A Patient-Specific Re-Engineered Cardiomyocyte Model Confirms the Circumstance-Dependent Arrhythmia Risk Associated with the African-Specific Common SCN5A Polymorphism (p.S1103Y): Implications for the Increased Sudden Deaths Observed in Black Individuals During the COVID-19 Pandemic

Samantha K. Hamrick, BS, CS John Kim, PhD, David J. Tester, BS, John R. Giudicessi, MD, PhD, Michael J. Ackerman, MD, PhD

PII: S1547-5271(21)02521-2
Reference: HRTHM 9093

To appear in: Heart Rhythm

Received Date: 30 August 2021
Revised Date: 14 December 2021
Accepted Date: 24 December 2021

Please cite this article as: Hamrick SK, Kim CJ, Tester DJ, Giudicessi JR, Ackerman MJ, A Patient-Specific Re-Engineered Cardiomyocyte Model Confirms the Circumstance-Dependent Arrhythmia Risk Associated with the African-Specific Common SCN5A Polymorphism (p.S1103Y): Implications for the Increased Sudden Deaths Observed in Black Individuals During the COVID-19 Pandemic, Heart Rhythm (2022), doi: https://doi.org/10.1016/j.hrthm.2021.12.029.

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A Patient-Specific Re-Engineered Cardiomyocyte Model Confirms the Circumstance-Dependent Arrhythmia Risk Associated with the African-Specific Common SCN5A Polymorphism (p.S1103Y): Implications for the Increased Sudden Deaths Observed in Black Individuals During the COVID-19 Pandemic

Short Title: Hamrick – p.S1103Y-SCN5A iPSC-CM model for drug-induced LQTS

Samantha K. Hamrick, BS\textsuperscript{1}, CS John Kim, PhD\textsuperscript{1}, David J. Tester, BS\textsuperscript{1}, John R. Giudicessi, MD, PhD, \textsuperscript{1,2} and Michael J. Ackerman, MD, PhD\textsuperscript{1}

Institutional affiliations: \textsuperscript{1}Departments of Cardiovascular Medicine (Division of Heart Rhythm Services), Pediatric and Adolescent Medicine (Division of Pediatric Cardiology), and Molecular Pharmacology & Experimental Therapeutics (Windland Smith Rice Sudden Death Genomics Laboratory), Mayo Clinic, Rochester, MN. \textsuperscript{2}Departments of Cardiovascular Medicine (Clinician-Investigator Training Program), Mayo Clinic, Rochester, MN.

Conflicts of Interest

MJA is a consultant for Abbott, ARMGO Pharma, Boston Scientific, Daiichi Sankyo, Invitae, LQT Therapeutics, Medtronic, and UpToDate. MJA and Mayo Clinic are involved in an equity/royalty relationship with AliveCor and Anumana. However, none of these entities were involved in this study in any manner. SKH, CSJK, DJT, JRG have no conflicts of interest to disclose.

Reprints and correspondence:

Michael J. Ackerman, M.D., Ph.D.

Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory

Guggenheim 501, Mayo Clinic, Rochester, MN 55905
ackerman.michael@mayo.edu, @MJAckermanMDPhD

Word count of manuscript: 3999 words, 3 figures, 11 references
ABSTRACT (word limit = 250, currently 239)

Background: During the early stages of the coronavirus disease 2019 (COVID-19) pandemic, a marked increase in sudden cardiac death (SCD) was observed. The p.S1103Y-SCN5A common variant, present in ~8% of individuals of African descent, may be a circumstance-dependent, SCD-predisposing, pro-arrhythmic polymorphism in the setting of hypoxia-induced acidosis or QT-prolonging drug use.

Objective: To ascertain the effects of acidosis and hydroxychloroquine (HCQ) on the action potential duration (APD) in a patient-specific induced pluripotent stem cell cardiomyocyte (iPSC-CM) model of p.S1103Y-SCN5A.

