Transcriptional profiles of genes related to electrophysiological function in *Scn5a*+− murine hearts

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**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+,1.5 (α) subunit, of the voltage-gated Na⁺ channel in cardiomyocytes. The key role of Na+,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+,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*+− 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*+− animals involved Ca²⁺ 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 Ca²⁺ regulation and examining their possible roles in BrS.

**KEYWORDS**
arrhythmia, Brugada syndrome, mechanisms, sodium channel, transcription
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 Na⁺ current (I_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_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⁺/− 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 (Guzadur 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 (Jeewaratanam 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⁺/− murine hearts (Dautova et al., 2010), prolongation of PR and QRS intervals (Jeewaratanam et al., 2010; Royer et al., 2005), and their sex-dependence, the latter revealing accentuated male over female phenotypes (Jeewaratanam 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 ± 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⁺/− mice were obtained through ex vivo removal. The atria were excised from the ventricles prior to snap-freezing and subsequent separate storage at −80°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 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⁺/K⁺-ATPase

The Na⁺/K⁺-ATPase is a heterotrimeric protein. Its Atp1a (α) subunit is targeted to the plasma membrane (Geering, 2001) by the K⁺-occluding (Lutsenko & Kaplan, 1993) Atp1b (β) subunit. Its activity in exporting three Na⁺ for every two K⁺ it imports (Post & Jolly, 1957) is modulated by the FXYD subunit (Garty & Karlish, 2006; Therien & Blostein, 2000). This contributes a minor intrinsic electrogenicity. However, it is the resulting K⁺ electrochemical gradient that maintains most of the negative resting
membrane potential \( (E_m) \), against its dissipation through expressed 2-pore domain \((K_{2p3.1}; Kcnk3)\) channels (Ketchum et al., 1995). The murine \( Scn5a^{+/−} \) 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; Vandenbergh, 1987).

