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Jean-baptiste Reisqs, Adrien Moreau, Azzouz Charrabi, Yvonne Sleiman, Albano C. Meli, Gilles Millat, Veronique Briand, Philippe Beauverger, Sylvain Richard, Philippe Chevalier

► To cite this version:

Jean-baptiste Reisqs, Adrien Moreau, Azzouz Charrabi, Yvonne Sleiman, Albano C. Meli, et al.. The PPAR γ pathway determines electrophysiological remodelling and arrhythmia risks in DSC2 arrhythmogenic cardiomyopathy. *Clinical and Translational Medicine*, 2022, 12 (3), 10.1002/ctm2.748 . hal-03613345

HAL Id: hal-03613345

<https://hal.science/hal-03613345>

Submitted on 18 Mar 2022

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Jean-Baptiste Reisqs^{1,2}
Adrien Moreau³
Azzouz Charrabi³
Yvonne Sleiman³
Albano C. Meli³
Gilles Millat^{1,4,5}
Veronique Briand²
Philippe Beauverger²
Sylvain Richard³
Philippe Chevalier^{1,4}

The PPAR γ pathway determines electrophysiological remodelling and arrhythmia risks in DSC2 arrhythmogenic cardiomyopathy

Dear Editor,

Arrhythmogenic cardiomyopathy (ACM) is a rare, life-threatening genetic disease frequently associated with mutations in desmosomal genes.¹ Histopathological hallmark includes fibrofatty replacement of myocardial tissue, potentially consisting of cardiomyocytes transdifferentiation into adipocytes.² The ACM involves electromechanical disorders and risks of developing arrhythmias and sudden cardiac death. We recently evidenced early electrical modifications of human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM) obtained from an ACM patient with a missense mutation (c.394C>T) in the *DSC2* gene encoding desmocollin 2 (DSC2-hiPSC-CMs). These modifications are risk factors for triggering arrhythmias independently of fibrofatty replacement of myocardial tissue.³ We now show that PPAR γ , a master regulator of the cardiomyocytes transdifferentiation into adipocytes, is critical early in the pro-arrhythmogenic pathogenesis in ACM-DSC2-hiPSC-CMs.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of heart samples revealed a higher PPAR γ gene expression level in the ACM-heart bearing the *DSC2* mutation than in the control heart (Figure S1). We derived hiPSCs from the same ACM-DSC2 patient, differentiated them into hiPSC-CMs, and cultured them for 60 days as described.³ We found a similar higher expression of PPAR γ and of two pro-adipogenic target genes, perilipin and adiponectin, in DSC2-hiPSC-CMs versus control-hiPSC-CMs (Figure 1A–C). Despite lower expression, the pro-cardiac myosin light chain 2 ventricular (MLC2v) validated the cardiomyocyte phenotype of DSC2-hiPSC-CMs (Figure 1D). To confirm the involvement of PPAR γ in the genes switch, we challenged DSC2-hiPSC-CMs with T0070907 (T007), which functions as a transcriptionally corepressor-selective PPAR γ inverse agonist.⁴ Incubation with T007 (1 μ M), for 40 days (D20–D60) during the maturation phase, corrected the expres-

sion levels of PPAR γ , perilipin, adiponectin and MLC2v (Figure 1A–D). These results corroborated the PPAR γ -dependent pro-adipogenic switch in the DSC2-hiPSC-CMs, linking with previous studies.^{2,5,6}

We next investigated the effects of T007 on the excitation–contraction coupling using a phase-contrast video-based analysis of the hiPSC-CM monolayer's contractile function. We defined several contractile parameters: the beat rate, contraction time, relaxation duration, the resting time between two contractions, asynchronous rate and differentiating factor (Figure 2A–F). For each analysed video, the asynchronous time defines the time-fraction of the area spent in asynchrony. The differentiating factor reflects the linear combination of determinant contractile properties ensuring the best discrimination between the control-hiPSC-CMs and the DSC2-hiPSC-CMs.

The mutation increased the beat and asynchronous rates (Figure 2A,B) and reduced the contraction time, relaxation and resting duration in DSC2-hiPSC-CMs versus control-hiPSC-CMs (Figure 2C–E). Incubation with T007 prevented these modifications. The differentiating factor was similar to that of the control-hiPSC-CMs (Figure 2F), showing that PPAR γ pathway inhibition counteracts the pathogenesis process. Interestingly, the PPAR γ agonist (GW1929) did not mimic the effect of the mutation on the differentiating factor and action potential (AP) duration in control-hiPSC-CMs (Figure S2A) in line with the negligible effect of PPAR γ agonists on basal transcription.⁴ A significant PPAR γ expression level may be required for ligand-mediated effects.

