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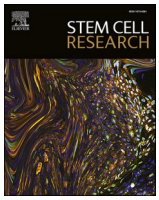

Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line (ESi107-A) from a transthyretin amyloid cardiomyopathy (ATTR-CM) patient carrying a p. Ser43Asn mutation in the TTR gene

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A B S T R A C T

Transthyretin (TTR) amyloid cardiomyopathy (ATTR-CM) is a life-threatening disease caused by the abnormal production of misfolded TTR protein by liver cells, which is then released systemically. Its amyloid deposition in the heart is linked to cardiac toxicity and progression toward heart failure. A human induced pluripotent stem cell (iPSC) line was generated from peripheral blood mononuclear cells (PBMCs) from a patient suffering familial transthyretin amyloid cardiomyopathy carrying a c.128G>A (p.Ser43Asn) mutation in the TTR gene. This iPSC line offers a useful resource to study the disease pathophysiology and a cell-based model for therapeutic discovery.

Resource table

Unique stem cell line identifier	ESi107-A
Alternative name(s) of stem cell line	ATTR-CM PBiPS1-Sv4F-3, TAC PBiPS1-Sv4F-3
Institution	Barcelona Stem Cell Bank (B-SCB). Regenerative Medicine Programme. Institut d'Investigació Biomèdica de Bellvitge (IDIBELL). CIMA University of Navarra
Contact information of distributor	Olalla Iglesias-García (oiglesias@unav.es) Anna Veiga (aveiga@idibell.cat)
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 55 Sex: Female Ethnicity if known: White-Caucasian

(*continued on next column*)

Resource table (*continued*)

Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Transgene free (CytoTune™-iPS 2.0 Sendai Reprogramming Kit)
Genetic Modification	No modification
Type of Genetic Modification	Inherited
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-/q-PCR
Associated disease	Transthyretin cardiac amyloidosis
Gene/locus	TTR, exon 2, c. 128G>A (p.Ser43Asn)
Date archived/stock date	11-07-2022; 19-07-2022
Cell line repository/bank	https://www.isciii.es/QueHacemos/Servicios/BIOBANCOS/BNLC/Paginas/LineasiPS.aspx https://hpscreg.eu/cell-line/ESi107-A

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Resource table (*continued*)

Ethical approval	The generation of the line was approved by the Ethical Committee of Investigation of the University of Navarra (Approval no. 2021.140)

1. Resource utility

This human induced pluripotent stem cell (iPSC) line is a useful tool for studies of disease pathophysiology, and to be used as an advanced cell-based disease model to advance the understanding of the mechanisms underlaying the disease, as well as for discovering new treatments for patients with cardiac amyloidosis (Table 1).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field microscopy	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunofluorescence	OCT4, SSEA3, SSEA4, NANOG, SOX2, TRA-1–60, TRA-1–81	Fig. 1 panel B
	Quantitative analysis RT-qPCR	LIN28, POUF5F1 (OCT4), REX1, NANOG, DPPA4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XX Resolution 500	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	10 sites tested, all matching	Available with the authors
Mutation analysis (IF APPLICABLE)	Sanger Sequencing	Heterozygous mutation of TTR p.Ser43Asn	Fig. 1 panel F
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma testing by VenorTM GeM Detection Kit, PCR-based.	Negative	Supplementary Fig. S1B
Differentiation potential	Directed differentiation	Confirmation of protein expression of ectodermal (TUJ1, GFAP), mesodermal (ASMA, GATA4) and endodermal (ALPHA1, FOXA2) markers	Fig. 1 panel E
List of recommended germ layer markers	**Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer**	Ectoderm: TUJ1/GFAP; Endoderm: AFP/FOXA2 Mesoderm: ASMA/GATA-4	IF with specific antibodies
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

2. Resource details

Transthyretin (TTR) amyloid cardiomyopathy (ATTR-CM) is an infiltrative disorder caused by the deposition of insoluble TTR amyloid fibrils in the myocardium. ATTR-CM is a prevalent and deadly disease which currently lacks and optimal therapy (Gertz et al., 2015). This stems from the lack of basic knowledge on the mechanisms that trigger the disease and drive its progression, which in turn derives from the absence of optimal models in which to delve into this. Currently, the only available source for human cardiomyocytes (CMs) are human pluripotent stem cells, and amongst these, hiPSC stand out due to the capacity to derive them from individuals from specific disease phenotypes, representing a powerful resource to study the disease pathophysiology and to develop new therapeutic options.

