Atrial Transcriptional Profiles of Molecular Targets Mediating Electrophysiological Function in Aging and Pgc-1β Deficient Murine Hearts

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Background: Deficiencies in the transcriptional co-activator, peroxisome proliferative activated receptor, gamma, coactivator-1β are implicated in deficient mitochondrial function. The latter accompanies clinical conditions including aging, physical inactivity, obesity, and diabetes. Recent electrophysiological studies reported that Pgc-1β−/− mice recapitulate clinical age-dependent atrial pro-arrhythmic phenotypes. They implicated impaired chronotropic responses to adrenergic challenge, compromised action potential (AP) generation and conduction despite normal AP recovery timecourses and background resting potentials, altered intracellular Ca²⁺ homeostasis, and fibrotic change in the observed arrhythmogenicity.

Objective: We explored the extent to which these age-dependent physiological changes correlated with alterations in gene transcription in murine Pgc-1β−/− atria.

Methods and Results: RNA isolated from murine atrial tissue samples from young (12–16 weeks) and aged (>52 weeks of age), wild type (WT) and Pgc-1β−/− mice were studied by pre-probed quantitative PCR array cards. We examined genes encoding sixty ion channels and other strategic atrial electrophysiological proteins. Pgc-1β−/− genotype independently reduced gene transcription underlying Na⁺-K⁺-ATPase, sarcoplasmic reticular Ca²⁺-ATPase, background K⁺ channel and cholinergic receptor function. Age independently decreased Na⁺-K⁺-ATPase and fibrotic markers. Both factors interacted to alter Hcn4 channel activity underlying atrial automaticity. However, neither factor, whether independently or interactively, affected transcription of cardiac Na⁺, voltage-dependent K⁺ channels, surface or intracellular Ca²⁺ channels. Nor were gap junction channels, β-adrenergic receptors or transforming growth factor-β affected.

Conclusion: These findings limit the possible roles of gene transcriptional changes in previously reported age-dependent pro-arrhythmic electrophysiological changes.
observed in Pgc-1β−/− atria to an altered Ca²⁺-ATPase (Atp2a2) expression. This directly parallels previously reported arrhythmic mechanism associated with p21-activated kinase type 1 deficiency. This could add to contributions from the direct physiological outcomes of mitochondrial dysfunction, whether through reactive oxygen species (ROS) production or altered Ca²⁺ homeostasis.

**Keywords:** peroxisome proliferator activated receptor-γ (PPARγ), coactivator-1 transcriptional coactivator (Pgc-1), quantitative PCR, ion channels, mitochondria, arrhythmias

**INTRODUCTION**

Atrial arrhythmias constitute a major public health problem. Atrial fibrillation (AF), affects 1–3% of the developed world population. This prevalence will likely increase to ~9 and ~18 million cases in the United States and Europe, respectively, by 2060 (Miyasaka et al., 2006). Chronic AF increases incidences of both morbidity, often as stroke, and all-cause mortality.

Age is a major risk factor for AF, affecting 0.1, 4, and 20% of individuals aged <55, 60–70 and >80 years, respectively. So are metabolic factors, arising from physical inactivity, obesity, diabetes mellitus, and metabolic syndrome. Themselves age-dependent, these account for ~60% of current upward trends in AF incidence (Miyasaka et al., 2006). Both age and metabolic deficiency in turn are associated with mitochondrial dysfunction (Vianna et al., 2006). Age is associated with mitochondrial DNA damage and compromised respiratory chain function (Krishnan et al., 2007); obese mice on high fat diets show defective mitochondrial electron transport chain complex 1 (Wang X. et al., 2015).

Acute and chronic mitochondrial dysfunction have cardiac pro-arrhythmic effects (Akar and O’Rourke, 2011). This indicates abnormalities in one or more of the ordered processes of action potential (AP) initiation, recovery, excitation and propagation. These in turn would reflect altered ion channel function or expression or structural, fibrotic or cardiomyopathic change altering AP conduction, findings reported in experimental diabetes (Russo and Frangogiannis, 2016) and metabolic syndrome (Ho et al., 2017).

Peroxisome proliferator activated receptor-γ (PPARγ) and coactivator-1 transcriptional coactivators (Pgc-1) are important cellular energetic regulators. They are abundant in oxidative tissues, including cardiac and skeletal muscle, and brown adipose tissue (Sonoda et al., 2007). They regulate mitochondrial biogenesis and mass (Villena, 2015). They also influence mitochondrial function, increasing expression of genes related to fatty acid β-oxidation, the tricarboxylic acid cycle and electron transport (Arany et al., 2005). Their expression is impaired in metabolic conditions including obesity, insulin resistance, type 2 diabetes and in first-degree relatives of diabetic patients (Scheuermann-Freestone et al., 2003). Pgc-1α levels respond to physiological stimuli such as fasting, exercise and cold temperatures, adapting tissues to changes in energy demand. Pgc-1β appears involved in baseline energetic balance (Villena, 2015).