Methods: iPSC-CMs were generated from a 14-year-old p.S1103Y-SCN5A-positive African American male. The patient’s variant-corrected iPSC-CMs (isogenic control, IC) were generated using CRISPR/Cas9 technology. FluoVolt voltage sensing dye was used to assess APD90 values in p.S1103Y-SCN5A-iPSC-CMs compared to IC before and after an acidic state (pH 6.9) or 24 hours of treatment with 10 µM HCQ.

Results: Under baseline conditions (pH 7.4), there was no difference in APD90 values of p.S1103Y-SCN5A versus isogenic control iPSC-CMs (p = NS). In the setting of acidosis (pH 6.9), there was a significant increase in fold-change of APD90 in p.S1103Y-SCN5A iPSC-CMs compared to IC iPSC-CMs (p < 0.0001). Similarly, with 24h 10 µM HCQ treatment, the fold-change of APD90 was significantly higher in p.S1103Y-SCN5A iPSC-CMs compared to IC iPSC-CMs (p < 0.0001).
Conclusions: Although the African-specific p.S1103Y-SCN5A common variant had no effect on APD90 under baseline conditions, the physiologic stress of either acidosis or HCQ treatment significantly prolonged the APD90 in patient-specific, re-engineered heart cells.

Key words: DI-LQTS, drug-induced long QT syndrome; iPSC-CM, induced pluripotent stem cell-derived cardiomyocyte; LQTS, long QT syndrome; and p.S1103Y-SCN5A, p.Ser1103Tyr-SCN5A.
INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for coronavirus disease 2019 (COVID-19), has caused over 4.5 million deaths worldwide according to the WHO COVID-19 Dashboard (as of August 30, 2021). Early in the COVID-19 pandemic, the re-purposing of hydroxychloroquine (HCQ), a disease-modifying anti-rheumatic and anti-malarial drug, to fight COVID-19 was explored due to its antiviral properties in vitro. The FDA granted emergency use authorization on March 28, 2020. However, HCQ results in heart rate-corrected QT interval (QTc) prolongation and therefore has the potential to cause drug-induced (DI)-QT prolongation, DI-torsades de pointes (TdP), and even DI-sudden cardiac death (SCD) in some individuals. In fact, on April 24, 2020, the FDA cautioned against the use of HCQ for COVID-19 treatment outside of a hospital setting or a clinical trial due to these risks of heart rhythm problems. HCQ has since been proven to be an ineffective treatment for COVID-19 in the RECOVERY trial.

The p.S1103Y-SCN5A common genetic variant found in the SCN5A-encoded Na\(_\text{v}1.5\) sodium channel, is present in ~8-10% of individuals of African descent and may be a circumstance-dependent, SCD-predisposing, pro-arrhythmic polymorphism in the setting of hypoxia-induced acidosis or during exposure to a QT-prolonging medication. Human embryonic kidney (HEK) cell in vitro heterologous over-expression studies of p.S1103Y-SCN5A have demonstrated a pathophysiological persistent late sodium current phenotype in the setting of an acidotic state akin to SCN5A-mediated type 3 long QT syndrome (LQTS, LQT3). Acidosis can be caused by a severe hypoxic state, such as is seen in some COVID-19 patients. This common, ethnic-specific genetic variant potentially exacerbates health disparities already caused by the pandemic. Severe hypoxic states are also seen in many other conditions and infections, and the
genetic variant is potentially contributing to other long-standing pre-existing health disparities and perhaps many future ones.

In light of the FDA’s past Emergency Use Authorization of HCQ, we ascertained the effects of acidosis and HCQ on the cardiac action potential duration (APD) in a patient-specific induced pluripotent stem cell cardiomyocyte (iPSC-CM) model of p.S1103Y-SCN5A.

METHODS

Case description and generation of patient-specific and isogenic control (IC) iPSC lines

The heterozygous p.S1103Y-SCN5A missense variant was identified incidentally in a 14-year-old black male without QT-prolongation (QTc=394 ms). Blood samples were obtained from the patient following informed consent in accordance with this Mayo Clinic IRB (09-006465) approved study.