In the present report, a human iPSC line (ESi107-A) was reprogrammed from peripheral blood mononuclear cells (PBMCs) from a patient of ATTR-CM carrying a genetic variant on the TTR gene. PBMCs were isolated by density gradient centrifugation. PBMCs were reprogrammed by non-integrating methodology using the Cytotune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) containing the four Yamanaka factors, OCT4, SOX2, KLF4 and c-MYC. The generated line was called ESi107-A and clones demonstrated the characteristic morphology of human iPSCs, with tightly compacted cells, a high nuclear-to-cytoplasma ratio and well-defined borders (Fig. 1A). The absence of the Sendai-based reprogramming vectors has been verified by RT-PCR after 10 passages (Supplementary Fig. S1A). The pluripotency of the clone ESi107-A was confirmed by immunofluorescent analysis of the pluripotency-associated markers OCT4, SOX2, NANOG, TRA-1–60, TRA-1–81, SSEA-3 and SSEA-4 (Fig. 1B), alkaline phosphatase activity (Fig. 1A) and RT-qPCR (Fig. 1C). The human iPSC line showed a normal 46, XX karyotype, analyzed by G-banding analysis (Fig. 1D). The capacity of *in vitro* differentiation towards the three germ layers was determined by embryoid body (EB) formation and differentiation, followed by immunofluorescence-based detection of the definite endoderm markers α-fetoprotein (AFP) and forkhead box A2 (FOXA2), the ectodermal markers βIII-tubulin (TUJ1), glial fibrillary acidic protein (GFAP) and neurofilament, and the mesodermal markers α-smooth muscle actin (ASMA) and GATA binding protein 4 (GATA4) (Fig. 1E). The presence of the mutation p.Ser43Asn was confirmed by gDNA extraction and sequencing (Fig. 1F). The iPSC identity was proved by microsatellite analysis and short tandem repeats were compared with the ones from the patient́s peripheral blood.

3. Materials and methods

3.1. Ethical approval

All procedures were approved by the University of Navarra Ethical Committee and by the Advisory Committee for Human Tissue and Cell Donation and Use, according to Spanish and European Union legislation.

3.2. Cell culture

Patient́s PBMCs were isolated by density gradient centrifugation and purified with multiple washes of RPMI medium, supplemented with 10% fetal calf serum (FCS), 50U/ml Penicillin, 50ug/ml Streptomycin and 2 mM L-glutamine and frozen in FCS containing 10% DMSO.

3.3. ESi107-A generation

Patient PBMCs were reprogrammed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Scientific). A total of 5×10^5 cells were infected in RPMI medium supplemented with 10% FBS, 1X P/S, 1X Glutamax and cytokines (100 ng/ml SCF, 100 ng/ml Ftl3L, 20 ng/ml IL-3 and 20 ng/ml IL-6) and 4 μg/ml Polybrene in an ultralow attachment dish o/n. iPSCs were cultured using Matrigel™-coated culture dishes

