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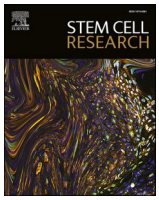

Lab Resource: Single Cell Line

Generation of the CSSi020-A (14437) iPSC line from a patient carrying a copy number variation (CNV) in the 17p11.2 chromosome region

Angela Maria Giada Giovenale [a,b,1], Elisa Maria Turco [a,1], Martina Mazzoni [a], Ilaria Ferrone [a], Barbara Torres [e], Laura Bernardini [e], Edvige Vulcano [b], Daniela Ferrari [b], Roberta Onesimo [g], Stefano D'Arrigo [f], Giuseppe Zampino [g,h], Maria Pennuto [c,d], Alessandro De Luca [e], Angelo Luigi Vescovi [a], Jessica Rosati [a,*]

[a] Cellular Reprogramming Unit, Fondazione IRCCS Casa Sollievo della Sofferenza, Viale dei Cappuccini, 71013 San Giovanni Rotondo, FG Italy
[b] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
[c] Veneto Institute of Molecular Medicine (VIMM), via Orus 2, 35129 Padova, Italy
[d] Department of Biomedical Sciences, University of Padova, via Ugo Bassi 58/B, 35131 Padova, Italy
[e] Medical Genetics Unit, Fondazione IRCCS Casa Sollievo della Sofferenza, Viale dei Cappuccini, 71013 San Giovanni Rotondo, Italy
[f] Department of Pediatric Neuroscience, Fondazione IRCCS Istituto Neurologico Carlo Besta, Via Giovanni Celoria, 11, 20133 Milano, Italy
[g] Rare Diseases and Birth Defects Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Largo Agostino Gemelli 8, 00168 Rome, Italy
[h] Dipartimento di Scienze della Vita e Sanità Pubblica, Università Cattolica del S. Cuore, Largo Francesco Vito, 1, 00168 Rome, Italy

A B S T R A C T

Smith-Magenis syndrome (SMS) is a complex neurodevelopmental disorder with a birth incidence of 1:25,000. SMS is caused by haploinsufficiency of the retinoic acid-induced retinoic acid1 (RAI1) gene, determined by an interstitial deletion of ~ 3.7 Mb (17p11.2, including the RAI1 gene) in 90 % of cases and a mutation on the RAI1 gene in only 10 % of cases. We generated and characterized a human pluripotent stem cell line (hIPSCs) derived from primary fibroblasts of a 17-year-old woman carrying a 17p11.2 deletion including the RAI1 gene.

Resource Table:

Unique stem cell line identifier	*CSSi020-A (14437)*
Alternative name(s) of stem cell line	*SMSdel1 cl Q*
Institution	Fondazione IRCCS Casa Sollievo della Sofferenza
Contact information of distributor	Jessica ROSATI; j.rosati@css-mendel.it
Type of cell line	*iPSC*
Origin	*human*
Additional origin info requiredfor human ESC or iPSC	*Age: 17 Sex: femaleEthnicity : Caucasian*
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	*Chr17:16761815–20462723 x1, build hg19*
Date archived/stock date	*October 2021*
Cell line repository/bank	*https://hpscreg.eu/cell-line/CSSi020-A*
Ethical approval	*Casa Sollievo della Sofferenza Ethical Committee, approval number:* 136/*CE*

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1. Resource utility

Smith-Magenis syndrome (SMS) is a neurodevelopmental disorder characterized by cognitive and behavioral symptoms, obesity and sleep disorders. We generated an iPSC line from fibroblasts of a patient with SMS carrying a 3.3 MB deletion (Table 1). *It will be used to create a model system for studying the disease.*

* Corresponding author.
E-mail address: j.rosati@css-mendel.it (J. Rosati).
[1] These two authors contribute equally to the work.

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	*Normal*	Fig. 1 *panel A*
Phenotype	*Immunocytochemistry*	*Staining of pluripotency markers: Oct4 and Tra1-60*	Fig. 1 *panel D*
	Quantitative analysis *RT-qPCR*	*Expression of pluripotency markers: OCT4, LIN28, L-MYC, SOX2*	Fig. 1 *panel E and panel F*
Genotype	Karyotype (G-banding) and resolution	*46XX, Resolution 450–500*	Fig. 1 *panel B*
Identity	STR analysis	*All the 17 sites tested matched*	*submitted in archive with journal*
Mutation analysis (IF APPLICABLE)	*CNV analysis*	*SNP-array*	Fig. 1 *panel C*
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. 1 *panel G, panel H and panel 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*	

2. Resource details

Smith-Magenis syndrome (SMS, OMIM #182290) is a rare neurobehavioral disorder caused by haploinsufficiency of Retinoic Acid-Induced Gene 1 (RAI1) which is determined by 17p11.2 chromosomal deletion (90 %) or a point-mutation directly on RAI1 gene (10 %).

