outflow tract than in its remaining myocardium (Nagase et al., 2002), predisposing to re-entrant excitation (Morita et al., 2003).

Murine models permit investigations relating particular mutations to their phenotypic consequences. The *Scn5a*<sup>+/-</sup> mouse recapitulates some of the clinical- and age-dependent features of BrS (Papadatos et al., 2002) and related proarrhythmic disorders, such as progressive cardiac conduction defect (Guzadhur et al., 2012; Probst et al., 2003; Tan et al., 2001) despite its differing myocardial and chamber volumes, heart rates, regional ion channel distributions (Zimmer et al., 2014), and ventricular action potential waveforms. The parallels extended to associations between *Scn5a* haploinsufficiency and age-related fibrotic changes (Jeevaratnam et al., 2010; Nademanee et al., 2015) that may reflect noncanonical roles of voltage-gated sodium channels in cardiac homeostasis (Abriel, 2010; McNair et al., 2004).

The BrS phenotype has been associated with close to 300 distinct genomic mutations (Kapplinger et al., 2010) though in most their causal relationships with the arrhythmic phenotype are not directly apparent (Hosseini et al., 2018). This abundance and diversity of disease-causing variants suggests that that aging interacts with a polygenic, rather than Mendelian, background in producing BrS phenotypes. Previous studies had examined

the age-related factors in development of arrhythmic risk in *Scn5a*<sup>+/-</sup> murine hearts (Dautova et al., 2010), prolongation of PR and QRS intervals (Jeevaratnam et al., 2010; Royer et al., 2005), and their sex-dependence, the latter revealing accentuated male over female phenotypes (Jeevaratnam et al., 2010). Fewer had examined the effects of *Scn5a* haploinsufficiency on non-electrophysiological aspects of cardiomyocyte function. This study explores the possible contributions of transcriptional alterations involving genes potentially related to electrophysiological phenotypes following heterozygotic *Scn5a* deletion in a murine model.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Replacement of the *Scn5a* gene's second exon with an SA-GFP-PGK neomycin cassette produced heterozygous genotypes in five male and three female mice aged  $11 \pm 3$  months, bred on a 129sv background, as described for the previously established BrS model (Papadatos et al., 2002). Mice were housed at a facility with a 12-h light/dark cycle at 21°C with access to sterile chow (RM3 Maintenance Diet; SDS), water, bedding, and environmental stimuli, in accordance with the Animals (Scientific Procedures) Act 1986, United Kingdom Home Office regulations. All procedures therefore also complied with the Guide for the Care and Use of Laboratory Animals, United States National Institutes of Health (NIH Publication No. 85-23, revised 1996). Animals were sacrificed by cervical dislocation (Schedule 1, UK Home Office Regulations).

### 2.2 | Tissue samples

Hearts from four wild type (WT) and four *Scn5a*<sup>+/-</sup> mice were obtained through ex vivo removal. The atria were excised from the ventricles prior to snap-freezing and subsequent separate storage at  $-80^{\circ}\text{C}$ .

### 2.3 | RNA isolation

RNA was isolated using the Monarch RNA isolation kit (New England Biolabs). Ventricular tissue was weighed and chopped into small pieces from which 30 mg was taken to protection buffer and homogenized with a Stuart handheld homogenizer until smooth. For atria the entire tissue sample was used. The manufacturer's protocol was followed including removal of genomic DNA. The resultant RNA quantity and quality were evaluated by

Bioanalyzer analysis following the manufacturer's protocol (Agilent RNA 6000 Nano Kit; Agilent Technologies) and all RNA samples exhibited RNA integrity number values  $>7$ .

## 2.4 | cDNA preparation

cDNA was prepared with the aid of High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's protocol. One microgram of RNA was used for each sample. The cDNA was tested with SYBR Green qPCR for efficient reverse transcription and lack of genomic DNA, as described previously (Edling et al., 2019).

## 2.5 | TaqMan array assay

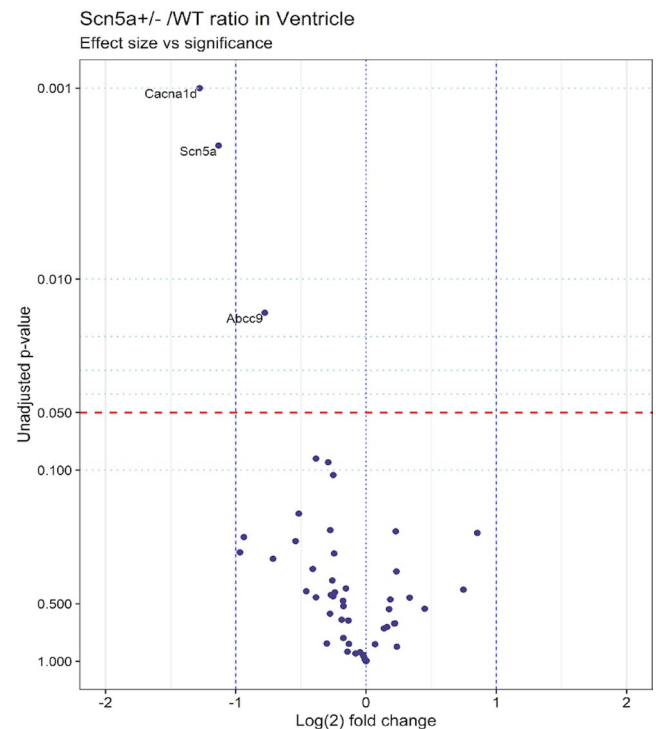
Thermo Fisher custom TaqMan array cards were used to examine the gene expression of selected genes as described in the Section 3. Fifty-five genes were assayed in all 16 samples (4 samples/group), and an additional 7 genes (*Gja1*, *Gja5*, *Hcn2*, *Scn1b*, *Scn2b*, *Scn3b*, and *Scn4b*) were assayed in 12 samples (3 samples/group). All assays on the cards were present in triplicate and pre-validated by Thermo Fisher. The cards were run on a Quant 7 cycler following the manufacturer's protocol without modifications.

## 2.6 | Data analysis and statistical testing

With the QuantStudio software threshold set at 0.2 fluorescence units and the baseline range automatically assigned, data were imported to Microsoft Excel for the application of the  $2^{-\Delta\Delta CT}$  method (Livak & Schmittgen, 2001). Determining the geometric mean of the Cq values of two housekeeping genes, *Actb* and *Gapdh*, made it possible to normalize and obtain fold changes from the transcript of each gene. Application of Student's independent *t*-tests on the processed data generated an estimate of the type I error rate, and, by extension, *p* value, of each change, for analysis of statistical significance.

## 3 | RESULTS

Of the 62 genes encoding systematically selected diverse cardiac electrophysiological or homeostatic functions (Huang, 2017), the Student's *t*-test to a  $p < 0.05$  significance level of each normalized fold change, demonstrated significant changes in concentrations of mRNAs

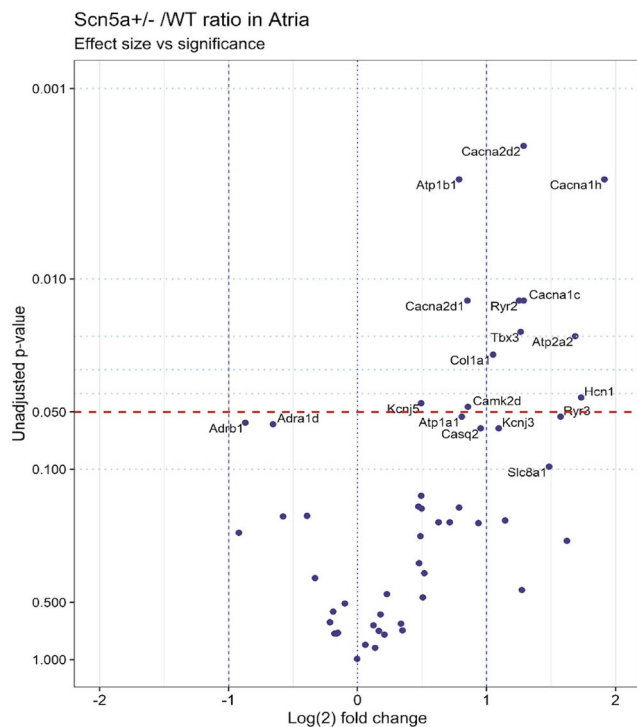


**FIGURE 1** Volcano plot of differentially expressed pre-selected genes, contrasting transcription in ventricular *Scn5a*<sup>+/-</sup> to wild type (WT). The y-axis indicates unadjusted *p* values based on Student's *t*-tests, while the x-axis indicates the  $\log_2$  of each normalized fold change. Gene transcriptional with *p* values  $< 0.1$  are labeled in the plot. The red line indicates significance level  $p = 0.05$

transcribed from 15 distinct genes. Of these, *Scn5a* expression was expectedly halved in ventricular, but was contrastingly not significantly downregulated in atrial tissue suggestive of feedback mechanisms increasing the expression of the WT allele. Of the 14 remaining genes showing an altered expression, none were shared by both atria and ventricles (Figures 1 and 2), with most exclusive to atrial tissue (Figure 2). Notably, of the statistically significant changes in gene expression, all those in the atria were upregulations, and all those in the ventricles were downregulations.

## 3.1 | Na<sup>+</sup>/K<sup>+</sup>-ATPase

The Na<sup>+</sup>/K<sup>+</sup>-ATPase is a heterotrimeric protein. Its *Atp1a* ( $\alpha$ ) subunit is targeted to the plasma membrane (Geering, 2001) by the K<sup>+</sup>-occluding (Lutsenko & Kaplan, 1993) *Atp1b* ( $\beta$ ) subunit. Its activity in exporting three Na<sup>+</sup> for every two K<sup>+</sup> it imports (Post & Jolly, 1957) is modulated by the FXD subunit (Garty & Karlisch, 2006 ; Therien & Blostein, 2000). This contributes a minor intrinsic electrogenicity. However, it is the resulting K<sup>+</sup> electrochemical gradient that maintains most of the negative resting



**FIGURE 2** Volcano plot of differentially expressed pre-selected genes, contrasting transcription in atrial *Scn5a*<sup>+/-</sup> to wild type (WT). The y-axis indicates unadjusted *p* values based on Student's *t*-tests, while the x-axis indicates the log<sub>2</sub> of each normalized fold change. Gene transcriptional changes with *p* values < 0.1 are labeled in the plot. The red line indicates significance level *p* = 0.05

membrane potential ( $E_m$ ), against its dissipation through expressed 2-pore domain ( $K_{2p3.1}$ ; *Kcnk3*) channels (Ketchum et al., 1995). The murine *Scn5a*<sup>+/-</sup> atria showed significant, 111%, increase in *Atp1b1* mRNA levels and doubled though not significant ( $p = 0.053$ ) *Atp1a1* levels relative to WT (Table 1).

### 3.2 | Ion channels controlling $E_m$

In phase 4 of the atrial or ventricular cardiomyocyte action potential,  $E_m$  is stabilized to approximately -90 mV. Although the electrochemical gradients required to maintain  $E_m$  depend on  $Na^+/K^+$ -ATPase activity, it is the inwardly rectifying  $K^+$  channels (Kir) making the major contribution to its steady-state value. Of these, the resulting inwardly rectifying  $I_{K1}$  conductance permits  $K^+$  influx, and prevents  $K^+$  efflux when  $E_m$  is more negative and positive, respectively, than its resting value. The underlying regionally heterogeneous tetrameric (Wang et al., 1998) Kir2.1 (*Kcnj2*), Kir2.2 (*Kcnj12*), and Kir2.3 (*Kcnj4*) owe their inward rectification to intracellular polyamines (Ficker et al.,

1994; Lopatin et al., 1994), and, to a lesser extent,  $Mg^{2+}$  (Matsuda et al., 1987; Vandenberg, 1987).

