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Original Paper

Impact of the DSP-H1684R Genetic Variant on Ion Channels Activity in iPSC-Derived Cardiomyocytes

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Key Words

Arrhythmias • Cardiomyocytes • Desmoplakin • Induced pluripotent stem cells • Ion channels Progressive cardiac conduction disease

Abstract

Background/Aims: Mutations of desmosomal genes are known to cause arrhythmogenic cardiomyopathy characterized by arrhythmias and sudden cardiac death. Previously, we described a novel genetic variant H1684R in desmoplakin gene (DSP), associated with a progressive cardiac conduction disease (PCCD). In the present study, we aimed to investigate an effect of the DSP-H1684R genetic variant on the activity of ion channels. Methods: We used cardiomyocytes derived from induced pluripotent stem cells (iPSC cardiomyocytes) from a patient with DSP-H1684R genetic variant and from two healthy donors. Immunofluorescent staining and western blot analyses were used to characterize patient-specific cardiomyocytes. By the whole-cell voltage-clamp technique we estimated the activity of voltage-gated sodium, calcium, and potassium channels that are responsible for action potential generation and its shape. Action potentials' parameters were measured using whole-cell current-clamp technique. Results: In patient-specific cardiomyocytes we observed both lower amplitudes of currents through sodium Na, 1.5 channels and L-type calcium channels, but higher amplitude of current through transient-outward potassium channels in comparison to donor cardiomyocytes. Current-clamp measurements revealed shortening of action-potential in DSP-H1684R-carrying iPSC cardiomyocytes. Therefore, observed alterations in the channels activity might have a great impact on the properties of action potential and development of PCCD.

K. Gusev and A. Khudiakov contributed equally to this work.

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Conclusion: Our results show that desmoplakin genetic variants, besides conduction slowing caused by structural heart remodeling, could affect multiple ion channel activity aggravating arrhythmia manifestation in PCCD.

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Introduction

Progressive cardiac conduction disease (PCCD) is a rare genetic disorder characterized by alterations in cardiac conduction through the His-Purkinje system and often resulting in complete atrial-ventricular block and widening of QRS complexes [1]. PCCD has a complex genetic background and clinically overlaps with several structural and electrical heart diseases [2]. Recently, we described a family carrying a new H1684R variant in the *DSP* gene encoding desmoplakin protein [3]. The *DSP*-H1684R genetic variant was associated with PCCD and a high risk of sudden death. Morphological examination of endomyocardial biopsy revealed a marked myocardial fibrosis, without any structural myocardial changes [3].

DSP is a large desmosomal protein linking the intermediate filaments network to plakoglobin and cadherins [4]. In cardiomyocytes, desmosomes are localized within intercalated discs (IDs) mediating the coupling of individual cells and the proper propagation of action potential (AP) [5, 6]. Desmosomes share many accessory proteins with other components of IDs – gap junctions, adherens junctions, and ion channels (for a review see Green et al. [7]). Mutations in components of desmosome were reported to affect the electrical activity of cardiomyocytes and lead to arrhythmia development [5, 6].

Mutations of desmosomal genes including *DSP* are well known to cause arrhythmogenic cardiomyopathy (ACM), a rare inherited heart disease characterized by fibrofatty replacement of cardiomyocytes, arrhythmias, and sudden cardiac death [6, 8, 9]. Arrhythmias and conduction disturbances arise early in the history of ACM often preceding structural changes in the heart. The link between desmosomal proteins and the electrical function of the heart is actively investigated. Particularly, mutations in plakophilin-2 (*PKP2*) gene or its downregulation were shown to affect sodium current through Na_v1.5 channel [10, 11]. A recent comprehensive study on human induced pluripotent stem cells-derived cardiomyocytes (iPSC cardiomyocytes) performed by El-Battrawy et al. showed a reduction in I_{Na} and modulation of potassium currents in ACM patient with G638A mutation in desmoglein-2 (*DSG2*) gene [12].

Hitherto, electrical activity of cells carrying *DSP* pathogenic variants has not been yet investigated despite many clinical phenotypes described in association with these variants [13–18]. Specific deletion of *DSP* in the murine cardiac conduction system led to the rapid development of lethal arrhythmias in the absence of cardiac dysfunction [19]. Zhang et al. showed that silencing the *DSP* gene in HL-1 cells decreased sodium currents [20]. However, in the murine model haploinsufficiency of *DSP* did not affect I_{Na} [21].