The hetero-octameric sarcloemmal 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 \( G_{i/o} \)-linked \( M2AChRs \) 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 \( Scn5a^{+/−} \) 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, \( Scn5a^{+/−} \) atria showed significant increases in \( Kcnj5 \) transcription levels and also a trend \((p = 0.061)\) toward markedly higher \( Kcnj3 \) 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 \((I_{Na/Ca})\) 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 \( Hcn2 \)
| Functional group                                                                 | Gene       | WT Mean ± SEM | SCN5A+/− Mean ± SEM | Atria WT Mean ± SEM | SCN5A+/− Mean ± SEM |
|---------------------------------------------------------------------------------|------------|---------------|----------------------|---------------------|---------------------|
| **Na⁺/K⁺-ATPase activity**                                                      | Atp1a1     | 1.0 ± 0.0     | 0.8 ± 0.0            | 1.0 ± 0.0           | 2.2 ± 0.5           |
|                                                                                 | Atp1a2     | 1.0 ± 0.1     | 0.9 ± 0.5            | 1.0 ± 0.2           | 1.2 ± 0.4           |
|                                                                                 | Atp1b1     | 1.0 ± 0.0     | 1.2 ± 0.1            | 1.0 ± 0.0           | 2.1 ± 0.3           |
| **Ion channels controlling resting membrane potential (P-IV)**                  | Abcc8      | 1.0 ± 0.2     | 0.5 ± 0.0            | 1.0 ± 0.1           | 1.0 ± 0.1           |
|                                                                                 | Abcc9      | 1.0 ± 0.9     | 0.6 ± 0.5            | 1.0 ± 0.1           | 1.2 ± 0.1           |
|                                                                                 | Kcnj3      | 1.0 ± 0.0     | 1.2 ± 0.0            | 1.0 ± 0.1           | 2.1 ± 0.2           |
|                                                                                 | Kcnj5      | 1.0 ± 0.0     | 0.8 ± 0.0            | 1.0 ± 0.1           | 1.4 ± 0.1           |
|                                                                                 | Kcnj8      | 1.0 ± 0.1     | 0.9 ± 0.2            | 1.0 ± 0.1           | 0.9 ± 0.1           |
|                                                                                 | Kcnj11     | 1.0 ± 0.2     | 0.7 ± 0.1            | 1.0 ± 0.1           | 0.9 ± 0.2           |
|                                                                                 | Kcnj12     | 1.0 ± 0.1     | 0.8 ± 0.1            | 1.0 ± 0.1           | 1.1 ± 0.2           |
|                                                                                 | Kcnk3      | 1.0 ± 0.1     | 1.0 ± 0.1            | 1.0 ± 0.1           | 1.0 ± 0.3           |
| **Ion channels initiating excitation**                                           | Hcn1       | 1.0 ± 0.1     | 1.8 ± 0.2            | 1.0 ± 0.0           | 3.3 ± 0.1           |
|                                                                                 | Hcn2       | 1.0 ± 0.1     | 0.8 ± 0.1            | 1.0 ± 0.1           | 1.0 ± 0.1           |
|                                                                                 | Hcn4       | 1.0 ± 0.3     | 0.6 ± 0.1            | 1.0 ± 0.0           | 3.1 ± 0.2           |
| **Ion channels permitting I_{Na} (P-0)**                                        | Scn5a      | 1.0 ± 0.1     | 0.5 ± 0.1            | 1.0 ± 0.1           | 0.9 ± 0.1           |
|                                                                                 | Scn7a      | 1.0 ± 0.1     | 0.9 ± 0.1            | 1.0 ± 0.1           | 1.7 ± 0.3           |
| **Subunits modulating the kinetic profile of I_{Na}**                           | Scn1b      | 1.0 ± 0.4     | 0.5 ± 0.1            | 1.0 ± 0.2           | 1.7 ± 0.4           |
|                                                                                 | Scn2b      | 1.0 ± 0.2     | 1.1 ± 0.1            | 1.0 ± 0.1           | 1.2 ± 0.4           |
|                                                                                 | Scn3b      | 1.0 ± 0.3     | 1.4 ± 0.2            | 1.0 ± 0.3           | 1.1 ± 0.1           |
|                                                                                 | Scn4b      | 1.0 ± 0.1     | 2.6 ± 0.5            | 1.0 ± 0.2           | 1.4 ± 0.3           |
| **Ion channels controlling repolarization (P-III)**                             | Kcn4       | 1.0 ± 0.3     | 0.9 ± 0.1            | 1.0 ± 0.0           | 1.4 ± 0.4           |
|                                                                                 | Kcn3       | 1.0 ± 0.1     | 1.0 ± 0.1            | 1.0 ± 0.0           | 1.6 ± 0.2           |
|                                                                                 | Kcne1l     | 1.0 ± 0.1     | 1.0 ± 0.1            | 1.0 ± 0.0           | 2.4 ± 0.3           |
|                                                                                 | Kcnh2      | 1.0 ± 0.1     | 1.0 ± 0.1            | 1.0 ± 0.0           | 0.8 ± 0.2           |
|                                                                                 | Kcnn1      | 1.0 ± 0.1     | 1.0 ± 0.1            | 1.0 ± 0.0           | 0.9 ± 0.1           |
|                                                                                 | Kcnn2      | 1.0 ± 0.1     | 1.1 ± 0.2            | 1.0 ± 0.5           | 1.2 ± 0.2           |
| **Ion channels permitting surface I_{Ca}**                                      | Cacna1c    | 1.0 ± 0.4     | 1.0 ± 0.5            | 1.0 ± 0.0           | 2.4 ± 0.1           |
|                                                                                 | Cacna1d    | 1.0 ± 0.0     | 0.4 ± 0.0            | 1.0 ± 0.2           | 1.6 ± 0.2           |
|                                                                                 | Cacna1g    | 1.0 ± 0.1     | 0.9 ± 0.2            | 1.0 ± 0.1           | 1.1 ± 0.2           |
|                                                                                 | Cacna1h    | 1.0 ± 0.0     | 0.8 ± 0.0            | 1.0 ± 0.0           | 0.3 ± 0.1           |
| **Subunits modulating surface I_{Ca} trafficking and kinetics**                | Cacnb2     | 1.0 ± 0.1     | 0.9 ± 0.3            | 1.0 ± 0.2           | 1.9 ± 0.7           |
|                                                                                 | Cacna2d1   | 1.0 ± 0.1     | 1.0 ± 0.1            | 1.0 ± 0.0           | 1.8 ± 0.1           |
|                                                                                 | Cacna2d2   | 1.0 ± 0.0     | 0.7 ± 0.0            | 1.0 ± 0.1           | 2.4 ± 0.1           |
| **Intracellular ion channels, transporters, and enzymes controlling Ca²⁺ homeostasis** | Atp2a2     | 1.0 ± 0.5     | 1.4 ± 0.6            | 1.0 ± 0.1           | 3.2 ± 0.3           |
|                                                                                 | Camk2d     | 1.0 ± 0.0     | 0.9 ± 0.1            | 1.0 ± 0.0           | 1.8 ± 0.1           |
|                                                                                 | Casq2      | 1.0 ± 0.9     | 0.5 ± 0.1            | 1.0 ± 0.0           | 1.9 ± 0.2           |
|                                                                                 | Ryr2       | 1.0 ± 0.1     | 0.8 ± 0.1            | 1.0 ± 0.0           | 2.4 ± 0.1           |
|                                                                                 | Ryr3       | 1.0 ± 0.0     | 1.0 ± 0.0            | 1.0 ± 0.1           | 3.0 ± 0.3           |
|                                                                                 | Slc8a1     | 1.0 ± 0.2     | 1.3 ± 0.4            | 1.0 ± 0.1           | 2.8 ± 0.6           |