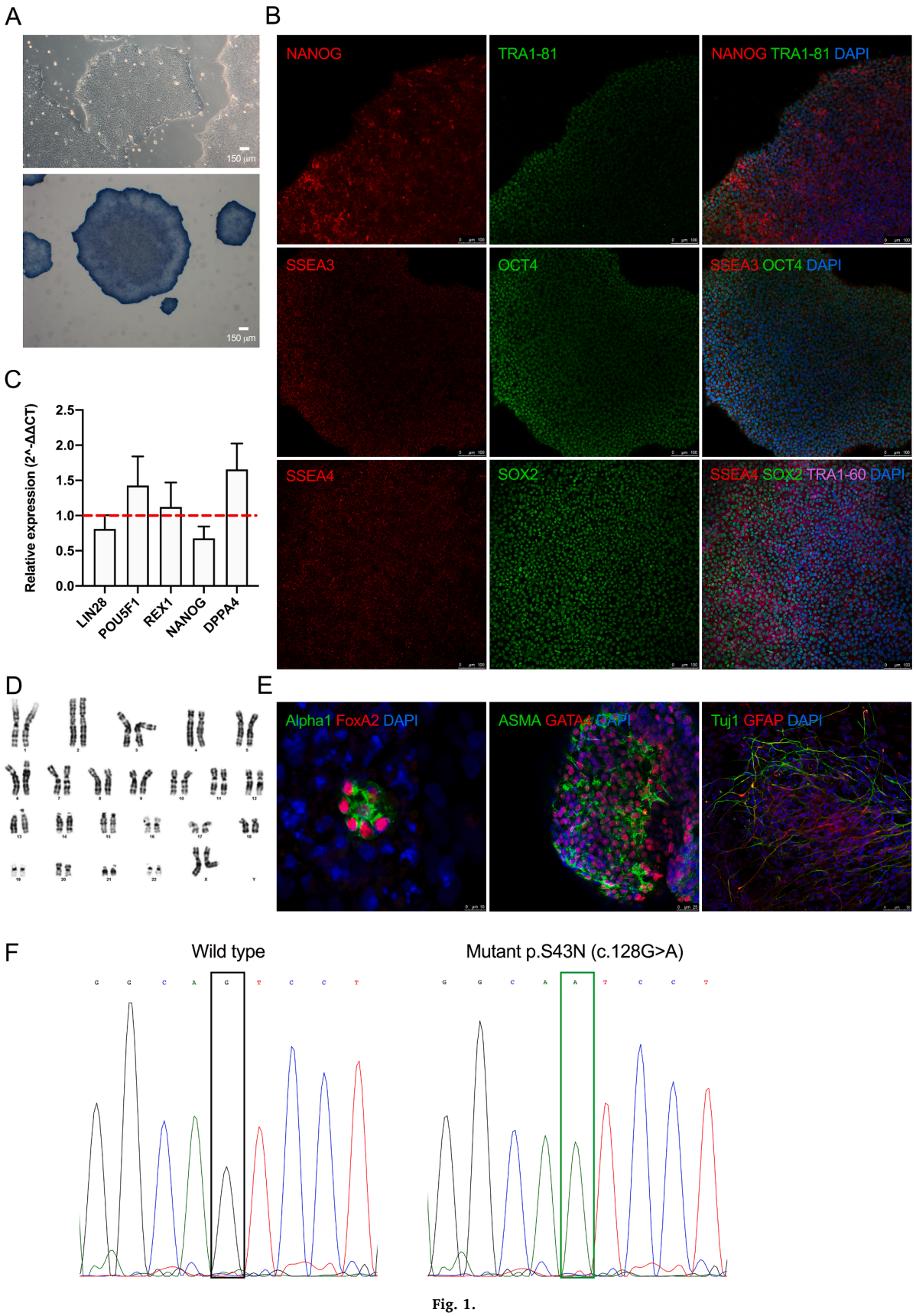
A
150 µm
150 µm
B
NANOG
TRA1-81
NANOG TRA1-81 DAPI
SSEA3
OCT4
SSEA3 OCT4 DAPI
SSEA4
SOX2
SSEA4 SOX2 TRA1-60 DAPI
C
Relative expression (2^-ΔΔCT)
2.5
2.0
1.5
1.0
0.5
0.0
LIN28
POU5F1
REX1
NANOG
DPPA4
D
E
Alpha1 FoxA2 DAPI
ASMA GATA4 DAPI
Tuj1 GFAP DAPI
F
Wild type
Mutant p.S43N (c.128G>A)
G G C A G T C C T
G G C A A T C C T

