Stem Cell Research 94 (2026) 104022

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Single Cell Line

Generation and characterization of the hiPSC line CSSi023-A (16154) from a patient with ADOA caused by an OPA1 variant

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A B S T R A C T

Autosomal Dominant Optic Atrophy plus syndrome (ADOA, OMIM #125250) is a mitochondrial optic neuropathy characterized by progressive degeneration of retinal ganglion cells (RGCs), leading to worsening visual impairment. The disease is caused by pathogenic variants in the Optic Atrophy 1 (OPA1) gene, a member of the guanosine triphosphatase (GTPase) family that plays a central role in mitochondrial fusion and fission, mitophagy regulation, and mitochondrial DNA (mtDNA) maintenance. To model this disorder, we generated and characterized a human induced pluripotent stem cell (hiPSC) line from primary fibroblasts obtained from a patient affected by ADOA syndrome.

1. Resource Table

Unique stem cell line identifier	CSSi023-A (16154)
Alternative name(s) of stem cell line	ADOA/OPA1 cl G
Institution	IRCCS Casa Sollievo della Sofferenza
Contact information of distributor	Jessica Rosati; j.rosati@operapadrepio.it
Type of cell line	hiPSCs
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 46 Sex: Female Ethnicity if known: Caucasian/Italian
Cell Source	Dermal Fibroblasts
Clonality	Clonal
Method of reprogramming	Non integrating episomal vectors
Genetic Modification	NO
Type of Genetic Modification	NO
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	qRT-PCR
Associated disease	Autosomal Dominant Optic Atrophy (ADOA)
Gene/locus	NM_015560.2: c.[124C > T]
Date archived/stock date	March 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/CSSi023-A
Ethical approval	IRCCS Fondazione Stella Maris, Comitato Etico Pediatrico Regione Toscana, CEPR:53/2020

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https://doi.org/10.1016/j.scr.2026.104022
Received 24 April 2026; Received in revised form 25 May 2026; Accepted 30 May 2026
Available online 1 June 2026

2. Resource utility

Variants in the Optic Atrophy 1 (OPA1) gene impair multiple mitochondrial functions, although the underlying molecular mechanisms remain largely unclear. For this reason, developing patient-specific cellular models, directly derived from affected individuals, is essential to investigate the functional consequences of OPA1 variants and to better understand disease pathogenesis.

3. Resource details

Autosomal Dominant Optic Atrophy syndrome (ADOA, OMIM #125250) is a rare autosomal dominant mitochondrial optic neuropathy characterized by progressive degeneration of retinal ganglion cells (RGCs), leading to a severe and progressive loss of vision (Lenaers et al. 2012). Mutations in the OPA1 gene (NM_015560.2) are causative of the disease. In this context, we generated a stable human induced pluripotent stem cell (hiPSC) line, CSSi023-A (16154), derived from primary dermal fibroblasts of a 46-year-old female patient carrying a heterozygous OPA1 variant, p.His42Tyr (c.124C>T) (Zanfardino et al. 2024). Fibroblasts were reprogrammed by nucleofection using three non-integrating pCXLE vectors encoding human reprogramming factors (hSOX2, hKLF4, hL-MYC, hLIN28, shp53, hOCT3/4). Four weeks post-nucleofection, emerging colonies displaying a stem cell-like morphology were selected (Fig. 1A). After P6, initial characterization confirmed a normal karyotype (46, XX) (Fig. 1B). Sanger sequencing further verified the persistence of the heterozygous OPA1 variant (c.124C>T) in both hiPSCs and parental fibroblasts (Fig. 1C), confirming genetic fidelity. Following P12, stemness validation was initiated. qRT-PCR analysis demonstrated complete silencing of exogenous reprogramming transgenes (Fig. 1D) and confirmed the expression of endogenous pluripotency-associated genes, including hOCT4, hSOX2, hL-MYC, hKLF4, hLIN28 (Fig. 1E). Immunocytochemical analyses further validated the pluripotent state, showing strong expression of the cytoplasmic marker TRA-1-60 and the nuclear marker OCT4 (Fig. 1F). To assess functional pluripotency, hiPSCs were induced to form human embryoid bodies (hiEBs) by manual aggregation and culture under non-adherent conditions (Fig. 1G). After 14 days, hiEBs were analyzed for the expression of markers representative of the three germ layers, confirming spontaneous differentiation capacity (Fig. 1H). For all RT-PCR comparisons, the previously established hiPSC line CSSi018-A (14192) was used as reference (Casamassa et al. 2024).

