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REPROGRAMMING EXPERIENCE OF BLOOD MONONUCLEAR CELLS INTO INDUCED PLURIPOTENT STEM CELLS (IPSCS) IN A PATIENT WITH CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA (CPVT)

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Abstract

Introduction. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a severe inherited arrhythmia linked to RYR2 mutations. CPVT is a major cause of sudden cardiac death in the young. Here we outline the experience of induced pluripotent stem cells (iPSCs) generation from a healthy donor and a patient with CPVT. By establishing iPSCs from affected individuals, this study can reveal mechanisms of disease pathophysiology and the potential of iPSC technology to develop cardiogenetic research and personalized medicine.

Aim. To investigate experimental methods used to generate and validate iPSCs derived from a healthy donor with wild-type RYR2 and a patient with RYR2 mutation.

Materials and methods. A 33-year-old female patient with CPVT was genetically screened using Sanger sequencing, revealing a de novo RYR2 mutation (c.13892A>T; p.D4631V). Peripheral blood mononuclear cells of a healthy donor and a patient were isolated by density gradient centrifugation and reprogrammed into iPSCs using the Sendai virus method. Cells were cultured under feeder-free conditions in Essential 8™ Flex medium. Pluripotency was confirmed via several methods, such as immunocytochemistry and G-banding karyotyping.

Results. The isolated cells showed optimal morphology, with cell colonies displaying smooth, well-defined edges and a rounded shape. The cells within the colonies were densely packed. Immunostaining confirmed the expression of the pluripotency marker TRA-1-60. Karyotype analysis revealed that all derived cell lines maintained stable chromosomal integrity.

Conclusions. iPSCs were successfully generated from a healthy donor to serve as a "control" for comparison with patient-derived iPSCs carrying a de novo heterozygous RYR2 mutation (c.13892A>T; p.D4631V). This iPSC-based model offers a valuable platform for investigating CPVT pathophysiology and testing potential therapeutic approaches.

Keywords: *iPSCs, CPVT, cardiac arrhythmia, mutation, disease modeling, personalized medicine*.

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Резюме

ОПЫТ РЕПРОГРАММИРОВАНИЯ МОНОНУКЛЕАРНЫХ КЛЕТОК КРОВИ В ИНДУЦИРОВАННЫЕ ПЛЮРИПОТЕНТНЫЕ СТВОЛОВЫЕ КЛЕТКИ (ИПСК) У ПАЦИЕНТА С КАТЕХОЛАМИНЕРГИЧЕСКОЙ ПОЛИМОРФНОЙ ЖЕЛУДОЧКОВОЙ ТАХИКАРДИЕЙ (КПЖТ)

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Введение. Катехоламинергическая полиморфная желудочковая тахикардия (КПЖТ) - это тяжелая наследственная аритмия, связанная с мутациями RYR2. КПЖТ — одна из основных причин внезапной сердечной смерти у молодых людей. Здесь мы описываем опыт генерации индуцированных плюрипотентных стволовых клеток (иПСК) от здорового донора и пациента с КПЖТ. Благодаря получению иПСК от пациентов это исследование может раскрыть механизмы патофизиологии заболевания и потенциал технологии иПСК для развития кардиогенетических исследований и персонализированной медицины.

Цель. Исследовать экспериментальные методы, используемые для создания и валидации иПСК, полученных от здорового донора с диким типом RYR2 и пациента с мутацией RYR2.

Материалы и методы. Пациентка 33 лет с КПЖТ прошла генетический скрининг с использованием секвенирования по Сэнгеру, выявив мутацию RYR2 de novo (c.13892A>T; p.D4631V). Мононуклеарные клетки периферической крови здорового донора и пациента были выделены центрифугированием в градиенте плотности и перепрограммированы в иПСК с использованием метода вируса Сендай. Клетки культивировались в условиях без фидера в среде Essential 8™ Flex. Плюрипотентность была подтверждена несколькими методами, такими как иммуноцитохимия и кариотипирование с G-бэндингом.