The hetero-octameric sarcolemmal and mitochondrial ATP-sensitive  $K^+$  channel ( $K_{ATP}$ ) makes a smaller, nevertheless still significant, contribution to  $I_{K1}$ . ATP binding to the four Kir6.2 (*Kcnj11*), and to a lesser extent to the Kir6.1 (*Kcnj8*) subunits (Seino, 1999) reduces its open probability. In contrast, ATP binding to the four SUR2A (*Abcc9*), or especially in the atria, SUR1 (*Abcc8*) subunits (Seino & Miki, 2004) facilitates its own hydrolysis to ADP, activating the channel in the presence of  $Mg^{2+}$ . The dependence of such a mechanism of inward rectification on  $[ATP]_i$  enables ischemic preconditioning in the face of transitory periods of ischemia (O'Rourke, 2000). Finally, GIRK4 (*Kcnj5*) constitutes one of the most modulable fractions of the resting  $I_{K1}$ . Binding of vagally released ACh to  $G_i$ -linked  $M_2$ AChRs expressed by the sinoatrial node (SAN) promotes the dissociation of its component  $\beta\gamma$  complex and  $\alpha$  subunit of the trimeric G protein. This increases the open probability of the GIRK4 pore hyperpolarizes resting  $E_m$  and so reduces heart rate (Wickman et al., 1998).

Murine *Scn5a*<sup>+/-</sup> ventricles, but not their atria showed significant, 42% reductions in *Abcc9* mRNA levels relative to WT, but no other significant differences in transcription of genes controlling  $E_m$ . In contrast, *Scn5a*<sup>+/-</sup> atria showed significant increases in *Kcnj5* transcription levels and also a trend ( $p = 0.061$ ) toward markedly higher *Kcnj5* level relative to WT (Table 1).

### 3.3 | Ion channels initiating excitation

Cardiac pacemaker cell automaticity depends at least in part on tetrameric hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (Brown & DiFrancesco, 1980). The membrane hyperpolarization following the preceding action potential removes the auto-inhibitory effect of the HCN voltage-sensitive domain increasing its pore open probability, actions further enhanced by cAMP binding (Flynn & Zagotta, 2018). A voltage clock component of pacing activity results from the balance between its consequent inward  $I_f$  current with a coincident  $K^+$  efflux resulting in membrane depolarization. In addition, a  $Ca^{2+}$  clock driven by depolarizing sarcolemmal  $Na^+/Ca^{2+}$  exchange current ( $I_{NCX}$ ) stimulated by the resultant rhythmic sarcoplasmic reticular (SR) release of  $Ca^{2+}$  (Bogdanov et al., 2001) contributes to the regulation of the pacemaker frequency. The resulting SAN excitation is propagated successively to the atrioventricular node (AVN), bundle of His and Purkinje fibers, and then to the ventricular cardiomyocytes.

*Hcn4* is the predominant SAN Hcn isoform (Baruscotti et al., 2011) and atrioventricular bundle; *Hcn1* and *Hcn2*



**TABLE 1** Heat map showing the statistical significance of changes in the expression of functional groups of genes tested by TaqMan array assay for transcriptional alterations in *Scn5a*<sup>+/-</sup>, relative to wild-type, murine hearts. Mean expression value with wild type normalised to 1.0 and standard error of the mean (SEM) in italics. Yellow indicates no change, green indicates downregulation and red indicates upregulation of expression. Darker shades of red/green reflect larger magnitudes of fold changes in gene transcription

Functional group	Gene	Ventricles				Atria			
		WT		SCN5A <sup>+/-</sup>		WT		SCN5A <sup>+/-</sup>	
		Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
Na <sup>+</sup> /K <sup>+</sup> -ATPase activity	Atp1a1	1.0	<i>0.0</i>	0.8	<i>0.0</i>	1.0	<i>0.0</i>	2.2	<i>0.5</i>
	Atp1a2	1.0	<i>1.1</i>	0.9	<i>0.5</i>	1.0	<i>0.2</i>	1.2	<i>0.4</i>
	Atp1b1	1.0	<i>0.0</i>	1.2	<i>0.1</i>	1.0	<i>0.0</i>	2.1	<i>0.3</i>
Ion channels controlling resting membrane potential (P-IV)	Abcc8	1.0	<i>0.2</i>	0.5	<i>0.0</i>	1.0	<i>0.1</i>	1.0	<i>0.1</i>
	Abcc9	1.0	<i>0.9</i>	0.6	<i>0.5</i>	1.0	<i>0.1</i>	1.2	<i>0.1</i>
	Kcnj3	1.0	<i>0.0</i>	1.2	<i>0.0</i>	1.0	<i>0.1</i>	2.1	<i>0.2</i>
	Kcnj5	1.0	<i>0.0</i>	0.8	<i>0.0</i>	1.0	<i>0.1</i>	1.4	<i>0.1</i>
	Kcnj8	1.0	<i>0.1</i>	0.9	<i>0.2</i>	1.0	<i>0.1</i>	0.9	<i>0.1</i>
	Kcnj11	1.0	<i>0.2</i>	0.7	<i>0.1</i>	1.0	<i>0.1</i>	0.9	<i>0.2</i>
	Kcnj12	1.0	<i>0.1</i>	0.8	<i>0.1</i>	1.0	<i>0.1</i>	1.1	<i>0.2</i>
	Kcnk3	1.0	<i>0.1</i>	1.0	<i>0.1</i>	1.0	<i>0.1</i>	1.0	<i>0.3</i>
Ion channels initiating excitation	Hcn1	1.0	<i>0.1</i>	1.8	<i>0.2</i>	1.0	<i>0.0</i>	3.3	<i>0.1</i>
	Hcn2	1.0	<i>0.1</i>	0.8	<i>0.1</i>	1.0	<i>0.1</i>	1.0	<i>0.1</i>
	Hcn4	1.0	<i>0.3</i>	0.6	<i>0.1</i>	1.0	<i>0.0</i>	3.1	<i>0.2</i>
Ion channels permitting <i>I</i> <sub>Na</sub> (P-0)	Scn5a	1.0	<i>0.1</i>	0.5	<i>0.1</i>	1.0	<i>0.1</i>	0.9	<i>0.1</i>
	Scn7a	1.0	<i>0.1</i>	0.9	<i>0.1</i>	1.0	<i>0.1</i>	1.7	<i>0.3</i>
Subunits modulating the kinetic profile of <i>I</i> <sub>Na</sub>	Scn1b	1.0	<i>0.4</i>	0.5	<i>0.1</i>	1.0	<i>0.2</i>	1.7	<i>0.4</i>
	Scn2b	1.0	<i>0.2</i>	1.1	<i>0.1</i>	1.0	<i>0.1</i>	1.2	<i>0.4</i>
	Scn3b	1.0	<i>0.3</i>	1.4	<i>0.2</i>	1.0	<i>0.3</i>	1.1	<i>0.1</i>
	Scn4b	1.0	<i>0.1</i>	2.6	<i>0.5</i>	1.0	<i>0.2</i>	1.4	<i>0.3</i>
Ion channels controlling repolarization (P-III)	Kcna4	1.0	<i>0.3</i>	0.9	<i>0.1</i>	1.0	<i>0.0</i>	1.4	<i>0.4</i>
	Kcnd3	1.0	<i>0.1</i>	1.0	<i>0.1</i>	1.0	<i>0.0</i>	1.6	<i>0.2</i>
	Kcne11	1.0	<i>6.2</i>	1.2	<i>4.3</i>	1.0	<i>1.1</i>	2.4	<i>7.3</i>
	Kcnh2	1.0	<i>0.1</i>	1.0	<i>0.1</i>	1.0	<i>0.1</i>	0.8	<i>0.2</i>
	Kcnn1	1.0	<i>0.1</i>	1.0	<i>0.1</i>	1.0	<i>0.0</i>	0.9	<i>0.1</i>
	Kcnn2	1.0	<i>0.1</i>	1.1	<i>0.2</i>	1.0	<i>0.5</i>	1.2	<i>0.2</i>
Ion channels permitting surface <i>I</i> <sub>Ca</sub>	Cacna1c	1.0	<i>0.4</i>	1.0	<i>0.5</i>	1.0	<i>0.0</i>	2.4	<i>0.1</i>
	Cacna1d	1.0	<i>0.0</i>	0.4	<i>0.0</i>	1.0	<i>0.2</i>	1.6	<i>0.2</i>
	Cacna1g	1.0	<i>0.1</i>	0.9	<i>0.2</i>	1.0	<i>0.1</i>	1.1	<i>0.2</i>
	Cacna1h	1.0	<i>0.0</i>	0.8	<i>0.0</i>	1.0	<i>0.0</i>	3.8	<i>0.1</i>
Subunits modulating surface <i>I</i> <sub>Ca</sub> trafficking and kinetics	Cacnb2	1.0	<i>0.1</i>	0.9	<i>0.3</i>	1.0	<i>0.2</i>	1.9	<i>0.7</i>
	Cacna2d1	1.0	<i>0.1</i>	1.0	<i>0.1</i>	1.0	<i>0.0</i>	1.8	<i>0.1</i>
	Cacna2d2	1.0	<i>0.0</i>	0.7	<i>0.0</i>	1.0	<i>0.1</i>	2.4	<i>0.1</i>
Intracellular ion channels, transporters, and enzymes controlling Ca <sup>2+</sup> homeostasis	Atp2a2	1.0	<i>0.5</i>	1.4	<i>0.6</i>	1.0	<i>0.1</i>	3.2	<i>0.3</i>
	Camk2d	1.0	<i>0.0</i>	0.9	<i>0.1</i>	1.0	<i>0.0</i>	1.8	<i>0.1</i>
	Casq2	1.0	<i>0.9</i>	0.5	<i>0.1</i>	1.0	<i>0.0</i>	1.9	<i>0.2</i>
	Ryr2	1.0	<i>0.1</i>	0.8	<i>0.1</i>	1.0	<i>0.0</i>	2.4	<i>0.1</i>
	Ryr3	1.0	<i>0.0</i>	1.0	<i>0.0</i>	1.0	<i>0.1</i>	3.0	<i>0.3</i>
	Slc8a1	1.0	<i>0.2</i>	1.3	<i>0.4</i>	1.0	<i>0.1</i>	2.8	<i>0.6</i>

(Continues)

TABLE 1 (Continued)

Functional group	Gene	Ventricles				Atria			
		WT		SCN5A <sup>+/-</sup>		WT		SCN5A <sup>+/-</sup>	
		Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
Surface adrenergic receptors	Adra1a	1.0	8.6	0.8	2.1	1.0	0.7	0.5	0.3
	Adra1b	1.0	0.1	0.8	0.0	1.0	0.1	0.7	0.1
	Adra1d	1.0	0.1	1.0	0.1	1.0	0.1	0.6	0.1
	Adrb1	1.0	0.1	0.8	0.1	1.0	0.1	0.6	0.2
	Adrb2	1.0	0.6	0.8	1.0	1.0	0.3	1.3	1.5
Proteins, and their subunits, involved in the adenylyl cyclase pathway	Adcy4	1.0	0.1	1.2	0.1	1.0	0.1	1.4	0.1
	Adcy5	1.0	0.1	1.2	0.0	1.0	0.1	1.4	0.2
	Pde2a	1.0	0.1	1.0	0.1	1.0	0.1	0.9	0.1
	Pde4d	1.0	0.1	0.9	0.1	1.0	0.1	1.4	0.3
	Prkaca	1.0	0.2	0.8	0.1	1.0	0.1	0.8	0.2
	Prka1a	1.0	0.1	1.1	0.1	1.0	0.1	1.4	0.1
	Prka2a	1.0	0.1	0.9	0.2	1.0	0.1	1.4	0.1
	Prka2b	1.0	0.0	1.7	0.2	1.0	0.1	1.1	0.3
	Fibrotic markers	Colla1	1.0	0.1	1.1	0.1	1.0	0.1	2.1
Col3a1		1.0	0.3	0.8	0.2	1.0	0.1	2.2	0.6
Tgfb1		1.0	0.1	1.1	0.1	1.0	0.0	1.1	0.2
Gap junction connexins	Gja1	1.0	0.2	0.7	0.2	1.0	0.2	2.0	0.2
	Gja5	1.0	0.2	0.9	0.2	1.0	0.3	1.2	0.2
	Gjd3	1.0	0.6	0.8	0.4	1.0	0.1	1.3	0.8
Other genes	Tbx3	1.0	0.2	1.1	0.4	1.0	0.1	2.4	0.2
	Trpc1	1.0	0.1	0.7	0.1	1.0	0.1	1.4	0.1

SEM, standard error of mean; WT, wild type.

are also selectively expressed by the AVN and bundle branches (Herrmann et al., 2011). Despite lower global expression levels (Günther & Baumann, 2015), *Hcn3* also contributes to shaping ventricular cardiomyocyte action potential waveforms (Fenske et al., 2011). We observed little detectable alteration in expression of the *Hcn3* gene in either atria or ventricles. *Scn5a*<sup>+/-</sup> and WT ventricles showed no significant differences in *Hcn1*, *Hcn2*, or *Hcn4* mRNA concentrations. In contrast, *Scn5a*<sup>+/-</sup> atria showed increased (by 233%) transcription of *Hcn1* mRNA compared to WT (Table 1).

