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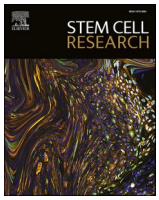

Generation and characterization of the CSSi021-A (15665) human induced pluripotent stem cell line from a Smith-Magenis syndrome patient with a heterozygous RAI1 mutation

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A B S T R A C T

Smith-Magenis syndrome (SMS) is a rare neurodevelopmental disorder caused by haploinsufficiency of the Retinoic Acid Induced 1 (RAI1) gene located at 17p11.2. It is estimated that approximately 90% of patients have a 17p11.2 deletion, including the RAI1 gene, while the remaining 10% exhibit a heterozygous mutation in the RAI1 gene. In this study, we report the generation of a human induced pluripotent stem cell (hiPSC) line derived from a 14-year-old female with an RAI1 mutation, which led to the onset of the SMS phenotype, starting from primary fibroblasts.

Resource Table:

Unique stem cell line identifier	CSSi021-A (15665)
Alternative name(s) of stem cell line	*RAI1-Q214X CL E*
Institution	IRCCS Casa Sollievo della Sofferenza
Contact information of distributor	Jessica Rosati; j.rosati@operapadrepio.it
Type of cell line	
Origin	*Human*
Additional origin info required for human ESC or iPSC	*Age: 14* *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	*Smith-Magenis syndrome*
Gene/locus	*NM_030665:c.[640C > T]*
Date archived/stock date	March 2023
Cell line repository/bank	https://hpscreg.eu/cell-line/CSSi021-A
Ethical approval	*Casa Sollievo della Sofferenza Ethical Committee, approval number: 136/CE*

1. Resource utility

Smith-Magenis syndrome is a rare neurodevelopmental disorder, with its molecular mechanisms still largely unknown, in part due to the

