Stem Cell Research 53 (2021) 102301

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Generation of the human iPSC line ESi082-A from a patient with macular dystrophy associated to mutations in the *CRB1* gene

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A B S T R A C T

Retinal dystrophies associated to mutations in the *CRB1* gene comprise a wide array of clinical presentations. A blood sample from a patient with a family history of *CRB1*-retinal dystrophy was used to prepare the iPSC line ESi082-A. The genotype of the donor, affected of a perifoveal-bilateral macular dystrophy includes one frameshift deletion and one hypomorphic allele. ESi082-A cell line has been characterized for pluripotency and will be used to prepare retinal cellular models to study the dysfunction leading to the disease.

1. Resource table

Unique stem cell line identifier	ESi082-A
Alternative name(s) of stem cell line	*CRB1*-MiPS4F1
Institution	Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain.
Contact information of distributor	Berta de la Cerda berta.delacerda@cabimer.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 56 Sex: Male Ethnicity: European descent
Cell Source	Peripheral blood mononucleated cells
Clonality	Clonal
Method of reprogramming	hOCT3/4, hSOX2, hc-MYC, and hKLF4 via non-integrative Sendai virus
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Macular dystrophy
Gene/locus	*CRB1*/ Chr. 1: 197,268,204–197,478,455 Mutations: c.613_619del (p.Ile205Aspfs*13) LOVD#CRB1_000175 c.498_506del (p.Ile167_Gly169del) CinVar ID#96659
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	10. 20. 2020
Cell line repository/bank	hpsreg.eu/cell-line/ESi082-A
Ethical approval	Cellular Reprogramming Board of Andalucía. Ethical Approval number: PR_01_2015

2. Resource utility

Most CRB1-associated retinal dystrophies present as Leber congenital amaurosis or retinitis pigmentosa but the hypomorphic allele CRB1 c.613_619del has been associated to macular dystrophy. The iPSC line ESi082-A will be used to obtain retinal cellular models by differentiation, enabling the study of the mechanisms of disease leading to cellular degeneration.

3. Resource details

Retinal dystrophies associated to *CRB1* mutations cover a wide spectrum of clinical presentations and currently it is unclear which are the factors leading to the different degrees of disease (Khan et al., 2018; Quinn et al., 2017). Cellular modelling using patient-derived iPSCs is a promising tool to uncover the mechanisms of disease. A patient diagnosed with macular dystrophy associated to a composite homozygous genotype in the *CRB1* gene was the donor of the biological sample to

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https://doi.org/10.1016/j.scr.2021.102301
Received 27 January 2021; Received in revised form 16 February 2021; Accepted 14 March 2021
Available online 18 March 2021