In the present study, we elucidated the impact of *DSP*-H1684R genetic variant on the activity of ion channels using patient-specific iPSC cardiomyocytes. Particularly, we studied the impact on sodium channel activity through $Na_v1.5$ channels, modulation of I_{Na} activation and inactivation. Moreover, we analysed the impact of *DSP*-H1684R on calcium and potassium currents testing the hypothesis that desmosome disturbance might lead to multiple channel dysfunction associated with arrhythmia and conduction disease development.

Materials and Methods

Generation of human-induced pluripotent stem cells

iPSC lines were generated and characterized as described previously [22]. Four lines were used in the study – two control lines from unrelated healthy donors (FAMRCi003-A and FAMRCi008-A) and two lines from the same PCCD patient carrying *DSP*-H1684R genetic variant (FAMRCi004-A and FAMRCi004-B). Experimental data from two patient-specific iPSC lines were analyzed together. Detailed information and char-

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acterization data could be found in hPSCreg database (https:// hpscreg.eu/). All iPSC lines were propagated in feeder-free conditions on Geltrex-coated plates in Essential 8 medium (Thermo Fisher Scientific) and passaged using ReleSR reagent (Stem Cell Technologies) or Tryple select (Thermo Fisher Scientific).

Cardiac differentiation

Cardiac differentiation of iPSCs was performed according to small molecules-mediated canonical Wnt pathway modulation protocol [23] with modifications. Briefly, differentiation was induced by switching Essential 8 medium to RPMI/ B27insmedium (Glutamax supplemented RPMI1640, B27 supplement without insulin, 100 U/ml penicillin, 100 µg/ ml streptomycin (Thermo Fisher Scientific)) containing 6 μM CHIR99021 (Selleckchem) for 48 h and then switching to RPMI/B27ins- medium containing 5 µM IWR1 (Stem Cell Technologies) for additional 48 h. On day 6 of differentiation medium was switched to RPMI/ B27 medium (Glutamax supplemented RPMI1640, B27 supplement, 100 U/ml penicillin, 100 µg/ml streptomycin (Thermo Fisher Scientific)). After day 14 of differentiation, metabolic selection of cardiomyocytes was performed as described previously [24]. For electrophysiological measurements, iPSC car-



Fig. 1. Characterization of iPSC cardiomyocytes generated from donor (D) and diseased patient's (PCCD) cells. A. A short scheme representing iPSC cardiomyocytes differentiation. B. Immunostaining of iPSC cardiomyocytes) for desmosome markers desmoplakin (DSP) and plakophilin-2 (PKP2); cytoskeleton marker troponin I type 3 (TNNI3); and DAPI-labeled nuclear DNA.C. Western blot showing expression of DSP and its summarized expression normalized to cardiac troponin T (TNNT2).

diomyocytes were dissociated using Tryple Select reagent (Thermo Fisher Scientific) and seeded as single cell suspension in RPMI/B27 medium. A short scheme representing iPSC cardiomyocytes differentiation is shown in Fig. 1A.

Immunocytochemistry

Cells were fixed with 4% PFA, permeabilized with 0.5% Triton X-100, blocked in 1% BSA and consequentially incubated with primary and secondary antibodies. Nuclei were stained with DAPI (Sigma). Used antibodies are listed in Supplementary Table S1 (for all supplementary material see www.cellphysiolbiochem.com). Images were captured using Zeiss AxioObserver Z1 microscope with Axiocam 506 mono camera. Zen software (Zeiss) was used for images processing.

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Western blot

Cells were lysed in a buffer containing 10 mM Tris (pH 8), 140 mM NaCl, 0.1% SDS, 1% Triton X-100, 0.5 mM EGTA, 1 mM EDTA, and protease inhibitors cocktail (Roche). Sample were boiled in the Laemmli sample buffer at 95 °C for 5 min. Proteins were resolved in SDS-PAGE and transferred to nitrocellulose membrane. Membrane was blocked with 5% non-fat milk and incubated with protein-specific primary antibody and then HRP-conjugated secondary antibody. Used antibodies are listed in Supplementary Table S1.