In vivo pluripotency was further confirmed through teratoma formation assays, followed by histological and immunohistochemical identification of derivatives of all three embryonic germ layers (Fig. 1I). In addition, after P9, genetic identity between parental fibroblasts and derived hiPSCs was validated by short tandem repeat (STR) profiling, confirming a matching DNA fingerprint (data available from the authors). All cell lines were routinely tested and confirmed negative for mycoplasma contamination (Supplementary File 1).

4. Materials and methods

4.1. Fibroblast culture and reprogramming method

Dermal fibroblasts were cultured in Dulbecco's Modified Eagle Medium High Glucose, 20 % of FBS, 1 % of L-Glutamine, NEAA and Penicillin-Streptomicin (Sigma Aldrich), at 37 °C and 5 % CO2. 3×10^5 fibroblasts, passage VI, were nucleofected with 1,5ug of 1:1:1 episomal mix of pCXLE-hUL (Addgene#27080), pCXLE-hSK (Addgene#27078) and pCXLE-hOCT4-shp53 (Addgene#27077) using 4D-Nucleofector™ (Lonza)(Program FF113). One week later, fibroblasts were plated on a dish pretreated with Matrigel (Corning) and cultured in NutristemXF medium (Biological industries). Colonies with stem cell-like morphology were amplified at 37 °C and 5 % CO2. iPSCs were tested periodically for the absence of mycoplasma contamination using an N-Garde Mycoplasma PCR kit.Table 1

4.2. qRT-PCR analyses

Total RNA was extracted using TRIzol reagent (Life Technologies). RNA integrity was assessed with RNA 6000 Nano LabChips (Agilent Technologies) and analyzed on an Agilent 2100 Bioanalyzer. Complementary DNA (cDNA) was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR was performed using SYBR Green-based primers for pluripotency markers and TaqMan probes for differentiation markers (Table 2). Gene expression levels were calculated using the 2^−ΔΔCT method. All reactions were run in triplicate, with β-ACTIN used as the endogenous reference gene.

5. Materials and methods

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5.2. qPCR analyses

Total RNAs were isolated using TRIzol reagent (Life Technologies). After validation of RNA integrity through RNA 6000 Nano LabChips (Agilent Technologies), processed on the Agilent 2100 Bioanalyzer, RNA was *retro*-transcripted by a High Capacity cDNA Reverse Transcription Kit (Applied Biosystem). SYBR Green primers for pluripotency and TaqMan primers for differentiation (Table 2) were used to perform qRT-PCR, analyzed through the 2–ΔΔCT method. Each reaction was run in triplicate using β-ACTIN as a reference gene.

5.3. Karyotype and STR analysis

For karyotyping, hiPSCs at P9 were cultured in Nutristem medium for 2–3 days. Karyotype analysis was carried out on GTG-banded metaphases (resolution 450–500). 30 metaphases were counted and three karyograms were analyzed. For STR analysis, the DNA was extracted using Dneasy blood and tissue kit (QIAGEN). PCR amplification of 17 distinct STRs was carried out using the QST*Rplusv2 kit (Elucigene Diagnostics), then PCR products were separated on an ABI Prism 3130 DNA sequencer and analyzed by GeneMapper version 4.0 (AppliedBiosystems) (Table STR).

