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Lab Resource: Multiple Stem Cell Lines

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A B S T R A C T

The Takotsubo syndrome (TTS) is characterized by acute transient left ventricular dysfunction in the absence of obstructive coronary lesions. An enhanced β-adrenergic signaling and higher sensitivity to catecholamine-induced-toxicity were identified as mechanisms associated with TTS. It is still elusive, whether TTS patients with recurrent events show similar underlying signaling pathomechanism. Induced pluripotent stem cell (iPSC)-lines were generated from skin fibroblasts of two independent female Takotsubo syndrome patients with a severe phenotype characterized by recurrent TTS events. For reprogramming, a non-integrative plasmid technique was used. All generated iPSCs maintained full pluripotency, genomic integrity, and spontaneous *in vitro* and *in vivo* differentiation capacity.

Resource Table:

Unique stem cell lines identifier	UMGi145-A UMGi146-A
Alternative names of stem cell lines	10-TTS (UMGi145-A) 11-TTS (UMGi146-A)
Institution	Clinic for Cardiology and Pneumonology, University Medical Center Goettingen
Contact information of distributor	PD Dr. rer. nat. Katrin Streckfuss-Bömeke katrin.streckfuss@med.uni-goettingen.de
Type of cell lines	iPSCs
Origin	human, female
Cell Source	skin fibroblasts
Clonality	clonal
Method of reprogramming	plasmids: pCXLE-hOct3/4-shp53-F (Addgene 27077), pCXLE-hSK (Addgene 27078), pCXLE-hUL (Addgene 27080)
Multiline rationale	Same disease, non-isogenic cell lines
Gene modification	No
Type of modification	N/A
Associated disease	Takotsubo syndrome (TTS)
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	10-TTS: December 29, 2015; 11-TTS: June 02, 2016
Cell line repository/bank	N/A
Ethical approval	Ethical committee of University Medical Center Goettingen (Az 21/1/11) and Ethical committee of University Zurich (KEK-No.: 2013-0075)

1. Resource utility

The Takotsubo syndrome (TTS) is a transient cardiac disease, which involves left ventricular apical akinesia. The pathomechanism of TTS is still not fully understood. However, a genetic predisposition is described. Therefore, we generated patient-specific induced pluripotent stem cell (ps-iPSC)-lines from two non-related TTS-patients with a severe phenotype, characterized as more than one TTS event.

2. Resource details

TTS is characterized by acute transient left ventricular dysfunction in the absence of obstructive coronary lesions. TTS patients show symptoms similar to the acute myocardial infarction. TTS is mostly triggered by high emotional or physical stress resulting in a high catecholamine level (Templin et al., 2015). The pathogenic mechanism leading to TTS is still not completely clarified. However, a β-adrenergic

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overstimulation and a genetic predisposition were shown to play an important role in TTS (Borchert et al., 2017). We isolated somatic cells from TTS-patients, reprogrammed them into ps-iPSCs and differentiated them into pure beating cardiomyocytes (iPSC-CMs). Using this established *in vitro* ps-iPSC-TTS model, we were able to identify an enhanced β-adrenergic signaling and higher sensitivity to catecholamine-induced toxicity as mechanisms associated with the TTS phenotype (Borchert et al., 2017). Until now it was not analyzed, whether TTS