Результаты. Изолированные клетки продемонстрировали оптимальную морфологию, при этом клеточные колонии имели гладкие, четко очерченные края и округлую форму. Клетки внутри колоний были плотно упакованы. Иммуноокрашивание подтвердило экспрессию маркера плюрипотентности TRA-1-60. Анализ кариотипа показал, что все полученные линии клеток сохраняли стабильную хромосомную целостность.

Выводы. иПСК были успешно получены от здорового донора, чтобы служить «контролем» для сравнения с iPSC, полученными от пациента, несущими гетерозиготную мутацию RYR2 de novo (c.13892A>T; p.D4631V). Эта модель на основе иПСК предлагает ценную платформу для исследования патофизиологии КПЖТ и тестирования потенциальных терапевтических подходов.

Ключевые слова: *иПСК, КПЖТ, сердечная аритмия, мутация, моделирование заболеваний, персонализированная медицина.*

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Түйіндеме

КАТЕХОЛАМИНЕРГИЯЛЫҚ ПОЛИМОРФТЫ ҚАРЫНШАЛЫҚ ТАХИКАРДИЯ (КПҚТ) БАР НАУҚАСТЫҢ ҚАН МОНОНУКЛЕАРЛЫ ЖАСУШАЛАРЫН ИНДУКЦИЯЛАНҒАН ПЛЮРИПОТЕНТТІ ДІҢ ЖАСУШАЛАРЫНА (ИПДЖ) ҚАЙТА БАҒДАРЛАМАЛАУ ТӘЖІРИБЕСІ

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Кіріспе. Катехоламинергиялық полиморфты қарыншалық тахикардия (КПҚТ) RYR2 мутацияларымен байланысты ауыр тұқым қуалайтын аритмия болып табылады. КПҚТ жас ересектердегі кенеттен жүрек өлімінің негізгі себебі болып табылады. Мұнда біз сау донордан және КПҚТ бар науқастан индукцияланған плюрипотентті дің жасушаларын (иПДЖ) генерациялау тәжірибемізді баяндаймыз. Зардап шеккен адамдардан иПДЖ алу арқылы бұл зерттеу аурудың патомеханизмдері туралы түсінік бере алады және иПДЖ технологиясының кардиогенетикалық зерттеулер мен дербес медицинаны дамыту мүмкіндігін көрсетеді.

Мақсаты. Жабайы типті RYR2 бар сау донордан және RYR2 мутациясы бар науқастан алынған иПДЖ генерациялау және тексеру үшін қолданылатын эксперименттік әдістерді зерттеу.

Материалдар мен әдістер. КПҚТ-мен ауыратын 33 жастағы әйел науқаста Сэнгер секвенирлеу арқылы генетикалық скрининг нәтижесінде жаңа RYR2 мутациясы анықталды (c.13892A>T; p.D4631V). Дені сау донор мен пациенттен алынған перифериялық қан мононуклеарлы жасушалары градиенті центрифугалау арқылы бөліп алынып, Сендай вирусы әдісімен иПДЖ-ға қайта бағдарламаланды. Жасушалар Essential 8™ Flex ортасында фидерсіз жағдайда өсірілді. Плюрипотенттілігі иммуноцитохимия және G-бэнд кариотиптеу сияқты әдістермен расталды.

Нәтижелері. Жасушалар оңтайлы морфологияны көрсетті, жасуша колониялары тегіс, шеттері анық және дөңгелек пішінді. Колониялардағы жасушалар тығыз орналасқан. Иммуноцитохимия әдісі TRA-1-60 плюрипотентті маркердің экспрессиясын көрсетті. Кариотиптік талдау барлық жасуша линиялары тұрақты хромосомалық тұтастықты сақтайтынын көрсетті.

Қорытынды. иПДЖ-да жаңа гетерозиготалы RYR2 мутациясы (c.13892A>T; p.D4631V) бар пациенттен алынған иПДЖ-мен салыстыру үшін «бақылау» ретінде қолдану үшін сау донордан сәтті алынды. Бұл иПДЖ негізіндегі модель КПҚТ патофизиологиясын зерттеу және ықтималды емдік әдістерді сынау үшін құнды платформаны ұсынады.