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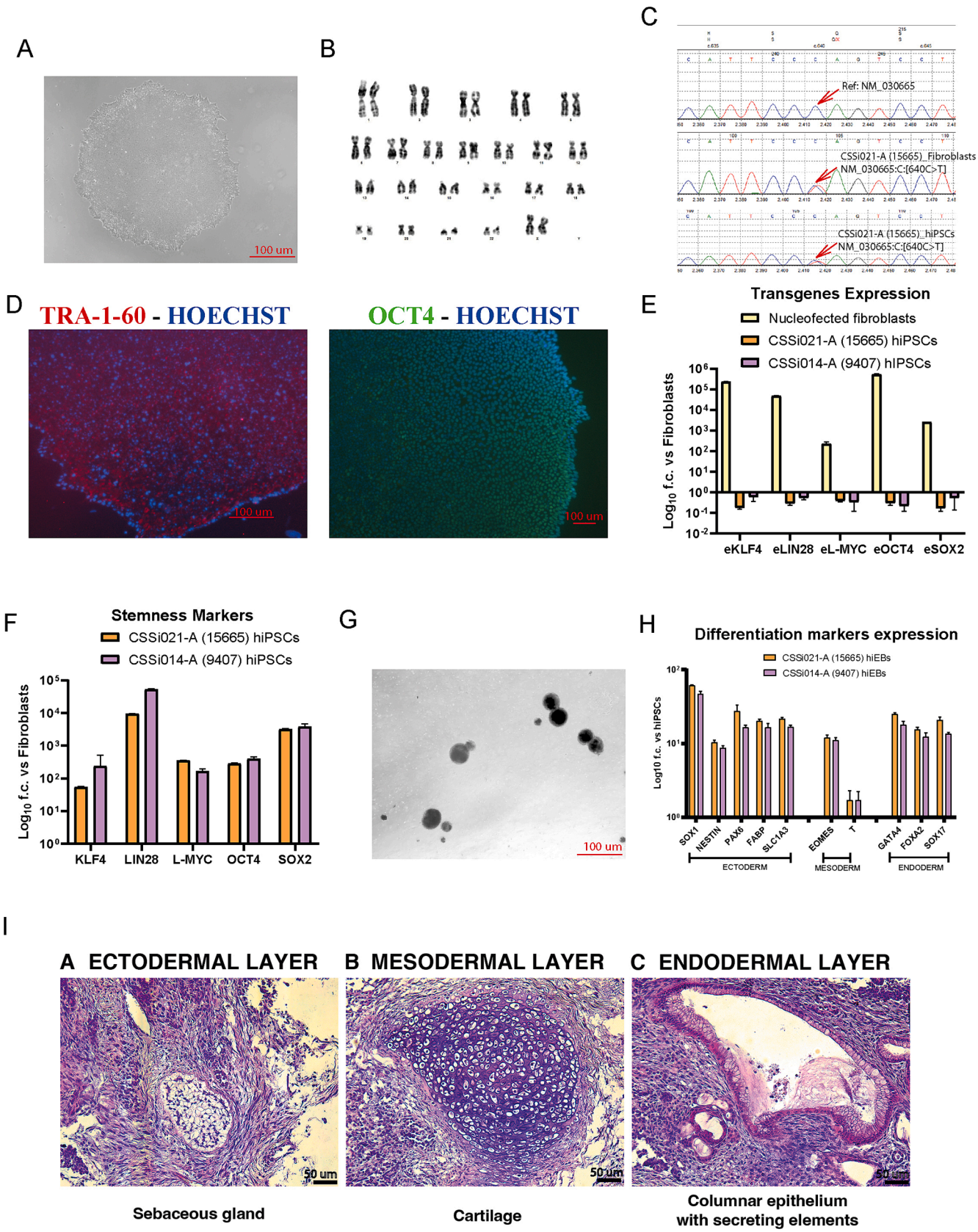
A
100 um
B
C
Ref: NM_030665
CSSi021-A (15665)_Fibroblasts
NM_030665:C:[640C>T]
CSSi021-A (15665)_hiPSCs
NM_030665:C:[640C>T]
D
TRA-1-60 - HOECHST
100 um
OCT4 - HOECHST
100 um
E
Transgenes Expression
Nucleofected fibroblasts
CSSi021-A (15665) hiPSCs
CSSi014-A (9407) hIPSCs
Log10 f.c. vs Fibroblasts
eKLF4 eLIN28 eL-MYC eOCT4 eSOX2
F
Stemness Markers
CSSi021-A (15665) hiPSCs
CSSi014-A (9407) hiPSCs
Log10 f.c. vs Fibroblasts
KLF4 LIN28 L-MYC OCT4 SOX2
G
100 um
H
Differentiation markers expression
CSSi021-A (15665) hiEBs
CSSi014-A (9407) hiEBs
Log10 f.c. vs hiPSCs
SOX1 NESTIN PAX6 FABP SLC1A3 EOMES T GATA4 FOXA2 SOX17
ECTODERM
MESODERM
ENDODERM
I
A ECTODERMAL LAYER
B MESODERMAL LAYER
C ENDODERMAL LAYER
50 um
50 um
50 um
Sebaceous gland
Cartilage
Columnar epithelium
with secreting elements

Fig. 1.

lack of an effective cellular model. Human induced pluripotent stem cells (hiPSCs) offer an ideal platform to study the underlying pathology of the disease and unlock critical insights into its mechanisms.

2. Resource Details

Smith-Magenis syndrome (SMS, OMIM #182290) is a complex neurobehavioral disorder caused by haploinsufficiency of the Retinoic Acid-Induced Gene 1 (RAI1) due to a 17p11.2 deletion (90 %) or a mutation in the RAI1 gene (10 %). With a prevalence of 1 in 25,000, SMS manifests with mental retardation, distinct behavioral features, craniofacial and skeletal abnormalities, hyperphagia, obesity, and sleep disturbances (A. C. M. Smith and Gropman 2021). While RAI1 is known to be a transcriptional factor, its precise role remains poorly understood (Carmona-Mora and Walz 2010; Atanesyan et al. 2012).