(Continues)
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+/− and WT ventricles showed no significant differences in Hcn1, Hcn2, or Hcn4 mRNA concentrations. In contrast, Scn5a+/− 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⁺ (Naᵥ) channels. The pore-forming (α) subunit of the predominant Naᵥ,1,5 (Scn5a) cardiac isoform, and possibly voltage sensing Naᵥ,2.1 (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 (β) subunits modulate the subsequent voltage-dependent return of Naᵥ,1,5 from its inactivated to its resting conformation. We here report no significant differences between Scn5a+/− 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
3.6 | Ion channels involved in I_{Ca}

The phase 2 plateau phases can typically extend over 300 ms. Here K\textsuperscript+ efflux arising from activation of I_{Kr}, I_{Kr}, and I_{Kur} is balanced by Ca\textsuperscript{2+} influx mediated by sarcomemal voltage-gated Ca\textsuperscript{2+} current. The predominant isoform of pore-forming (α) subunit of the underlying voltage-gated Ca\textsuperscript{2+} (Ca\textsubscript{v}) channels, Ca\textsubscript{v}.1.2 (Cacna1c) and Ca\textsubscript{v}.1.3 (Cacna1d), expressed to a lesser extent shows strong homologies with Na\textsubscript{v}.1.5 (Tanabe et al., 1988). It generates a surface L-type Ca\textsuperscript{2+} current (I_{Ca,L}) with a distinct, very slow, inactivation. Furthermore, the α subunit complexes with both Cacnb2 (β) and γ and Cacna2d1/2 (αγ2) subunits. αγ2 enhances β-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\textsubscript{v}.3.1 (Cacna1g) nor Ca\textsubscript{v}.3.2 (Cacna1h) require accessory subunits for expression and permission of surface T-type Ca\textsuperscript{2+} current (I_{Ca,T}).

In Scn5a\textsuperscript{+/−} 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\textsuperscript{+/−} atria showed significant increases, >80% in Cacna1c, Cacna1h, Cacna2d1, and Cacna2d2 expression (Table 1).

3.7 | Intracellular ion channels, transporters, and enzymes controlling Ca\textsuperscript{2+} homeostasis

In addition to surface Ca\textsuperscript{2+} fluxes, intracellular Ca\textsuperscript{2+} homeostasis involves Ca\textsuperscript{2+} movements between SR and cytosol. SR Ca\textsuperscript{2+}-release channel ryanodine receptors (Ryr) activate upon binding of the cytosolic Ca\textsuperscript{2+} derived from the initial I_{Ca,L} inducing a Ca\textsuperscript{2+}-induced Ca\textsuperscript{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\textsuperscript{2+} transport into the SR; Ca\textsuperscript{2+} binding to calsequestrin (Casq2) then reduces free [Ca\textsuperscript{2+}] in SR (Knollmann, 2009), facilitating SR membrane Ca\textsuperscript{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\textsuperscript{2+}/CaM-dependent kinase II (Camk2d) regulates Ca\textsuperscript{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\textsubscript{v} subtypes (Burel et al., 2017), K\textsubscript{v} (Li et al., 2007; Tessier et al., 1999; Wagner et al., 2009), and Ca\textsubscript{v} (Blaih et al., 2010) channels.