Fig. 1.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency markers	Mouse anti-OCT4	1:2	Santa Cruz, sc-5279	AB_628051
	Goat anti-NANOG	1:5	R&D Systems, AF1997	AB_355097
	Rabbit anti-SOX2	1:100	ABR, PA1-16968	AB_2195781
	Rat anti-SSEA3	1:1	Hybridoma Bank, MC-631	AB_528476
	Mouse anti-SSEA4	1:1	Hybridoma Bank, MC-813-	AB_528477
	Mouse anti-TRA-1–60	1:100	70	AB_2119183
	Mouse anti-TRA-1–81	1:100	Millipore, MAB4360	AB_177638
			Millipore, MAB4381	
Differentiation Markers	Mouse anti-TUJ1	1:40	Covance, MMS-435P	AB_2313773
	Rabbit anti-GFAP	1:1000	Dako, Z0334	AB_10013382
	Mouse anti-ASA	1:400	Sigma, A2172	AB_476695
	Rabbit anti-AFP	1:200	Agilent, A0008	AB_2650473
	Goat anti-FOXA2	1:50	R&D Systems, AF2400	AB_2294104
	Rabbit anti-GATA-4	1:25	Santa Cruz, sc-9053	AB_2247396
Secondary antibodies	AF488 Goat anti-Mouse	1:200	Jackson, 115-546-071	AB_2338865
	Cy3 Goat anti-Rat	1:200	Jackson, 112-165-020	AB_2338243
	AF488 Donkey anti-Rabbit	1:200	Jackson, 711-545-152	AB_2313584
	DyLight649 Goat anti-Mouse	1:200	Jackson, 115-495-075	AB_2338809
	AF488 Donkey anti-Goat	1:200	Jackson, 705-545-147	AB_2336933
	Cy3 Donkey anti-Mouse	1:200	Jackson, 715-165-140	AB_2340812
	Cy3 Donkey anti-Goat	1:200	Jackson, 705-165-147	AB_2340812
	AF488 Donkey anti-Mouse	1:200	Jackson, 715-545-151	AB_2307351
	Cy3 Donkey anti-Guinea pig	1:100	Jackson, 706-165-148	AB_2341099
	AF488 Goat anti-Mouse	1:200	Jackson, 115-546-071	AB_2338865
	Cy3 Goat anti-Mouse	1:200	Jackson, 115-165-075	AB_2338689
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
SeV viral vectors (RT-PCR)	SeV	181 bp	GGATCACTGGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG	
	Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA	
	c-Myc	532 bp	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG	
Pluripotency Markers (qPCR)	LIN28	97 bp	GGAGGCCAAGAAAGGGAATATGA/	
	POUF5F1		AACAATCTTGTGGCCACTTTGACA	
	(OCT4)	71 bp	GGAAGGAATTGGGAACACAAAGG/ AACTTCACCTTCCCTCCAACCA	
	REX1	108 bp	TGGAGCCTGTGTGAACAGAA/ CCACCTCCAGGCAGTAGTGA	
	NANOG	78 bp	CCTGTGATTTGTGGGCCTG/ GACAGTCTCCGTGTGAGGCAT	
	DPPA4	116 bp	TGCACTCTTCTTGCTTCCTG/ ATTCCCATTGGAGGCTTTTT	
House-Keeping Genes (qPCR)	GAPDH	189 bp	TGGTATCGTGGAAGGACTCATGA/ ATGCCAGTGAGCTTCCCGTTCAG	
Genotyping	TTR mutation (c.128G > A)	83 bp	TCCAAGTGTCCTCTGATGGT/ TTTCTGAACACATGCACGGC	
Targeted mutation analysis/ sequencing	Wild type: TCCAAGTGTCCTCTGATGGTCAAAGTTCTAGATGCTGTCCGAGGC**AGT**CCTGCCATCAATGTGGCCGTGCATGTGTTCAGAAA Mutant allele: TCCAAGTGTCCTCTGATGGTCAAAGTTCTAGATGCTGTCCGAGGC**AAT**CCTGCCATCAATGTGGCCGTGCATGTGTTCAGAAA	83 bp	N/A	

(BD Biosciences) and mTESR™1 (StemCell Technologies). Cells were routinely passaged using 0.5 mM EDTA (Invitrogen) at a splitting ~~in a~~ ratio of 1:4 – 1:8 once a week (Fig. 1A).