Finally, Pgc-1α, and Pgc-1β-deficient murine experimental models replicate clinically observed atrial pro-arrhythmic effects of energetic deficiencies. Recent reports implicated age-dependent impairments in chronotropic adrenergic responses, AP generation and conduction despite normal AP recoveries and background resting potentials. They also demonstrated altered intracellular Ca²⁺ homeostasis and fibrotic change (Gurung et al., 2011; Ahmad et al., 2018; Valli et al., 2017a,b, 2018a).

The present studies now explore for transcriptional alterations in the genes encoding the underlying electrophysiological mechanisms in atria of the Pgc-1β-deficient mice (Komen and Thorburn, 2014). Genes were selected and grouped according to the physiological processes underlying different physiological aspects of excitable activity (Huang, 2017). This adapts an approach first applied to rat as opposed to genetically modified mouse hearts (Atkinson et al., 2013). This would make it possible to determine the extent to which previously reported pro-arrhythmic changes reflect altered gene transcription, or whether they more likely follow alterations in subsequent stages in gene expression or even altered function in their resulting proteins.

**MATERIALS AND METHODS**

**Animals**

Experiments were approved by the University of Cambridge ethics review board under a United Kingdom project license for studies of cardiac arrhythmia. All procedures complied with United Kingdom Home Office regulations [Animals (Scientific Procedures) Act 1986]. The experiments also conformed to the Guide for the Care and Use of Laboratory Animals, United States National Institutes of Health (NIH Publication No. 85-23, revised 1996). Mice were housed in an animal facility at 21°C with 12-h light/dark cycles. Animals were fed sterile chow (RM3 Maintenance Diet, SDS, Witham, Essex, United Kingdom) and had free access to water, bedding and environmental stimuli. Mice were sacrificed by cervical dislocation. No recovery, anesthetic or surgical procedures were required. Wild Type (WT) C57/B6 and Pgc-1β−/− (The Jackson Laboratory, ME, United States) mice were bred for the experimental protocols. Mice were bred on a C57/B6 background to avoid possible strain-related confounds.

**Tissue Samples**

Hearts were obtained from four experimental groups, respectively, consisting of young WT, young Pgc-1β−/−, aged WT, and aged Pgc-1β−/− mice. Three hearts were studied from each group. The mice studied were littermates of animals whose hearts had undergone electrophysiological study reported
RNA was extracted from fresh frozen tissues, stored in −80°C, with the QiaGen RNEasy mini Plus kit. Cardiac atrial tissue were weighed and put on ice and subsequently homogenized in RLT buffer supplemented with beta-mercaptoethanol with a Stuart handheld homogenizer until completely smooth. Genomic DNA was eliminated by centrifugation through a column supplied with the kit prior to extraction of the RNA according to the manufacturer’s protocol. RNA integrity was assessed by using an Agilent bioanalyzer to obtain RNA integrity numbers (RIN) according to the manufacturer’s protocol. RNA samples with RINs above 8 were used for the study. The RNA was used to prepare cDNA with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer’s instructions. The efficiency of the cDNA protocol was tested by preparing the cDNA from a serial dilution of the RNA and then these cDNA samples were run on a qPCR confirming equal efficiency over a range of RNA concentrations. cDNA was also confirmed negative for genomic DNA contamination. Each custom made card contained 64 pre-validated assays in triplicate with a reaction volume of 1 μl. The cards were run exactly according to instructions specific for the cards. Briefly, the cDNA (100 ng/well) was mixed with 2× Mastermix from Thermo Fisher Scientific, 100 μl was loaded in each well slot on the cards. The cards were then spun down and sealed and run on a Quant 7 cyclers. The amplification conditions were: 50°C for 2 min and 95°C for 10 min for the initial DNA melting and inactivation of the RT reaction, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Analysis of the Taqman array card data was performed by using the Quant studio software and Microsoft Excel by calculating fold changes with the ddCT method as described by Livak and Schmittgen (2001). The threshold was set at 0.2 fluorescence units and the baseline range was set to automatic assignment. The geometric mean of the Cq values for the genes HPRT, Gapdh, and ActinB were used as references. P-values were calculated using two-way analysis of variance (ANOVA) and Student’s independent t-tests.

RESULTS

The studies explored transcription profiles in atria of young and aged, WT and Pgc-1β−/− murine hearts previously demonstrated to recapitulate cardiac pro-arrhythmic phenotypes associated with energetic deficiency. Quantitative PCR evaluated transcriptional backgrounds for genes strategic to pro-arrhythmic electrophysiological phenotypes that might in turn constitute potential pharmacological targets (Komen and Thorburn, 2014). Genes were selected and grouped according to the component physiological processes underlying excitable activity leading to arrhythmia (Huang, 2017), adapting approaches introduced in rat hearts (Atkinson et al., 2013). Samples obtained by isolating RNA from murine atrial tissue were studied by qPCR using Thermo Fisher Scientific custom Taqman array cards pre-probed with the 60 different genes of potential interest for cardiac function.