### 3.4 | Ion channels involved in $I_{Na}$

The steeper depolarizing, phase 0, of atrial and ventricular cardiomyocyte action potential compared to that of pacemaker cells, reflects their expression of voltage-gated  $Na^+$  ( $Na_v$ ) channels. The pore-forming ( $\alpha$ ) subunit of the predominant  $Na_v1.5$  (*Scn5a*) cardiac isoform, and possibly voltage sensing  $Na_v2.1$  (*Scn7a*) (Hiyama et al., 2002) comprises four domains (DI–DIV), each with six helical

segments (S1–S6). Cardiomyocyte depolarization causes a repulsion of basic Arg and Lys residues located at every third position of S4 (Schwartz & Stühmer, 1984) driving an outward rotation that activates a rapid first-order activation. The predominant component of the subsequent inactivation proceeds through a fast (2–10 ms) ball and chain interaction mediated by the IFM motif of the DIII–DIV loop (Goldin, 2003). The cytoskeleton-anchoring auxiliary (Isom, 2001; Malhotra et al., 2000, 2002) *Scn1b–Scn4b* ( $\beta$ ) subunits modulate the subsequent voltage-dependent return of  $Na_v1.5$  from its inactivated to its resting conformation. We here report no significant differences between *Scn5a*<sup>+/-</sup> and WT hearts in the levels of expression of these genes (Table 1), except *Scn5a* itself in the ventricles.

### 3.5 | Ion channels mediating action potential repolarization

The early rapid phase 1 and delayed phase 3 action potential repolarization phases that follow phase 0 depolarization and the phase 2 plateau, respectively, are driven by particular

voltage-gated  $K^+$  ( $K_v$ ) channel subtypes. Their  $K_v$   $\alpha$  subunits form pseudo-tetramers that show a similar steady-state voltage sensitivity of activation as, but markedly differing kinetics from, those of  $Na_v$ . These require all four  $\alpha$  subunits to undergo a simultaneous, even if cooperative, shifts from closed to intermediate to open conformations. There are marked heterogeneity between isoforms. The relatively rapidly activating  $K_v1.4$  (*Kcna4*),  $K_v4.2$  (*Kcnd2*), and  $K_v4.3$  (*Kcnd3*) mediate the early phase 1 A-type currents  $I_{to1,s}$  and  $I_{to1,f}$  respectively (Angelo et al., 2002).  $K_v7.1$  (*Kcnq1*) mediates the slowly activating phase 3 delayed-rectifier current ( $I_{Ks}$ ), whose voltage-dependence is modulated by co-assembled KCNE5 (*Kcne1*) (Angelo et al., 2002). The  $K_v11.1$  (*Kcnh2*) (Vandenberg et al., 2012) and  $K_v1.5$  (*Kcna5*) (Feng et al., 1997) activates in successfully shorter timeframes to result in the rapid ( $I_{Kr}$ ), and ultra-rapid atrial ( $I_{Kur}$ ) currents, respectively. Furthermore, in the atria (Skibsbjerg et al., 2014), the small conductance  $Ca^{2+}$ -activated  $K^+$  (SK) channels,  $K_{Ca2.1}$  (*Kcnn1*) and  $K_{Ca2.2}$  (*Kcnn2*) (Tuteja et al., 2005), further contribute to repolarization.

There were no significant differences in the concentrations of mRNA transcribed from any of their evaluated genes responsible for the repolarization between *Scn5a*<sup>+/-</sup> and WT mice in either ventricular or atrial tissue (Table 1).

### 3.6 | Ion channels involved in $I_{Ca}$

The phase 2 plateau phases can typically extend over 300 ms. Here  $K^+$  efflux arising from activation of  $I_{Ks}$ ,  $I_{Kr}$ , and  $I_{Kur}$  is balanced by  $Ca^{2+}$  influx mediated by sarcolemmal voltage-gated  $Ca^{2+}$  current. The predominant isoform of pore-forming ( $\alpha$ ) subunit of the underlying voltage-gated  $Ca^{2+}$  ( $Ca_v$ ) channels,  $Ca_v1.2$  (*Cacna1c*) and  $Ca_v1.3$  (*Cacna1d*), expressed to a lesser extent shows strong homologies with  $Na_v1.5$  (Tanabe et al., 1988). It generates a surface L-type  $Ca^{2+}$  current ( $I_{Ca-L}$ ) with a distinct, very slow, inactivation. Furthermore, the  $\alpha$  subunit complexes with both *Cacnb2* ( $\beta$ ) and  $\gamma$  and *Cacna2d1/2* ( $\alpha2\gamma$ ) subunits.  $\alpha2\gamma$  enhances  $\beta$ -mediated (Pragnell et al., 1994) channel trafficking (Felix et al., 1997) in addition to modulating the kinetic profile of  $I_{Ca-L}$ . In contrast, neither  $Ca_v3.1$  (*Cacna1g*) nor  $Ca_v3.2$  (*Cacna1h*) require accessory subunits for expression and permission of surface T-type  $Ca^{2+}$  current ( $I_{Ca-T}$ ).

In *Scn5a*<sup>+/-</sup> ventricles, *Cacna1d* transcript levels showed a significant decrease of 59% compared to WT, with no further significant changes in mRNA levels corresponding to the remaining genes above. *Scn5a*<sup>+/-</sup> atria showed significant increases, >80% in *Cacna1c*, *Cacna1h*, *Cacna2d1*, and *Cacna2d2* expression (Table 1).

### 3.7 | Intracellular ion channels, transporters, and enzymes controlling $Ca^{2+}$ homeostasis

In addition to surface  $Ca^{2+}$  fluxes, intracellular  $Ca^{2+}$  homeostasis involves  $Ca^{2+}$  movements between SR and cytosol. SR  $Ca^{2+}$ -release channel ryanodine receptors (Ryr) activate upon binding of the cytosolic  $Ca^{2+}$  derived from the initial  $I_{Ca}$ , inducing a  $Ca^{2+}$ -induced  $Ca^{2+}$  release that couples excitation to cardiomyocyte contraction (Fabiato, 1983). Ryr2 is the most common cardiac isoform, though Ryr3 (Perez et al., 2005) is also expressed. SERCA (Atp2a2) mediates active  $Ca^{2+}$  transport into the SR;  $Ca^{2+}$  binding to calsequestrin (Casq2) then reduces free [ $Ca^{2+}$ ] SR (Knollmann, 2009), facilitating SR membrane  $Ca^{2+}$  transport. Along with triadin (Caswell et al., 1991) and junctin (Jones et al., 1995), Casq2 also complexes with Ryrs (Zhang et al., 1997).  $Ca^{2+}$ /CaM-dependent kinase II (Camk2d) regulates  $Ca^{2+}$  homeostasis at all points along this axis (Rodriguez et al., 2003; Wehrens et al., 2004), as well as phosphorylating, and modulating the behavior of, several  $Na_v$  subtypes (Burel et al., 2017),  $K_v$  (Li et al., 2007; Tessier et al., 1999; Wagner et al., 2009), and  $Ca_v$  (Blaich et al., 2010) channels.

*Scn5a*<sup>+/-</sup> ventricles showed no significant changes in concentrations of mRNAs transcribed from the above relative to WT. In contrast, *Scn5a*<sup>+/-</sup> atria displayed significant *Atp2a2*, *Ryr2*, and *Camk2d* upregulation, with increases in expression of 222%, 138%, and 81%, respectively, as well as a trend ( $p < 0.1$ ) toward greater apparent *Ryr3* and *Casq2* transcription (Table 1).

### 3.8 | Surface adrenoceptors

Surface adrenoceptor (AR) activation further modulates cardiomyocyte action potential waveforms. Aside from the  $G_q$ -linked  $\alpha_1$ -AR (*Adra1a*; subtype A), neither *Adra1b* nor *Adra1d* (subtypes B and D) elicit differential net negative and positive inotropic effects in either the right or left ventricles (Wang et al., 2006). Cardiomyocytes express  $G_s$ -linked  $\beta$ -ARs.  $\beta_1$ -ARs (*Adrb1*) predominate, but  $Ca_v1.2$ -coupled  $\beta_2$ -ARs (*Adrb2*) also contribute to the generation of positive chronotropy, inotropy, and lusitropy, through mechanisms including  $G_i$ -mediated phospholipase  $A_2$  activation (Pavoine & Defer, 2005).

However, *Scn5a*<sup>+/-</sup> showed no significant atrial or ventricular differences in levels of mRNA transcribed from genes encoding these surface adrenoceptors from WT (Table 1).

### 3.9 | Proteins, and their subunits, involved in the adenylyl cyclase pathway

The adenylyl cyclase pathway, exemplified by  $\beta$ -AR signaling, mediates changes in chronotropy, inotropy, and lusitropy. Its central hub is the tetrameric enzyme phosphokinase A (Pearce et al., 2010). Of its two catalytic subunits, *Prkaca* encodes one subtype, and two autoinhibitory regulatory subunits—subdivided into types I, for example, *Prkar1a*, and II, for example, *Prkar2a* or *Prkar2b*. It is colocalized via A-kinase anchoring proteins (AKAPs) (Bauman & Scott, 2002) with phosphodiesterases (PDEs), including PDE2 (*Pde2a*) and PDE4 (*Pde4d*). This establishes a high cAMP turnover rate. Large fold changes in  $[cAMP]_i$  relieve the allosteric autoinhibition of catalytic subunits, such that the intact holoenzyme (Smith et al., 2017) can initiate a phosphorylation cascade.

Neither *Scn5a*<sup>+/-</sup> atria nor ventricles showed any significant differences in the concentration of RNA transcribed from genes encoding proteins, and their subunits, involved in the adenylyl cyclase pathway relative to WT.

### 3.10 | Fibrotic markers

Fibrosis involves replacement of myocardial with connective tissue resulting in the remodeling of cardiac chambers (Travers et al., 2016). It is driven by transforming growth factor- $\beta$  (TGF- $\beta$ ) (Walton et al., 2017) activating canonical and non-canonical pathways culminating in myofibroblast stimulation. This increases the formation relative to degradation of extracellular matrix. The resulting deposition of types I (*Col1a1*) and III (*Col3a1*) collagen fibrils creates scar tissue (Khalil et al., 2017).