In cardiomyocytes, the AP initiates the contraction. We studied the effect of T007 on the electrophysiological properties of DSC2-hiPSC-CMs using the patch-clamp technique. Compared to control-hiPSC-CMs, the DSC2-hiPSC-CMs exhibited a twofold shortening of the AP duration at 1.0 Hz and lost action potential duration (APD)

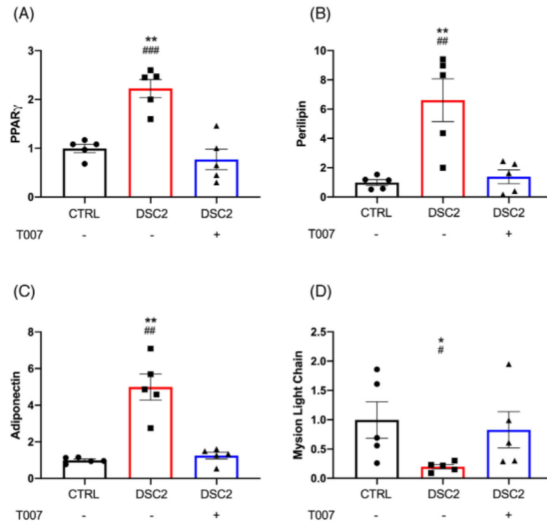


FIGURE 1 T0070907 modulates PPAR γ pathway and prevents the cardiomyocyte phenotype in DSC2 patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM). Fold change compared to control for (A) PPAR γ , (B) perilipin, (C) adiponectin and (D) myosin light chain (MLC) expression level, measured using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Error bars represent the standard error of the mean (SEM), $N = 5/\text{group}$. *Control versus DSC2, #DSC2 versus T007; * $p < .05$, ** $p < .01$, *** $p < .001$ (Tuckey multiple comparisons test)

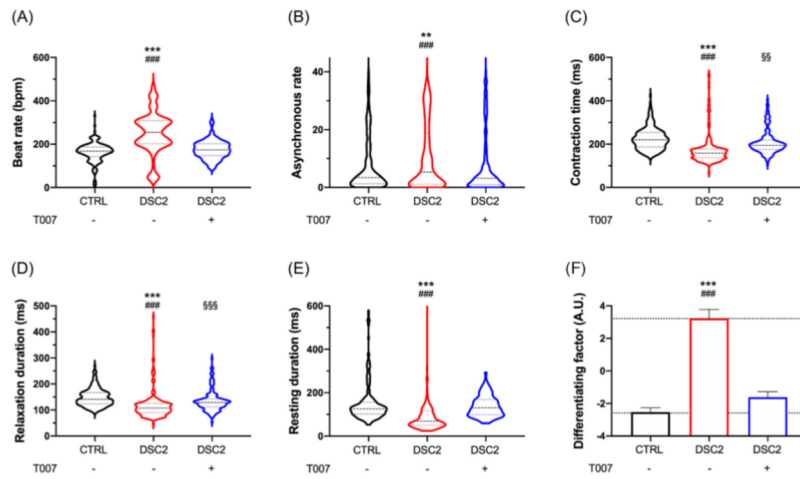


FIGURE 2 T0070907 prevents contractile disturbances in DSC2 patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs). The contractile activity was studied by video analysis in monolayer condition (control, $N = 249$ (black); patient, $N = 212$ (red); T0070907, $N = 197$ (blue)): beat rate (A), asynchronous rate (B), contraction time (C), relaxation duration (D), resting duration (E) and differentiating factor (F). Results are represented by violin plot with the median, first and third quartile. *Control versus DSC2, #DSC2 versus T007, \$\$\$ $p < .01$, *** $p < .001$ (Kruskal-Wallis test)