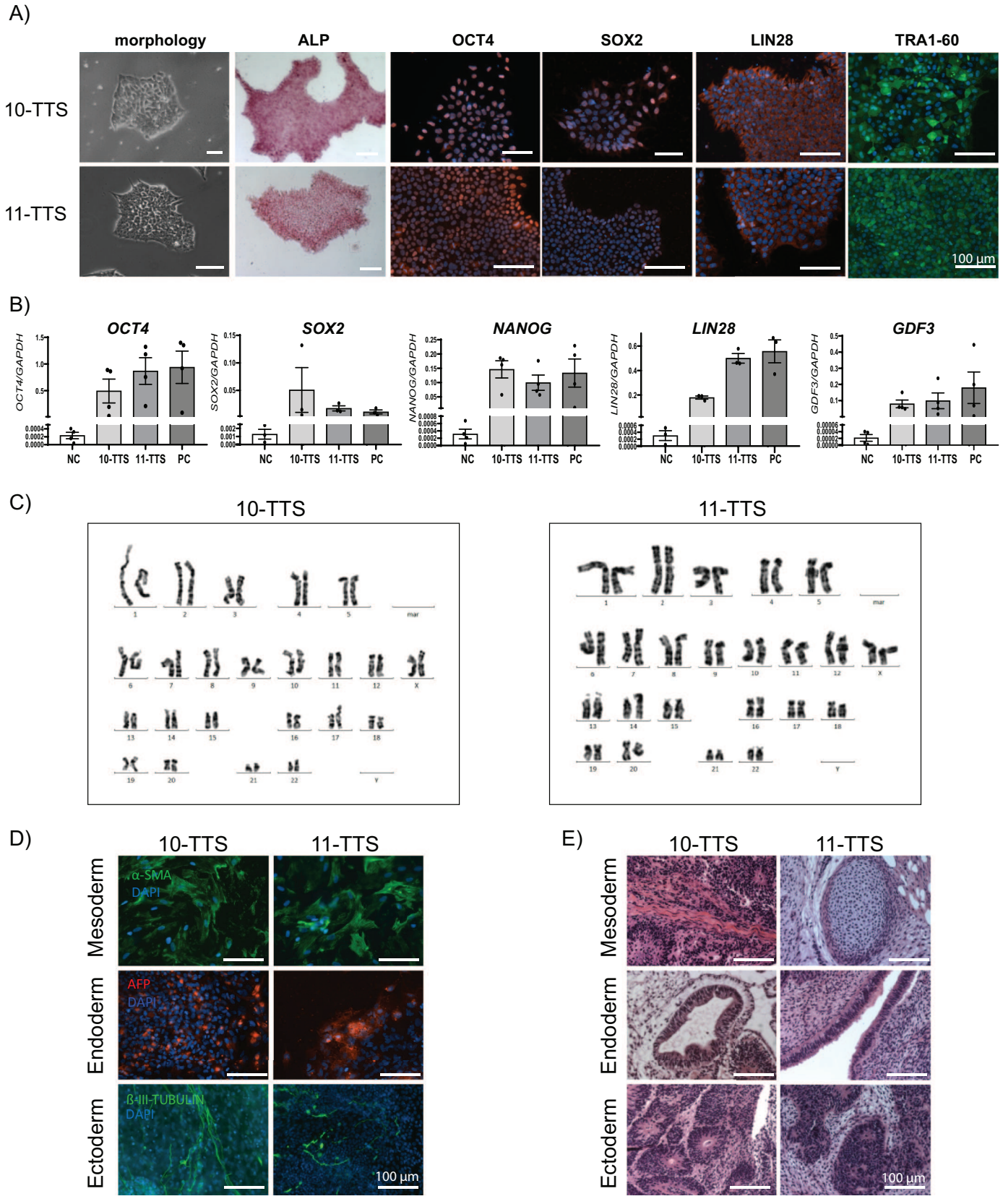

Fig. 1.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UMGi145-A	10-TTS	Female	84	Caucasian	N/A	Takotsubo syndrome
UMGi146-A	11-TTS	Female	62	Caucasian	N/A	Takotsubo syndrome

patients with recurrent TTS events show similar underlying signaling pathomechanism. To analyze this circumstance, we established ps-iPSC-TTS-lines from two non-related TTS patients with recurrent TTS events.

Therefore, skin fibroblasts of two independent female TTS patients (patient 10 and 11) were reprogrammed into ps-iPSC-line by using a non-integrating plasmid-system (Okita et al., 2011). All generated cell lines show pluripotency characteristics as shown in Fig. 1. They showed typical pluripotency morphology, were positive for alkaline phosphatase (ALP) staining and expressed typical pluripotency markers such as OCT4, SOX2, LIN28 and TRA1-60 on protein level (Fig 1A). Expression of pluripotency genes on mRNA level has been demonstrated by qPCR in comparison to fibroblasts as a negative control and an already published iPSC-line as positive control (Borchert et al., 2017) (Fig 1B). The generated iPSC lines were authenticated by STR analysis compared to their parental fibroblasts (archive of journal) and tested negative for mycoplasma contamination (Supplementary Fig 1A) as well as for episomal vectors by semiquantitative PCR (Supplementary Fig 1B). We used a primer pair for *EBNA-1* sequence derived from Epstein-Barr virus, since *EBNA-1* is part of the episomal vectors used for reprogramming (Okita et al., 2011). *EBNA* was expressed in the plasmids used for reprogramming, but not in the generated 10-TTS or 11-TTS lines (passage 25-82). To confirm the clearance of episomal plasmids in the generated iPSC lines, we used a primer pair against the vector-derived expression of *OCT4 (pla)* demonstrating expression of exogenous *OCT4* only in the plasmid pCXLE-hOCT3/4-shp53, but not in the generated cell lines. The sendai virus-generated iPSC-Line SV-iPSC NC served as negative control for plasmid-generated iPSCs (Supplementary Fig 1B).

Standard G-banding karyotype analysis was performed to test genomic integrity and resulted in a normal karyotype of 10-TTS (passage 40) and 11-TTS (passage 26) (Fig 1C). The spontaneous differentiation capacity of all generated iPSC lines was analyzed by embryoid body formation *in vitro* and teratoma formation in immunodeficient mice *in vivo*. The expression of proteins of all three germ layers as α-smooth muscle actin (α-SMA) (Mesoderm), α-fetoprotein (AFP) (Endoderm), and β-III-Tubulin (Ectoderm) were demonstrated *in vitro* (Fig 1D) and teratoma formation with tissue from all three germ layers was shown *in vivo* (Fig 1E).