Gene transcription fold changes were normalized to levels for the gene in question in young WT mice (Livak and Schmittgen, 2001). Table 1 lists the genes and the gene expression results obtained, and Figure 1 overviews expression patterns of relative effect sizes and the directions of the observed differences; red backgrounds indicate increased and green decreased expression relative to young WT. Their intensities are scaled to the highest values (darkest red) and lowest values (darkest greens) of the entire set of genes represented in the map with yellow in the middle indicating an absence or little change. These results are accompanied by the results of a two-way analysis of variance testing for independent and interacting effects of genotype and of age to a P < 0.05 significance level. Effects of age and genotype were sorted by functional gene group corresponding to the physiological processes described in previous reports on Pgc-1β−/− phenotypes. Control inclusion of Pparγ1b transcription levels confirmed highly significant independent effects of genotype.

Of genes related to (1) the energetically dependent Na+,
K+-ATPase-mediated membrane transport required to generate ionic gradients upon which excitability activity depends and (2) background K+ channels mediating the resting potential, we tested transcription activity for Na+-K+ ATPase α1 and α2 catalytic and accessory β1, β2 subunits (encoded by Atpa1, Atpa2, and Atpb1, respectively), the ATP-sensitive inward rectifier K+ channel Kir2.2 (Kcnj12) coupling resting potentials to intracellular nucleotide levels (Nichols, 2006), and the ATP-binding cassette (ABC) transporter subunits members 8 and 9 (Abcc8 and Abcc9), inwardly rectifying pore-forming K+ channels Kir6.1, Kir6.2, and Kir3.1 (Kcnj8, Kcnj11, and Kcnj3) and G protein-activated inward rectifier potassium channel 4, Kir3.4 (Kcnj5). The two-way ANOVA suggested independent effects of genotype decreasing Atp1a1, Atp1a2, Atp1b1, Kcnj8, and Abcc9 transcription, and independent effects of age decreasing Abcc9 transcription.

Of (3) voltage-dependent activating processes, the two-way ANOVA suggested independent effects of genotype decreasing Scn7α and interacting effects of genotype and age altering expression of Hcn4 known to mediate hyperpolarization-activated cyclic nucleotide–gated channels underlying SAN pacemaker currents If (Thollon et al., 2007). It did not detect altered transcription of the major, Scn5α, isoform concerned with cardiac Na+ current. Nor did the two-way ANOVA demonstrate either independent or interacting effects of genotype and age on (4) the surface membrane voltage-dependent L-type Ca2+ channels Cav1.2 and Cav1.3 (Cacna1c and Cacna1d), T-type, Cav3.1 (Cacna1g), and Cav3.2 (Cacna1h), and the accessory β2 (Cacnb2), α2/δ1 and α2/δ2 subunits (Cacna2d2). Of (5) molecules related to intracellular Ca2+ homeostasis...
### TABLE 1 | Gene groups examined for transcripational changes by Taqman PCR in WT and Pgc-1β−/− murine atria.