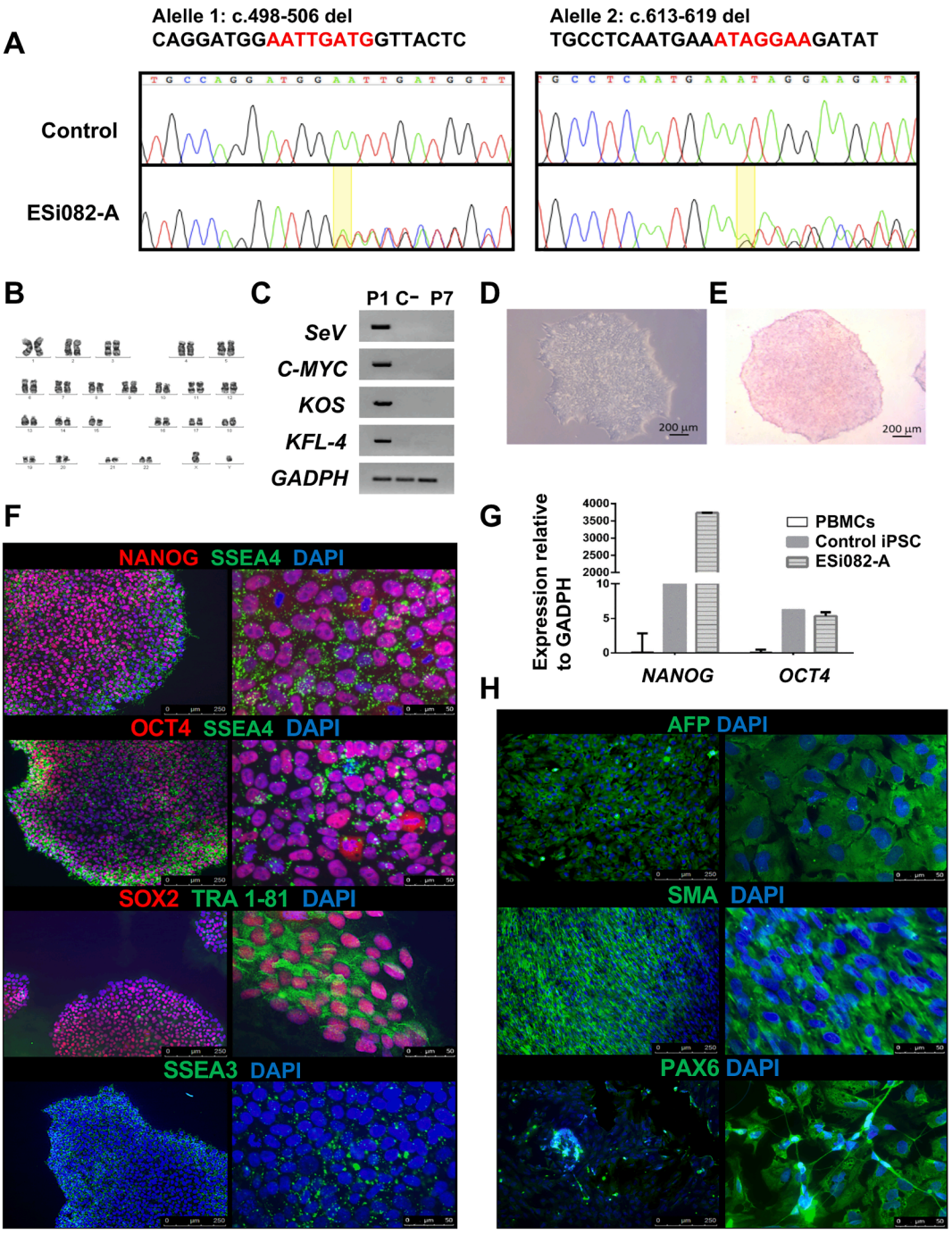

Fig. 1.

obtain the ESi082-A iPSC line. Mononucleated cells collected from peripheral blood (PBMCs) were reprogrammed to iPSCs using the non-integrative Sendai virus to transduce the reprogramming factors h*OCT3/4*, hc-*MYC*, h*KLF4*, and h*SOX2*. Identity of the cell line was confirmed by DNA fingerprinting, matching with the DNA of the original biological material (available with the authors). Moreover, DNA sequencing of ESi082-A compared to a normal control confirmed the presence of the two mutations in the *CRB1* gene (Fig. 1A). *CRB1*/c.613_619del mutation is the maternal allele, a mutation previously associated to Leber congenital amaurosis (http://databases.lovd.nl/shared/variants/CRB1), and *CRB1*/c.498_506del is the paternal allele, reported as pathogenic for macular dystrophy (Khan et al., 2018). The iPSC line was checked for normal 46, XY male karyotype (Fig. 1B) and for the typical stem cell morphology of compact flat colonies composed of cells with a high nuclear/cytosolic ratio (Fig. 1D). Clearance of viral capsid and ectopic reprogramming factors was apparent at passage 7, with no expression of these genes detected by RT-PCR (Fig. 1C). To characterize the pluripotency of cell line, expression of *NANOG* and *OCT4* genes was quantified by RT-qPCR. ESi082-A iPSC line presented a high level of expression for both genes compared to PBMCs of the patient, used as a negative control (Fig. 1G). A complete panel of pluripotency markers was evaluated by immunofluorescence, demonstrating nuclear expression for OCT4, NANOG and SOX2, and surface expression for SSEA3, SSEA4 and TRA-1-81 (Fig. 1F; right panels depict a magnification of each left panel). Additionally, elevated enzymatic activity for alkaline phosphatase was also shown for the iPSC line (Fig. 1E). The