Scn5a\textsuperscript{+/−} ventricles showed no significant changes in concentrations of mRNAs transcribed from the above relative to WT. In contrast, Scn5a\textsuperscript{+/−} 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\textsubscript{q}-linked α\textsubscript{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\textsubscript{q}-linked β-ARs. β\textsubscript{1}-ARs (Adrb1) predominate, but Ca\textsubscript{v}.1.2-coupled β\textsubscript{2}-ARs (Adrb2) also contribute to the generation of positive chronotropy, inotropy, and lusitropy, through mechanisms including G\textsubscript{i}-mediated phospholipase A\textsubscript{2} activation (Pavoine & Defer, 2005).

However, Scn5a\textsuperscript{+/−} 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 β-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]ₐ relieve the allosteric autoinhibition of catalytic subunits, such that the intact holoenzyme (Smith et al., 2017) can initiate a phosphorylation cascade.

Neither Scn5a⁺/− 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-β (TGF-β) (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⁺/− atria nor Scn5a⁺/− ventricles showed significant differences in Tgfb1 expression compared to WT. In contrast, Scn5a⁺/− 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 synctium 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 Gjd3 was reliably detected. However, there were no significant differences in the levels of the gap junction connexin mRNAs between Scn5a⁺/− 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⁺/− ventricles showed no significant changes in Tbx3 and Trpc1 mRNA levels relative to WT mice. In contrast, Scn5a⁺/− atria exhibited significant upregulations of the former, with a 140% increase (Table 1).

4 | DISCUSSION

This study examines the effects of diminished expression of Na1.5 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⁺/− 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 $E_{\text{rev}}$, such as Abcc9 (Hu et al., 2014) and Kcnj8; ion channels initiating excitation, such as Hcn4; subunits modulating $I_{\text{Na}}$ kinetics, such as Scn1b–3b (Hu et al., 2009; Riuró et al., 2013; Watanabe et al., 2008); ion channels controlling repolarization, such as Kcnr3 (Delpón et al., 2008), Kcne5, and Kcnd3; and surface $I_{\text{Ca,L}}$, such as Cav1.1c, Cav1.2b (Cordeiro et al., 2009), and Cav1.2d1. 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 Na1.5 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⁺/− genotype. The ventricular changes were limited to falls in the transcription of two unrelated genes: Abcc9 and Cav1.1d. The atrial Scn5a⁺/− genotype resulted in significant upregulations of 12 genes. Most of these changes clustered in the functional gene group regulating intracellular Ca²⁺ homeostasis, either involving surface
currents (Cacna1c, Cacna1h, Cacna2d1, and Cacna2d2) or cytosolic and SR proteins (Atp2a2, Camk2d, and Ryr2). In addition, Na₅.1.5 haploinsufficiency may increase atrial predisposition to fibrosis via Col1a1.

Here we relate the present findings to previously reported electrophysiological features of Scn5a⁺⁻ murine hearts. First, the reduced ventricular Nav1.5 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⁺⁻ murine hearts were mildly bradycardic and showed increased risks of SAN block with age, and attributed these to altered I₅Na rather than I₈ (Lei et al., 2005). This study correspondingly reported that Scn5a⁺⁻/⁻ hearts showed unaltered ventricular Hcn1, Hcn2, or Hcn4 expression and actual increases in atrial Hcn1 expression compared to WT mice. In addition, young Scn5a⁺⁻/⁻ hearts showed impaired atrial, AV, and ventricular conduction velocity (Papadatos et al., 2002) consistent with the reduced overall Na₅.1.5 mRNA reported previously (Leoni et al., 2010) and 54% reduced ventricular Na₅.1.5 mRNA reported here. Scn5a⁺⁻/⁻ 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 Kcnq2 and Kcnq3 expression and I_F(o) density to similar extents in both Scn5a⁺⁻/⁻ and WT hearts (Martin et al., 2012).