| gene | WT Young | WT Old | Pgc-1β−/− Young | Pgc-1β Old |
|------|----------|--------|-----------------|-----------|
| **Na⁺-K⁺ ATPase activity** | | | | |
| Atp1a1 | 1.00 | 0.07 | 1.03 | 0.11 |
| Atp1a2 | 1.00 | 0.29 | 1.37 | 0.24 |
| Atp1b1 | 1.00 | 0.22 | 0.94 | 0.13 |
| **Ion channels relating to the resting membrane potential** | | | | |
| Kcnj12 | 1.00 | 0.20 | 0.93 | 0.20 |
| Abcc8 | 1.00 | 0.17 | 1.10 | 0.05 |
| Abcc9 | 1.00 | 0.12 | 1.69 | 0.21 |
| Kcnj8 | 1.00 | 0.13 | 0.79 | 0.17 |
| Kcnj11 | 1.00 | 0.13 | 0.86 | 0.06 |
| Kcnj3 | 1.00 | 0.18 | 0.81 | 0.11 |
| Kcnj5 | 1.00 | 0.10 | 1.00 | 0.12 |
| Ocn3 | 1.00 | 0.09 | 0.93 | 0.11 |
| **Ion channels relating to the initiation of excitable activity** | | | | |
| Hcn1 | 1.00 | 0.04 | 0.57 | 0.16 |
| Hcn3 | 1.00 | 0.24 | 1.56 | 0.34 |
| Hcn4 | 1.00 | 0.43 | 0.32 | 0.03 |
| Scn5a | 1.00 | 0.15 | 0.69 | 0.19 |
| Scn7a | 1.00 | 0.13 | 0.88 | 0.21 |
| **Ca²⁺ homeostasis – surface** | | | | |
| Cacna1c | 1.00 | 0.15 | 0.80 | 0.13 |
| Cacna1d | 1.00 | 0.11 | 0.85 | 0.17 |
| Cacna1g | 1.00 | 0.30 | 0.85 | 0.09 |
| Cacna1h | 1.00 | 0.13 | 1.13 | 0.14 |
| Cacnb2 | 1.00 | 0.20 | 1.46 | 0.34 |
| Cacna2d1 | 1.00 | 0.12 | 1.13 | 0.12 |
| Cacna2d2 | 1.00 | 0.41 | 0.82 | 0.24 |
| **Ca²⁺ homeostasis – intracellular** | | | | |
| Ryr2 | 1.00 | 0.13 | 0.95 | 0.20 |
| Ryr3 | 1.00 | 0.25 | 2.07 | 1.33 |
| Atp2a2 | 1.00 | 0.17 | 1.21 | 0.11 |
| Slc8a1 | 1.00 | 0.10 | 2.16 | 0.72 |
| Casq2 | 1.00 | 0.10 | 1.00 | 0.21 |
| **Ion channels relating to repolarisation** | | | | |
| Kona4 | 1.00 | 0.14 | 0.67 | 0.08 |
| Kond3 | 1.00 | 0.31 | 0.78 | 0.13 |
| Konh2 | 1.00 | 0.23 | 0.69 | 0.14 |
| Konn1 | 1.00 | 0.22 | 0.94 | 0.27 |
| Konn2 | 1.00 | 0.08 | 1.06 | 0.14 |
| Konk3 | 1.00 | 0.18 | 1.45 | 0.13 |
| Kone1l | 1.00 | 0.28 | 0.60 | 0.16 |
| **Adrenergic and cholinergic receptors** | | | | |
| Chrm2 | 1.00 | 0.17 | 1.49 | 0.26 |
| Adra1a | 1.00 | 0.21 | 1.37 | 0.56 |
| Adra1b | 1.00 | 0.05 | 0.87 | 0.15 |
| Adra1d | 1.00 | 0.27 | 1.23 | 0.48 |
| Adrb1 | 1.00 | 0.24 | 0.75 | 0.21 |
| Adrb2 | 1.00 | 0.27 | 0.86 | 0.19 |
| **The cAMP pathway** | | | | |
| Adcy4 | 1.00 | 0.07 | 0.79 | 0.10 |
| Adcy5 | 1.00 | 0.23 | 0.82 | 0.11 |
| Pde2a | 1.00 | 0.18 | 0.99 | 0.14 |
| Pde4d | 1.00 | 0.09 | 0.67 | 0.04 |
| Prkaca | 1.00 | 0.18 | 0.79 | 0.06 |
| Prkar1a | 1.00 | 0.17 | 0.74 | 0.05 |
| Prkar2a | 1.00 | 0.14 | 0.79 | 0.15 |
| Prkar2b | 1.00 | 0.23 | 4.84 | 2.44 |
| Camk2d | 1.00 | 0.08 | 1.35 | 0.25 |

(Continued)
between cellular compartments, there were neither independent nor interacting effects on transcription of ryanodine receptor isoforms RyR2 (Ryr2) and RyR3 (Ryr3), the principal cardiac Na⁺-Ca²⁺ exchanger (Slc8a1), and cardiac SR Ca²⁺ binding protein (Casq2). However, there was a significant effect of genotype upon the transport mediating Ca²⁺-ATPase (Atp2a2).

The two-way ANOVA revealed no significant effects bearing on (6) transcription on voltage dependent K⁺ channels underlying AP recovery, including voltage sensitive transient outward current Iₒ, Kv1.4 (Kcnq4), and Kv4.3 (Kcnd3), rapid K⁺ current, IKr, Kv11.1 (Kcnh2), the recently characterized Ca²⁺-activated K⁺ channel KCa2.1 (Kcnm1) and KCa2.2 (Kcnm2) that have a selective atrial as opposed to ventricular occurrence thought to contribute to AP (Xu et al., 2003). This was also the case for the two-predomain TWIK-related acid-sensitive potassium channel 1, TASK-1/K 2P 3.1 (Kcnk3) (Olschewski et al., 2017) and the regulatory KCNE1 subunit (Kcne1).