Neither *Scn5a*<sup>+/-</sup> atria nor *Scn5a*<sup>+/-</sup> ventricles showed significant differences in *Tgfb1* expression compared to WT. In contrast, *Scn5a*<sup>+/-</sup> atria showed a significant upregulation of *Col1a1* mRNA by 107% relative to WT (Table 1).

### 3.11 | Gap junction connexins

The component cardiomyocytes of each cardiac chamber create an electrical syncytium through formation of gap junctions, each consisting of two hemichannels. The connexon is a hexamer of connexin subunits, of which Cx43 (*Gja1*) and Cx40 (*Gja5*) are the most important for ventricular (Verheule et al., 1997) and atrial propagation of excitation (Gollob et al., 2006), respectively. Although murine Cx30.2 occasionally heteromizes (Gemel et al., 2008) with such connexins in the AVN, expression of the human orthologue Cx31.9 (*Gjd3*) protein may be undetectable in the myocardium (Kreuzberg et al., 2009).

cDNA corresponding to *Gdj3* was reliably detected. However, there were no significant differences in the levels of the gap junction connexin mRNAs between *Scn5a*<sup>+/-</sup> and WT mice, in either atria or ventricles (Table 1).

### 3.12 | Other genes

T-box transcription factor 3 (*Tbx3*) and transient receptor potential canonical 1 (*Trpc1*) are prerequisites for specifying the atrioventricular conduction system (Bakker et al., 2008) and for governing the hypertrophic response in failing cardiomyocytes (Seth et al., 2009), respectively.

*Scn5a*<sup>+/-</sup> ventricles showed no significant changes in *Tbx3* and *Trpc1* mRNA levels relative to WT mice. In contrast, *Scn5a*<sup>+/-</sup> atria exhibited significant upregulations of the former, with a 140% increase (Table 1).

## 4 | DISCUSSION

This study examines the effects of diminished expression of Na<sub>v</sub>1.5 on the transcriptome of murine atrial and ventricular cardiomyocytes, and, in turn, the extent to which such significant changes reflect or explain electrophysiological observations. It surveys transcriptional changes in *Scn5a*<sup>+/-</sup> murine hearts hitherto used as an experimental model for BrS and related clinical conditions. Over 90% of the identified genomic mutations in BrS patients involve *Scn5a* (Chen et al., 1998). However, BrS has also been correlated with mutations in an additional 20 genes (Watanabe et al., 2011). These include genes related to ion channels controlling resting  $E_m$ , such as *Abcc9* (Hu et al., 2014) and *Kcnj8*; ion channels initiating excitation, such as *Hcn4*; subunits modulating  $I_{Na}$  kinetics, such as *Scn1b-3b* (Hu et al., 2009; Riuró et al., 2013; Watanabe et al., 2008); ion channels controlling repolarization, such as *Kcne3* (Delpón et al., 2008), *Kcne5*, and *Kcnd3*; and surface  $I_{Ca}$ , such as *Cacna1c*, *Cacna2b* (Cordeiro et al., 2009), and *Cacna2d1*. Such findings are consistent with a multigenic backdrop in BrS, that could potentially involve evolution of its phenotype in the aged organism (Antzelevitch et al., 2005).

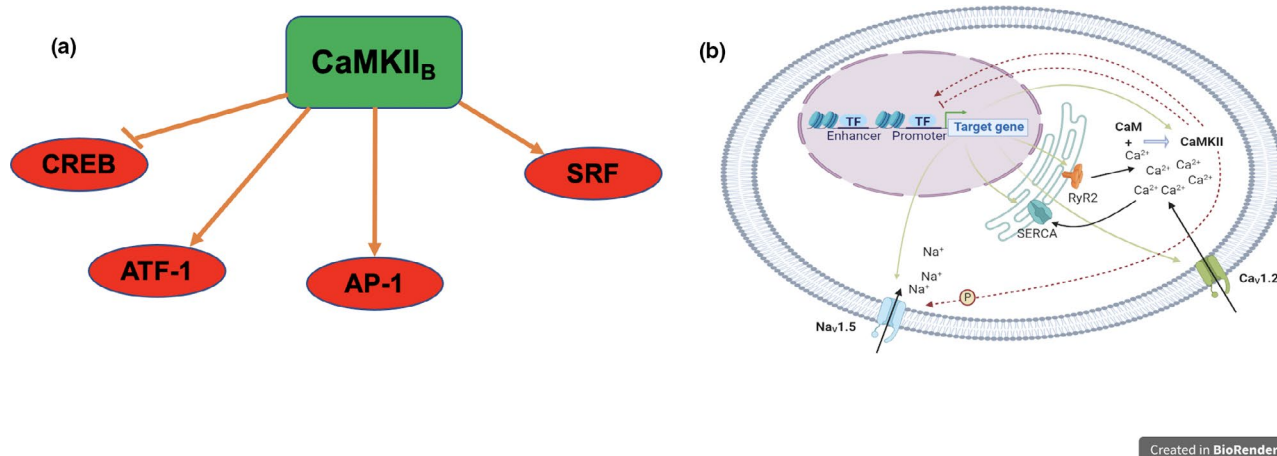
This study accordingly explores the effects of Na<sub>v</sub>1.5 haploinsufficiency on the transcriptome of atrial and ventricular cardiomyocytes, with a particular focus on the latter additional genes. Atria and ventricles showed differing transcriptional alterations arising from the *Scn5a*<sup>+/-</sup> genotype. The ventricular changes were limited to falls in the transcription of two unrelated genes: *Abcc9* and *Cacna1d*. The atrial *Scn5a*<sup>+/-</sup> genotype resulted in significant upregulations of 12 genes. Most of these changes clustered in the functional gene group regulating intracellular Ca<sup>2+</sup> homeostasis, either involving surface



currents (*Cacna1c*, *Cacna1h*, *Cacna2d1*, and *Cacna2d2*) or cytosolic and SR proteins (*Atp2a2*, *Camk2d*, and *Ryr2*). In addition,  $\text{Na}_v1.5$  haploinsufficiency may increase atrial predisposition to fibrosis via *Col1a1*.

Here we relate the present findings to previously reported electrophysiological features of *Scn5a*<sup>+/-</sup> murine hearts. First, the reduced ventricular  $\text{Na}_v1.5$  but normal or increased HCN expression reported here correlate with particular ventricular electrophysiological properties reported on earlier occasions. Previous physiological studies had reported that *Scn5a*<sup>+/-</sup> murine hearts were mildly bradycardic and showed increased risks of SAN block with age, and attributed these to altered  $I_{\text{Na}}$  rather than  $I_f$  (Lei et al., 2005). This study correspondingly reported that *Scn5a*<sup>+/-</sup> hearts showed unaltered ventricular *Hcn1*, *Hcn2*, or *Hcn4* expression and actual increases in atrial *Hcn1* expression compared to WT mice. In addition, young *Scn5a*<sup>+/-</sup> hearts showed impaired atrial, AV, and ventricular conduction velocity (Papadatos et al., 2002) consistent with the reduced overall  $\text{Na}_v1.5$  mRNA reported previously (Leoni et al., 2010) and 54% reduced ventricular  $\text{Na}_v1.5$  mRNA reported here. *Scn5a*<sup>+/-</sup> also showed accentuated QT dispersions, shortened ventricular action potential durations particularly involving the right ventricle recapitulating clinical observations (Ikeda, 2001) (Pitzalis et al., 2003), that may form the bases of both ST elevation and arrhythmia in BrS (Yan & Antzelevitch, 1999). These changes took place in an absence of altered expression in genes encoding ion channels controlling cardiomyocyte repolarization; the latter invite future investigations of increased right than left ventricular *Kcnd2* and *Kcnd3* expression and  $I_{\text{to}}$  density to similar extents in both *Scn5a*<sup>+/-</sup> and WT hearts (Martin et al., 2012).

Second, the present findings show relatively normal atrial  $\text{Na}_v1.5$  mRNA expression, inviting future studies investigating for regionally specific negative feedback loop regulating atrial *Scn5a* transcription. This would then parallel previous reports describing differing levels of right and left ventricular *Scn5a* transcription in both WT and mutant mice hearts (Martin et al., 2012). In contrast, *Scn5a*<sup>+/-</sup> atria particularly demonstrated altered mRNA levels of gene products involved in surface  $\text{Ca}^{2+}$  current function such as *Cacna1c*, *Cacna1h*, *Cacna2d1*, and *Cacna2d2*, and of intracellular proteins, such as *Camk2d* and *Ryr2*, involved in  $[\text{Ca}^{2+}]_i$  homeostasis with potential actions on  $I_{\text{Na}}$  (Table 1). In particular, the atrial upregulation of *Camk2d*, in *Scn5a*<sup>+/-</sup> atria could over activate CaMKII known to phosphorylate Ser1933 and Ser1944 in the C-terminal domain of  $\text{Na}_v1.5$ , interfering with  $\text{Ca}^{2+}$ /CaM-directed alteration of its inactivation kinetics (Burel et al., 2017), as well as independently hyperpolarizing its steady-state inactivation curve (Shah et al., 2006 ; Yoon et al., 2009). Furthermore, by phosphorylating Ser571 in its first intracellular loop, CaMKII augments the late  $I_{\text{Na}}$  ( $I_{\text{Na,L}}$ ) (Glynn et al., 2015). Similar events have been reported with excessive angiotensin II (Omar Velez Rueda et al., 2012) and reactive oxygen species (He & Zuo, 2015) in overactivating cardiomyocyte CaMKII. CaMKII also alters phosphorylation of targets such as RyR2 (Tian, 2004), the protein product of the *Ryr2* gene, itself also upregulated in *Scn5a*<sup>+/-</sup> atria. Moreover, gain-of-function mutations in *Ryr2* greatly reduce the protein expression of  $\text{Na}_v1.5$  in both atria (King et al., 2013) and ventricles (Ning et al., 2016), implying raised  $[\text{Ca}^{2+}]_i$ -mediated acute and chronic  $\text{Na}_v$  inhibition.



**FIGURE 3** Through phosphorylation,  $\text{Ca}^{2+}$ /CaM-activated kinase II (CaMKII) introduces post-translational modifications that influence the activities of various transcription factors (a). We posit a possible role for CaMKII, which post-translationally modulates  $\text{Na}_v1.5$ , in mediating the negative feedback loop and/or changes in the transcription of genes controlling cytosolic  $[\text{Ca}^{2+}]$  (b) in the atria of *Scn5a*<sup>+/-</sup> mice. AP-1, activator protein 1; ATF-1, cAMP-dependent transcription factor 1; CREB, cAMP response element-binding protein; SRF, serum response factor. “Subscript B” refer to CaMKII isoforms

Such a relationship between changes in  $\text{Na}_v1.5$  function and  $\text{Ca}^{2+}$  homeostasis may involve post-translational and/or transcriptional mechanisms. The  $\text{CaMKII}\delta_B$  isoform possesses a nuclear localization signal, providing a basis for its role in excitation-transcription coupling (Figure 3a). Differential stimulation of nuclear  $\text{CaMKII}_B$  influences a range of transcription factors, including CREB (Sun et al., 1994), ATF-1 (Shimomura et al., 1996), AP-1 (Antoine et al., 1996), and SRF (Flück et al., 2000); the last is crucial in both initiating and maintaining the pre-established cardiac transcriptional profile. This raises the intriguing possibility that  $\text{CaMKII}$  provides the missing link between the apparent negative feedback loop regulating atrial *Scn5a* transcription, and the upregulation of genes regulating intracellular  $\text{Ca}^{2+}$  homeostasis (Figure 3b).