Electrophysiological measurements

 I_{Na} measurements. Sodium currents (I_{Na}) were recorded using whole-cell patch-clamp. Microelectrodes were made from borosilicate glass using a puller (P-1000, Sutter Instrument). The electrode resistance was 1.5-2.5 MOhm. The series resistance was compensated by 85-90%. Data acquisition was performed using Axopatch 200B amplifier and Clampfit software version 10.3 (Molecular devices). Currents were acquired at 20 kHz and low-pass filtered at 5 kHz using an analog-to-digital interface (Digidata 1440A acquisition system, Molecular devices).

The extracellular solution for recording I_{Na} contained (mM): 140 NaCl, 1 MgCl2, 1.8 CaCl2, 10 HEPES, 10 Glucose (pH 7.4 CsOH). The intracellular solution contained (mM): 130 CsCl, 10 NaCl, 10 EGTA, 10 HEPES (pH 7.3 CsOH). During I_{Na} measurements, currents through L-type Ca²⁺ channels were blocked by 10 μ M nifedipine.

 $I_{v_{a}}$ amplitudes and properties were measured as previously reported [25]. Briefly, to measure peak $I_{_{N_2}}$, cells were hold at potential of -100 mV. Current-voltage (IV) relationship was assessed by depolarizing voltage steps from -80 to 60 mV during 40 ms at 1 Hz frequency. I_{Na} conductance (G) was calculated by the equation: $G = I_{Na}/(V-V_{rev})$, where V is the voltage test, V_{rev} is the reversal potential. Steady-state inactivation was tested by measuring the I_{Na} elicited by a 20 ms step to -15 mV after a conditioning pulse of 500 ms ranging from -120 mV to 0 mV in 5 mV step increments. Steady-state activation and inactivation curves were obtained as normalized G versus applied test voltage step. Steady-state activation and inactivation properties were obtained from individual corresponding curves fitted with the Boltzmann function: $G/G_{max} = 1/(1 + exp((V_{1/2} - V)/k))$, where G_{max} is the maximal sodium conductance, and $V_{1/2}$ is the voltage at which half of the channels are activated and k is the slope factor. The time course of recovery from inactivation was measured using double-pulse protocol of 500 ms conditioning pulse at -15 mV followed by a recovering hyperpolarization gap at -100 mV for a various duration from 1 ms to 3000 ms and 20 ms test pulse at -15 mV. I_{Na} elicited from the test pulse was normalized to that one from the first conditioning pulse and was plotted versus the recovery time. Curves were fitted with the double-exponential function: $y = A_{fast} x (1-exp(-t/t_{fast})) + A_{slow} x (1-exp(-t/t_{slow}))$, where A_{fast} and A_{slow} are the fractions of fast and slow inactivating components, respectively, and t_{fast} and t_{slow} are their time constants.

 $I_{Cal'}$ $I_{Kto'}$ AP measurements. Electrophysiological measurements of currents through L-type Ca²⁺ channels (I_{Cal}), transient-outward potassium channels (I_{Kto}), action potential (AP) were performed with an EPC10 USB patch clamp amplifier (HEKA). Data were recorded by PatchMaster acquisition software (HEKA). The micropipette resistance was 2–5 M Ω . I_{Cal} and I_{Kto} were measured by voltage-clamp mode in whole-cell configuration.

Bath solution contained (in mM): 140 NaCl, 5.4 KCl, 1 MgCl₂, 0.5 Na₂HPO₄, 1 CaCl₂, 10 Glucose, 10 HEPES-NaOH, pH 7.4. Pipette solution for I_{Cal} , I_{Kto} and AP measurements (in mM): 100 KAsp, 20 KCl, 5 Na₂ATP, 5.84 MgCl₂, 2.19 CaCl₂, 10 EGTA, 10 HEPES-KOH. Estimated concentration free Mg²⁺ 1mM, free Ca²⁺ 50 nM (according to WEBMAXC extended from 7/3/2009).

To measure $I_{cal'}$ cells were hold at potential of -80 mV. IV relationship was assessed by depolarization steps from -60 to +50 mV for 250 ms after 500 ms depolarization step to -40 mV to inactivate I_{Na} . Depolarizing steps were applied at 0.5 Hz frequency. The background current, calculated as steady-state current at potentials \leq -40mV and approximated with a linear fit to other potentials, was subtracted prior I_{Cal} peak estimation.