Of (7) receptor proteins related to cardiomyocyte cholinergic and adrenergic autonomic activation, the two-way ANOVA detected an independent effect of genotype decreasing muscarinic M2 (Chrm2) expression. There were no effects upon the three cardiac, α1A, α1B, and α1D (Adr1a, Adr1b, and Adra1d) adrenergic receptor subtypes, thought to protect against pathological remodeling in heart failure (O’Connell et al., 2013) or β1 and β2- adrenergic receptor subtypes (Adrb1 and Adrb2). The two-way ANOVA detected no effects on transcription of (8) cyclic AMP-dependent cellular messenger pathways involved in such autonomic signaling of which we explored cardiac adenylyl cyclase, types 4 and 5 (Adcy4 and Adcy5), of which type 5 accentuates cardiomyopathic changes on chronic catecholamine stimulation, cGMP-dependent and cAMP-specific 3’,5’-cyclic phosphodiesterases 2A and 4D (Pde2a and Pde4d), and the protein kinase A catalytic α-subunit (Prkaca). We did not observe altered transcription in cardiac Ca²⁺/calmodulin-dependent protein kinase, type II-δ (Camk2d) (Zhang et al., 2004).

The only alterations suggested for markers for (9) atrial fibrosis, concerned independent effects of age in reducing Col1a1, the major component of type I collagen, the fibrillar collagen found in most connective tissues. Transcription of the remaining cytokine transforming growth factor β (TGF-β1; Tgfb1) (Hao et al., 2011), connexin mCx30.2 (Gjd3) (Kreuzberg et al., 2005), and the collagen precursor for the collagen type III α1 chain (Col3a1) (Davies et al., 2014) were largely unchanged. The final set of genes tested are thought to act as (10) markers for a range of developmental, inflammatory and hypertrophic changes ultimately impacting AP generation and propagation, as well as the genes encoding Pgc-1β itself and the complementary Pgc-1α. Translation of both the transcriptional repressor known to affect particular components of the cardiac conduction system, Tbx3 (Sylva et al., 2014), and the major thick filament protein Myh6 (MHC-α) whose mutations are associated with late-onset hypertrophic cardiomyopathy, atrial sepal defects and sinus node disorder (Huang, 2017). Mutations in atrial natriuretic peptide (Nnpa) have been implicated in familial AF (Perrin and Gollob, 2012). The non-specific ion channel Trpc1 conducts both Ca²⁺ and Na⁺ with Ca²⁺ store depletion or activation of the phospholipase C system with associations with hypertrophic change showed increases in groups relative to young WT (Xu and Beech, 2001). The two-way ANOVA did not demonstrate any effects of age or genotype apart from interacting effects on the gene Pgc-1α gene known to complement the function of Pgc-1β−/− and on the gene Tbx3.

**TABLE 1 | Continued**

| gene                | WT       | WT       | Pgc-1β−/− | Pgc-1β−/− |
|---------------------|----------|----------|-----------|-----------|
|                      | Young    | Old      | Young     | Old       |
|                     | mean     | +/- sem  | mean      | +/- sem   |
| Fibrotic markers    |          |          |           |           |
| Tgfb1               | 1.00     | 0.15     | 0.79      | 0.15      |
| Gjd3                | 1.00     | 0.45     | 0.46      | 0.04      |
| Col1a1              | 1.00     | 0.09     | 0.55      | 0.09      |
| Col3a1              | 1.00     | 0.16     | 1.31      | 0.07      |
| Other genes         |          |          |           |           |
| Tbx3                | 1.00     | 0.17     | 0.74      | 0.05      |
| Myh6                | 1.00     | 0.17     | 0.63      | 0.11      |
| Nnpa                | 1.00     | 0.13     | 1.12      | 0.21      |
| Trpc1               | 1.00     | 0.24     | 1.67      | 0.15      |
| Pparc1a             | 1.00     | 0.03     | 1.55      | 0.13      |
| Pparc1b             | 1.00     | 0.15     | 0.77      | 0.21      |

**Figures 2–5** display volcano plots derived from statistical assessments of transcriptional differences in individual genes between atria from young and aged, WT and Pgc-1β−/− mice stratified by P-values (<0.01, <0.05 and <0.10, respectively) and plotted against their effect sizes. Comparisons were made between young Pgc-1β−/− and young WT (Figure 2), the effects of aging in WT (Figure 3) and Pgc-1β−/− (Figure 4), respectively, and the eventual differences between aged Pgc-1β−/− and aged WT (Figure 5). They thus provide graphical indications as to where the significant paired differences were to be found, plotting the data points in their entirety over the full range of their P-values, and identifying genes where they had P < 0.1. In all these comparisons, there were no alterations in transcription of genes bearing on Nav1.5, L or T-type Ca²⁺ channel, Ca²⁺ homeostasis, or repolarizing K⁺ channel function.
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FIGURE 1 | Heatmap visualizing differential expression between the sample groups grouped by electrophysiological function at the individual gene level combined with two-way ANOVA results examining for independent and interacting effects of Pgc-1β−/− genotype and age. All fold changes normalized to the mean of the expression in samples from young WT hearts. The heatmap is coded yellow for no change, red for increased and green for decreased in fold gene transcription.