Түйін сөздер: *иПДЖ, КПҚТ, жүрек аритмиясы, мутация, ауруды модельдеу, дербес медицина.*

Дәйексөз үшін:

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Introduction

Primary electrical heart disorders (PED), also referred as cardiac channelopathies, are a group of inherited genetic conditions that affect the electrical activity of cardiomyocytes. PED includes various diseases such as long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada syndrome, early repolarization syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), and idiopathic ventricular fibrillation [1]. One of the deleterious consequences of PED may be sudden cardiac death, especially in young individuals. According to the World Health Organization (WHO) sudden cardiac death is an unexpected death that occurs within the first 1 hour of symptom onset. The genetic basis of the disease includes specific rare mutations in genes encoding cardiac ion channels or their subunits. Mutations in ion channel genes include sodium, potassium, calcium channels, and mutations in regulatory proteins [2]. CPVT is an extremely severe type of inherited arrhythmia characterized by a typically normal electrocardiogram (ECG) and triggered by physical or emotional stress. The main arrhythmogenic mechanism consists of unusual calcium release from the sarcoplasmic reticulum (SR). In a healthy heart, calcium (Ca^{2+}) transients occur following the initiation of an action potential, as calcium influx through L-type calcium channels triggers calcium release from the SR. In contrast, in CPVT, mutations in the associated genes alter the function of the encoded proteins, resulting in spontaneous Ca^{2+} transients [3]. CPVT is marked by sudden cardiac arrest and accounts for nearly 15% of sudden cardiac deaths in young individuals. If left undiagnosed, it is highly fatal, with a mortality rate reaching up to 50% by the age of 35. The main disease-causing gene, which occurs in approximately 60-70% of patients with CPVT is the gene encoding ryanodine receptor 2 (RYR2). Most RYR2 mutations are missense variants that result in the accumulation of calcium and its sudden release into the cytoplasm. Such release triggers delayed afterdepolarizations, leading to ventricular arrhythmias. Genetic analysis revealed other mutations in genes like CASQ2, TRDN, TECRL, KCNJ1, CALM1, CALM2, CALM3, SCN5A, PKP2, and ANK2, but their prevalence is less than 5% [4]. In the frame of our previous research projects, we studied 35 patients, 33 of whom were diagnosed with idiopathic ventricular tachycardia by mutations in the RYR2 gene. We observed a novel heterozygous missense mutation in the RYR2 gene (c.13892A>T; p.D4631V; case #239) with high pathogenic potential (high in-silico, de-novo prediction scores, reference databases negative) in a patient with classic clinical features of CPVT. The de novo penetrance of this variant should be considered high due to the early age of onset, characteristic, and severe clinical course [5]. It is challenging to functionally prove the relationship of such deleterious mutations with the phenotypic manifestations of the disease. Only patient-specific models in inherited cardiac diseases may partially unveil the mechanism of disease formation and progression. Induced pluripotent stem cell (iPSC) technology may serve as a promising tool for mirroring a patient's genetic blueprint.

The development of induced pluripotent stem cells (iPSCs) by Shinya Yamanaka in 2006 marked a groundbreaking achievement, made possible by the collective contributions of past and present researchers in related scientific fields [6]. Human induced pluripotent stem cell (hiPSC) technology has advanced significantly since Yamanaka and colleagues first demonstrated that human fibroblasts could be reprogrammed by the forced expression of four transcription factors: octamer-binding protein 3/4 (OCT3/4; also known as POU5F1), SOX2, c-MYC (Myc proto-oncogene protein), and Krüppel-like factor 4 (KLF4). This pioneering method was subsequently validated by other research groups, either using the same set of factors or modified versions. Like human embryonic stem cells (hESCs), hiPSCs exhibit the key properties of self-renewal and pluripotency, enabling differentiation into derivatives of all three germ layers. Unlike hESCs, however, hiPSCs are generated without the use of human embryos, thereby avoiding many of the ethical controversies that have historically impeded hESC research. Additionally, because hiPSCs can be derived from individual patients, they retain unique genomic characteristics, making them valuable for modeling patient-specific disease phenotypes [7-10].

