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LETTER TO EDITOR

The PPARγ 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 PPARγ, 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 PPARγ 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 PPARγ 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 PPARγ in the genes switch, we challenged DSC2-hiPSC-CMs with T0070907 (T007), which functions as a transcriptionally corepressor-selective PPARγ inverse agonist.[4] Incubation with T007 (1 μM), for 40 days (D20–D60) during the maturation phase, corrected the expression levels of PPARγ, perilipin, adiponectin and MLC2v (Figure 1A–D). These results corroborated the PPARγ-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 PPARγ pathway inhibition counteracts the pathogenesis process. Interestingly, the PPARγ 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 PPARγ agonists on basal transcription.[4] A significant PPARγ 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)

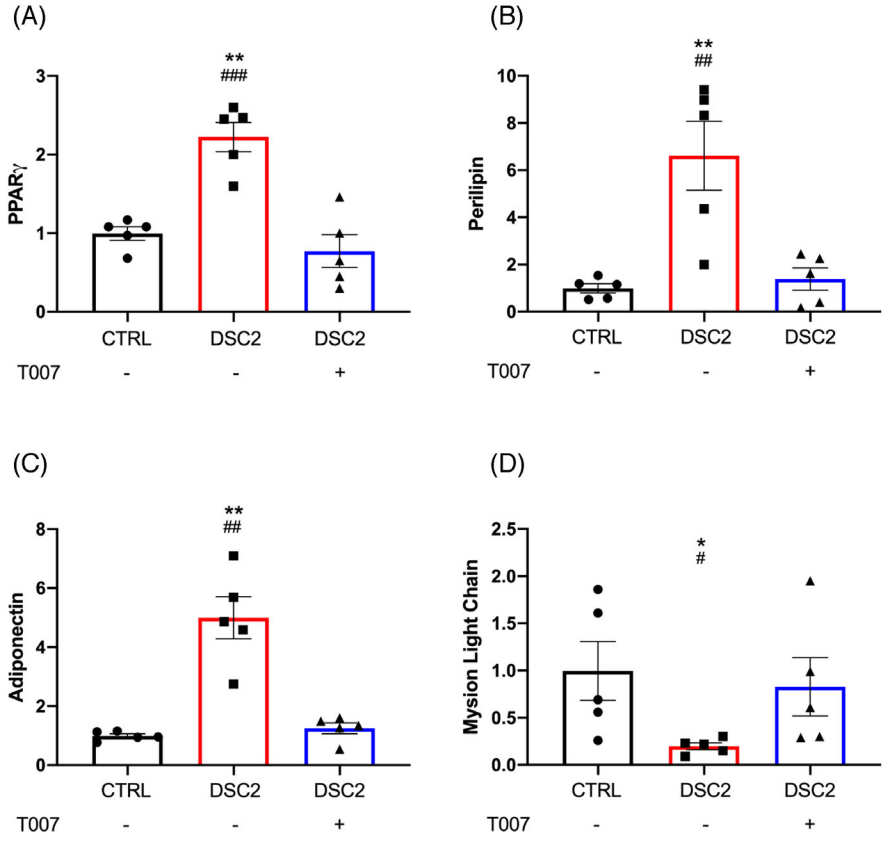

FIGURE 1 T0070907 modulates PPARγ pathway and prevents the cardiomyocyte phenotype in DSC2 patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM). Fold change compared to control for (A) PPARγ, (B) perilipin, (C) adiponectin and (D) myosin light chain (MLC) expression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