This was with the exception of Atp2a2 in Figure 5, and Ryr3 in Figure 4. Of the remaining genes analyzed:

(1) Compared to young WT hearts, young Pgc-1β−/− demonstrated (P < 0.05) reduced transcription in the following genes concerned with resting potential maintenance (Figure 2): inwardly rectifying Kir6.1 (Kcnj8) and regulatory subunits of the ATP-sensitive K+ channel 9 (Abcc9). There was also a small though significant Ppargc1a increase suggesting a compensation for the reduced Ppargc1b transcription. Of genes bearing on activation of electrophysiological activity, Scn7a, but not the principal Scn5a isoform, showed altered transcription. Of molecules underlying intracellular Ca2++ homeostasis, the trend (P < 0.1) toward reduced cardiac RyR2-Ca2+ SR channel (Ryr2) transcription was in a direction contrasting with previously reported increased SR Ca2++ release phenotypes (Gurung et al., 2011). Patterns of β-adrenergic receptor subtype transcription showed no change, in contrast with the previously reported chronotropic incompetence associated with Pgc-1β−/− (Lelliott et al., 2006). Similarly, there were no alterations in markers for fibrotic change (Tgfb1, Gjd3, Col1a1, and Col3a1), although there was a reduced transcription of α1B-adrenergic receptor subtypes (Adra1b) known to mediate protective and adaptive functions preventing pathological remodeling in cardiac failure through Gq/11 signaling (O’Connell et al., 2013) and of the transcriptional repressor Tbx3. There was increased transcription of the complementary Pgc-1α (Ppargc1a) involved in adjustments to altered metabolic demand.

(2) Figure 3 explores the effects of aging in WT atria. There was again a Ppargc1a increase compensating for the reduced Ppargc1b transcription. In contrast to the comparisons between young Pgc-1β−/− and young WT, aging increased (P < 0.05) transcription of the ATP-sensitive K+ channel 9 regulatory subunit (Abcc9), concerned with resting potential maintenance. The trend toward reduced transcription in HCN channels only concerned Hcn1 rather than the principal Hcn4 isoform. A decreased Pde4d proved to be exclusive to the WT mice, and consequently had not been detected when analyzed with the Pgc-1β−/− in the ANOVA. However, there were no changes concerning Nav1.5, molecules related to L or T-type Ca2+ channel function or repolarising K+ channels, or of RyR2 or other molecules controlling intracellular Ca2+ homeostasis. There was increased transcription of the complementary Pgc-1α (Ppargc1a) involved in adjustments to altered metabolic demand. Transcription of the Col1a1 marker for fibrotic change was reduced. There was a trend toward decreased transcription of TRPC channels involved in signaling cascades mediating cardiac hypertrophy and remodeling (Eder and Molkentin, 2011; Freichiel et al., 2017) was increased.

(3) Figure 4 illustrates effects of aging in Pgc-1β−/− suggestive of particular effects of Pgc-1β−/− on age-related remodeling changes. Aging Pgc-1β−/− showed features
Young \textit{Pgc1}β^{-/−} vs young WT gene expression ratio

![Volcano plot of differentially expressed genes comparing young \textit{Pgc1}β^{-/−} with young WT. Plots of stratified \textit{P}-values against expression fold changes for results expressing young \textit{Pgc1}β^{-/−} normalized to young-WT.]

not shown by aging WT: there was increased transcription of the RyR3 ryanodine receptor isoform (Ryr3) and Tbx3, and trends toward increased transcription of the principal HCN4 variant of the channels concerned with sinoatrial pacemaker current. There was also trends toward decreased transcription of KCa2.2 (Kcnm2) and increased transcription of adrenergic receptor subtype Adra1d, thought to protect against pathological remodeling in heart failure. Aging \textit{Pgc1}β^{-/−} did not show the decreased translation of Col1a1 or Abcc9 in contrast to aging WT.

(4) Figure 5 compares transcriptional profiles in aged \textit{Pgc1}β^{-/−} with those of aged WT constituting the end situations arising from the processes represented in Figures 3, 4. This revealed a \textit{Ppargc1a} decrease in contrast to its increase in some of the remaining comparisons. In aged \textit{Pgc1}β^{-/−} compared to aged WT, there was a markedly increased transcription of the principal Hcn4 isoform concerned with sinoatrial pacemaker current and decreased transcription of the SR Ca^{2+}-ATPase subunit Atp2a2 and of \textit{Pgc1}α (\textit{Ppargc1a}). There were trends toward decreased transcription of the type I collagen marker Col3a1, TWIK-related acid-sensitive potassium channel 1 (Kcnk3), the ATP-sensitive K^{+} channel 9 (Abcc9), and the cholinergic M2 receptor (Chrm2).