This article **aims** to summarize the experimental methodologies employed in the generation and characterization of iPSCs derived from a healthy donor and a patient exhibiting a CPVT phenotype. Within the scope of this article, we wanted to share our experience in the

implementation of this complex but promising technology in the field of cardiogenetics with the scientific community of Kazakhstan. We are pioneering the use of iPSC technology in Kazakhstan, with the expectation that it will contribute to the advancement of personalized medicine in the field of cardiovascular disorders.

Materials and methods

CPVT case and genetic analysis

The research was performed in accordance with the principles of the Declaration of Helsinki. The research protocol was approved at a meeting of the Local Ethics Committee of the Private Institution "National Laboratory Astana", protocol No. 05-2022 dated 10.21.2022. Informed written consent was obtained from the participant. The CPVT case included a 33-year-old female individual. The onset of symptoms occurred at the age of 13, characterized by recurrent episodes of syncope and the emergence of distinctive ECG patterns, including mono- and polymorphic ventricular premature beats, which progressed to bidirectional ventricular tachycardia and episodes of polymorphic ventricular tachycardia. We performed Sanger sequencing of hot-spot regions of the RYR2 gene for this patient. Our targeted genomic sequencing revealed de-novo heterozygous missense RYR2 mutation (c.13892A>T; p.D4631V) in a 33-year-old female patient with CPVT.

Isolation of peripheral blood mononuclear cells (PBMC)

PBMCs were isolated from whole blood using a density gradient centrifugation method with Histopaque-1077 (Sigma-Aldrich). Briefly, the blood was diluted with PBS and layered carefully over Histopaque-1077, followed by centrifugation at 400 × g for 30 minutes at room temperature without brake. The mononuclear cell layer was collected, washed twice with PBS, and centrifuged to remove platelets and residual plasma. The isolated PBMCs were counted, viability assessed, and the cells were either used immediately for reprogramming or cryopreserved in freezing medium containing 10% DMSO and FBS.

Reprogramming of PBMC

Peripheral blood mononuclear cells (PBMCs) were reprogrammed into induced pluripotent stem cells (iPSCs) using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Life Technologies). After isolation, PBMCs were cultured in StemPro™-34 medium (Life Technologies) supplemented with cytokines to promote cell expansion and viability. On day 4, cells were transduced with Sendai virus vectors encoding the reprogramming factors OCT4, SOX2, KLF4, and c-MYC at a multiplicity of infection (MOI) of 7:7:7, respectively. The transduction was performed in 24-well plates and centrifuged at 750 × g for 45 minutes. Following transduction, cells were maintained in the same medium for an additional 2 days. On day 6, transduced cells were transferred onto Matrigel-coated 6-well plates. Media were changed, and emerging iPSC colonies were manually picked between days 18–21 and cultured in Essential 8™ medium (Life Technologies) under feeder-free conditions. All procedures were performed under sterile conditions.

Immunocytochemistry (ICC)

For pluripotency marker analysis, fixed iPSC were stained using immunocytochemistry. The TRA-1-60 Alexa Fluor™ 488 antibody (Life Technologies) was diluted 1:50 and added to the cell culture medium. Then incubated with the diluted antibody solution at 37°C for 30 minutes in a humidified incubator with 5% CO_2. Following incubation, cells were gently washed three times with FluoroBrite DMEM to remove unbound antibody. Imaging was performed using a Celena X fluorescence microscope to confirm the expression of pluripotency markers.

Karyotyping

To check chromosomal stability, iPSCs were subjected to G-banding karyotyping. Cells were treated with colcemid to arrest them in metaphase, followed by hypotonic treatment with 0.075 M KCl. After fixation with methanol:acetic acid (3:1), cells were dropped onto chilled slides and air-dried. Slides were stained with Giemsa and analyzed under a microscope to determine karyotypic stability.