3. Materials and methods

All cells were cultured under humidified conditions at 37°C and 5% CO_2 saturation.

3.1. Somatic cell isolation

3.5-4 mm skin biopsies of the donors were taken aseptically and placed in DMEM containing penicillin (100 U/mL)/streptomycin (100 µg/mL). The biopsy was cut into pieces and transferred in dishes containing fibroblast growth medium (DMEM supplemented with 10 % FCS, 1x NEAA, Glutamine (2 mmol/L), β-mercaptoethanol (50 µmol/L), penicillin (50 U/mL)/streptomycin (50 µg/mL), bFGF (10 ng/mL)). Transduction experiments were performed before passage 3.

3.2. Generation and culture of ps-iPSCs

For plasmid-based integration-free reprogramming, 4×10^5 cells were used for electroporation with the NHDF Nucleofector Kit (Lonza). Suspended cells and 1-2 µg of the plasmids pCXLE-hSK, pCXLE-hUL and pCXLEhOct3/4-shp53-F were used per experiment (Borchert et al., 2017). Electroporation was done with the nucleofector II from Lonza (program P22 or U23). Cells were plated in fibroblast medium with 5 µmol/L per survival factor and 500 µmol/L sodium butyrate. 7 days after transfection the fibroblasts were passaged on Geltrex for picking iPS-like colonies 2-3 weeks later. The iPSCs were cultivated with E8 medium (life technologies) and passaged every 3-4 days with versene (0.48 mmol/L EDTA).

3.3. In vitro differentiation of ps-iPSCs

iPSCs were cocultured with mitotically inactivated mouse embryonic fibroblasts in hES medium (DMEM/F12 supplemented with 10 % KOSR, 1x NEAA and 1x β-mercaptoethanol). With a confluency of 90% the cells were scraped into big clusters and passaged to an uncoated 6 cm dish. Embryoid bodies (EBs) formation arised in suspension. After 2 days medium was changed to Iscove-Diff Medium (Iscove basal medium, 5% FCS, 1x NEAA and 450 µmol/L monothioglycerol). After 8 days of cultivation, EBs were replated and analyzed after further 8 or 25 days (Tables 1 and 2).

3.4. Immunocytochemical staining

Cells were fixed with 4% paraformaldehyde at room temperature for 20 minutes and blocked with 1% BSA. Primary antibody staining was performed overnight at 4°C. Secondary antibodies were provided in 1% BSA for 1 hour at 37°C. Nuclei staining was executed with 4.6-diamino-2-phenylindole (DAPI, 0.2 ng/mL). Samples were mounted with VectaShield. Used antibodies are listed in Table 3.

3.5. Semi-quantitative and quantitative polymerase chain reaction

RNA was isolated with the SV Total RNA Isolation System (Promega). For cDNA synthesis DNase-treated RNA (200 ng), Murine Leukaemia Virus Reverse Transcriptase and Oligo $d(T)_{16}$ (Applied Biosystems) were used. *Semi-quantitative* PCR was performed using GoTaq polymerase. The PCR products were separated by electrophoresis on 2.5% agarose gels. Quantitative PCR was performed using the iQ™ SYBR® Green Supermix (Bio-Rad) and the iQ™5 real-time PCR detection system (Bio-Rad). All primers were listed in Table 3. *GAPDH* was used as a housekeeping gene.

3.6. Karyotype analysis

iPSCs (50 – 60% confluent) were handled with Demecolcine (16 h, 100 ng/mL, life technologies) and treated with 0.075 M KCl for 45 min at 37°C. Cells were fixed (Methanol:Acetic acid (3:1)), dropped onto slides and dried for 72 h at RT. Chromosomes were stained with 9.3 % Giemsa (Sigma Adrich). 15 metaphase spreads/cell line were analyzed by IKAROS 5.8.13 software (Metasystems) in the Department of Haematology and Medical Oncology, UMG.