Peripheral blood mononuclear cells (PBMC) were reprogrammed by episome. Blood Media was used to grow PBMCs for 3 to 7 days in a 5% CO₂, 37°C, and humidified incubator. Briefly, Blood Media is composed of IMDM (Gibco, 12440053), 20% KnockOut Serum Replacement (Gibco, 10828010), IGF-1 (Peprotech, 100-11), EPO (Peprotech, 100-64), IL-3 (Peprotech, 200-03), and hSCF (Peprotech, 300-07). DNA for transfection was prepared with pCXLE-hOCT3/4-shp53-F (OCT3/4) (Addgene, 27077), pCXLE-hSK (SOX2/KLF4) (Addgene, 27078), pCXLE-hUL (L-MYC) (Addgene, 27080), and PCXWB-EBNA1 (EBNA-1) (Addgene, 37624). The P3 Primary Cell 4D-Nucleofector X Kit (Lonza, V4XP-3024) and the Lonza 4D Nucleofector machine were used to reprogram the PBMCs. Afterwards, the PBMCs were placed in a 37°C, 5% CO₂, and humidified incubator. Media was changed to 50:50 of Blood Media and ES Media on day 2 after transfection. ES Media is composed of KnockOut DMEM (Gibco, 10829018) with 20% KnockOut Serum Replacement. On day 4, media was replenished with ES.
Media only. On day 7, media was changed to mTeSR1 (Stemcell Technologies, 85850) only with 1% Antibiotic-Antimycotic (Gibco, 15240-062). mTeSR1 media was changed every other day until seeing visible iPSC colonies. Colonies were picked at around 21 days post-transfection with the Yamanaka factors.

The patient-derived p.S1103Y-SCN5A variant iPSCs were “gene-corrected” using CRISPR/Cas9 technology to generate an isogenic control line by Applied StemCell (Milpitas, California).

**Cardiomyocyte differentiation**

The iPSCs were cultured in mTeSR1 in 6-well plates (Falcon, 353046) with Matrigel (Corning, 356278) coating and incubated at 37°C and 5% CO₂. The mTeSR1 media was changed daily. At 80-90% confluence, iPSCs were disaggregated with ReLeSR (Stemcell Technologies, 05872), seeded into 24-well plates (Corning, 3524), cultured in mTeSR1 with 10 μM ROCK inhibitor (Tocris, 1254), and allowed to grow for 2-4 days until 80-90% confluent. For differentiation, the culture medium was changed to RPMI 1640 medium (Gibco, 11875-093) supplemented with B27-minus insulin (Gibco, A18956-01) containing 5 μM CHIR99021 (Sigma, SML1046) for 48 hr (day 2). After 48 hr, the medium was changed to RPMI-B27-minus insulin containing 5 μM IWP2 (Sigma, I0536) and incubated until day 4. On day 4, the medium was changed back to RPMI 1640 medium supplemented with B27-minus insulin and cells were maintained in this media until beating iPSC-CMs appeared, typically around day 10 or day 12. One week following the initial observation of beating, the media was changed, and the iPSC-CMs were maintained in RPMI 1640 medium supplemented with B27 (Gibco, 17504-044).

**Dissociation of iPSC-CMs**

The iPSC-CM aggregate cultures were maintained in RPMI 1640 medium supplemented with B27 in 24-well plates. At differentiation day 30, iPSC-CMs were subjected to enzymatic dissociation
using CM dissociation media (Stemcell Technologies, 05025) to obtain single cell suspensions of CMs. The iPSC-CMs were first washed with 1 mL of PBS for 30 sec. Following aspiration of the PBS, 250 μL of CM dissociation media was added to each well. After 2 min of incubation at RT, the CM dissociation media was aspirated, and the cells were incubated for 3 min in a 5% CO₂ incubator at 37°C. Following incubation, 500 μL of DMEM (Corning, 10-013-CV) with 20% FBS (Gibco, 10437-028) was added to each well. The solution was triturated 4-6 times with a 1000 μL pipet tip and cells were transferred to a Matrigel-coated 35 mm glass bottom dish (MatTek, P35G-1.5-10-C) and cultured at 37°C, 5% CO₂ for 24 hr. The media was then changed to RPMI 1640 medium supplemented with B27 and stored in a 5% CO₂ incubator at 37°C until use.