Results

Peripheral blood mononuclear cells (PBMCs) were successfully isolated using Histopaque-1077–based density gradient centrifugation method, which was selected as the optimized protocol due to its improved yield, clarity of cell layer separation, and processing efficiency. In this method, whole blood was diluted 1:1 with sterile PBS and gently layered onto Histopaque-1077, followed by centrifugation at 400 × g for 30 minutes at room temperature without brake. This approach resulted in a well-defined buffy coat layer containing PBMCs. Following two rounds of washing with PBS and centrifugation at 250 × g for 10 minutes, cells exhibited high viability, minimal erythrocyte contamination, and formed a clean pellet. The simplified layering technique and avoidance of mechanical disruption helped preserve cell morphology and integrity. Overall, the optimized method provided good results in terms of PBMC purity, yield, and ease of isolation, and was therefore selected for subsequent experiments. Peripheral blood mononuclear cells (PBMCs) were successfully reprogrammed into induced pluripotent stem cells (iPSCs) using Sendai virus vectors, which demonstrated efficiency, reproducibility, and cell viability. In this reprogramming method, freshly isolated PBMCs were cultured in a defined medium without prior cell-type selection, and transduced directly with Sendai virus vectors carrying the Yamanaka factors. Colonies with typical iPSC morphology emerged between days 18–21, and were manually picked for expansion. This method required minimal manipulation, allowing for a workflow with reduced handling time and lower contamination risk. Furthermore, the use of Essential 8 medium under feeder-free conditions supported robust colony growth and maintenance of the TRA-1-60 pluripotency marker. Overall, the modified protocol of reprogramming proved to be more efficient and practical, yielding a higher reprogramming success rate and healthier iPSC colonies suitable for downstream applications such as disease modeling and cardiomyocyte differentiation. Therefore, this method was selected as the optimized reprogramming approach in our study. As a result of reprogramming, we were able to obtain iPSC colonies with good morphology, well-defined edges, and shape (Fig.1). As a validation, we stained iPSCs with the pluripotency marker TRA-1-60 and identified expression of the marker under the fluorescent microscope (Fig.2). Moreover, karyotype examination of iPSCs showed stable chromosomal integrity (Fig.3).

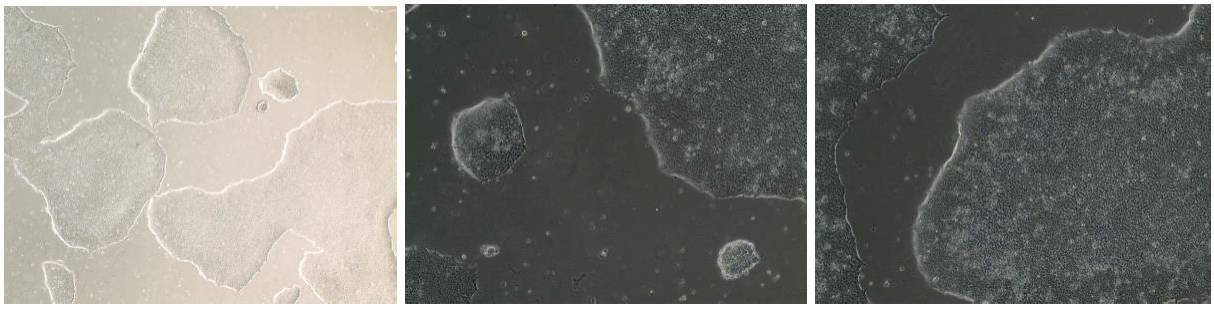

Figure 1. Stable induced pluripotent stem cells with optimal morphology.