Third, we here report that although *Scn5a*<sup>+/-</sup> atria and ventricles showed similar connexin expression levels and similar TGF- $\beta$  expression, *Scn5a*<sup>+/-</sup> atria showed a significantly upregulated *Col1a1* transcription relative to WT. This would parallel their interstitial fibrotic phenotype (Coronel et al., 2005; Frustaci et al., 2005) exacerbated by aging (Jeevaratnam et al., 2016).

The precise mechanistic links between the *Scn5a*<sup>+/-</sup> genotype, cardiac chamber-specific transcriptional changes, and the BrS phenotype remains to be fully elucidated. However, this study reveals a clear link between  $\text{Na}_v1.5$  expression and calcium homeostasis in the atrial cardiomyocytes, with *Camk2d* a (speculative, but) possible mediator (Figure 3b). Moreover, the absence of gene expression alterations in the ventricular tissue might suggest that the level of  $\text{Na}_v1.5$  is not as critical as in the atria and that the compensatory mechanisms are either not activated or required.

Nonetheless, this tentative hypothesis must be regarded with caution. First, to avoid undermining the statistical power of this study, the relatively small sample of *Scn5a*<sup>+/-</sup> hearts was not divided into distinct groups of left and right atria and ventricles. Yet the lack of distinction made between the different sides of the hearts' chambers may have obscured a potential laterality in gene expression, which, especially in light of functional data demonstrating left/right differences in the electrophysiology of BrS patient hearts (Pitzalis et al., 2003), merits future investigation. Second, and relatedly, to best simulate the effects of age-related structural, molecular, and electrophysiological changes in human hearts on BrS risk and onset (Jeevaratnam et al., 2010; Nademanee et al., 2015; Papadatos et al., 2002), the *Scn5a*<sup>+/-</sup> hearts studies were exclusively those of aged mice, though future study could also add younger *Scn5a*<sup>+/-</sup> hearts as a control group. However, given similar sex-related effects on BrS risk and *Scn5a*<sup>+/-</sup> murine heart function (Jeevaratnam et al., 2010), the lack of distinction made

between the sexes (to avoid underpowering the study) likewise may have obscured sex-specific changes in atrial or ventricular gene expression, again meriting further investigation. Finally, future study may benefit from western blotting to both verify whether translation reflects transcriptional changes, and to provide mechanistic insights into, for instance, changes in protein trafficking with *Scn5a* heterozygosity and differences therein between left and right atria and ventricles.

Despite these limitations, this study of cardiomyocyte transcriptional profiles in a murine *Scn5a*<sup>+/-</sup> model provides a path for further investigation into the molecular mechanisms underlying common arrhythmic disorders.

## CONFLICT OF INTEREST

None to declare.

## AUTHOR CONTRIBUTIONS

Michael Takla, Charlotte E. Edling, Kevin Zhang, and Samantha C. Salvage undertook the lab experiments, quality control, and technical troubleshooting; Michael Takla, Charlotte E. Edling, Khalil Saadeh, and Gary Tse undertook the data analysis; Michael Takla, Charlotte E. Edling, and Khalil Saadeh wrote the first draft of the manuscript. Christopher L.-H. Huang and Kamalan Jeevaratnam designed the study, provided supervision, and secured funding for the work. All authors reviewed all subsequent drafts of the manuscript.

## ORCID

Christopher L.-H. Huang  <https://orcid.org/0000-0001-9553-6112>

Kamalan Jeevaratnam  <https://orcid.org/0000-0002-6232-388X>

## REFERENCES

- Abriel, H. (2010). Cardiac sodium channel Nav1.5 and interacting proteins: Physiology and pathophysiology. *Journal of Molecular and Cellular Cardiology*, 48, 2–11. <https://doi.org/10.1016/j.yjmcc.2009.08.025>
- Amin, A. S., Asghari-Roodsari, A., & Tan, H. L. (2010). Cardiac sodium channelopathies. *Pflügers Archiv - European Journal of Physiology*, 460, 223–237. <https://doi.org/10.1007/s00424-009-0761-0>
- Angelo, K., Jespersen, T., Grunnet, M., Nielsen, M. S., Klaerke, D. A., & Olesen, S.-P. (2002). KCNE5 induces time- and voltage-dependent modulation of the KCNQ1 current. *Biophysical Journal*, 83, 1997–2006. [https://doi.org/10.1016/S0006-3495\(02\)73961-1](https://doi.org/10.1016/S0006-3495(02)73961-1)
- Antoine, M., Gaidon, C., & Loeffler, J. P. (1996).  $\text{Ca}^{2+}$ /calmodulin kinase types II and IV regulate c-fos transcription in the AtT20 corticotroph cell line. *Molecular and Cellular Endocrinology*, 120, 1–8. [https://doi.org/10.1016/0303-7207\(96\)03806-3](https://doi.org/10.1016/0303-7207(96)03806-3)
- Antzelevitch, C., Brugada, P., Brugada, J., & Brugada, R. (2005). Brugada syndrome: From cell to bedside. *Current Problems*

- in *Cardiology*, 30, 9–54. <https://doi.org/10.1016/j.cpcardiol.2004.04.005>
- Bakker, M. L., Boukens, B. J., Mommersteeg, M. T. M., Brons, J. F., Wakker, V., Moorman, A. F. M., & Christoffels, V. M. (2008). Transcription factor Tbx3 is required for the specification of the atrioventricular conduction system. *Circulation Research*, 102, 1340–1349. <https://doi.org/10.1161/CIRCRESAHA.107.169565>
- Baruscotti, M., Bucchi, A., Viscomi, C., Mandelli, G., Consalez, G., Gneschi-Rusconi, T., Montano, N., Casali, K. R., Micheloni, S., Barbuti, A., & DiFrancesco, D. (2011). Deep bradycardia and heart block caused by inducible cardiac-specific knockout of the pacemaker channel gene Hcn4. *Proceedings of the National Academy of Sciences United States of America*, 108, 1705–1710. <https://doi.org/10.1073/pnas.1010122108>
- Bauman, A. L., & Scott, J. D. (2002). Kinase- and phosphatase-anchoring proteins: harnessing the dynamic duo. *Nature Cell Biology*, 4, E203–E206. <https://doi.org/10.1038/ncb0802-e203>
- Blaich, A., Welling, A., Fischer, S., Wegener, J. W., Kostner, K., Hofmann, F., & Moosmang, S. (2010). Facilitation of murine cardiac L-type Cav1.2 channel is modulated by calmodulin kinase II-dependent phosphorylation of S1512 and S1570. *Proceedings of the National Academy of Sciences United States of America*, 107, 10285–10289. <https://doi.org/10.1073/pnas.0914287107>
- Bogdanov, K. Y., Vinogradova, T. M., & Lakatta, E. G. (2001). Sinoatrial nodal cell ryanodine receptor and Na<sup>+</sup>-Ca<sup>2+</sup> exchanger: Molecular partners in pacemaker regulation. *Circulation Research*, 88, 1254–1258. <https://doi.org/10.1161/hh1201.092095>
- Brown, H., & DiFrancesco, D. (1980). Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *Journal of Physiology*, 308, 331–351. <https://doi.org/10.1113/jphysiol.1980.sp013474>
- Burel, S., Coyan, F. C., Lorenzini, M., Meyer, M. R., Lichti, C. F., Brown, J. H., Loussouarn, G., Charpentier, F., Nerbonne, J. M., Townsend, R. R., Maier, L. S., & Marionneau, C. (2017). C-terminal phosphorylation of NaV1.5 impairs FGF13-dependent regulation of channel inactivation. *Journal of Biological Chemistry*, 292, 17431–17448. <https://doi.org/10.1074/jbc.M117.787788>
- Caswell, A. H., Brandt, N. R., Brunschwig, J. P., & Purkerson, S. (1991). Localization and partial characterization of the oligomeric disulfide-linked molecular weight 95 000 protein (triadin) which binds the ryanodine and dihydropyridine receptors in skeletal muscle triadic vesicles. *Biochemistry*, 30, 7507–7513. <https://doi.org/10.1021/bi00244a020>
- Chen, Q., Kirsch, G. E., Zhang, D., Brugada, R., Brugada, J., Brugada, P., Potenza, D., Moya, A., Borggrefe, M., Breithardt, G., Ortiz-Lopez, R., Wang, Z., Antzelevitch, C., O'Brien, R. E., Schulze-Bahr, E., Keating, M. T., Towbin, J. A., & Wang, Q. (1998). Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature*, 392, 293–296. <https://doi.org/10.1038/32675>
- Chockalingam, P., Clur, S. A. B., Breur, J. M. P. J., Kriebel, T., Paul, T., Rammeloo, L. A., Wilde, A. A. M., & Blom, N. A. (2012). The diagnostic and therapeutic aspects of loss-of-function cardiac sodium channelopathies in children. *Heart Rhythm*, 9, 1986–1992. <https://doi.org/10.1016/j.hrthm.2012.08.011>
- Cordeiro, J. M., Marieb, M., Pfeiffer, R. Calloe, K., Burashnikov, E., & Antzelevitch, C. (2009). Accelerated inactivation of the L-type calcium current due to a mutation in CACNB2b underlies Brugada syndrome. *Journal of Molecular and Cellular Cardiology*, 46, 695–703. <https://doi.org/10.1016/j.yjmcc.2009.01.014>
- Coronel, R., Casini, S., Koopmann, T. T. Wilms-Schopman, F. J. G., Verkerk, A. O., de Groot, J. R., Bhuiyan, Z., Bezzina, C. R., Veldkamp, M. W., Linnenbank, A. C., van der Wal, A. C., Tan, H. L., Brugada, P., Wilde, A. A. M., & de Bakker, J. M. T. (2005). Right ventricular fibrosis and conduction delay in a patient with clinical signs of Brugada syndrome: A combined electrophysiological, genetic, histopathologic, and computational study. *Circulation*, 112, 2769–2777. <https://doi.org/10.1161/CIRCULATIONAHA.105.532614>
- Dautova, Y., Zhang, Y., Grace, A. A., & Huang, C. L. H. (2010). Atrial arrhythmogenic properties in wild-type and Scn5a<sup>+/-</sup> murine hearts. *Experimental Physiology*, 95, 994–1077. <https://doi.org/10.1113/expphysiol.2010.053868>
- Delpón, E., Cordeiro, J. M., Núñez, L., Thomsen, P. E. B., Guerschicoff, A., Pollevick, G. D., Wu, Y., Kanters, J. K., Larsen, C. T., Hofman-Bang, J., Burashnikov, E., Christiansen, M., & Antzelevitch, C. (2008). Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. *Circulation Arrhythmia Electrophysiology*, 1(3), 209–218. <https://doi.org/10.1161/CIRCEP.107.748103>
- Edling, C. E., Fazmin, I. T., Chadda, K. R., Ahmad, S., Valli, H., Grace, A. A., Huang, C. L.-H., & Jeevaratnam, K. (2019). Ageing in Pgc-1β<sup>-/-</sup> mice modelling mitochondrial dysfunction induces differential expression of a range of genes regulating ventricular electrophysiology. *Bioscience Reports*, 39. <https://doi.org/10.1042/BSR20190127>
- Fabiato, A. (1983). Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *American Journal of Physiology-Cell Physiology*, 245, C1–C14. <https://doi.org/10.1152/ajpcell.1983.245.1.C1>
- Felix, R., Gurnett, C. A., De Waard, M., & Campbell, K. P. (1997). Dissection of functional domains of the voltage-dependent Ca<sup>2+</sup> channel α2δ subunit. *Journal of Neuroscience*, 17, 6884–6891. <https://doi.org/10.1523/JNEUROSCI.17-18-06884.1997>
- Feng, J., Wible, B., Li, G. R., Wang, Z., & Nattel, S. (1997). Antisense oligodeoxynucleotides directed against Kv1.5 mRNA specifically inhibit ultrarapid delayed rectifier K<sup>+</sup> current in cultured adult human atrial myocytes. *Circulation Research*, 80(4), 572–579. <https://doi.org/10.1161/01.RES.80.4.572>
- Fenske, S., Mader, R., Scharr, A., Pappazios, C., Cao-Ehlker, X., Michalakis, S., Shaltiel, L., Weidinger, M., Stieber, J., Feil, S., Feil, R., Hofmann, F., Wahl-Schott, C., & Biel, M. (2011). HCN3 contributes to the ventricular action potential waveform in the murine heart. *Circulation Research*, 109, 1015–1023. <https://doi.org/10.1161/CIRCRESAHA.111.246173>
- Ficker, E., Taglialatela, M., Wible, B. A., Henley, C., & Brown, A. (1994). Spermine and spermidine as gating molecules for inward rectifier K<sup>+</sup> channels. *Science*, 266, 1068–1072. <https://doi.org/10.1126/science.7973666>
- Flück, M., Booth, F. W., & Waxham, M. N. (2000). Skeletal muscle CaMKII enriches in nuclei and phosphorylates myogenic factor SRF at multiple sites. *Biochemical and Biophysical Research Communications*, 270, 488–494. <https://doi.org/10.1006/bbrc.2000.2457>
- Flynn, G. E., & Zagotta, W. N. (2018). Insights into the molecular mechanism for hyperpolarization-dependent activation of