 I_{kto} was measured similarly to the previously described protocol [26]. The cells for I_{kto} evaluation were hold at -80 mV and the depolarization pulse of +60 mV and the duration of 500 ms was applied at 0.25 Hz frequency. The current at +60 mV for 500 ms after preliminary depolarization to -20 mV for 320 ms was used to subtract other background currents. To measure how I_{kto} amplitude depends on the duration of preliminary depolarization to -20 mV, the duration of this depolarizing step was varied from 5 to 320 ms.

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The recorded currents were normalized to the cell capacitance obtained during automatic compensation of cell capacitance and series resistance by HEKA amplifier.

To measure APs, recordings were performed using current-clamp mode in whole-cell configuration. A holding current was fixed in the way to hold cell at the potential around -70 mV. To trigger APs, an additional current of 3 ms duration was injected at 1 Hz frequency. A set of 10 APs was recorded, and 5th-10th APs were used to obtain average AP and to analyse its parameters.

All measurements were performed at room temperature in a blinded manner.

Data analysis

Data were analysed using IgorPro software (v.8.04, Wavemetrics). Statistical comparisons were made either using one-way ANOVA with Bonferroni correction (for normal distributions with homogenous variances) or with Kruskal-Wallis H-test with pairwise Mann-Whitney analysis. Discrete values were compared by Fisher t-test. Pooled data are given as mean \pm SEM, with n representing the number of cells tested. Values of p < 0.05 were considered significant and labeled as (*), p < 0.01 and p < 0.001 were labeled as (**) and (***), correspondingly.

Results

Characterization of cardiomyocytes carrying DSP-H1684R genetic variant

Patient-specific iPSC were obtained from a patient with a familiar PCCD associated with *DSP*-H1684R genetic variant absent in ExAc0.2 and gnomAD databases (https://exac.broadinstitute.org, https://gnomad.broadinstitute.org). According to the 2018 American College of Medical Genetics and Genomics (ACMG) guidelines, this variant was classified as likely pathogenic.

Patient-specific iPSC cardiomyocytes were obtained by differentiation of iPSC using the Wnt/b-catenin signaling pathway modulation protocol with subsequent metabolic purification. iPSC cardiomyocytes used in this study were differentiated for 24 days. Human iPSC cardiomyocytes were immunostained for the cardiac sarcomeric protein TNNI3 (cardiac troponin I) and desmosomal markers – DSP and PKP2. The distribution of DSP and PKP2 proteins did not differ between control iPSC cardiomyocytes and PCCD iPSC cardiomyocytes (Fig. 1B). Western blot analysis also did not reveal any difference in the expression of DSP between iPSC cardiomyocytes from diseased and healthy individuals (Fig. 1C). In summary, we found no morphological differences between cardiomyocytes from diseased and healthy individuals.

Measurements of $I_{Na'}$, I_{CaL} and I_{Kto}

Differentiated iPSC cardiomyocytes from the patient with *DSP*-H1684R genetic variant were compared to cells derived from 2 healthy donors. In our electrophysiological experiments, the average cell capacitance for the iPSC cardiomyocytes did not differ significantly between groups and varied in the range of 15-17 pF (PCCD: 17±1 pF, n=54, p=0.335; D1:17±2 pF, n=20; D2: 15±1pF, n=25).

To reveal if the conduction slowing in the patient with *DSP*-H1684R genetic variant is attributed to I_{Na} , we studied ion channel activity in iPSC cardiomyocytes. Measurements of sodium current through Na_v1.5 channel in human iPSC cardiomyocytes revealed a decrease in I_{Na} density in iPSC cardiomyocytes carrying *DSP*-H1684R genetic variant as compared to healthy donors (Fig. 2A, B). The peak amplitude of I_{Na} decreased from -420±54 pA/pF (n=7, D1), -471±40 pA/pF (n=13, D2) in donor cells to -226±28 pA/pF in PCCD cells (n=12, PCCD). The shape of IV-relationship did not change.