We collected skin fibroblasts from a patient with a heterozygous mutation, NM_030665:c.[640C > T], which generates a premature stop codon, resulting in a truncated protein (p.(GLN214*). Reprogramming was achieved using non-integrative vectors containing the reprogramming factors lin28, Oct4, c-Myc, Klf4, and Sox2. We selected iPSC colonies that displayed a uniform, flat, and stem cell-like morphology (Fig. 1A) and characterized them. Short tandem repeat (STR) analysis confirmed the genetic identity and stability of the iPSCs, showing identical DNA profiles to the parental fibroblasts (Submitted to Journal in archive), and the karyotype was confirmed as normal (46,XX) (Fig. 1B). Genomic DNA sequencing confirmed the presence of the disease-related mutation (p.Q214*; c.640C > T) in the iPSCs (Fig. 1C). Immunofluorescence staining for the surface marker TRA-1–60 and the nuclear marker OCT4 confirmed their expression (Fig. 1D).

After 15 passages in vitro, qRT-PCR analysis revealed the loss of exogenous reprogramming factors and the activation of endogenous genes (KLF4, LIN28, OCT4, L-MYC, and SOX2) (Fig. 1E-F). We used fibroblast cells as a negative control and a previously published hiPSC line (CSSi014-A (9407) (Casamassa et al. 2022)) as a reference control.

We further characterized the ability of the iPSCs to differentiate into all three embryonic germ layers. In vitro, the iPSCs formed embryoid bodies (EBs), which were analyzed for differentiation markers at 14 days using qRT-PCR (Fig. 1G-H). For in vivo analysis, the iPSCs were injected into an immunodeficient mouse, where they formed a teratoma—a tumor containing all three germ layers, as confirmed by immunohistochemical analysis (Fig. 1I). Throughout all procedures, the cells were regularly tested for Mycoplasma contamination (Supplementary File 1), with no contamination detected.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Staining of stemness markers: Oct4 and Tra1-60	Fig. 1 panel D
	Quantitative analysis RT-qPCR	Expression of stemness markers: OCT4, LIN28, L-MYC, SOX2, KLF4	Fig. 1 panel E and F
Genotype	Karyotype (G-banding) and resolution	46 XX, Resolution 450–500	Fig. 1 panel B
Identity	STR analysis	All the 17 sites tested matched	Submitted to Journal in archive
Mutation analysis (IF APPLICABLE)	Sequencing	NM_030665:c.[640C > T]	Fig. 1 panel C
Microbiology and virology	Mycoplasma	Mycoplasma tested by N-Garde Mycoplasma PCR kit (EuroClone) isNegative	Fig. 1 supplementary
Differentiation potential	Embryoid body formation and Teratoma formation	Embryoid bodies morphology, expressed genes in embryoid bodies: SOX1, NESTIN, PAX6, EOMES, T, GATA4, FOXA2, SOX17. Three germ layers in the teratoma.	Fig. 1 panel G, H and 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	

3. Materials and methods

3.1. 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 IV, 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). iPSCs were tested periodically for the absence of mycoplasma contamination using an N-Garde Mycoplasma PCR kit.

3.2. 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 (Table 1) 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.

3.3. Karyotype analysis

For karyotyping, iPSCs at passage IX 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.