**Live cell imaging for action potential duration (APD) measurement**

The iPSC-CMs were cultured on 35 mm glass bottom dishes that were pre-coated with Matrigel (Corning, 356278) at 37°C, 5% CO₂. For imaging, cells were incubated at 37°C, 5% CO₂ for 20 min in Tyrode’s solution (Alfa Aesar, J67607K2) containing a fluorescent voltage sensitive dye, FluoVolt (Invitrogen, F10488). The Tyrode’s/FluoVolt solution contained 0.5 μL FluoVolt dye, 5 μL PowerLoad concentrate and 4 mL Tyrode’s solution. They were then washed three times in fresh Tyrode’s solution. During imaging, the dishes were kept in a heated 37°C stage-top environment chamber supplied with 5% CO₂. Imaging of voltage-induced cellular APD was taken under a 40X-water objective using a Nikon Eclipse Ti light microscope. Time-lapse videos of multiple, individual beating iPSC-CMs, paced at 1 Hz were recorded at a speed of 20 ms per frame for 20 sec at 15% LED power. Single regions of interest were selected for every beating iPSC-CM captured in the recordings. The iPSC-CMs were either i) untreated (pH 7.4 without HCQ), ii) in an acidotic condition of pH 6.9, or iii) treated with 10 µM HCQ in normal pH 7.4 for 24h. The raw data was exported to Excel software (Microsoft, Redmond, WA) and then analyzed
with an in-house developed Excel-based program. To calculate APD90 fold-change, each APD90 value of a treated iPSC-CM was divided by the average baseline APD90 of each data set (for example, one IC-C1 iPSC-CM treated with HCQ APD90 divided by IC-C1 iPSC-CMs average baseline APD90). An APD90 fold-change value close to 1.0 was considered no change.

**Immunocytochemistry**

Cells were fixed with 4% paraformaldehyde for 10 min at RT followed by being washed 3 times with PBS. The cells were then permeabilized/blockaded with 0.1% Triton X-100/PBS (PBST)/5% goat serum for 1 hr at RT, and subsequently incubated in primary antibody solution made of PBST/5% goat serum containing 1 µg/mL of Oct-4 (Invitrogen, PA5-27438) and SSEA-4 (ThermoFisher, MA1-021) primary antibodies for iPSCs, or 1 µg/mL of cTnT (Proteintech, 15513-1-AP) or 1 µg/ml of SCN5A (Santa Cruz Biotechnology, sc-271255) primary antibody for iPSC-CMs, at 4°C overnight. The next day, cells were washed 3 times with PBST/5% goat serum at RT before being incubated in PBST/5% goat serum with a 1:200 dilution of Alexa Fluor 568 IgG (H+L) Cross-Adsorbed Goat anti-Mouse (Invitrogen, A-11004) and/or Alexa Fluor 488 IgG (H+L) Cross-Adsorbed Goat anti-Rabbit (Invitrogen, A-11008) secondary antibodies at RT for 1 hr; DAPI was added to each secondary antibody solution at dilution of 1 µg/mL. After secondary antibody incubation, cells were washed 3 times with PBST and finally covered in 500 µL of PBS for imaging. Images were acquired on a Zeiss LSM 780 confocal microscope in the Mayo Microscopy and Flow Cytometry Cell Analysis Core Facility.

**Statistical analysis**

All data points are shown as the mean value and bars represent the standard error of the mean. A Student’s t-test (two tailed) was performed to determine statistical significance between two
groups and a one-way ANOVA and Tukey post-hoc test was performed for comparisons among three or more groups. A p<0.05 was considered to be significant.

RESULTS

Generation of patient-specific iPSCs and CRISPR/Cas9-engineered isogenic control (IC) iPSCs

The heterozygous p.S1103Y-SCN5A missense variant was identified incidentally in a 14-year-old black male who presented for a second opinion cardiology evaluation for hypertrophic cardiomyopathy (HCM) and was ultimately dismissed with insufficient evidence for a clinical diagnosis for HCM. The patient had a normal electrocardiogram displaying a heart-rate corrected QTc=394 ms.