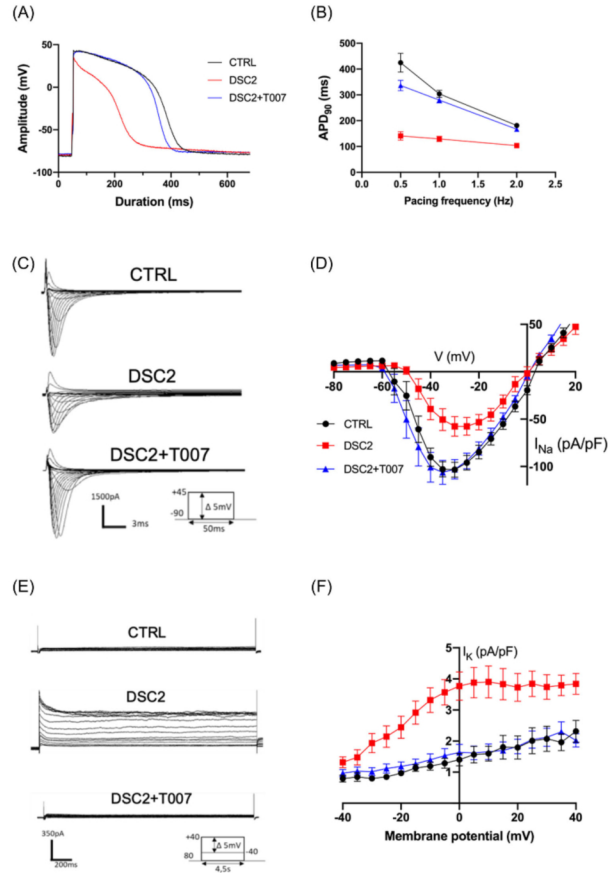


FIGURE 3 Electrical activity of control and patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) with or without T0070907. (A) Action potentials (APs) of hiPSC-CM from control (black), DSC2 patient in absence of T0070907 (red) and after 40 days of incubation with 1 μ M of T0070907 (blue). (B) AP duration at 90% of repolarisation (APD_{90}) as a function of pacing rate (control, $N = 42$; DSC2, $N = 40$; DSC2 + T0070907, $N = 40$). (C) Whole-cell Na^+ current traces of control (top), patient-specific hiPSC-CMs (middle) and after incubation of T007 (bottom). (D) Averaged current density-voltage ($I-V$) relationship of I_{Na} (control, $N = 23$; DSC2, $N = 21$; DSC2 + T0070907, $N = 23$). (E) Whole-cell I_K currents raw traces of control (top), patient-specific (middle) and after incubation of T0070907 (bottom) hiPSC-CMs. (F) $I-V$ curves of I_K associated currents (control, $N = 15$; DSC2, $N = 18$; DSC2 + T007, $N = 14$). Error bars represent the standard error of the mean (SEM). All differences are statistically different, $p < .001$, DSC2 versus control and versus DSC2 + T0070907 (Tukey multiple comparison test)

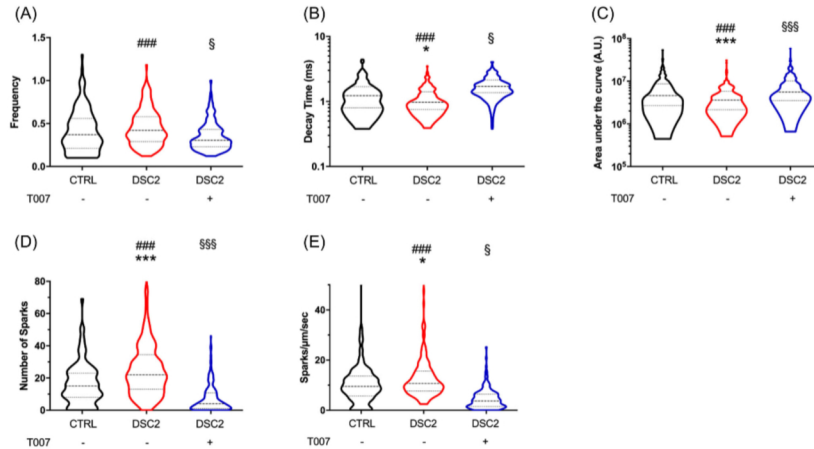


FIGURE 4 Spontaneous Ca^{2+} handlings of control, patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) and incubated with T0070907. The Ca^{2+} transient activity (control, $N = 207$ (black); patient, $N = 222$ (red); T0070907, $N = 197$ (blue)) were studied. Spontaneous frequency (A), decay time (B) and area under the curve (AUC) (C). The Ca^{2+} sparks were studied by measuring the number of sparks in each cell (D) and the frequency of sparks (E). The violin plot represented the median, first and third quartile, and a Kruskal–Wallis test was performed. * Control versus DSC2, [†]DSC2 versus T007, [‡]control versus T007; [§] $p < .05$, ^{***,##,SSS} $p < .001$ (Tukey multiple comparisons test were performed)