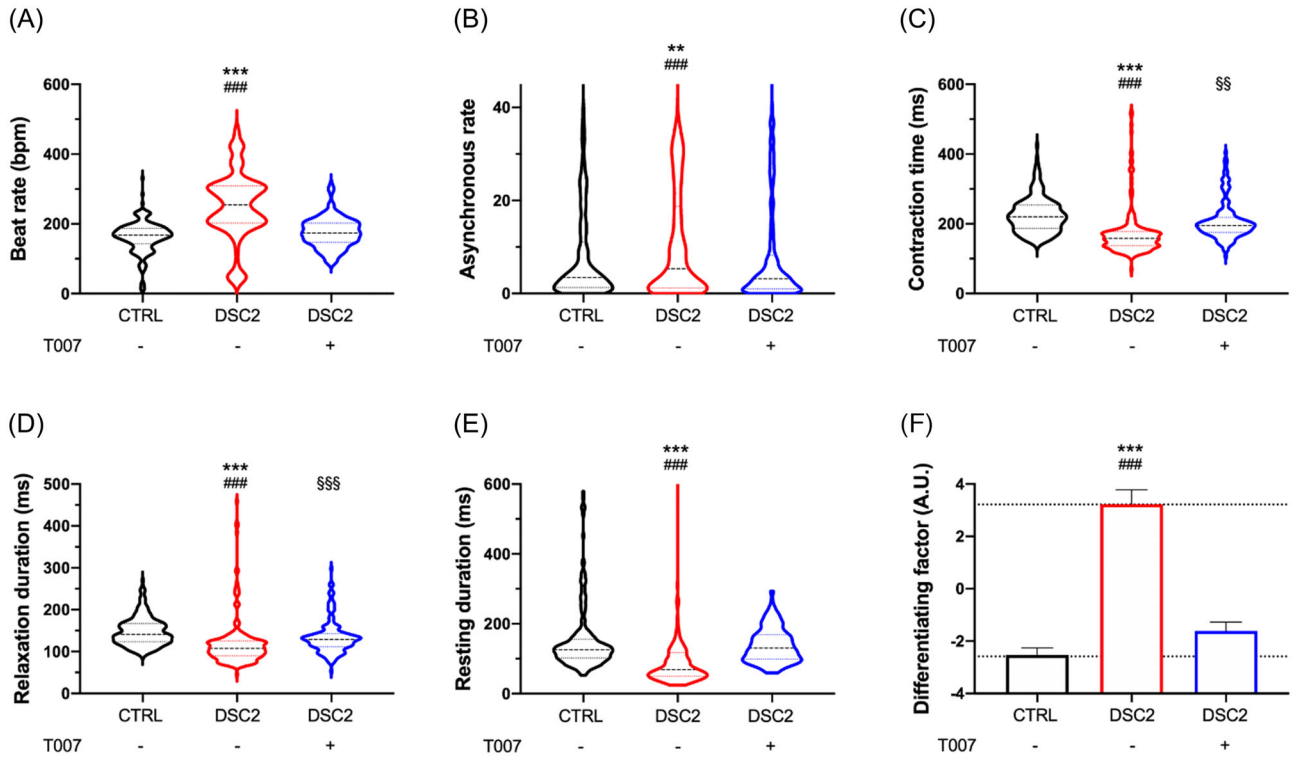

FIGURE 2 T0070907 prevents contractile disturbances in DSC2 patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs). The contractile activity was studied by video analysis in monolayer condition (control, $N = 249$ (black); patient, $N = 212$ (red); T0070907, $N = 197$ (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, §control versus T007; $^{**,\S\S}p < .01$, $^{***,\#\#\#,\S\S\S}p < .001$ (Kruskal–Wallis test)