Following generation of patient-specific (p.S1103Y containing) and CRISPR/Cas9 S1103Y variant corrected isogenic control (IC) iPSCs and iPSC-CMs, immunofluorescence imaging showed that the iPSCs contained the pluripotency markers Oct-4 and SSEA-4, and the iPSC-CMs contained the cardiac marker cTnT (Figure 1A). Sanger sequencing confirmed that the p.S1103Y-SCN5A variant was in the patient line and that the IC was free of this variant (Figure 1B). Using quantitative IF, we selected cardiac specific marker harboring cells and calculated the mean value of fluorescent intensity of SCN5A staining divided by area. The IF imaging showed there was no significant difference in SCN5A protein expression levels between p.S1103Y-SCN5A iPSC-CMs and IC iPSC-CMs (p=NS, Figure 2).

Patient-specific p.S1103Y-SCN5A iPSC-CMs are more sensitive to the APD-prolonging effects of either acidosis or hydroxychloroquine (HCQ)

Fluorescent voltage sensing dye, FluoVolt, was used to assess APD90 in p.S1103Y-SCN5A iPSC-CMs compared to ICs at i) baseline (pH 7.4), ii) after an acidic state (pH 6.9), or iii) after 24
hours of treatment with 10 µM HCQ at normal pH 7.4. Representative APD90 tracings are shown in Figure 3A and 3B. Under baseline conditions (pH 7.4), there was no difference in APD90 values of p.S1103Y-SCN5A iPSC-CMs (clone 1, 398 ± 7 ms, n=21) versus IC (clone 1, 395 ± 6 ms, n=21, p = NS, Figure 3C). The baseline APD90 measurements were also similar between p.S1103Y-SCN5A iPSC-CMs (clone 2, 364 ± 3 ms, n=20) and IC (clone 2, 379 ± 3 ms, n=20, p=NS, Figure 3C).

However, at pH 6.9, the APD90 was prolonged significantly in p.S1103Y-SCN5A iPSC-CMs (clone 1, 398 ± 7 ms [n=21] vs 477 ± 6 ms [n=15], Δ+79 ms, p < 0.0001) but not in the IC iPSC-CMs (clone 1, 395 ± 6 ms, [n=21] vs 377 ± 6 ms [n=15], Δ-18 ms, p = NS). This was also observed in p.S1103Y-SCN5A clone 2 and IC clone 2, where the APD90 was significantly prolonged in the S1103Y iPSC-CMs (clone 2, 364 ± 3 ms, [n=20] vs 440 ± 5 ms [n=19], Δ+76 ms, p < 0.0001) following an acidotic state, but not in the IC (clone 2, 379 ± 3 ms [n=20] vs 371 ± 2 ms [n=19], Δ-8 ms, p = NS).

We also calculated the APD90 fold-change. To calculate APD90 fold-change, each APD90 value of treated iPSC-CMs was divided by the average baseline APD90 of each data set (for example, IC-clone 1 acidosis APD90 divided by IC-clone 1 average baseline APD90). An APD90 fold-change value close to 1.0 was considered no change. Under the setting of acidosis (pH 6.9), there was a significant increase in fold-change of the APD90 only in the two clones of p.S1103Y-SCN5A (Clone 1, 1.199 ± 0.014, Clone 2, 1.209 ± 0.013) compared to the two IC clones’ iPSC-CMs (Clone 1, 0.956 ± 0.015, Clone 2, 0.979 ± 0.006, p<0.0001, Figure 3D).