5.4. Immunofluorescence staining

hiPSCs were fixed using 4 % paraformaldehyde for 20 min at RT. Clones were blocked in PBS with 20 % Normal Goat Serum for TRA-1–60 staining and with the addition of 0.1 % Triton X-100 for OCT4 staining, for 1 h at RT. Primary antibodies (Table 2), diluted in blocking buffer were incubated O/N at 4 °C. Alexa-Fluor-conjugated secondary antibodies were added for 1,5h at RT. Cellular nuclei were stained with Hoechst. Images were taken using a Nikon C2 fluorescence microscope.

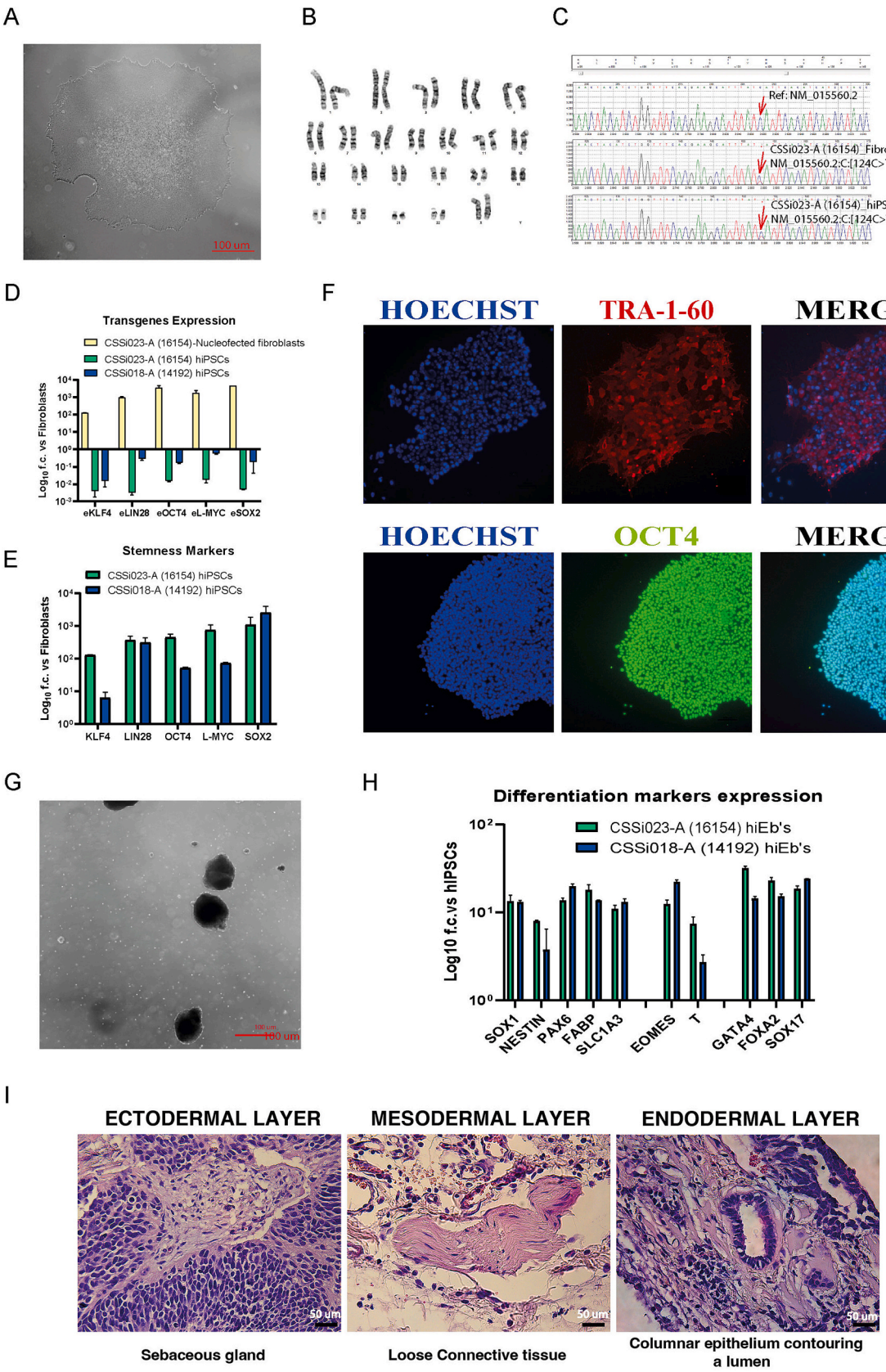

Fig. 1. Characterization of CSSi023-A (16154) A. Phase contrast image of the iPSC morphology. B. Cytogenetic analysis showing a normal karyotype (46, XX). C. DNA sequencing analysis of ADOA/OPA1 gene :c.124C>T mutation. Red arrows indicate mutation site. D. qRT-PCR analysis of transgene expression showing the loss of episomal vectors during iPSC amplification. E. Expression analysis of stemness markers. F: Representative immunofluorescent images of iPSCs marked for stem cell markers, OCT4 (green) and TRA-1-60 (red). Nucleus is labelled with Hoechst 33342 (blue). G. Phase contrast image of embryoid bodies. H. Expression analysis of differentiation markers. I. Teratoma showing a normal ectodermal, mesodermal and endodermal differentiation.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Immunocytochemistry	Staining of stemness markers: TRA-1–60 and OCT4	Fig. 1F
	Quantitative analysis RT-qPCR	Expression of stemness markers: hOCT4, hLIN28, hL-MYC, h SOX2, hKLF4	Fig. 1D-E
Genotype	Karyotype (G-banding) and resolution	46 XX, Resolution 450–500	Fig. 1B