**DISCUSSION**

The present studies applied quantitative PCR to explore for age-dependent transcriptional changes in genes strategic
to electrophysiological phenotypes underlying atrial pro-
arrhythmic tendency in murine hearts deficient in the
transcriptional coactivator peroxisome proliferator activated
receptor-γ coactivator-1β (PGC-1β). Pgc-1β−/− hearts have
proved useful experimental models in studies of atrial
arrhythmogenic mechanisms associated with clinical conditions
arising from energetic, particularly mitochondrial, functional
deficiencies. Of these, the most widespread are atrial arrhythmias,
particularly AF for which age and metabolic factors constitute
major risk factors through their associations with mitochondrial
dysfunction and its associated impaired oxidative capacity
(Vianna et al., 2006). Pgc-1α and Pgc-1β offer potential
therapeutic targets. The PPAR-α agonist fenofibrate, inhibited
atrial metabolic remodeling in AF (Liu W.Y. et al., 2016; Zhao
et al., 2016). Rosiglitazone reduced atrial interstitial fibrosis 
and AF promotion in diabetic rabbits by modulating oxidative stress 
and inflammation (Lin et al., 2014). The specific PPARγ ligands
the thiazolidinediones (TZDs) improve clinical insulin sensitivity
in type 2 diabetes mellitus (Liu A. et al., 2016). They may also
decrease myocardial fibrosis and improve cardiac function
(Liu A. et al., 2016).

Recent electrophysiological studies had demonstrated age-
dependent atrial arrhythmic phenotypes in murine Pgc-1β−/−
hearts relating these to abnormal electrophysiological properties,
Ca2+ homeostasis and fibrotic change (Valli et al., 2017a,b, 
2018a; Ahmad et al., 2018). The present quantitative PCR studies
went on to explore for altered transcription of genes selected
to encode the ion channels, other transport proteins, markers
for autonomic and energetic function and developmental,
inflammatory, and fibrotic and hypertrophic changes that
may underly those reported physiological changes. It was
therefore possible to determine whether the electrophysiological
differences (Valli et al., 2017a,b, 2018a; Ahmad et al., 2018)
correlated with correspondingly altered patterns of protein
transcriptional change, or otherwise. Whilst not distinguishing
surface from internal membrane protein expression, they
revealed differing patterns of transcriptional change in proteins
related to each process. This approach had previously successfully
been applied to studies in rat as opposed to genetically
modified mouse hearts (Atkinson et al., 2013). Thus the previous
electrophysiological studies in young and old, WT and Pgc-
1β−/−, hearts reported:

![FIGURE 3](image_url)
(1) Impaired sino-atrial pacing and negative dromotropic responses suggesting defective atrioventricular conduction following adrenergic activation particularly in intact aged Pgc-1β−/− mice (Ahmad et al., 2018) and impaired chronotropic responses to adrenergic stimulation (Lelliott et al., 2006). In contrast, 2-way ANOVA detected no significant differences between groups in transcription of autonomic β-adrenergic receptors, but did detect genotypic effects decreasing the cholinergic Chrm2. There were no differences in HCN4 transcription mediating pacemaker currents. Differences suggested by the volcano plots did not bear on β-adrenergic responses; young Pgc-1β−/− showing lower Adra1b expression than young WT, and old WT showing lower Pde4d expression than young WT. Aged Pgc-1β−/− showed increased Hcn4 expression compared to aged WT, contrasting with the compromised SAN response to adrenergic challenge shown by Pgc-1β−/−.

In intact Langendorff-perfused hearts, there were (2) similar resting potentials between experimental groups (Valli et al., 2017b). Yet markers for molecules concerned with resting potential generation indicated reduced transcription in several genes related to resting potential. The two-way ANOVA demonstrated that the Pgc-1β−/− genotype decreased the markers of Na+-K+-ATPase transcription Atp1a1, ATP1a2, and Atp1b1, and those of the K+ channels Abcc9 and Kcnj8. The volcano plots indicated that young Pgc-1β−/− showed reduced Abcc9 and Kcnj8 relative to young WT, and aged WT, increased Abcc9 relative to young WT.