modulation according to pacing rate (Figure 3A,B). The voltage-gated Na^+ current (I_{Na}) induces AP fast depolarisation, playing a master role in cardiac excitability. The DSC2-hiPSC-CMs exhibited a twofold decrease in I_{Na} density and a rightward shift in its voltage-dependent activation and steady-state inactivation (Figures 3C,D and S3). Consistently, the expression level of the *SCN5A* gene coding for the cardiac Na_v channels (I_{Na}) was decreased (Figure S4). Voltage-gated K^+ currents play a crucial role in AP repolarisation. Compared to control-hiPSC-CMs, DSC2-hiPSC-CMs exhibited a 3.5-fold increase in K^+ current density, accounting for the AP shortening (Figure 3E,F). RT-qPCR analysis revealed an increase of +227% in the *KCNH2* gene coding for I_{Kr} and +220% in the *KCNQ1* gene coding for I_{Ks} in DSC2-hiPSC-CMs compared to control-hiPSC-CMs (Figure S4). Exposure of DSC2-hiPSC-CMs to T007 prevented the differences with control-hiPSC-CMs (Figures 3A–F and S3–S5).

We evaluated the Ca^{2+} transients of spontaneously beating hiPSC-CMs using the fluorescent non-ratiometric Fluo-4 Ca^{2+} -sensitive dye. The DSC2-hiPSC-CMs (vs. control-hiPSC-CMs) exhibited increased Ca^{2+} transient frequency but decreased decay time and global Ca^{2+} mobilised area under the curve (AUC) during the Ca^{2+}

transient (Figure 4A–C) in coherence with impaired contractility. They also exhibited an increase in the number and frequency of pro-arrhythmic abnormal spontaneous diastolic microscopic Ca^{2+} events (Ca^{2+} sparks), reflecting Ca^{2+} leak from the sarcoplasmic reticulum through the ryanodine receptor. Treatment of the DSC2-hiPSC-CMs with T007 attenuated all these effects (Figure 4D,E).

Late T007 incubation (D55–D60) could not reverse the variant-specific modifications of the AP in DSC2-hiPSC-CMs (Figure S5). In addition, when we cultured DSC2-hiPSC-CMs in the presence of T007 between D20 and D60 and then removed the drug at D100, the cells exhibited the typical disease phenotype (Figure S5). Therefore, early and continuous exposure of DSC2-hiPSC-CMs to T007 is required to maintain a wild-type-like cellular electrophysiological signature (Figure S6).

In conclusion, the repressing effect of T007 on PPAR γ transcriptional activity maintained regular electrical activity and Ca^{2+} handling in hiPSC-CMs bearing the *DSC2* (c.394C>T) mutation. T007 also lowered early pro-arrhythmic events intrinsic to cardiomyocytes, likely to contribute to rhythm disturbance independently of fibrofatty replacement of myocardial tissue in ACM

patients (Figure S7). The risk of malignant ventricular tachyarrhythmias relies both on (i) shortened AP repolarisation, responsible for short QT (and JTC) intervals in a cohort of patients,³ caused by high *KCNH2* (I_{Kr}) and *KCNQ1* (I_{Ks}) expressions and (ii) and abnormal occurrence of Ca^{2+} sparks known to promote arrhythmias independently of any change in the AP and QT interval.⁷ Combining a short AP (QT) with disturbed Ca^{2+} is likely to increase the pro-arrhythmic risk. Our results align with different studies in transgenic mice linking PPAR γ activation, cardiomyocyte lipid accumulation, dilated cardiomyopathy, changes in electrophysiological profile and intracellular Ca^{2+} handling, and arrhythmogenic risks, although differences between mice and humans may limit some interpretations.^{6,8-10} Overall, our study provides new comprehensive insights directly in human cardiomyocytes regarding the role of PPAR γ in genetic ACM. Repression of PPAR γ transcription may offer a unique opportunity for ACM treatment.

ACKNOWLEDGEMENTS

We thank the 'Montpellier Ressources Imagerie' (MRI) platform (<https://www.mri.cnrs.fr/fr/>). A CIFRE grant from Sanofi R&D supported this work to Jean-Baptiste Reisqs. Fond Marion Elisabeth Brancher supported the post-doctoral fellowship of Adrien Moreau.

FUNDING INFORMATION

A CIFRE grant from Sanofi R&D supported this work to Jean-Baptiste Reisqs. Fond Marion Elisabeth Brancher supported the post-doctoral fellowship of Adrien Moreau.