Second, the present findings show relatively normal atrial Na₅.1.5 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⁺/- atria particularly demonstrated altered mRNA levels of gene products involved in surface Ca²⁺ current function such as Cacna1c, Cacna1h, Cacna2d1, and Cacna2d2, and of intracellular proteins, such as Camk2d and Ryr2, involved in [Ca²⁺]i homeostasis with potential actions on I₅Na (Table 1). In particular, the atrial upregulation of Camk2d, in Scn5a⁺⁻/- atria could over activate CaMKII known to phosphorylate Ser1933 and Ser1944 in the C-terminal domain of Na₅.1.5, interfering with Ca²⁺/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 I₅Na (I₅Na.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⁺⁻/- atria. Moreover, gain-of-function mutations in Ryr2 greatly reduce the protein expression of Na₅.1.5 in both atria (King et al., 2013) and ventricles (Ning et al., 2016), implying raised [Ca²⁺]i-mediated acute and chronic Na₅ inhibition.

**FIGURE 3** Through phosphorylation, Ca²⁺/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 Na₅.1.5, in mediating the negative feedback loop and/or changes in the transcription of genes controlling cytosolic [Ca²⁺]i (b) in the atria of Scn5a⁺⁻/⁻ 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 Na\textsubscript{1.5} function and Ca\textsuperscript{2+} homeostasis may involve post-translational and/or transcriptional mechanisms. The CaMKII\textsubscript{B} isoform possesses a nuclear localization signal, providing a basis for its role in excitation-transcription coupling (Figure 3a). Differential stimulation of nuclear CaMKII\textsubscript{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 (Flick et al., 2000); the last is crucial in both initiating and maintaining the pre-established cardiac transcriptional profile. This raises the intriguing possibility that CaMKII provides the missing link between the apparent negative feedback loop regulating atrial CaMKII and the upregulation of genes regulating intracellular Ca\textsuperscript{2+} homeostasis (Figure 3b).

Third, we here report that although Scn5a\textsuperscript{+/−} atria and ventricles showed similar connexin expression levels and similar TGF-β expression, Scn5a\textsuperscript{+/−} atria showed a significantly upregulated Colla1 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\textsuperscript{+/−} genotype, cardiac chamber-specific transcriptional changes, and the BrS phenotype remains to be fully elucidated. However, this study reveals a clear link between Na\textsubscript{1.5} 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 Na\textsubscript{1.5} 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\textsuperscript{+/−} 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\textsuperscript{+/−} hearts studies were exclusively those of aged mice, though future study could also add younger Scn5a\textsuperscript{+/−} hearts as a control group. However, given similar sex-related effects on BrS risk and Scn5a\textsuperscript{+/−} 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\textsuperscript{+/−} 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.

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**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](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](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). KCN5E 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., Gaiddons, C., & Loeffler, J. P. (1996). Ca\textsuperscript{2+}/calmodulin kinase type II and IV regulate c-fos transcription in the ATr20 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
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., Visconi, C., Mandelli, G., Consalez, G.,
Gneccchi-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-
arresting proteins: harnessing the dynamic duo. Nature Cell
Biology, 4, E203–E206. https://doi.org/10.1038/ncc0802-e203

Blair, 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 calmod-
ulin 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+-Ca2+ ex-
changer: 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 FGFl3-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 oligo-
meric disulfide-linked molecular weight 95 000 protein (trial-
din) which binds the ryanodine and dihydropyridine receptors
in skeletal muscle triadic vesicles. Biochemistry, 30, 7507–7513.
https://doi.org/10.1021/bi02044a020

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., Rammelooy, 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., Calloc, 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.
ymjcc.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 elec-
trophysiological, 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+/- murine
hearts. Experimental Physiology, 95, 994–1077. https://doi.
org/10.1111/exphys.2010.053868