Identity	STR analysis	All the 17 sites tested matched	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	NM_015560.2c. [124C > T]	Fig. 1C
Microbiology and virology	Mycoplasma	Mycoplasma tested by N-Garde Mycoplasma PCR kit (EuroClone) is Negative	Fig. 1 supplementary
Differentiation potential	Embryoid body formation and Teratoma formation	Embryoid bodies morphology, Genes expressed in embryoid bodies: SOX1, NESTIN, PAX6, EOMES, T, GATA4, FOXA2, SOX17. Proof of teratoma three germ layers formation.	Fig. 1G-H-I
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

5.5. Pluripotency assay

hiPSCs, after XIV passage, were transferred in floating conditions in 25 cm flasks. Nutristem-XF medium was gradually switched with DMEM F-12, 20 % Knock-out serum replacement (Gibco), 0.1 mM β-mercaptoethanol, 1 × NEAA, 50 U/ml Penicillin-Streptomycin, 2 mM l-glutamine. After 14 days, hiEBs were collected and characterized.

5.6. Sequencing

Genomic DNA was extracted from fibroblasts and iPSC using QIAamp DNA Blood Mini Kit (Qiagen). NM_015560.2: exon 2 was PCR-amplified by using specific primers (Table 2). The purified amplicon was sequenced by BigDye terminator v.3.1 Cycle Sequencing kit on ABI 3100 Genetic Analyzer (Applied Biosystems).

5.7. Teratoma formation and animal ethics

For teratoma formation, dyspase-treated hiPSCs derived from 6-well plates were resuspended in 100 µl Matrigel and injected into NOD/SCID mice according to ethical guidelines. After 9 weeks, the teratomas were removed for histological analysis with haematoxylin/eosin.

All animal care and experimental procedures were carried out according to the current national and international animal ethics guidelines and were approved by the Italian Ministry of Health (protocol