Decrease in I_{Na} amplitude could be determined not only by expression level of $Na_v 1.5$, but also by the modulation of the channel properties. Moreover, knock-out in another desmosome gene *PKP2* was previously shown to influence I_{Na} electrophysiologycal properties [10, 11]. To check if the decrease in I_{Na} amplitude was due to the changes in biophysical properties of the channel, we measured the activation, steady-state inactivation, and recovery from

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Fig. 2. Effect of DSP-H1684R genetic variant on the amplitude and properties of I_{N_2} . A. Representative traces of I_{Na} in donor iPSC cardiomyocytes (D1, black; D2, grey) and in PCCD cardiomyocytes (red). B. Current-voltage curves depicting the peak amplitude of I_{Na} at the indicated voltages. C. Bar plot of maximal I_{Na} amplitude obtained during measurements of current-voltage relationship. D. Properties of sodium currents - activation, steady-state inactivation and recovery from inactivation. Detailed values for



the parameters of I_{Na} properties are shown in Table 1. Asterisk reflects significance (p<0.05), for details see Materials and Methods.

inactivation in cells with *DSP*-H1684R variant. None of these parameters was changed compared to control cells (Fig. 2C; Table 1).

In ventricular myocytes, generation and propagation of AP through the heart is defined mostly by I_{Na} while currents through L-type Ca²⁺ channels (I_{CaL}) lead to Ca²⁺ entry and contraction of the ventricular cardiomyocytes. However, in pacemaker cells I_{CaL} plays an important role in the triggering of AP [27]. Our measurements revealed that I_{CaL} was also decreased in iPSC cardiomyocytes with *DSP*-H1684R genetic variant (Fig. 3A, B). The peak amplitude decreased from -12±1.5 pA/pF (n=20, D1), -15.9 ± 1.7 pA/pF (n=25, D2) in donor cells to -6.7 ± 0.7 pA/ pF in patient cells (n=54, PCCD) (Fig. 3C).

After cardiomyocyte is depolarized, it takes time for plasma membrane poten-

Table 1. Properties of I_{Na} , measured in donor and PCCD iPSC cardiomyocytes

	D1	D2	PCCD		
Steady-state activation					
$V_{1/2}$, mV	-35.2 ± 1.2	-32.9 ± 1.0	-31.5 ± 1.0		
k, mV	6.6 ± 0.4	5.5 ± 0.3	5.8 ± 0.2		
n	13	11	12		
Steady-state	e inactivation				
V _{1/2} , mV	-70.3 ± 1.0	-71.5 ± 2.4	-67.6 ± 1.5		
k, mV	8.1 ± 0.3	8.5 ± 0.3	8.1 ± 0.3		
n	13	11	12		
Recovery fr	om inactivation				
τ_{fast} , ms	31.7 ± 11.3	32.5 ± 8.6	28.4 ± 8.7		
A _{fast}	0.75 ± 0.06	0.75 ± 0.06	0.83 ± 0.05		
τ_{slow} , ms	436 ± 34	242 ± 38	317 ± 72		
A _{slow}	0.30 ± 0.05	0.29 ± 0.04	0.23 ± 0.04		
n	10	6	9		

tial to recover. There are several potassium currents participating in this process [26, 27]. The first recovery phase, phase 1 of the cardiac action-potential, is determined by transient outward potassium current (I_{Kto}). We measured I_{Kto} as an outward current at +60 mV which is inactivated by pre-step depolarization to -20 mV within 100-300 msec. These measurements allowed us to observe the increase in I_{Kto} (Fig. 3D, E). I_{Kto} increased from 2.2 ± 0.2 pA/ pF (n=20, D1), 1.9 ± 0.4 (n=24, D2) in donor iPSC cardiomyocytes to 4.4 ± 0.8 pA/pF (n=49, PCCD) in PCCD cells (Fig. 3F). Corresponding time of I_{Kto} inactivation was unchanged (D1: 75 ± 19.8 ms, n=18; D2: 73.7 ± 14.2 ms, n=19; PCCD: 70.8 ± 8.7 ms, n=42; Fig. 3G).

Altogether, we observed multiple disturbances in the activity of different ion channels in iPSC cardiomyocytes carrying *DSP*-H1684R genetic variant.