Delpón, E., Cordeiro, J. M., Núñez, L., Thomsen, P. E. B., Guerichetto,
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−/− mice modelling mitochondrial dysfunction
induces differential expression of a range of genes regulating
ventricular electrophysiology. Bioscience Reports, 39. https:
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/ajpcel
l.1983.245.1.C1

Felix, R., Gurnett, C. A., De Ward, M., & Campbell, K. P. (1997).
Dissection of functional domains of the voltage-dependent
Ca2+ channel z2δ subunit. Journal of Neuroscience, 17, 6884–
6891. https://doi.org/10.1523/JNEUROSCI.17-08-6884.1997

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+ 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., Schar, A., Paparizos, C., Cao-Ehlicher, X.,
Michalakis, S., Shaltiel, L., Weidinger, M., Steiber, 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:
https://doi.org/10.1161/CIRCRESAHA.111.246173

Ficker, E., Taglialetela, M., Wible, B. A., Henley, C., & Brown, A.
(1994). Spermine and spermidine as gating molecules for in-
ward rectifier K+ channels. Science, 266, 1068–1072. https:
https://doi.org/10.1126/science.797366

Flück, M., Booth, F. W., & Waxham, M. N. (2000). Skeletal muscle
CaMKII enriches in nuclei and phosphorylates myogenic fac-
tor SRF at multiple sites. Biochemical and Biophysical Research
Communications, 270, 488–494. https://doi.org/10.1006/brbc.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

Frustraci, A., Priori, S. G., Pieroni, M., Chimenti, C., Napolitano, C., Rivolta, I., Sanna, T., Bellocchi, 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 β 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

Gollob M. H., Jones D. L., Krahn A. D., Danis L., Gong X. Q., Shao Q., Liu X., Veint 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. https://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., Bjerrregaard, P., Tobwin, J. A., & Chairman, 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

Guazhdurh, L., Liang, W., Parcheysy, 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+/- 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., Szabowska, 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 β3 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., Borggreve, 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 electrophysiologic 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., Enjoji, 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 β subunits: Anything but auxiliary. *Neuroscientist*, 7, 42–54. https://doi.org/10.1077/10735840100700108

Jeevaratnam, K., Guzadhur, 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., Guzadhur, L., Duehmke, R. M., Lei, M., Grace, A. A., & Huang, C. L. H. (2010). Differences in sino-attrial and atrio-ventricular function with age and sex attributable to the Scn5a+/- 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., Huyhnh, T., Lee, S.-J., Karch, J., & Molkentin, J. D. (2017). Fibroblast-specific TGF-β-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/cvr141

Knöllmann, 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., Segschneider, 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 electrophysiological 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

Kusano, K. F., Taniyama, M., Nakamura, K., Miura, D., Banba, K., Nagase, S., Morita, H., Nishii, N., Watanabe, A., Tada, T., Murakami, M., Miyaï, 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+ 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-ΔΔCt 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 β-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 β 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 β1 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., Guzadhrur, L., Brice, N., Grace, A. A., Schwiening, C., Terracciano, C. M., & Huang, C. L. H. (2012). Reduced Na+ and higher K+ channel expression and function contribute to right ventricular origin of arrhythmias in Scn5a+/–+ mice. Open Biology, 2(6), 120072. https://doi.org/10.1098/rsob.120072

Matsuda, H., Saigusa, A., & Irisawa, H. (1987). Ohmic conduction through the inwardly rectifying K channel and blocking by internal Mg2+. 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

McNair, W. P., Ku, L., Taylor, M. R. G., Fain, P. R., Dao, D., Wolfel, E., Mestroni, L., & Familial Cardiomyopathy Registry Research Group (2004). SCNSA mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. Circulation, 110(15), 2163–2167. https://doi.org/10.1161/01.CIR.0000014458.58660.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., Kusanov, K. F., Emori, T., Matsushita, 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., Boonness, N., Vitayakitsirikul, V., Ratanarapee, S., Sharma, S., van der Wal, A. C., Christiansen, M., Tan, H. L., Wilde, A. A., Nomami, A., Sheppard, M. N., Veerakul, G., & Behr, E. R. (2015). Fibrosis, connexin43, and conduction abnormalities in the Brugada syndrome. Journal of the American College of Cardiology. https://doi.org/10.1016/j.jacc.2015.08.862