3.7. Teratoma formation

Two 6 cm dishes (70% confluent) of the generated iPSC-lines were injected subcutaneously into 8-week-old immunodeficient mice. Two months after injection resulting teratomas were harvested, fixed in

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal stem cell-like morphology	Fig. 1 panel A
Phenotype	Qualitative analysis	Positive immuncytochemistry stainings of pluripotency markers: OCT4, SOX2, LIN28, TRA1-60	Fig. 1 panel A
	Quantitative analysis (RT-qPCR)	The expression pattern of *OCT4, SOX2, NANOG, LIN28*, and *GDF3* from 10-TTS and 11-TTS correlate with the expression pattern of a published iPSC-line. Skin fibroblasts (NC) show low expression of pluripotency genes.	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	*46XX, Resolution 300*	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A STR analysis: 16 independent PCR-systems were tested. 10-TTS and 11-TTS match to the specific host fibroblasts in all analyzed points	N/A submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative Mycoplasma testing by luminescence	Supplementary Figure 1
Differentiation potential	Embryoid body formation and teratoma formation	Differentiation in all three germ layers. 10-TTS and 11-TTS show expression of α-smooth muscle actin, β-III-tubulin and α-feto protein. Both cell lines are capable to generate teratoma formation in immunodeficient mice.	Figure 1 panel D and E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

Antibody		Dilution	Company Cat # and RRID
Pluripotency marker	Goat anti-OCT3/4 IgG	1:40	R&D, Minneapolis, Minnesota, USA, Cat# AF1759, RRID:AB_354975
Pluripotency marker	Mouse anti-SOX2 IgG2a	1:50	R&D, Minneapolis, Minnesota, USA, Cat# MAB2018, RRID:AB_358009
Pluripotency marker	Goat anti-LIN28 IgG	1:300	R&D, Minneapolis, Minnesota, USA, Cat# AF3757, RRID:AB_2234537
Pluripotency marker	Mouse anti-TRA1-60 IgM	1:200	Abcam, Cambridge, United Kingdom, Cat# ab16288, RRID:AB_778563
Germlayer marker	Rabbit anti-AFP IgG	1:100	Dako, Hamburg, Germany, Cat# A0008, RRID:AB_2650473
Germlayer marker	Mouse anti-α-SMA IgG2a	1:3000	Sigma Aldrich, St. Louis, Missouri, USA, Cat# A2547, RRID:AB_476701
Germlayer marker	Mouse anti-β-III-Tubulin	1:1000	BioLegend, San Diego, California, USA, Cat# MMS-435P, RRID:AB_2313773
Secondary antibody	Cy3 goat-anti-mouse IgG + IgM	1:300	Jackson ImmunoResearch, Cambridge, UK, Cat# 111-165-045, RRID:AB_2338003
Secondary antibody	Alexa Fluor 647 donkey- anti-rabbit IgG	1:1000	Thermo Fisher Scientific, Waltham, Massachusetts, USA; Cat# A-31573, RRID:AB_2536183
Secondary antibody	Alexa Fluor 555 donkey-anti-goat IgG	1:1000	Thermo Fisher Scientific, Waltham, Massachusetts, USA, Cat# A-21432, RRID:AB_2535853
Secondary antibody	Alexa Fluor 488 donkey-anti-mouse IgG	1:1000	Thermo Fisher Scientific, Waltham, Massachusetts, USA, Cat# A-21202, RRID:AB_141607
Secondary antibody	Alexa Fluor 488 goat-anti-mouse IgG + IgM	1:500	Thermo Fisher Scientific, Waltham, Massachusetts, USA, Cat# A-10680, RRID:AB_2534062

Primers

	Target	Forward/Reverse primer (5′-3′)
Pluripotency marker (quantitative PCR)	*GDF3*	TTCGCTTTCTCCCAGACCAAGGTTTC/ TACATCCAGCAGGTTGAAGTGAACAGCACC
Pluripotency marker (quantitative PCR)	*LIN28*	AGTAAGCTGCACATGGAAGG/ ATTGTGGCTCAATTCTGTGC
Pluripotency marker (quantitative PCR)	*NANOG*	AGTCCCAAAGGCAAACAACCCACTTC/ ATCTGCTGGAGG CTGAGGTATTTCTGTCTC
Pluripotency marker (quantitative PCR)	*OCT4*	GACAACAATGAAAATCTTCAGGAGA/ TTCTGGCGCCGGTTACAGAACCA
Pluripotency marker (quantitative PCR)	*SOX2*	GCTACAGCATGATGCAGGACCA/ TCTGCGAGCTGGTCATGGAGTT
House-Keeping gene (quantitative PCR, semi-quantitative PCR)	*GAPDH*	GTCTCCTCTGACTTCAACAGCG/ ACCACCCTGTTGCTGTAGCCAA
Episomal vector expression (semi-quantitative PCR)	*EBNA-1*	ATCAGGGCCAAGACATAGAGATG/ GCCAATGCAACTTGGACGTT
Episomal vector expression (semi quantitative-PCR)	*OCT3/4 (pla)*	CATTCAAACTGAGGTAAGGG/ TAGCGTAAAAGGAGCAACATAG

formalin and analyzed with hematoxylin and eosin staining.

3.8. Mycoplasma test

Mycoplasma tests are performed every 10 passages via luminescence with the Lonza Mycoalert Plus-Kit.

3.9. STR analysis

STR analysis was performed by Eurofins Genomics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101746.

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