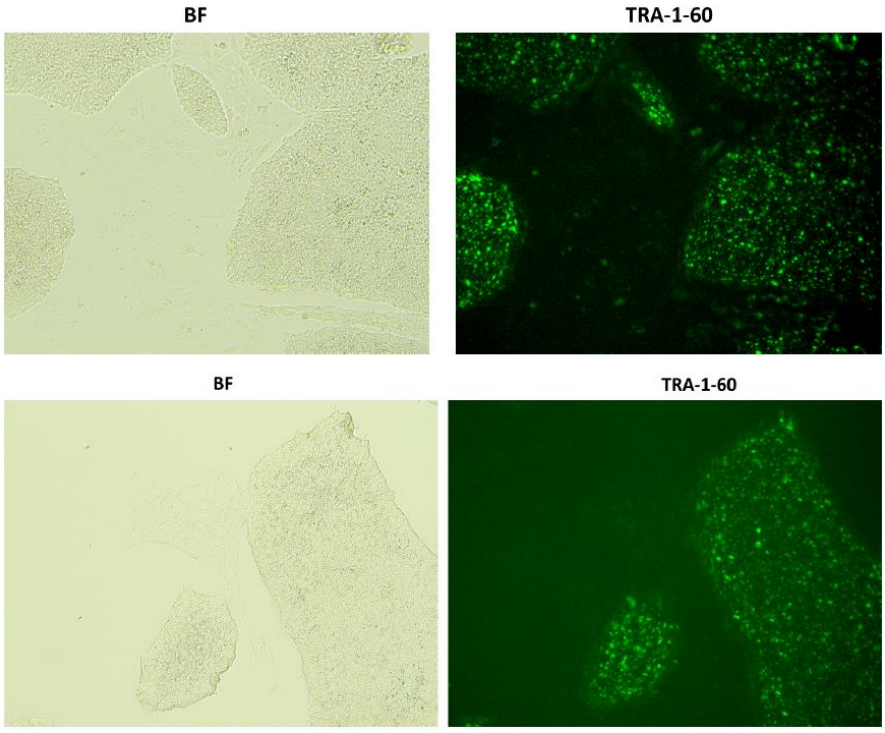

Figure 2. Immunocytochemistry of iPSCs for TRA-1-60 pluripotency marker.

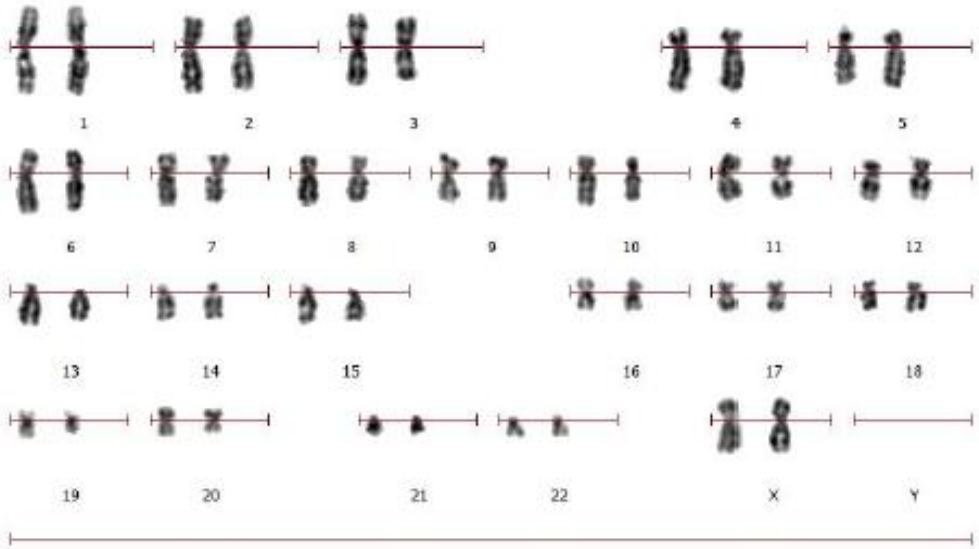

Figure 3. Karyotyping analysis of iPSCs.