Nagase, S., Kusanov, K. F., Morita, H., Fujimoto, Y., Kakishita, M., Nakamura, K., Emori, T., Matsushita, 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

Ning, F., Luo, L., Ahmad, S., Valli, H., Jeevaratnam, K., Wang, T., Guzadhrur, L., Yang, D., Fraser, J. A., Huang, C. L.-H., Ma, A., & Salvage, S. C. (2016). The RyR2-P2328S mutation down-regulates Nav1.5 producing arrhythmic substrate in murine
ventricles. Pflugers Arch - 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/japplphysiol.01383.2011

Papadatos, G. A., Wallerstein, P. M. R., Head, C. E. G., Ratcliff, R., Brady, P. A., Benndorf, K., Samurez, R. C., Trezise, A. E. O., Huang, C. L.-H., Vandenberg, J. J., 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 β2-adrenergic signaling new role for the cPLA2. Cellular Signalling, 17, 141–152. https://doi.org/10.1016/j.cellsign.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., Lopez, J. R., & Allen, P. D. (2005). Expression levels of RyR1 and RyR3 control resting free Ca2+ in skeletal muscle. American Journal of Physiology - Cell Physiology, 288(3), C640–C649. https://doi.org/10.1152/ajpcell.00407.2004

Pitzalis, M. V., Anacletio, M., Iacovelli, M., Forleo, C., Guida, P., Troccoli, R., Massari, F., Mastropaques, 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

Pragnell, M., De Waard, M., Mori, Y., Tanabe, T., Snutch, T. P., & Campbell, K. F. (1994). Calcium channel β-subunit binding to a conserved motif in the I-II cytoplasmic linker of the α1-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

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 β2 subunit reveals SCN2B as a new candidate gene for Brugada syndrome. Human Mutation, 34(7), 961–966. https://doi.org/10.1002/humu.22528

Rodríguez, 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. R., Le Bouter, S., Marionneau, C., Grivel-Charhibili, 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, P. (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., Balser, 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

Skibsbye, L., Poulet, C., Dines, 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., Veesler, D., Byrne, D. P., Vonderach, M., Strashnov, I., Eyers, C. E., Eyers, P. A., Langeberg, L. K., & Scott, J. D. (2017). Local protein kinase A action proceeds through intact holoenzymes. Science, 358, 1288–1293. https://doi.org/10.1126/science.aaj1669

Sun, P., Enslen, H., Myung, P. S., & Maurer, R. A. (1994). Differential activation of CREB by Ca2+/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., Beauport-Krol, G. C. M., van Tintelen, P. J., van den Berg, M. P., Wilde, A. A. M., & Balser, 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 Ca2+/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 Ca2+-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 myoccardium. *Circulation Research, 80*, 673–681. https://doi.org/10.1161/01.RES.80.5.673

Wagner, S., Hacker, E., Grandi, E., Weber, S. L., Dykhova, N., Sossalla, S., Sowa, T., Fabritz, L., Kirchhof, P., Bers, D. M., & Maier, L. S. (2009). Ca/cammodulin 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-β 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 α1-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.-I., Demolombe, S., Probst, V., Anselme, F., Escande, D., Wiesfeld, A. C. P., Pfeuffer, A., Kääb, S., Wichmann, H.-E., Hasdemir, C., Aizawa, Y., Wilde, A. M. M., Roden, D. M., ... Bezzina, C. R. (2008). Sodium channel β1 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., Nagami A., Ohkubo K., Kawata H., Hayashi Y., Ishikawa T., Makijama T., Nagao S., Yagihara N., Takehara N., Kawamura Y., Sato A., Okamura K., Hosaka Y., Sato M., Fukae S., Chinusushi 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., Lehnert, S. E., Reiken, S. R., & Marks, A. R. (2004). Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanoide receptor. *Circulation Research, 94*, e61–e70. https://doi.org/10.1161/01.res.0000125626.33738.e2

Wickman, K., Nemec, 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/S0896-6773(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/gscp.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−/− murine hearts. *Physiological Reports, 9*, e15043. https://doi.org/10.14814/phy2.15043