3.4. Immunofluorescence staining

iPSCs 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.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Stemness Markers	Rabbit anti-OCT4; Mouse anti-TRA-1–60	0.1111 0.1111	Life technologies (A13998); Life technologies(411000)	RRID: AB 2534182;RRID: AB_2533494
Secondary antibodies	Anti-Rabbit AlexaFluor 488; Anti-Mouse AlexaFluor555	0.7361 0.7361	Invitrogen (A11034); Invitrogen(A21422)	RRID: AB_2576217; RRID: AB_2535844
SYBR green Primers used for qRT-PCR	**Primers** **Target**	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal Plasmids (qRT-PCR)	eOCT4	83 bp	Fwd: CAT TCA AAC TGA GGT AAG GG	
		205 bp	Rev: TAG CGT AAA AGG AGC AAC ATA G	
	eL-MYC		Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C	
	eSOX2		Rev: TAG CGT AAA AGG AGC AAC ATA G	
			Fwd: GGC TGA GAA GAG GAT GGC TAC	
	eKLF4	112 bp	Rev: TTT GTT TGA CAG GAG CGA CAA T	
			Fwd: TTC ACA TGT CCC AGC ACT ACC AGA	
			Rev: TTT GTT TGA CAG GAG CGA CAA T	
			Fwd: CCA CCT CGC CTT ACA CAT GAA GA	
			Rev: TAG CGT AAA AGG AGCAAC ATA G	
Stemness Markers (qRT-PCR)	Oct-04	179 bp	Fwd: TTG CTG CAG AAG TGG GTG GA	
			Rev: TGG CTG ATC TGC TGC AGT GT	
	l-MYC	169 bp		
			Fwd: TGA GAG GCG GCC AAA AGG AA	
			Rev: CAG CGG ACA TGA GGC TAC CA	
	SOX2	142 bp		
			Fwd: GCG AAC CCA AGA CCC AGG CCT GCT CC	
			Rev: CAG GGG GTC TGC TCG CAC CGT GAT G	
	KLF4	80 bp		
			Fwd: TTC ACA TGT CCC AGC ACT ACC AGA	
			Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC	
		166 bp	Fwd: TCT CAA GGC ACA CCT GCG AA	
			Rev: CCT GGA AAA TGC TCG GTC GC	
House-Keeping Genes (qRT-PCR)	β-ACTIN	203 bp	Fwd: GGC ATCCTC ACC CTGAAG TA	
			Rev: GGG GTGTTG AAG GTCTCA AA	
TaqMan primers used for qRT-PCR	**Target**		**Probe**	
Differentationmarkers	SOX1		Hs01057642_s1	
	NESTIN		Hs04187831_g1	
	PAX6		Hs00240871_m1	
	T		Hs00610080_m1	
	EOMES		Hs00172872_m1	
	GATA4		Hs00171403_m1	
	FOXA2		Hs00232764_m1	
	SOX17β-ACTIN		Hs00751752_s1Hs 99999903_m1	
Targeted mutation analysis/sequencing	RAI1	229 bp	Fwd: TCACTCCCTGCACGTCCA	
			Rev: GGCAGTCGGTGCTGTGCAAC	

3.5. Pluripotency assay

iPSCs, at XVI passage, were picked up and 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 fourteen days, EBs were ready for the characterization. To evaluate the in vivo teratoma formation, iPSCs, combined with 100ul Matrigel, were injected into the flank of immunodeficient mice. The teratoma was used for histological analysis with hematosilin/eosin staining.

3.6. STR analysis

Dneasy blood and tissue kit (QIAGEN) was used for DNA extraction. 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).

3.7. Sequencing

Genomic DNA was extracted from fibroblasts and iPSC using QIAamp DNA Blood Mini Kit (Qiagen). IDS 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).

CRediT authorship contribution statement

Angela Maria Giada Giovenale: Writing – original draft, Resources, Data curation. **Elisa Maria Turco:** Resources. **Ilaria Ferrone:** Resources. **Chiara Giacometti:** Resources. **Silvia Tomaselli:** Resources. **Edvige Vulcano:** Resources. **Daniela Ferrari:** Resources. **Ornella Candido:** Resources. **Laura Bernardini:** Resources. **Alessandro De Luca:** Resources. **Nadia Trivieri:** Resources. **Elena Binda:** Resources. **Roberta Onesimo:** Resources. **Stefano D'Arrigo:** Resources. **Giuseppe Zampino:** Resources. **Maria Pennuto:** Resources, Funding acquisition. **Angelo Luigi Vescovi:** Conceptualization. **Jessica 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.

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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.2025.103726.

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