- HCN channels. *Proceedings of the National Academy of Sciences United States of America*, 115, E8086–E8095. <https://doi.org/10.1073/pnas.1805596115>
- Frustaci, A., Priori, S. G., Pieroni, M., Chimenti, C., Napolitano, C., Rivolta, I., Sanna, T., Bellocci, F., & Russo, M. A. (2005). Cardiac histological substrate in patients with clinical phenotype of Brugada syndrome. *Circulation*, 112, 3680–3687. <https://doi.org/10.1161/CIRCULATIONAHA.105.520999>
- Garty, H., & Karlish, S. J. D. (2006). Role of FXYD proteins in ion transport. *Annual Review of Physiology*, 68, 431–459. <https://doi.org/10.1146/annurev.physiol.68.040104.131852>
- Geering, K. (2001). The functional role of  $\beta$  subunits in oligomeric P-type ATPases. *Journal of Bioenergetics and Biomembranes*, 33, 425–438.
- Gemel, J., Lin, X., Collins, R., Veenstra, R. D., & Beyer, E. C. (2008). Cx30.2 can form heteromeric gap junction channels with other cardiac connexins. *Biochemical and Biophysical Research Communications*, 369, 388–394. <https://doi.org/10.1016/j.bbrc.2008.02.040>
- Glynn, P., Musa, H., Wu, X., Unudurthi, S. D., Little, S., Qian, L., Wright, P. J., Radwanski, P. B., Gyorke, S., Mohler, P. J., & Hund, T. J. (2015). Voltage-gated sodium channel phosphorylation at Ser571 regulates late current, arrhythmia, and cardiac function in vivo. *Circulation*, 132, 567–577. <https://doi.org/10.1161/CIRCULATIONAHA.114.015218>
- Goldin, A. L. (2003). Mechanisms of sodium channel inactivation. *Current Opinion in Neurobiology*, 13, 284–290. [https://doi.org/10.1016/S0959-4388\(03\)00065-5](https://doi.org/10.1016/S0959-4388(03)00065-5)
- Gollob M. H., Jones D. L., Krahn A. D., Danis L., Gong X. Q., Shao Q., Liu X., Veinot J. P., Tang A. S. L., Stewart A. F. R., Tesson F., Klein G. J., Yee R., Skanes A. C., Guiraudon G. M., Ebihara L., & Bai D. (2006). Somatic Mutations in the Connexin 40 Gene (GJA5) in Atrial Fibrillation. *New England Journal of Medicine*, 354, (25), 2677–2688. <http://dx.doi.org/10.1056/nejmoa052800>
- Günther, A., & Baumann, A. (2015). Distinct expression patterns of HCN channels in HL-1 cardiomyocytes. *BMC Cell Biology*, 16. <https://doi.org/10.1186/s12860-015-0065-5>
- Gussak, I., Antzelevitch, C., Bjerregaard, P., Towbin, J. A., & Chaitman, B. R. (1999). The Brugada syndrome: Clinical, electrophysiologic and genetic aspects. *Journal of the American College of Cardiology*, 33, 5–15. [https://doi.org/10.1016/S0735-1097\(98\)00528-2](https://doi.org/10.1016/S0735-1097(98)00528-2)
- Guzadhur, L., Jiang, W., Pearcey, S. M., Jeevaratnam, K., Duehmke, R. M., Grace, A. A., Lei, M., & Huang, C. L.-H. (2012). The age-dependence of atrial arrhythmogenicity in Scn5a<sup>+/-</sup> murine hearts reflects alterations in action potential propagation and recovery. *Clinical and Experimental Pharmacology and Physiology*, 39, 518–527. <https://doi.org/10.1111/j.1440-1681.2012.05706.x>
- He, F., & Zuo, L. (2015). Redox roles of reactive oxygen species in cardiovascular diseases. *International Journal of Molecular Sciences*, 16, 27770–27780. <https://doi.org/10.3390/ijms161126059>
- Herrmann, S., Layh, B., & Ludwig, A. (2011). Novel insights into the distribution of cardiac HCN channels: An expression study in the mouse heart. *Journal of Molecular and Cellular Cardiology*, 51, 997–1006. <https://doi.org/10.1016/j.yjmcc.2011.09.005>
- Hiyama, T. Y., Watanabe, E., Ono, K., Inenaga, K., Tamkun, M. M., Yoshida, S., & Noda, M. (2002). Nax channel involved in CNS sodium-level sensing. *Nature Neuroscience*, 5, 511–512. <https://doi.org/10.1038/nn856>
- Hosseini, S. M., Kim, R., Udupa, S., Costain, G., Jobling, R., Liston, E., Jamal, S. M., Szybowska, M., Morel, C. F., Bowdin, S., Garcia, J., Care, M., Sturm, A. C., Novelli, V., Ackerman, M. J., Ware, J. S., Hershberger, R. E., Wilde, A. A. M., & Gollob, M. H. (2018). Reappraisal of reported genes for sudden arrhythmic death. *Circulation*, 138, 1195–1205. <https://doi.org/10.1161/CIRCULATIONAHA.118.035070>
- Hu, D., Barajas-Martinez, H., Burashnikov, E., Springer, M., Wu, Y., Varro, A., Pfeiffer, R., Koopmann, T. T., Cordeiro, J. M., Guerchicoff, A., Pollevick, G. D., & Antzelevitch, C. (2009). A mutation in the  $\beta$ 3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. *Circulation: Cardiovascular Genetics*, 2, 270–278. <https://doi.org/10.1161/CIRCGENETICS.108.829192>
- Hu, D., Barajas-Martinez, H., Terzic, A., Park, S., Pfeiffer, R., Burashnikov, E., Wu, Y., Borggreffe, M., Veltmann, C., Schimpf, R., Cai, J., Nam, G.-B., Deshmukh, P., Scheinman, M., Preminger, M., Steinberg, J., López-Izquierdo, A., Ponce-Balbuena, D., Wolpert, C., ... Antzelevitch, C. (2014). ABCC9 is a novel Brugada and early repolarization syndrome susceptibility gene. *International Journal of Cardiology*, 171, 431–442. <https://doi.org/10.1016/j.ijcard.2013.12.084>
- Huang, C. L.-H. (2017). Murine electrophysiological models of cardiac arrhythmogenesis. *Physiological Reviews*, 97, 283–409. <https://doi.org/10.1152/physrev.00007.2016>
- Ikeda, T., Sakurada, H., Sakabe, K., Sakata, T., Takami, M., Tezuka, N., Nakae, T., Noro, M., Enjoi, Y., Tejima, T., Sugi, K., & Yamaguchi, T. (2001). Assessment of noninvasive markers in identifying patients at risk in the Brugada syndrome: Insight into risk stratification. *Journal of the American College of Cardiology*, 37(6), 1628–1634.
- Isom, L. L. (2001). Sodium channel  $\beta$  subunits: Anything but auxiliary. *Neuroscientist*, 7, 42–54. <https://doi.org/10.1177/107385840100700108>
- Jeevaratnam, K., Guzhadhur, L., Goh, Y. M., Grace, A. A., & Huang, C. L.-H. (2016). Sodium channel haploinsufficiency and structural change in ventricular arrhythmogenesis. *Acta Physiologica*, 216, 186–202. <https://doi.org/10.1111/apha.12577>
- Jeevaratnam, K., Zhang, Y., Guzhadhur, L., Duehmke, R. M., Lei, M., Grace, A. A., & Huang, C. L. H. (2010). Differences in sino-atrial and atrio-ventricular function with age and sex attributable to the Scn5a<sup>+/-</sup> mutation in a murine cardiac model. *Acta Physiologica*, 200(1), 23–33. <https://doi.org/10.1111/j.1748-1716.2010.02110.x>
- Jones, L. R., Zhang, L., Sanborn, K., Jorgensen, A. O., & Kelley, J. (1995). Purification, primary structure, and immunological characterization of the 26-kDa calsequestrin binding protein (junctin) from cardiac junctional sarcoplasmic reticulum. *Journal of Biological Chemistry*, 270, 30787–30796. <https://doi.org/10.1074/jbc.270.51.30787>
- Kapplinger, J. D., Tester, D. J., Alders, M., Benito, B., Berthet, M., Brugada, J., Brugada, P., Fressart, V., Guerchicoff, A., Harris-Kerr, C., Kamakura, S., Kyndt, F., Koopmann, T. T., Miyamoto, Y., Pfeiffer, R., Pollevick, G. D., Probst, V., Zumhagen, S., Vatta, M., ... Ackerman, M. J. (2010). An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Hear Rhythm*, 7, 33–46. <https://doi.org/10.1016/j.hrthm.2009.09.069>
- Ketchum, K. A., Joiner, W. J., Sellers, A. J., Kaczmarek, L. K., & Goldstein, S. A. N. (1995). A new family of outwardly rectifying