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal morphology	Fig. 1D
Phenotype	Qualitative analysis	Positive staining for pluripotency markers: OCT4, NANOG, SOX2, TRA-1-81, SSEA3, SSEA4 and alkaline phosphatase.	Fig. 1F, 1E
	Quantitative analysis	RT-qPCR analysis of the expression for NANOG and OCT4	Fig. 1G
Genotype	Karyotype (G-banding) and resolution	46, XY, Resolution 450–500	Fig. 1B
Identity	Microsatellite PCR (mPCR) OR STR analysis	STR analysis. 10 sites tested. All matched mPCR not done	Available with the authors N/A
Mutation analysis	Sequencing	chr1:197,201,504: c.613_619del c.498_506del	Fig. 1A
	Southern Blot OR WGS	Not done	N/A
Microbiology and virology	Mycoplasma	Negative *Mycoplasma* detection. Tested by luminescence	Supplementary Fig. 1
Differentiation potential	In vitro embryoid body formation	Positive immunostaining for mesodermal muscle actin, endodermal alpha fetoprotein and ectodermal PAX6	Fig. 1H
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not done	N/A
Genotype additional info	Blood group genotyping	Not done	N/A

ability of ESi082-A to generate the three germ layers was evaluated by inducing the formation of embryoid bodies *in vitro* and then confirming the expression of specific markers by immunofluorescence: endodermal α-fetoprotein (AFP), mesodermal smooth-muscle actin (SMA) and ectodermal PAX6 (Fig. 1H; right panels depict a magnification of each left panel). Characterization is summarized in Table 1.

4. Materials and methods

4.1. Mutation sequencing

Genomic DNA from iPSCs was isolated using the QIAamp DNA Blood mini kit (Qiagen). Primers for amplification and Sanger sequencing of the genomic region flanking the mutation sites are described in Table 2.

4.2. Peripheral blood mononuclear cells (PBMCs) primary culture

PBMCs were isolated from 4 mL of peripheral blood using Vacutainer CPT tubes (BD) and cultured in expansion medium (EM: QBSF-60 (Quality Biological) with 50 μg/ml of ascorbic acid (Sigma-Aldrich); 50 ng/ml of SCF (Stemcell Technologies); 10 ng/ml IL-3 (Stemcell Technologies); 2 U/mL of EPO (Stemcell Technologies); 40 ng/ml IGF-1 (Stemcell Technologies); 1 μM Dexamethasone (Sigma-Aldrich) and 1% Pen/strep (Gibco)).

4.3. Reprogramming PBMCs to iPSCs

$2.5x10^4$ PBMCs were transduced with reprogramming factors (CytoTune®-IPS Reprogramming Kit (Life Technologies)) for 24 h. Cells were washed and kept in EM medium for 48 h. $1x10^5$ cells were seeded on feeders (irradiated hFF (ATCC CRL2429)) and cultured in QBSF-60 with 50 μg/ml of ascorbic acid and 1% pen/strep. On day 15, medium was changed to hESCM: Knock Out DMEM-F12 (Gibco); 20% Knock Out serum (Gibco); 1% glutamine (Gibco); 1% NEAA (Gibco); 0.23 mM β-mercaptoethanol (Gibco); 1% pen-strep and 10 ng/μl bFGF (Peprotech)). Colonies with stem-like morphology were manually isolated in the days 21 to 27 post-transduction. Clone 1, named ESi082-A, was adapted to grow in mTeSR1 (Stemcell Technologies) onto Matrigel.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:400	Cell Signaling Technology Cat# 2840, RRID: AB_2167691
	Rabbit anti-NANOG	1:400	Cell Signaling Technology Cat# 4903, RRID: AB_10559205
	Rabbit anti-SOX2	1:400	Cell Signaling Technology Cat# 3579, RRID:AB_2195767
	Mouse anti-SSEA4	1:100	Millipore Cat# MAB 4304, RRID: AB_177629
	Mouse anti-TRA-1-81	1:100	Stemgent Cat# 09–0069, RRID: AB_2119069
	Rat anti-SSEA3	1:100	Thermo Fisher Cat# MA 1–020, RRID: AB_2536682
Differentiation Markers	Rabbit anti-PAX6	1:500	Covance Cat# PRB-278P, RRID: AB_291612
	Mouse anti-SMA	1:300	Sigma-Aldrich Cat# A5228, RRID: AB_262054
	Mouse anti-AFP	1:20	R & D Systems Cat# MAB1368, RRID: AB_357658
Secondary antibodies	Donkey anti-Rabbit 594	1:500	Thermo Fisher Scientific Cat# A-21207, RRID: AB_141637
	Donkey anti-Mouse 488	1:500	Thermo Fisher Scientific Cat# A-21202, RRID: AB_141607
	Rabbit anti-Rat	1:500	Invitrogen Cat# A21210, RRID: AB_2535796