CONFLICT OF INTERESTS

Sanofi, a global biopharmaceutical company focused on human health, employs Jean-Baptiste Reisqs, Veronique Briand and Philippe Beauverger. All other authors have nothing else to disclose. All authors read and approved the final version of the manuscript and ensure it was the case.

Jean-Baptiste Reisqs^{1,2}
 Adrien Moreau³
 Azzouz Charrabi³
 Yvonne Sleiman³
 Albano C. Meli³
 Gilles Millat^{1,4,5}
 Veronique Briand²
 Philippe Beauverger²
 Sylvain Richard³
 Philippe Chevalier^{1,4}

¹Neuromyogene Institute, Claude Bernard University, Lyon 1, Villeurbanne, France

²Cardiovascular and Metabolism Research, Sanofi R&D, Chilly Mazarin, France

³Université de Montpellier, INSERM, CNRS, PhyMedExp, Montpellier, France

⁴Hospices Civils de Lyon, Lyon, Service de Rythmologie, France

⁵Laboratoire de Cardiogénétique Moléculaire, Centre de Biologie et Pathologie Est, Bron, France

Correspondence

Sylvain Richard, INSERM U1046, CNRS UMR9214, Université de Montpellier, PhyMedExp, CHU Arnaud de Villeneuve, Bâtiment Crastes de Paulet, 371 Avenue du doyen Gaston Giraud, 34295 Montpellier Cedex 5, France.

Email: sylvain.richard@inserm.fr

Philippe Chevalier, Service de Rythmologie Cardiaque, CHU de Lyon HCL, GH Est-Hôpital Louis Pradel 59 Boulevard Pinel, 69677 Bron Cedex, France.

Email: philippe.chevalier@chu-lyon.fr

Jean-Baptiste Reisqs, Adrien Moreau, Sylvain Richard and Philippe Chevalier contributed equally as junior and senior investigators, respectively.

ORCID

Adrien Moreau  <https://orcid.org/0000-0003-3100-0807>

Sylvain Richard  <https://orcid.org/0000-0001-9460-6705>

REFERENCES

- Awad MM, Calkins H, Judge DP. Mechanisms of disease: molecular genetics of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Nat Clin Pract Cardiovasc Med*. 2008;5:258-267. <https://doi.org/10.1038/ncpcardio1182>
- Ma D, Wei H, Lu J, et al. Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J*. 2013;34:1122-1133. <https://doi.org/10.1093/eurheartj/ehs226>
- Moreau A, Reisqs J-B, Delanoe-Ayari H, et al. Deciphering DSC2 arrhythmogenic cardiomyopathy electrical instability: from ion channels to ECG and tailored drug therapy. *Clin Transl Med*. 2021;11:e319. <https://doi.org/10.1002/ctm2.319>
- Brust R, Shang J, Fuhrmann J, et al. A structural mechanism for directing corepressor-selective inverse agonism of PPAR γ . *Nat Commun*. 2018;9:4687. <https://doi.org/10.1038/s41467-018-07133-w>
- Garcia-Gras E, Lombardi R, Giocondo MJ, et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. *J Clin Invest*. 2006;116:2012-2021. <https://doi.org/10.1172/JCI27751>
- Kim C, Wong J, Wen J, et al. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature*. 2013;494:105-110. <https://doi.org/10.1038/nature11799>
- Thireau J, Pasquie J-L, Martel E, et al. New drugs vs. old concepts: a fresh look at antiarrhythmics. *Pharmacol Ther*.

- 2011;132:125-145. <https://doi.org/10.1016/j.pharmthera.2011.03.003>
8. Son N-H, Park T-S, Yamashita H, et al. Cardiomyocyte expression of PPARgamma leads to cardiac dysfunction in mice. *J Clin Invest.* 2007;117:2791-2801. <https://doi.org/10.1172/JCI30335>
9. Morrow JP, Katchman A, Son N-H, et al. Mice with cardiac overexpression of peroxisome proliferator-activated receptor γ have impaired repolarization and spontaneous fatal ventricular arrhythmias. *Circulation.* 2011;124:2812-2821. <https://doi.org/10.1161/CIRCULATIONAHA.111.056309>
10. Xie Y, Gu Z-J, Wu M-X, et al. Disruption of calcium homeostasis by cardiac-specific over-expression of PPAR- γ in mice: a role in ventricular arrhythmia. *Life Sci.* 2016;167:12-21. <https://doi.org/10.1016/j.lfs.2016.10.014>