Fig. 3. Effect of DSP-H1684R genetic variant on the amplitude I_{CaL} and I_{Kto}. A. Representative traces of I_{CaL} in donor iPSC cardiomyocytes (D1, black; D2, grey) and in PCCD cardiomyocytes (red). B. Current-voltage curves depicting the peak amplitude of I_{cal} at the indicated voltages. C. Bar plot of maximal I_{CaL} amplitude obtained during measurements of current-voltage relationship. D. Representative traces of I_{Kto} in donor



iPSC cardiomyocytes and in PCCD iPSC cardiomyocytes. E. Inactivation curves depicting the dependence of the peak amplitude of I_{Kto} measured at +60 mV on the duration of depolarizing prepulse to -20 mV. F. Maximal I_{Kto} amplitude reflecting amplitude of outward current at +60 mV without depolarization minus amplitude of outward current at +60 mV with 320 ms depolarization prepulse to -20 mV. G. Time of exponential inactivation of I_{Kto} amplitude as dependence from the duration of depolarizing prepulse to -20 mV. Asterisk reflects significance (p<0.05), for details see Materials and Methods.

Fig. 4. Effect of DSP-H1684R genetic variant on the action-potentials (APs). A. Representative action-potentials in donor iPSC cardiomyocytes (D1, black; D2, grey) and in PCCD cardiomyocytes (red) superimposed for the direct comparison.



B. Averaged parameters of action-potential characteristics – probability for triggering AP, which reflects proportion of cells where AP was triggered; amplitude of AP; maximal velocity of AP; action-potential duration measured at 50% of its amplitude. Asterisk reflects significance (p<0.05), for details see Materials and Methods.

Action potential measurements

To analyse, how the changes in the sodium, calcium, and potassium currents affect the AP in iPSC cardiomyocytes carrying *DSP*-H1684R variant, we applied a holding current in order to fix membrane potential around -70 mV close to the holding potential of adult cardiomyocytes. This fixation was performed since iPSC cardiomyocytes being not completely mature cells exhibit a decreased holding potential, that prevents AP triggering [28]. The APs were initiated by applying injecting current for 3 ms with 1 Hz frequency. As shown on the Fig. 4, *DSP*-H1684R genetic variant demonstrated a suppressive effect on the AP. However, we did not succeed in triggering the AP in all cells. AP triggering probability was 83% (13/14 cells) and 100% (24/24 cells) in D1 and D2 groups, respectively. Meanwhile, in PCCD cells AP triggering probability was 73% (33/45 cells), significantly smaller than in donor cells. Moreover, the amplitude of AP was lowered in PCCD cells (D1: 102.1 ± 5.6 mV; D2: 102.8 ± 1.6 mV; PCCD: 94.4 ± 3 mV, Fig. 4). Despite the reduction of I_{Na} the maximal upstroke veloc-

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ity (Vmax) of AP did not differ significantly (D1: $35.3 \pm 10.9 \text{ mV/ms}$; D2: $29.3 \pm 5.6 \text{ mV/ms}$; PCCD: $21 \pm 4.3 \text{ mV/ms}$). However, the duration of APs at 50% of maximal amplitude was reduced in cells with *DSP*-H1684R genetic variant (D1: $486 \pm 99 \text{ ms}$; D2 287 ± 21 ms; PCCD: $232 \pm 22 \text{ ms}$). Thus, down-regulation of I_{Na} and I_{CaL} together with up-regulation of I_{Kto} resulted in a pronounced AP modulation.

Discussion

Pathogenic variants in desmosomal genes are predominant causes of arrhythmogenic cardiomyopathy development. However, *DSP* variants account only for 2-12% of cases, probably due to the fact that *DSP* mutations lead to the more penetrant phenotype associated with sudden cardiac death [13, 14, 17]. Recently, pathogenic variants in the *DSP* gene were also linked to dilated cardiomyopathy, left ventricular non-compaction cardiomyopathy, and cardiac conduction disorders, thus indicating the complexity of molecular mechanisms involving the *DSP* [3, 18, 29, 30]. All above-mentioned disorders are prone to have early manifestations with life-threatening arrhythmias in the absence of the structural heart defects. Moreover, analysis of the murine model with *DSP* haploinsufficiency and *DSP* mutation carriers demonstrated that electrophysiological manifestation of disease could precede histological changes [21].