- potassium channel proteins with two pore domains in tandem. *Nature*, 376, 690–695. <https://doi.org/10.1038/376690a0>
- Khalil, H., Kanisicak, O., Prasad, V., Correll, R. N., Fu, X., Schips, T., Vagnozzi, R. J., Liu, R., Huynh, T., Lee, S.-J., Karch, J., & Molkenkin, J. D. (2017). Fibroblast-specific TGF- $\beta$ -Smad2/3 signaling underlies cardiac fibrosis. *Journal of Clinical Investigation*, 127, 3770–3783. <https://doi.org/10.1172/JCI94753>
- King, J., Wickramarachchi, C., Kua, K., Du, Y., Jeevaratnam, K., Matthews, H. R., Grace, A. A., Huang, C. L.-H., & Fraser, J. A. (2013). Loss of Nav1.5 expression and function in murine atria containing the RyR2-P2328S gain-of-function mutation. *Cardiovascular Research*, 99, 751–759. <https://doi.org/10.1093/cvr/cvt141>
- Knollmann, B. C. (2009). New roles of calsequestrin and triadin in cardiac muscle. *Journal of Physiology*, 587, 3081–3087. <https://doi.org/10.1113/jphysiol.2009.172098>
- Kreuzberg, M. M., Liebermann, M., Segsneider, S., Dobrowolski, R., Dobrzynski, H., Kaba, R., Rowlinson, G., Dupont, E., Severs, N. J., & Willecke, K. (2009). Human connexin31.9, unlike its orthologous protein connexin30.2 in the mouse, is not detectable in the human cardiac conduction system. *Journal of Molecular and Cellular Cardiology*, 46, 553–559. <https://doi.org/10.1016/j.yjmcc.2008.12.007>
- Kurita, T., Shimizu, W., Inagaki, M., Suyama, K., Taguchi, A., Satomi, K., Aihara, N., Kamakura, S., Kobayashi, J., & Kosakai, Y. (2002). The electrophysiologic mechanism of ST-segment elevation in Brugada syndrome. *Journal of the American College of Cardiology*, 40, 330–334. [https://doi.org/10.1016/S0735-1097\(02\)01964-2](https://doi.org/10.1016/S0735-1097(02)01964-2)
- Kusano, K. F., Taniyama, M., Nakamura, K., Miura, D., Banba, K., Nagase, S., Morita, H., Nishii, N., Watanabe, A., Tada, T., Murakami, M., Miyaji, K., Hiramatsu, S., Nakagawa, K., Tanaka, M., Miura, A., Kimura, H., Fuke, S., Sumita, W., ... Ohe, T. (2008). Atrial fibrillation in patients with Brugada syndrome. Relationships of gene mutation, electrophysiology, and clinical backgrounds. *Journal of the American College of Cardiology*, 51, 1169–1175. <https://doi.org/10.1016/j.jacc.2007.10.060>
- Lei, M., Goddard, C., Liu, J., Léoni, A. L., Royer, A., Fung, S. S. M., Xiao, G., Ma, A., Zhang, H., Charpentier, F., Vandenberg, J. I., Colledge, W. H., Grace, A. A., & Huang, C. L. H. (2005). Sinus node dysfunction following targeted disruption of the murine cardiac sodium channel gene Scn5a. *Journal of Physiology*, 567(2), 387–400. <https://doi.org/10.1113/jphysiol.2005.083188>
- Li, J., Marionneau, C., Koval, O., Zingman, L., Mohler, P. J., Nerbonne, J. M., & Anderson, M. E. (2007). Calmodulin kinase ii inhibition enhances ischemic preconditioning by augmenting ATP-sensitive K<sup>+</sup> current. *Channels*, 1, 387–394. <https://doi.org/10.4161/chan.5449>
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>- $\Delta\Delta$ CT</sup> method. *Methods*, 25, 402–408. <https://doi.org/10.1006/meth.2001.1262>
- Lopatin, A. N., Makhina, E. N., & Nichols, C. G. (1994). Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. *Nature*, 372, 366–369. <https://doi.org/10.1038/372366a0>
- Lutsenko, S., & Kaplan, J. H. (1993) An essential role for the extracellular domain of the Na, K-ATPase  $\beta$ -subunit in cation occlusion. *Biochemistry*, 32, 6737–6743. <https://doi.org/10.1021/bi00077a029>
- Malhotra, J. D., Kazen-Gillespie, K., Hortsch, M., & Isom, L. L. (2000). Sodium channel  $\beta$  subunits mediate homophilic cell adhesion and recruit ankyrin to points of cell-cell contact. *Journal of Biological Chemistry*, 275, 11383–11388. <https://doi.org/10.1074/jbc.275.15.11383>
- Malhotra, J. D., Koopmann, M. C., Kazen-Gillespie, K.A., Fettman, N., Hortsch, M., & Isom, L. L. (2002). Structural requirements for interaction of sodium channel  $\beta$ 1 subunits with ankyrin. *Journal of Biological Chemistry*, 277, 26681–26688. <https://doi.org/10.1074/jbc.M202354200>
- Martin, C. A., Siedlecka, U., Kemmerich, K., Lawrence, J., Cartledge, J., Guzadhur, L., Brice, N., Grace, A. A., Schwiening, C., Terracciano, C. M., & Huang, C. L. H. (2012). Reduced Na<sup>+</sup> and higher K<sup>+</sup> channel expression and function contribute to right ventricular origin of arrhythmias in Scn5a<sup>+/2</sup> mice. *Open Biology*, 2(6), 120072. <https://doi.org/10.1098/rsob.120072>
- Matsuda, H., Saigusa, A., & Irisawa, H. (1987). Ohmic conductance through the inwardly rectifying K channel and blocking by internal Mg<sup>2+</sup>. *Nature*, 325, 156–159. <https://doi.org/10.1038/325156a0>
- Matsuo, K., Akahoshi, M., Nakashima, E., Suyama, A., Seto, S., Hayano, M., & Yano, K. (2001). The prevalence, incidence and prognostic value of the Brugada-type electrocardiogram: A population-based study of four decades. *Journal of the American College of Cardiology*, 38, 765–770. [https://doi.org/10.1016/S0735-1097\(01\)01421-8](https://doi.org/10.1016/S0735-1097(01)01421-8)
- McNair, W. P., Ku, L., Taylor, M. R. G., Fain, P. R., Dao, D., Wolfel, E., Mestroni, L., & Familial Cardiomyopathy Registry Research Group (2004). SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. *Circulation*, 110(15), 2163–2167. <https://doi.org/10.1161/01.CIR.0000144458.58660.BB>
- Meregalli, P. G., Wilde, A. A. M., & Tan, H. L. (2005). Pathophysiological mechanisms of Brugada syndrome: Depolarization disorder, repolarization disorder, or more? *Cardiovascular Research*, 67, 367–378. <https://doi.org/10.1016/j.cardiores.2005.03.005>
- Morita, H., Morita, S. T., Nagase, S., Satoshi, Banba, K., Nishii, N., Tani, Y., Watanabe, A., Nakamura, K., Kusano, K. F., Emori, T., Matsubara, H., Hina, K., Kita, T., & Ohe, T. (2003). Ventricular arrhythmia induced by sodium channel blocker in patients with Brugada syndrome. *Journal of the American College of Cardiology*. <https://doi.org/10.1016/j.jacc.2003.06.004>
- Nademanee, K., Raju, H., De Noronha, S. V., Papadakis, M., Robinson, L., Rothery, S., Makita, N., Kowase, S., Boonmee, N., Vitayakritsirikul, V., Ratanarapee, S., Sharma, S., van der Wal, A. C., Christiansen, M., Tan, H. L., Wilde, A. A., Nogami, A., Sheppard, M. N., Veerakul, G., & Behr, E. R. (2015). Fibrosis, connexin-43, and conduction abnormalities in the Brugada syndrome. *Journal of the American College of Cardiology*, 66, 1976–1986. <https://doi.org/10.1016/j.jacc.2015.08.862>
- Nagase, S., Kusano, K. F., Morita, H., Fujimoto, Y., Kakishita, M., Nakamura, K., Emori, T., Matsubara, H., & Ohe, T. (2002). Epicardial electrogram of the right ventricular outflow tract in patients with the Brugada syndrome: Using the epicardial lead. *Journal of the American College of Cardiology*, 39, 1992–1995. [https://doi.org/10.1016/S0735-1097\(02\)01888-0](https://doi.org/10.1016/S0735-1097(02)01888-0)
- Ning, F., Luo, L., Ahmad, S., Valli, H., Jeevaratnam, K., Wang, T., Guzadhur, L., Yang, D., Fraser, J. A., Huang, C. L.-H., Ma, A., & Salvage, S. C. (2016). The RyR2-P2328S mutation downregulates Nav1.5 producing arrhythmic substrate in murine