Discussion

Primary electrical cardiac disorders or channelopathies are inherited genetic disorders affecting the electrical activity of cardiomyocytes, posing a risk of malignant arrhythmias that can result in sudden cardiac death. Channelopathies include heterogeneous diseases such as long QT syndrome (LQTS), short QT syndrome (SQTS), idiopathic ventricular fibrillation (IVF), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and early repolarization syndromes (ERS). CPVT is commonly linked to

mutations in genes involved in calcium handling within cardiac cells, such as the ryanodine receptor gene (RYR2) and the calsequestrin gene (CASQ2). If left untreated, CPVT carries a high risk of mortality, with around 30% of affected individuals experiencing at least one cardiac arrest and up to 80% suffering one or more syncopal episodes. In some cases, sudden death may be the initial presentation of the disease [11,12]. According to the guideline of European Society of Cardiology, "upgrade of genetic counseling and testing" is in the list of 10 novel key aspects of management of ventricular arrhythmias and prevention of sudden cardiac death. Future advancements in the evaluation of genetic variants are essential, as the number of variants of uncertain significance (VUS) and likely pathogenic variants continues to grow with the increasing use of genetic testing [13]. A number of specific genes have been strongly implicated in the pathophysiology of channelopathies. However, not all genetic variants within these genes are well characterized, especially those classified as VUS. From a fundamental research perspective, the underlying disease mechanisms are yet to be elucidated. In these terms, disease modeling tools such as induced pluripotent stem cell-derived cardiomyocytes can serve as an appropriate technology for studying the molecular basis of the disease. Here, we report the successful generation of induced pluripotent stem cells (iPSCs) from a healthy donor to serve as a control in disease modeling of catecholaminergic polymorphic ventricular tachycardia (CPVT). The induction of iPSCs from peripheral blood mononuclear cells (PBMCs) using the Sendai virus reprogramming method demonstrated efficiency and reproducibility. The colonies displayed classical iPSC morphology, expressed the pluripotency marker TRA-1-60, and maintained normal karyotype stability. These findings are consistent with previous reports indicating the efficacy of Sendai virus-based reprogramming systems in generating integration-free, pluripotent stem cells with reliability for downstream applications [14-16]. The case of the CPVT patient carrying a de novo heterozygous missense mutation in the RYR2 gene (c.13892A>T; p.D4631V) provides an opportunity to reveal pathogenic mechanisms associated with RYR2 dysfunction. RYR2 is a tetrameric protein embedded in the membrane of the sarcoplasmic reticulum (SR), where it facilitates ion release during systole. It is tethered to calsequestrin-2 (CASQ2) through associated regulatory proteins. The pathophysiology of CPVT is characterized by impaired calcium homeostasis resulting from mutations in critical calcium-handling proteins, such as RYR2 and CASQ2 [17,18]. As a rare but fatal arrhythmia with a normal resting ECG, CPVT represents significant diagnostic and therapeutic challenges. Therefore, an induced pluripotent stem cell (iPSC)-based model derived from the patient's genetic background may serve as a valuable platform for investigating the mechanisms underlying arrhythmia onset, disturbances in calcium handling, and individualized responses to pharmacological interventions [19,20].

Future differentiation of both control and mutant iPSC lines into cardiomyocytes will enable electrophysiological assessments and calcium imaging studies, providing functional validation of the disease phenotype and response to candidate drugs. Such patient-specific cardiomyocytes may also uncover genotype–phenotype relationships, contributing to improved risk stratification.

Conclusion

This study reports the successful generation of induced pluripotent stem cells (iPSCs) from a healthy donor, which will further be used as a "control" sample to compare it with the patient's iPSCs carrying RYR2 mutation. Currently, generation of iPSCs from patient with CPVT, carrying a de novo heterozygous missense mutation in the RYR2 gene (c.13892A>T; p.D4631V) is ongoing. This iPSCs model provides a valuable platform for studying the pathophysiology of CPVT and evaluating potential therapeutic interventions. Further experiments will be conducted to obtain patient-derived cardiomyocytes and assess their electrophysiological responses, calcium handling, and contractile functions.

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