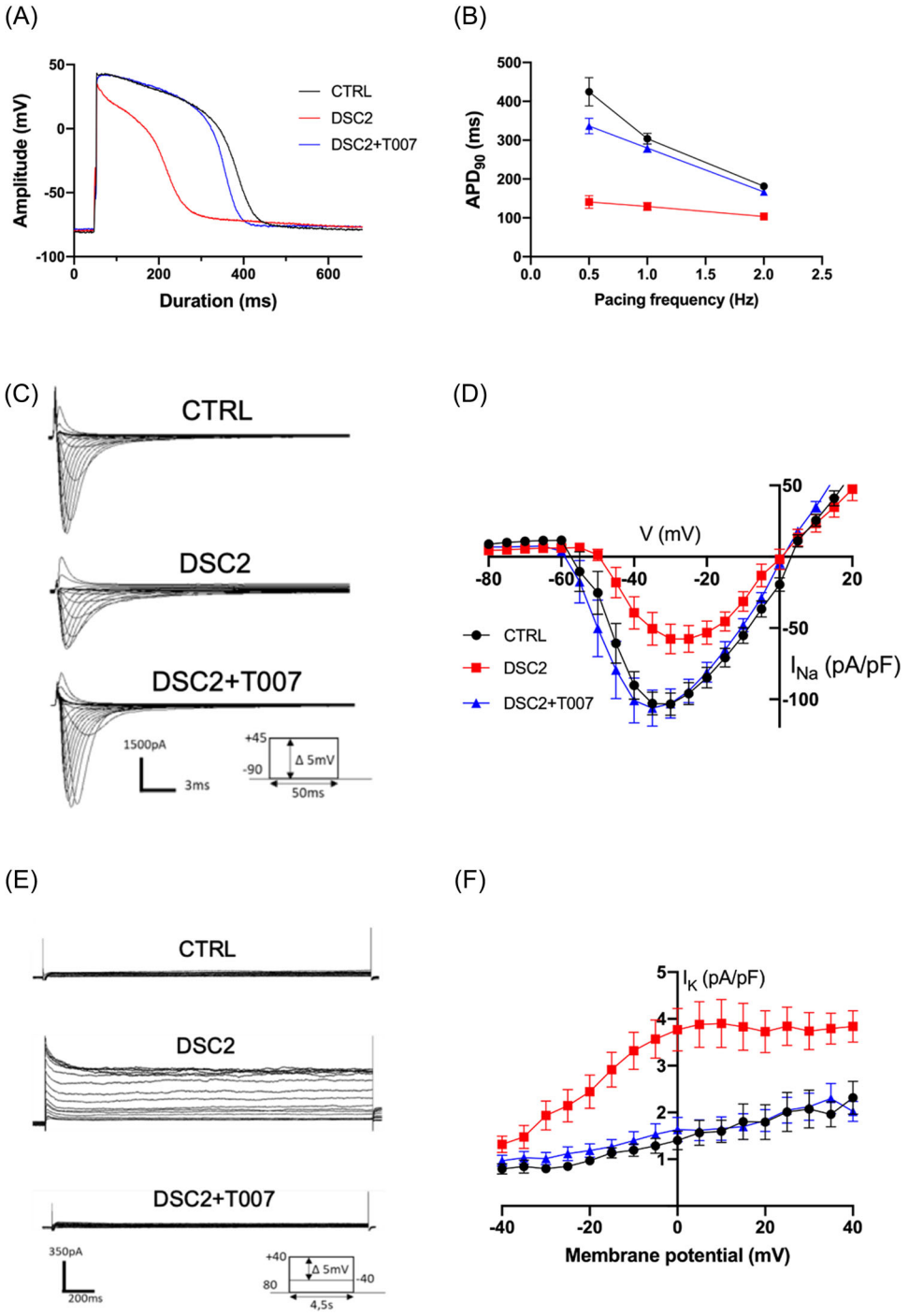

FIGURE 3 Electrical activity of control and patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) with or without T0070907. (A) Action potentials (APs) of hiPSC-CM from control (black), DSC2 patient in absence of T0070907 (red) and after 40 days of incubation with 1 μM 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 (Tuckey multiple comparison test)

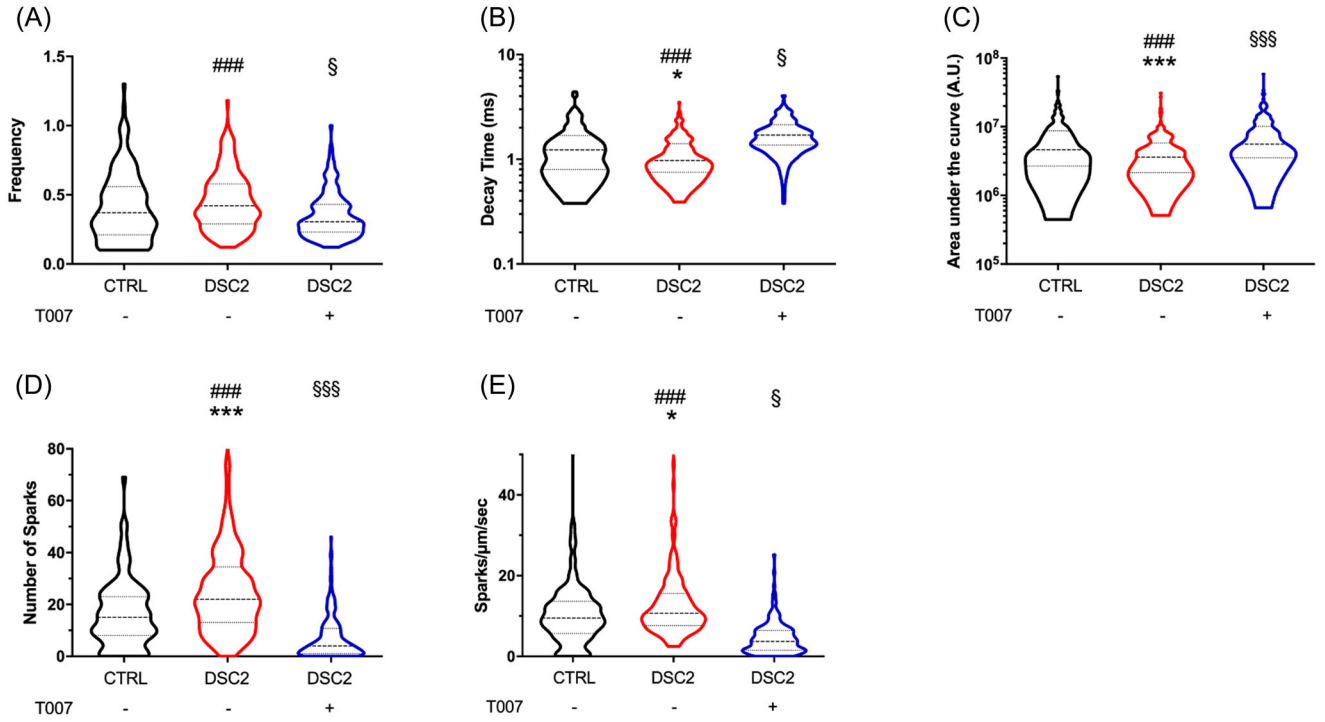

FIGURE 4 Spontaneous Ca^{2+} handlings of control, patient-specific human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) and incubated with T0070907. The Ca^{2+} transient activity (control, $N = 207$ (black); patient, $N = 222$ (red); T0070907, $N = 197$ (blue)) were studied. Spontaneous 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; $^{*,\S}p < .05$, $^{***,\#\#\#,\S\S\S}p < .001$ (Tuckey 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-arrhythmogenic 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 PPARγ 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-arrhythmogenic 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-arrhythmogenic risk. Our results align with different studies in transgenic mice linking PPARγ 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 PPARγ in genetic ACM. Repression of PPARγ transcription may offer a unique opportunity for ACM treatment.

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CONFLICT OF INTERESTS

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