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit anti-OCT4; Mouse anti-TRA-1–60	1:100 1:100	Life technologies (A13998); Life technologies (411000)	RRID: AB 2534182; RRID: AB_2533494
Secondary antibodies	Anti-Rabbit AlexaFluor 488; Anti-Mouse AlexaFluor 555	1:1000 1:1000	Invitrogen (A11034); Invitrogen (A21422)	RRID: AB_2576217; RRID: AB_2535844
	Primers			
SYBR green Primers used for qPCR	**Target**	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal Plasmids (qPCR)	eOCT4	83 bp	Fwd: CAT TCA AAC TGA GGT AAG GG Rev: TAG CGT AAA AGG AGC AAC ATA G	
	eLIN28	205 bp	Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C Rev: TAG CGT AAA AGG AGC AAC ATA G	
	eL-MYC	80 bp	Fwd: GGC TGA GAA GAG GAT GGC TAC Rev: TTT GTT TGA CAG GAG CGA CAA T	
	eSOX2	66 bp	Fwd: TTC ACA TGT CCC AGC ACT ACC AGA Rev: TTT GTT TGA CAG GAG CGA CAA T	
	eKLF4	112 bp	Fwd: CCA CCT CGC CTT ACA CAT GAA GA Rev: TAG CGT AAA AGG AGC AAC ATA G	
Stemness Markers (qPCR)	OCT4	179 bp	Fwd: TTG CTG CAG AAG TGG GTG GA Rev: TGG CTG ATC TGC TGC AGT GT	
	LIN28	169 bp	Fwd: TGA GAG GCG GCC AAA AGG AA Rev: CAG CGG ACA TGA GGC TAC CA	
	L-MYC	142 bp	Fwd: GCG AAC CCA AGA CCC AGG CCT GCT CC Rev: CAG GGG GTC TGC TCG CAC CGT GAT G	
	SOX2	80 bp	Fwd: TTC ACA TGT CCC AGC ACT ACC AGA Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC	
	KLF4	166 bp	Fwd: TCT CAA GGC ACA CCT GCG AA Rev: CCT GGA AAA TGC TCG GTC GC	
House-Keeping Genes (qPCR)	β-ACTIN	203 bp	Fwd: GGC ATCCTC ACC CTGAAG TA Rev: GGG GTGTTG AAG GTCTCA AA	
TaqMan primers used for qPCR	**Target**		**Probe**	
Differentation markers	SOX1		Hs01057642_s1	
	NESTIN		Hs04187831_g1	
	PAX6		Hs00240871_m1	
	T		Hs00610080_m1	
	EOMES		Hs00172872_m1	
	GATA4		Hs00171403_m1	
	FOXA2		Hs00232764_m1	
	SOX17		Hs00751752_s1	

(continued on next page)

Table 2 (*continued*)

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
	β-ACTIN		Hs 99999903_m1	
Targeted mutation analysis/ sequencing	OPA1	269 bp	Fwd: GGG CTG TGT TTC CTT TAG TAT ATT GC Rev: GGA AGG TTT GTC AGA GAA GAG AAC TGC	

number #751/2019-PR). We used (n = 3) female Nude-Foxn 1nu mice and we analyzed 1 teratoma. The animals were monitored weekly to assess the presence and growth of the teratoma, which were measured using a precision caliper (maximum permitted size of the teratoma: 12 mm in the largest diameter). Daily monitoring of animal included: general welfare status and any skin alterations in the injection area (ulcerations, scratches). Human endpoints included: signs or symptoms of toxicity (persistent and continuous weight loss, difficulty walking, significant skin ulcers).

CRediT authorship contribution statement

Angela Maria Giada Giovenale: Writing – original draft, Resources, Data curation. **Ilaria Ferrone:** Resources. **Silvia Tomaselli:** Resources. **Elisa Maria Turco:** Supervision, Resources. **Martina Mazzoni:** Resources. **Barbara Torres:** Resources. **Paola Zanfardino:** Resources. **Edvige Vulcano:** Resources. **Daniela Ferrari:** Resources. **Devid Damiani:** Resources. **Filippo M. Santorelli:** Resources. **Alessandro De Luca:** Resources. **Maria Pennuto:** Resources. **Angelo Luigi Vescovi:** Supervision. **Vittoria Petruzzella:** Writing – review & editing. **Jessica Diana Rosati:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization.

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.

Acknowledgements

This work was supported by a grant from the Italian Ministry of Health, R25-5 × 1000 to JR; a grant from Fondazione Prosolidar, 508-2021_IT to ALV and JR.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2026.104022.

Data availability

No data was used for the research described in the article.

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