Recently, we described the *DSP*-H1684R genetic variant associated with a progressive cardiac conductance disease and a high risk of sudden death [3]. We suggested that this variant might result in desmosome remodeling and the abnormal electrical activity of cardiomyocytes. To elucidate the consequences of the *DSP*-H1684R, we generated iPSC-derived cardiomyocytes from a patient [22]. As a control cells, we used iPSC cardiomyocytes from two different healthy donors. Our results showed that distribution of desmoplakin, plakophilin-2, and troponin I in *DSP*-H1684R cells did not differ from the control cells (Fig. 1B). However, the electrical activity of patient iPSC-cardiomyocytes was altered compared to donor (Fig. 4). Moreover, several currents defining generation of AP and its shape were affected (Fig. 2, 3). This was in agreement with previous studies of the *DSG2* gene mutation, resulted in ACM development [12].

The *DSP*-H1684R genetic variant reduced the amplitude of $Na_v 1.5$ current (Fig. 2A-C). Similar results were previously reported for other desmosomal genes [10, 11, 12, 20]. $Na_v 1.5$ current reduction was not related to the changes in I_{Na} activation, steady-state inactivation or recovery from inactivation (Fig. 2D, Table 1), thus suggesting the lower abundance of the $Na_v 1.5$ channel on the plasma membrane of iPSC cardiomyocytes. This observation was in accordance with the reduction of $Na_v 1.5$ trafficking to the intercalated disc area associated with *PKP2* deficiency reported earlier [10].

Interestingly, L-type calcium current was also significantly decreased in our *DSP* model (Fig. 3), indicating the abnormal calcium signaling in diseased cardiomyocytes. caused already on the level of Ca^{2+} entry. While we buffered free cytosolic Ca^{2+} in our conditions with a Ca^{2+} buffer (10 mM EGTA), obtained results are not related to the Ca^{2+} dependent modulation of L-type calcium channels, such as Ca^{2+} inactivation [27].

Surprisingly, in parallel with I_{CaL} decrease, we observed an increase in $I_{Kto'}$, that was an opposite to the effect found in the *DSG2* mutation [12]. It could indicate the fact that disturbance of desmosomes resulting in similar phenotype and similar reduction of $I_{Na'}$, could affect other ion channels in a different way.

The reduction of sodium and calcium ion currents could have an impact on the AP triggering, especially in cardiac pacemaker cells, where both I_{Na} and I_{CaL} are involved in phase 0 of AP [27]. In fact, having the abnormalities in I_{Na} , I_{CaL} and I_{Kto} , iPSC cardiomyocytes derived from PCCD patient also demonstrated disturbed APs. We observed that AP amplitude and its triggering were suppressed in *DSP*-H1684R cells, and it was also accompanied by the decline in AP duration (Fig. 4).

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Our study has a number of limitations that should be mentioned. IPSC cardiomyocytes represent an immature cardiac phenotype which is different from that of mature cardiomyocytes. We used two iPSC lines as controls derived from two unrelated healthy donors without any history of cardiovascular disorders and no familial history of cardiac disease. However, this approach cannot completely exclude the presence of any silent pathological mutations in control iPSC lines, so the use of isogenic gene edited iPSC lines is considered to be a best practice to demonstrate that observed effects are mediated by patient-specific mutations.

Conclusion

Altogether, our results demonstrate that *DSP* disease-causing variants could have an impact on the multiple ion currents in the cardiomyocytes. These currents overall suppress AP generation and duration which could result in a progressive cardiac conductance disease, development of arrhythmogenic cardiomyopathy, and lead to the sudden cardiac death.

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Author Contributions

Gusev, Khudiakov, Kaznacheyeva, and Kostareva designed the experiments and edited the manuscript. Gusev, Zaytseva, and Makeenok performed and analysed electrophysiological data. Khudiakov, and Perepelina performed and analysed Western blot, immunostaining experiments. Khudiakov, Perepelina and Kostareva generated and characterized iPSC cardiomyocytes used in the study. All authors contributed to the data collections and manuscript writing. All authors read and approved the final manuscript. Gusev and Khudiakov contributed equally to this work.

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Statement of Ethics

The study was performed in accordance with the ethical standards of the Almazov National Medical Research Centre ethical committee (approval Nº13/19.06.2014) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

Disclosure Statement

The authors declare no competing interests.

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