- ventricles. *Pflugers Archiv - European Journal of Physiology*, 468, 655–665. <https://doi.org/10.1007/s00424-015-1750-0>
- O'Rourke, B. (2000). Myocardial K(ATP) channels in preconditioning. *Circulation Research*, 87, 845–855.
- Omar Velez Rueda, J., Palomeque, J., & Mattiazzi, A. (2012). Early apoptosis in different models of cardiac hypertrophy induced by high renin-angiotensin system activity involves CaMKII. *Journal of Applied Physiology*, 112, 2110–2120. <https://doi.org/10.1152/jappphysiol.01383.2011>
- Papadatos, G. A., Wallerstein, P. M. R., Head, C. E. G., Ratcliff, R., Brady, P. A., Benndorf, K., Saumarez, R. C., Trezise, A. E. O., Huang, C. L.-H., Vandenberg, J. I., Colledge, W. H., & Grace, A. A. (2002). Slowed conduction and ventricular tachycardia after targeted disruption of the cardiac sodium channel gene Scn5a. *Proceedings of the National Academy of Sciences United States of America*, 99, 6210–6215. <https://doi.org/10.1073/pnas.082121299>
- Pavoine, C., & Defer, N. (2005). The cardiac  $\beta$ 2-adrenergic signalling a new role for the cPLA2. *Cellular Signalling*, 17, 141–152. <https://doi.org/10.1016/j.cellsig.2004.09.001>
- Pearce, L. R., Komander, D., & Alessi, D. R. (2010). The nuts and bolts of AGC protein kinases. *Nature Reviews Molecular Cell Biology*, 11, 9–22. <https://doi.org/10.1038/nrm2822>
- Perez, C. F., López, J. R., & Allen, P. D. (2005). Expression levels of RyR1 and RyR3 control resting free  $Ca^{2+}$  in skeletal muscle. *American Journal of Physiology - Cell Physiology*, 288(3), C640–C649. <https://doi.org/10.1152/ajpcell.00407.2004>
- Pitzalis, M. V., Anaclerio, M., Iacoviello, M., Forleo, C., Guida, P., Troccoli, R., Massari, F., Mastropasqua, F., Sorrentino, S., Manghisi, A., & Rizzon, P. (2003). QT-Interval prolongation in right precordial leads: An additional electrocardiographic hallmark of Brugada syndrome. *Journal of the American College of Cardiology*, 42, 1632–1637. <https://doi.org/10.1016/j.jacc.2003.07.005>
- Post, R. L., & Jolly, P. C. (1957). The linkage of sodium, potassium, and ammonium active transport across the human erythrocyte membrane. *Biochimica et Biophysica Acta*, 3002, 118–128. [https://doi.org/10.1016/0006-3002\(57\)90426-2](https://doi.org/10.1016/0006-3002(57)90426-2)
- Pragnell, M., De Waard, M., Mori, Y., Tanabe, T., Snutch, T. P., & Campbell, K. P. (1994). Calcium channel  $\beta$ -subunit binds to a conserved motif in the I-II cytoplasmic linker of the  $\alpha$ 1-subunit. *Nature*, 368, 67–70. <https://doi.org/10.1038/368067a0>
- Probst, V., Kyndt, F., Potet, F., Trochu, J.-N., Mialet, G., Demolombe, S., Schott, J.-J., Baró, I., Escande, D., & Le Marec, H. (2003). Haploinsufficiency in combination with aging causes SCN5A-linked hereditary lenègre disease. *Journal of the American College of Cardiology*, 41, 643–652. [https://doi.org/10.1016/S0735-1097\(02\)02864-4](https://doi.org/10.1016/S0735-1097(02)02864-4)
- Riuró, H., Beltran-Alvarez, P., Tarradas, A., Selga, E., Campuzano, O., Vergés, M., Pagans, S., Iglesias, A., Brugada, J., Brugada, P., Vázquez, F. M., Pérez, G. J., Scornik, F. S., & Brugada, R. (2013). A missense mutation in the sodium channel  $\beta$ 2 subunit reveals SCN2B as a new candidate gene for Brugada syndrome. *Human Mutation*, 34(7), 961–966. <https://doi.org/10.1002/humu.22328>
- Rodriguez, P., Bhogal, M. S., & Colyer, J. (2003). Stoichiometric phosphorylation of cardiac ryanodine receptor on serine 2809 by calmodulin-dependent kinase II and protein kinase A. *Journal of Biological Chemistry*, 278, 38593–38600. <https://doi.org/10.1074/jbc.M301180200>
- Royer, A., Van Veen, T. A. B., Le Bouter, S., Marionneau, C., Griol-Charhbil, V., Léoni, A. L., Steenman, M., van Rijen, H. V. M., Demolombe, S., Goddard, C. A., Richer, C., Escoubet, B., Jarry-Guichard, T., Colledge, W. H., Gros, D., de Bakker, J. M. T., Grace, A. A., Escande, D., & Charpentier, F. (2005). Mouse model of SCN5A-linked hereditary Lenègre's: Disease age-related conduction slowing and myocardial fibrosis. *Circulation*, 111(14), 1738–1746. <https://doi.org/10.1161/01.CIR.0000160853.19867.61>
- Schwartz, L. M., & Stühmer, W. (1984). Voltage-dependent sodium channels in an invertebrate striated muscle. *Science*, 225, 523–525. <https://doi.org/10.1126/science.6330898>
- Seino, S. (1999). ATP-sensitive potassium channels: A model of heteromultimeric potassium channel/receptor assemblies. *Annual Review of Physiology*, 61(1), 337–362. <https://doi.org/10.1146/annurev.physiol.61.1.337>
- Seino, S., & Miki, T. (2004). Gene targeting approach to clarification of ion channel function: Studies of Kir6.x null mice. *Journal of Physiology*, 554, 295–300. <https://doi.org/10.1113/jphysiol.2003.047175>
- Seth, M., Zhang, Z. S., Mao, L., Graham, V., Burch, J., Stiber, J., Tsiokas, L., Winn, M., Abramowitz, J., Rockman, H. A., Birnbaumer, L., & Rosenberg, P. (2009). TRPC1 channels are critical for hypertrophic signaling in the heart. *Circulation Research*, 105, 1023–1030. <https://doi.org/10.1161/CIRCRESAHA.109.206581>
- Shah, V. N., Wingo, T. L., Weiss, K. L., Williams, C. K., Balsler, J. R., & Chazin, W. J. (2006). Calcium-dependent regulation of the voltage-gated sodium channel hH1: Intrinsic and extrinsic sensors use a common molecular switch. *Proceedings of the National Academy of Sciences United States of America*, 103, 3592–3597. <https://doi.org/10.1073/pnas.0507397103>
- Shimomura, A., Ogawa, Y., Kitani, T., Fujisawa, H., & Hagiwara, M. (1996). Calmodulin-dependent protein kinase II potentiates transcriptional activation through activating transcription factor 1 but not cAMP response element-binding protein. *Journal of Biological Chemistry*, 271, 17957–17960. <https://doi.org/10.1074/jbc.271.30.17957>
- Skibsbjerg, L., Poulet, C., Diness, J. G., Bentzen, B. H., Yuan, L., Kappert, U., Matschke, K., Wettwer, E., Ravens, U., Grunnet, M., Christ, T., & Jespersen, T. (2014). Small-conductance calcium-activated potassium (SK) channels contribute to action potential repolarization in human atria. *Cardiovascular Research*, 103, 156–167. <https://doi.org/10.1093/cvr/cvu121>
- Smith, F. D., Esseltine, J. L., Nygren, P. J., Veessler, D., Byrne, D. P., Vonderach, M., Strashnov, I., Eysers, C. E., Eysers, P. A., Langeberg, L. K., & Scott, J. D. (2017). Local protein kinase A action proceeds through intact holoenzymes. *Science*, 356, 1288–1293. <https://doi.org/10.1126/science.aaj1669>
- Sun, P., Enslin, H., Myung, P. S., & Maurer, R. A. (1994). Differential activation of CREB by  $Ca^{2+}$ /calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. *Genes & Development*, 8, 2527–2539. <https://doi.org/10.1101/gad.8.21.2527>
- Tan, H. L., Bink-Boelkens, M. T. E., Bezzina, C. R., Viswanathan, P. C., Beaufort-Krol, G. C. M., van Tintelen, P. J., van den Berg, M. P., Wilde, A. A. M., & Balsler, J. R. (2001). A sodium-channel mutation causes isolated cardiac conduction disease. *Nature*, 409, 1043–1047. <https://doi.org/10.1038/35059090>
- Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., & Numa, S. (1988). Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature*, 328, 313–318. <https://doi.org/10.1038/328313a0>

- Tessier, S., Karczewski, P., Krause, E. G., Pansard, Y., Acar, C., Lang-Lazdunski, M., Mercadier, J. J., & Hatem, S. N. (1999). Regulation of the transient outward  $K^+$  current by  $Ca^{2+}$ /calmodulin-dependent protein kinases II in human atrial myocytes. *Circulation Research*, *85*(9), 810–819. <https://doi.org/10.1161/01.RES.85.9.810>
- Therien, A. G., & Blostein, R. (2000). Mechanisms of sodium pump regulation. *American Journal of Physiology-Cell Physiology*, *279*, C541–C566. <https://doi.org/10.1152/ajpcell.2000.279.3.C541>
- Tian, X. (2004). Mechanisms by which SCN5A mutation N1325S causes cardiac arrhythmias and sudden death in vivo. *Cardiovascular Research*, *61*(2), 256–267. <https://doi.org/10.1016/j.cardiores.2003.11.007>
- Travers, J. G., Kamal, F. A., Robbins, J., Yutzey, K. E., & Blaxall, B. C. (2016). Cardiac fibrosis. *Circulation Research*, *118*(6), 1021–1040. <https://doi.org/10.1161/CIRCRESAHA.115.306565>
- Tuteja, D., Xu, D., Timofeyev, V., Lu, L., Sharma, D., Zhang, Z., Xu, Y., Nie, L., Vázquez, A. E., Young, J. N., Glatter, K. A., & Chiamvimonvat, N. (2005). Differential expression of small-conductance  $Ca^{2+}$ -activated  $K^+$  channels SK1, SK2, and SK3 in mouse atrial and ventricular myocytes. *American Journal of Physiology – Heart & Circulatory Physiology*, *289*(6), H2714–H2723. <https://doi.org/10.1152/ajpheart.00534.2005>
- Vandenberg, C. A. (1987). Inward rectification of a potassium channel in cardiac ventricular cells depends on internal magnesium ions. *Proceedings of the National Academy of Sciences United States of America*, *84*, 2560–2564. <https://doi.org/10.1073/pnas.84.8.2560>
- Vandenberg J. I., Perry M. D., Perrin M. J., Mann S. A., Ke Y., & Hill A. P. (2012). hERG  $K^+$  Channels: Structure, Function, and Clinical Significance. *Physiological Reviews*, *92*(3), 1393–1478. <http://dx.doi.org/10.1152/physrev.00036.2011>
- Verheule, S., Van Kempen, M. J. A., te Welscher, P. H. J. A., Kwak, B. R., & Jongsma, H. J. (1997). Characterization of gap junction channels in adult rabbit atrial and ventricular myocardium. *Circulation Research*, *80*, 673–681. <https://doi.org/10.1161/01.RES.80.5.673>
- Wagner, S., Hacker, E., Grandi, E., Weber, S. L., Dybkova, N., Sossalla, S., Sowa, T., Fabritz, L., Kirchhof, P., Bers, D. M., & Maier, L. S. (2009). Ca/calmodulin kinase II differentially modulates potassium currents. *Circulation: Arrhythmia and Electrophysiology*, *2*, 285–294. <https://doi.org/10.1161/CIRCEP.108.842799>
- Walton, K. L., Johnson, K. E., & Harrison, C. A. (2017). Targeting TGF- $\beta$  mediated SMAD signaling for the prevention of fibrosis. *Frontiers in Pharmacology*, *8*. <https://doi.org/10.3389/fphar.2017.00461>
- Wang G. Y., McCloskey D. T., Turcato S., Swigart P. M., Simpson P. C., & Baker A. J. (2006). Contrasting inotropic responses to  $\alpha 1$ -adrenergic receptor stimulation in left versus right ventricular myocardium. *American Journal of Physiology-Heart and Circulatory Physiology*, *291*, (4), H2013–H2017. <http://dx.doi.org/10.1152/ajpheart.00167.2006>
- Wang, Z., Yue, L., White, M., Pelletier, G., & Nattel, S. (1998). Differential distribution of inward rectifier potassium channel transcripts in human atrium versus ventricle. *Circulation*, *98*, 2422–2428. <https://doi.org/10.1161/01.CIR.98.22.2422>
- Watanabe, H., Koopmann, T. T., Le Scouarnec, S., Yang, T., Ingram, C. R., Schott, J.-J., Demolombe, S., Probst, V., Anselme, F., Escande, D., Wiesfeld, A. C. P., Pfeufer, A., Kääh, S., Wichmann, H.-E., Hasdemir, C., Aizawa, Y., Wilde, A. A. M., Roden, D. M., ... Bezzina, C. R. (2008). Sodium channel  $\beta 1$  subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *Journal of Clinical Investigation*, *118*, 2260–2268. <https://doi.org/10.1172/JCI33891>
- Watanabe H., Nogami A., Ohkubo K., Kawata H., Hayashi Y., Ishikawa T., Makiyama T., Nagao S., Yagihara N., Takehara N., Kawamura Y., Sato A., Okamura K., Hosaka Y., Sato M., Fukae S., Chinushi M., Oda H., Okabe M., Makita N. (2011). Electrocardiographic Characteristics and SCN5A Mutations in Idiopathic Ventricular Fibrillation Associated With Early Repolarization. *Circulation: Arrhythmia and Electrophysiology*, *4*(6), 874–881. <http://dx.doi.org/10.1161/circep.111.963983>
- Wehrens, X. H. T., Lehnart, S. E., Reiken, S. R., & Marks, A. R. (2004).  $Ca^{2+}$ /calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. *Circulation Research*, *94*, e61–e70. <https://doi.org/10.1161/01.res.0000125626.33738.e2>
- Wickman, K., Nemeč, J., Gendler, S. J., & Clapham, D. E. (1998). Abnormal heart rate regulation in GIRK4 knockout mice. *Neuron*, *20*, 103–114. [https://doi.org/10.1016/S0896-6273\(00\)80438-9](https://doi.org/10.1016/S0896-6273(00)80438-9)
- Yan, G. X., & Antzelevitch, C. (1999). Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation*, *100*, 1660–1666. <https://doi.org/10.1161/01.CIR.100.15.1660>
- Yoon, J. Y., Ho, W. K., Kim, S. T., & Cho, H. (2009). Constitutive CaMKII activity regulates  $Na^+$  channel in rat ventricular myocytes. *Journal of Molecular and Cellular Cardiology*, *47*, 475–484. <https://doi.org/10.1016/j.yjmcc.2009.06.020>
- Zhang, L., Kelley, J., Schmeisser, G., Kobayashi, Y. M., & Jones, L. R. (1997). Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor: Proteins of the cardiac junctional sarcoplasmic reticulum membrane. *Journal of Biological Chemistry*, *272*, 23389–23397. <https://doi.org/10.1074/jbc.272.37.23389>
- Zimmer, T., Haufe, V., & Blechschmidt, S. (2014). Voltage-gated sodium channels in the mammalian heart. *Global Cardiology Science and Practice*, *2014*, 449–463. <https://doi.org/10.5339/gcsp.2014.58>

**How to cite this article:** Takla, M., Edling, C. E., Zhang, K., Saadeh, K., Tse, G., Salvage, S. C., Huang, C. L.-H., & Jeevaratnam, K. (2021). Transcriptional profiles of genes related to electrophysiological function in *Scn5a*<sup>+/-</sup> murine hearts. *Physiological Reports*, *9*, e15043. <https://doi.org/10.14814/phy2.15043>