3.4. Genomic DNA extraction and genotyping

Genomic DNA was isolated using QIAamp® DNA Mini Kit (Qiagen, Cat #51304). DNA was amplified using Platinum® Taq DNA Polymerase HF (Invitrogen) by specific primers (Table 2) with the following conditions: 94 °C 5′, [94 °C 30″, 60 °C 30″, 68 °C 30″]×40, 68 °C 30′. The presence of the c. 128G>A mutation in TTR gene was analyzed by Sanger sequencing in 15 colonies of TOPO-TA cloned PCR product (83 bp) (TOPO TA cloning kit Invitrogen) (Fig. 1F).

3.5. RNA extraction and RT-qPCR

Total RNA was extracted using Trizol and cDNA was synthesized with SuperScript II reverse transcriptase, following manufactureŕs instructions (Thermo Fisher Scientific). RT-PCR reaction was carried out using PowerUp™SYBR® Green Master Mix (Applied Byosystems) underwent 40 rounds of amplification on a QuantStudio 5 Real-Time PCR System (Applied Biosystems). Gene expression levels were normalized using Gapdh and data are presented in comparison with ~~to~~ an established iPSC line (CBiPS1sv-4F-40) (Fig. 1C). Silencing of the exogenous reprogramming factors was analysed in PCR products on a 2% agarose gel (Supplementary Fig. S1A).

3.6. Immunofluorescence and alkaline phosphatase staining on cultured cells

Cells were fixed with 4% PFA, blocked and permeabilized with TBS + 0.5% Triton X-100 + 6% donkey serum. Primary antibodies were incubated overnight at 4 °C in TBS + 0.1% Triton X-100 + 6% donkey serum and secondary antibodies for 2 h at 37 °C. Nuclei were stained with 4′,6-diamino2-fenilindol. Confocal images were taken using a Leica TSC SPE/SP5 microscope (Marti et al., 2013). (Fig. 1B and Fig. 1E). Antibodies used are listed in Table 2. Alkaline phosphatase activity was assayed with Sigma AB0300 kit following the manufactureŕs instructions on fixed cells.

3.7. Trilineage differentiation

In vitro differentiation was tested by EB formation for 21–28 days, using the following differentiation media: 50% Neurobasal medium, 50% DMEM/F12, 1 % N2, 1% B27, 1% Glutamax and 1% Penicillin-Streptomycin (Ectoderm); Knockout-DMEM, 10% FBS, 1% NEAA, 0.1% β-mercaptoethanol, 1% Glutamax and 1% Penicillin-Streptomycin (Endoderm); Endoderm medium supplemented with 0.5 mM ascorbic acid (Mesoderm).

3.8. Karyotype determination

iPSC colonies at passage 12 were treated with colcemid (KaryoMAX colcemid, Gibco), trypsinized, incubated with hypotonic solution (KCl, Gibco) and fixed in Carnoy fixative. Genomic integrity was evaluated by G-banded metaphase karyotype analysis of 20 metaphase spreads at Hospital Sant Joan de Déu, Barcelona (Fig. 1D).

3.9. Short tandem repeat (STR) analysis

Genomic DNA obtained from patients' PBMCs and from iPSCs and STRs were amplified using the GenePrint 10 system (Promega). Amplified samples were analyzed by capillary electrophoresis by a Genetic Analyzer 3130 (Applied Biosystems).

3.10. Mycoplasma detection

Mycoplasma was detected using Venor GeM Mycoplasma detection kit (Supplementary Fig. S1B).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Olalla Iglesias Garcia reports financial support was provided by Pfizer Inc.

Data availability

Data will be made available on request.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103189.

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