Similarly, the APD90 was prolonged significantly in p.S1103Y-SCN5A iPSC-CMs (clone 1, 398± 7 ms [n=21] vs 504 ± 9 ms [n=15], Δ+106 ms, p < 0.0001; clone 2, 365± 3 ms [n=19] vs
404 ± 4 ms [n=17], Δ+39 ms, p < 0.0001) but not in the IC iPSC-CMs (clone 1, 395 ± 6 ms [n=21] vs 394 ± 6 ms [n=15], Δ-1 ms, p = NS; clone 2, 422 ± 4 ms [n=20] vs 422 ± 3 ms [n=20], Δ0 ms, p < 0.0001) following 24 h treatment with 10 µM HCQ. The fold-change of the APD90 was significantly higher in the p.S1103Y-SCN5A clone 1 and clone 2 iPSC-CMs (1.268 ± 0.022, 1.109 ± 0.011) compared to IC clone 1 or clone 2 iPSC-CMs (0.999 ± 0.015, 1.001 ± 0.008, p<0.0001, Figure 3E).

**DISCUSSION**

African Americans who possess the p.S1103Y-SCN5A variant have an increased risk of acquired arrhythmias. This variant is also associated with an increased risk of sudden unexplained death (SUD) and sudden infant death syndrome (SIDS) in black individuals.6,8,9 Tester et al. found that this variant was overrepresented in an autopsy-negative SUD cohort.8 p.S1103Y-SCN5A was also overrepresented in African American SIDS cases by Van Norstrand et al.9 Similarly, Plant et al. determined that African American infants who are homozygous for p.S1103Y-SCN5A were at a 24-fold increased risk of developing SIDS.6 Common risk factors for SIDS include apnea and/or respiratory acidosis.6

While it has been shown previously in a HEK cell model that acidosis can affect cells with this p.S1103Y-SCN5A variant,6 we confirmed these findings in a patient-specific, re-engineered heart cell model. Our data also establish that the p.S1103Y-SCN5A variant was necessary to produce the significant APD prolongation observed in the patient’s iPSC-CMs following HCQ treatment. Therefore, HCQ is another potential risk factor for individuals with the p.S1103Y-SCN5A variant because it can cause DI-arrhythmias and DI-LQTS. HCQ is currently being used to treat malaria, systemic lupus erythematosus, and rheumatoid arthritis where its cardiac safety profile is substantial.3,10
Given its prevalence, and the increased risk of DI-arrhythmias and the potential DI-SCD associated with this drug, genetic testing for p.S1103Y-SCN5A may be warranted before the initiation of HCQ and other QT-prolonging drugs in those of African descent. Importantly, a baseline 12-lead ECG alone may not be suitable for screening as p.S1103Y-SCN5A does not appear to affect the APD90 under baseline conditions. In emergency situations, where treatment with any known QT-prolonging drug needs to begin immediately, in-hospital, post-treatment QT monitoring should be performed. With mobile ECG devices capable of accurately assessing the QTc in nearly any setting, consideration of routine pre- and post-treatment QTc monitoring will soon become practically and financially feasible. Due to p.S1103Y-SCN5A’s circumstance-dependent risk, this is particularly important for patients of African descent with concomitant QT risk factors or those at-risk for metabolic or respiratory acidosis, including those with moderate-to-severe manifestations of COVID-19.

The iPSC-CM model used herein has advantages over the non-cardiac HEK cell-based models previously utilized. HEK cell models lack i) the majority of structural and interacting proteins involved in the cardiac APD and ii) the personalized, precision medicine nature of iPSC-CM models. iPSC-CMs provide a more physiologically relevant system for patient-specific studies. This specific p.S1103Y-SCN5A iPSC-CM model would be useful to test the effects of other QT-prolonging drugs, exercise-induced arrhythmias, and/or the cytokine storm phenomenon that can occur during COVID-19 infection. This model could be applied to test potential therapies for these conditions, as well.

CONCLUSIONS

Although the African-specific p.S1103Y-SCN5A common variant had no effect on APD under baseline conditions, the physiologic stress of either acidosis or HCQ treatment prolonged
significantly the APD in patient-specific, re-engineered heart cells. Further epidemiological studies are needed to determine if this African-specific p.S1103Y-SCN5A common polymorphism and its circumstance-dependent reduction in repolarization reserve have contributed to the increased rate of SCD and the racial outcome disparities observed during the COVID-19 pandemic.

ACKNOWLEDGEMENTS

We would like to thank the team in the Mayo Clinic Microscopy and Cell Analysis Core facility in Rochester, MN, for their dedication to quality assistance and care with the equipment.