Primers		
	Target, size (bp)	Forward/Reverse primer (5′-3′)
SeV genome silencing (RT-PCR)	SeV, 181	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS, 528	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
	KLF4, 410	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA
	cMYC, 532	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG
House-Keeping Gene (RT-PCR)	GAPDH, 86	ACGACCCCTTCATTGACCTCAACT/ ATATTTCTCGTGGTTCACACCCAT
Pluripotency markers (RT-qPCR)	NANOG	Hs02387400g1
	OCT4	Hs01654807s1
House-keeping gene (RT-qPCR)	GADPH	Hs02786624g1
Targeted mutation analysis/ sequencing	Allele c.498–506 del, 262	GCAAGAACTCCTGCCAAC/ AGGCATGTAGCCTCGTTC
	Allele c.498–506 del, 170	TACTCCTGCTTCTGTGTCC/ GTGATTCAGATATGATCACACTTACC

4.4. RT-PCR and qPCR for detection of viral clearance and pluripotency markers

1 μg of total RNA isolated with RNeasy Mini Kit (Qiagen) was retro-transcribed using QuantiTect Reverse Transcription Kit (Qiagen). RT-

PCR reaction was performed using MyTaq DNA Polymerase (Bioline GmbH) with the primers listed in Table 2. For Q-PCR, triplicate reactions were prepared with TaqMan Gene expression Master Mix and probes listed in Table 2.

4.5. Immunofluorescence analysis

Cells grown in glass coverslips were washed with PBS, fixed in 4% PFA for 15 min, washed twice in PBS and blocked with 2% donkey serum in TPBS (0.2% Triton-X100/PBS) for 1 h. Primary antibody was incubated for 1 h in 1% BSA in TPBS, washed 3 times in TPBS and incubated with secondary antibody diluted in 1% BSA in TPBS for 1 h. After 3 washes, preparations were finished with mounting medium with DAPI. Antibodies are listed in Table 2.

4.6. Alkaline phosphatase

Enzymatic activity was detected using AP staining kit II (Stemgent).

4.7. In vitro differentiation

iPSC colonies were manually collected and cultured in non-adherent conditions in hESCM without bFGF for 7 days. Embryoid bodies were manually collected and seeded onto gelatinized-glass coverslips, cultured for one week in EBM (DMEM/F12, 10% FBS, 1% GlutaMAX, 1% MEM NEAA, 1% Pen/Strep) and analyzed by immunofluorescence using the antibodies in Table 2.

4.8. Karyotype analysis

On passage 11, 30 metaphases were counted and genome integrity was analyzed by G-banding at 400–550 band resolution in Biobanco del SSPA, Granada, Spain.

4.9. Fingerprinting

STR analysis was performed form iPSC and PBMC genomic DNA using the GenePrint® 10 System (Promega) in Biobanco del SSPA, Granada, Spain.

4.10. Mycoplasma detection

MycoAlert™ PLUS *Mycoplasma* Detection Kit (Lonza) was used to test for *Mycoplasma* (Suppl. Fig. 1).

Funding

This work was supported by Andalusian Ministry of Health, Equality and Social Policies (PI-0099-2018).

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102301.

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