(3)-(6) Pgc-1β−/− showed (3) age-dependent slowing of AP conduction associated with reduced AP upstroke rates (dV/dt)max implicating reduced Na+ channel function (Valli et al., 2017a). In contrast, (4) AP repolarization rates reflected in action potential durations (APDs) remained similar between experimental groups (Valli et al., 2017b). Loose-patch clamp studies related the above findings to (5) reduced depolarizing, Na+, but (6) normal repolarizing voltage-dependent and inward rectifier K+ current in both young and aged Pgc-1β−/− genotype (Valli et al., 2018a). In contrast to (3) and (5), both two-way ANOVA and volcano plots demonstrated no differences between groups for Nav1.5 (Scn5a), or surface L- or T-type Ca2+ channel related genes relevant to initiation or maintenance of AP depolarization. However, in accord with (4) and (6), markers for voltage-dependent K+ channels underlying AP repolarization duration were indistinguishable between groups culminating in indistinguishable outcomes between aged Pgc-1β−/− and aged WT.
Finally, \( Pgc-1\beta^{-/-} \) was associated with (7) \( Ca^{2+} \) homeostatic changes reflected in diastolic SR \( Ca^{2+} \) release events, altered \( Ca^{2+} \) current characteristics and consequent early and delayed afterdepolarization phenomena (Gurung et al., 2011). In contrast, both two-way ANOVA and the volcano plots revealed no systematic changes in markers for \( Ca^{2+} \) homeostatic change mediating RyR2 (\( Ryr2 \)), Na\(^+\)/Ca\(^{2+}\) exchange (\( Slc8a1 \)) or calsequestrin (\( Casq2 \)). However, there were independent genotypic effects on sarcoplasmic reticular \( Ca^{2+} \) ATPase (\( Atp2a2 \)). These manifested as diminished \( Atp2a2 \) transcription in old \( Pgc-1\beta^{-/-} \) compared to aged WT (but not young \( Pgc-1\beta^{-/-} \)) hearts, with increased expression of Ryr3 (but not Ryr2) in old \( Pgc-1\beta^{-/-} \) compared to young \( Pgc-1\beta^{-/-} \).

(8) The \( Pgc-1\beta^{-/-} \) genotype also was responsible for producing an age-dependent fibrotic change itself contributing to slowed AP conduction (Jeevaratnam et al., 2011). In contrast, both two-way ANOVA and volcano plots demonstrated that age independently reduced expression of \( Col1a1 \) though there were no effects whether independent or interacting of \( Pgc-1\beta^{-/-} \) in the remaining (\( Tgf\beta1, Gjd5, Col3a1 \)) transcription markers. However, young \( Pgc-1\beta^{-/-} \) showed reduced \( \alpha_{1B} \)-adrenergic receptor (\( Adra1b \)) transcription compared to young
WT known to mediate protective and adaptive functions preventing pathological remodeling in cardiac failure through Gq/11 signaling (O’Connell et al., 2013). Of examined markers for a range of developmental, inflammatory and hypertrophic changes, two-way ANOVA demonstrated no differences bearing on Tbx3, Myh6, or Nppa although Tbx3 was increased in aged relative to young Pgc-1β−/−.

Finally, two way ANOVA demonstrated that both genotype and age independently and interactively exerted transcription changes in the complementary Pgc-1α gene (Ppargc1a). There was increased transcription in aged WT compared to young WT, and in young Pgc-1β−/− compared to young WT but decreased transcription in aged Pgc-1β−/− compared to aged WT.

The present findings together limit roles for transcriptomic changes in the age-dependent pro-arrhythmic phenotypic features of Pgc-1β−/− to altered Ca2+-ATPase (Atp2a2) expression. However, this is compatible with a previously reported arrhythmic mechanism demonstrated in p21-activated kinase type 1 deficient hearts on an earlier occasion (Wang et al., 2014; Wang Y. et al., 2015). These could add to contributions from direct physiological consequences of altered mitochondrial dysfunction. Thus the latter increases reactive oxygen species (ROS) production that acutely affects voltage-dependent Na+ and K+ channels (Wang et al., 2004; Liu et al., 2010), ryanodine receptors and gap junctions (Terentyev et al., 2008; Brown and O’Rourke, 2010; Sovari et al., 2013). Both transcriptomically and physiologically produced alterations in Ca2+ homeostasis could reduce Nav1.5 function (King et al., 2013; Ning et al., 2016), in turn associated with increased TGF-β activity, fibrotic change (Brooks and Conrad, 2000; Rosenkranz et al., 2002; Dai et al., 2009; Hafner et al., 2010; Hao et al., 2011), and disrupted gap junction function (van Veen et al., 2005; Chilton et al., 2007; Xie et al., 2009). Both Nav1.5 and gap junction changes may contribute pro-arrhythmic conduction changes observed in Pgc-1β−/− hearts.

ETHICS STATEMENT

Experiments were approved by the University of Cambridge ethics review board under a United Kingdom project license for studies of cardiac arrhythmia. All procedures complied with the United Kingdom Home Office regulations [Animals (Scientific Procedures) Act 1986]. The experiments also conformed to the Guide for the Care and Use of Laboratory Animals, United States National Institutes of Health (NIH Publication No. 85-23, revised 1996).

AUTHOR CONTRIBUTIONS

CE, KC, and IF performed the genome transcription studies, HV and SA performed the specimen preparation, CH and KJ conceived and designed the study and wrote the manuscript.

FUNDING

We thank the Medical Research Council (MR/M001288/1), Wellcome Trust (105727/Z/14/Z), British Heart Foundation (PG/14/79/31102 and PG/15/12/31280), Sudden Adult Death Syndrome (SADS) United Kingdom Society, and the Fundamental Research Grant Scheme (FRGS/2014/SKK01/PERDANA/02/1) (Ministry of Education, Malaysia) for their generous support.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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