FUNDING SOURCES

This work was supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program.
REFERENCES


Figure 1. Generation of patient-specific iPSCs and CRISPR/Cas9-engineered isogenic control (IC) iPSCs

In Panel A, brightfield images show the classic morphology of both IC (top left) and patient (p.S1103Y, bottom left) iPSCs. Immunofluorescence imaging shows that the IC and patient iPSCs have the pluripotent markers Oct4 and SSEA4 (top and bottom middle, green=Oct4, red=SSEA4, blue=DAPI), and the iPSC-CMs have the cardiac marker cTnT (top and bottom right, green=cTnT, blue=DAPI). In Panel B, Sanger sequencing shows that the patient’s p.S1103Y variant (c.3308 C>A, bottom) was appropriately “gene-corrected” using CRISPR/Cas9-based approaches to create the IC (top).
Figure 2. SCN5A Expression

Panel A shows cardiac marker cTnT (left) and SCN5A expression (middle) (green=cTnT, red=SCN5A, blue=DAPI) in isogenic control (IC, top) versus patient iPSC-CMs (bottom). In Panel B, the bar graph shows that there was no significant difference in SCN5A expression between IC and patient iPSC-CMs.
Figure 3. Patient-specific p.S1103Y-SCN5A iPSC-CMs are more sensitive to the APD-prolonging effects of acidosis and hydroxychloroquine (HCQ). Panel A shows representative tracings from FluoVolt imaging of isogenic control (IC) and p.S1103Y variant-containing 30-day-old iPSC-CMs. FluoVolt raw data was analyzed using a lab-created software to determine the APD90s of the iPSC-CM lines. The APD90s were measured under the conditions of either baseline (BL, pH 7.4, untreated), acidosis (pH 6.9), or HCQ use (10 µM for 24 h) at normal pH 7.4. Cells were paced at 1 Hz. APD90 tracings from the IC iPSC-CMs showed no significant difference between BL, pH 6.9, or HCQ. However, APD90 tracings from the p.S1103Y-SCN5A iPSC-CMs showed marked APD prolongation during exposure to either pH 6.9 or HCQ. In Panel B, the representative merge tracings show BL condition as black, acidosis condition as red, and HCQ condition as blue (IC=left column, p.S1103Y=right column). Panel C shows the average
APD90 of the IC clone 1 (C1, n=21 cells) versus p.S1103Y C1 (n=21 cells) and IC clone 2 (C2, 20 cells) versus p.S1103Y C2 (n=20 cells) iPSC-CMs. Panels D and E represent APD90 fold-changes for acidosis- and HCQ-treated iPSC-CMs, respectively. Each treated iPSC-CM APD90 value was divided by the average baseline APD90 value, for both IC and patient iPSC-CMs, respectively (Panel C: IC-C1 n=15 cells, IC-C2 n=19 cells, p.S1103Y-C1 n=15 cells, p.S1103Y-C2 n=19 cells, Panel D: IC-C1 n=15 cells, IC-C2 n=19 cells, p.S1103Y-C1 n=15 cells, p.S1103Y-C2 n=17 cells). Individual circles indicate each fold-change data point. The red dashed line indicates a baseline ratio of 1.0, which represents each group’s average baseline APD90. One-way ANOVA with Tukey multiple comparison was used for all statistical analyses (ns: p>0.05, ****: p<0.0001).
Figure 1

(A) Brightfield, Oct-4/SSEA-4, cTnT

(B) Genomic Control, p.S1103Y

Control

Oct-4/SSEA-4

100 µm

cTnT

50 µM

20 µM

p.S1103Y

Genetic Control

G C C T C C T C T

p.S1103Y

G C C T A C T C T
Figure 2

A

Isogenic Control

20 µM

p.S1103Y

B

Fluorescent Value of SCN5A

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Figure 3

A

Baseline
Acidosis
HCQ

Isogenic Control

C

ns

ns

APD90 (ms)

D

APD90 Fold-Change - Acidosis

E

APD90 Fold-Change - HCQ

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