SMS phenotype shows mental and behavioral features, craniofacial and skeletal abnormalities, infant obesity and an alteration of circadian rhythm (Rinaldi et al. 2022). The primary fibroblast line, derived from skin biopsy, carries a heterozygous deletion of 3.7 MB (17p11.2) which starts from nucleotide position 16,761,815 and ends to position 20,426,723 including RAI1 gene. RAI1 is the causative gene of Smith-Magenis Syndrome but its roles are still largely unknown (Carmona-Mora and Walz 2010). Reprogramming was achieved through non-integrative vectors containing factors as lin28, oct4, c-myc, klf4 and sox2. We selected and characterized iPSC colonies with a circular, uniform, stem cell-like shape (Fig. 1A). To confirm the absence of chromosomal rearrangements, we analyzed the karyotype of iPSCs, which was normal (46, XX) (Fig. 1B). The presence of the 3.3 Mb deletion was confirmed in both iPSCs and starting fibroblasts (Fig. 1C). We performed immunofluorescence staining with an antibody against the surface marker TRA-1–60 and the nuclear marker OCT4 (Fig. 1D). We verified the absence of exogenous reprogramming factors and the turn-on of endogenous ones (KLF4, LIN28, OCT4, L-MYC and SOX2) through qRT-PCR (Fig. 1E-F). Pluripotency was confirmed both in vitro and in vivo. In vitro, iPSCs spontaneously aggregated into embryoid bodies (EBs) that, after 14 days of differentiation, expressed genes belonging to all three embryonic layers (Fig. 1G-H). This analysis was performed using fibroblast cells as a negative control and a previously published hiPSCs line, CSSi013-A (9360) (D'Anzi et al., 2022), as a reference control. In vivo, hiPSCs, introduced into an immunodeficient mouse, formed a teratoma that was analyzed by immunohistochemical analysis that demonstrated the presence of the three embryonic layers (Fig. 1I). Finally, short tandem repeats (STR) analysis showed that the DNA profile of the parental fibroblasts was the same as that of the derived iPSCs (data available from the authors). All cells were periodically tested as negative for Mycoplasma contamination (Supplementary File 1).

3. Materials and methods

3.1. Fibroblast culture and reprogramming method

SMS fibroblasts were maintained in culture in High Glucose Dulbecco's Modified Eagle Medium supplemented with 20 % FBS, 1 % L-Glutamine, Penicillin-Streptomicin, Non-Essential Amino Acids (SigmaAldrich), at 37 °C and 5 % CO2. Fibroblasts (passage IV) were nucleofected with the following episomal plasmids pCXLE-hUL (Addgene #27080), pCXLE-hSK (Addgene #27078) and pCXLE-hOCT4-shp53 (Addgene #27077) using 4D-Nucleofector™ X-unit (Lonza), FF113 program and P2 buffer. Then, fibroblasts were plated into a Matrigel-pretreated dish (Corning) and grown in NutristemXF medium (Biological-Industries). Colonies were picked and expanded. The hiPSCs were tested for the absence of mycoplasma contamination using N-grade Mycoplasma PCR kit.

3.2. Immunofluorescence staining

iPSCs were fixed using 4 % paraformaldehyde for 20 min *at RT and blocked in PBS with 20 % Normal Goat Serum for TRA-1–60 staining and 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. Secondary antibodies were added for 1,5 h at RT. Cellular nuclei were stained with Hoechst. Images were taken using a Nikon C2 fluorescence microscope.*

3.3. Karyotype analysis

iPSCs were cultured in Nutristem XF medium for 2–3 days and then treated with a 0.1 µg*/ml COLCEMID solution (ThermoFisher Scientific) for 60* min *at 37 °C to obtain metaphases. Karyotype analysis was carried out on GTG-banding. Thirty metaphases were counted.*

3.4. qPCR analyses

Total RNA was isolated using TRIzol reagent (Life Technologies). After acquisition of RNA integrity through RNA 6000 Nano LabChips (Agilent Technologies), we produced cDNA using High Capacity cDNA Reverse Transcription Kit (Applied-Biosystem). Specific primers for stemness, pluripotency and episomal genes (Table 2) were used to perform qPCR expression analysis, each reaction in triplicate with β-ACTIN gene as reference. For gene expression we adopted 2–ΔΔCT method.

3.5. Embryoid bodies and teratoma formation assay

For the embryoid body formation, the hIPSCs were picked-up and transferred in floating conditions. Nutristem-XF was gradually switched into DMEM F-12, 20 % Knock-out serum replacement (Gibco), 0.1 mM β-mercaptoethanol, and 1 % of NEAA, Penicillin-Streptomicin, and l-glutamine in 3 days. After fourteen days, EBs were collected. For teratoma formation, about 1 million of hiPSCs, combined with Matrigel, were injected into immunodeficient mice. Once formed the teratomas were collected for

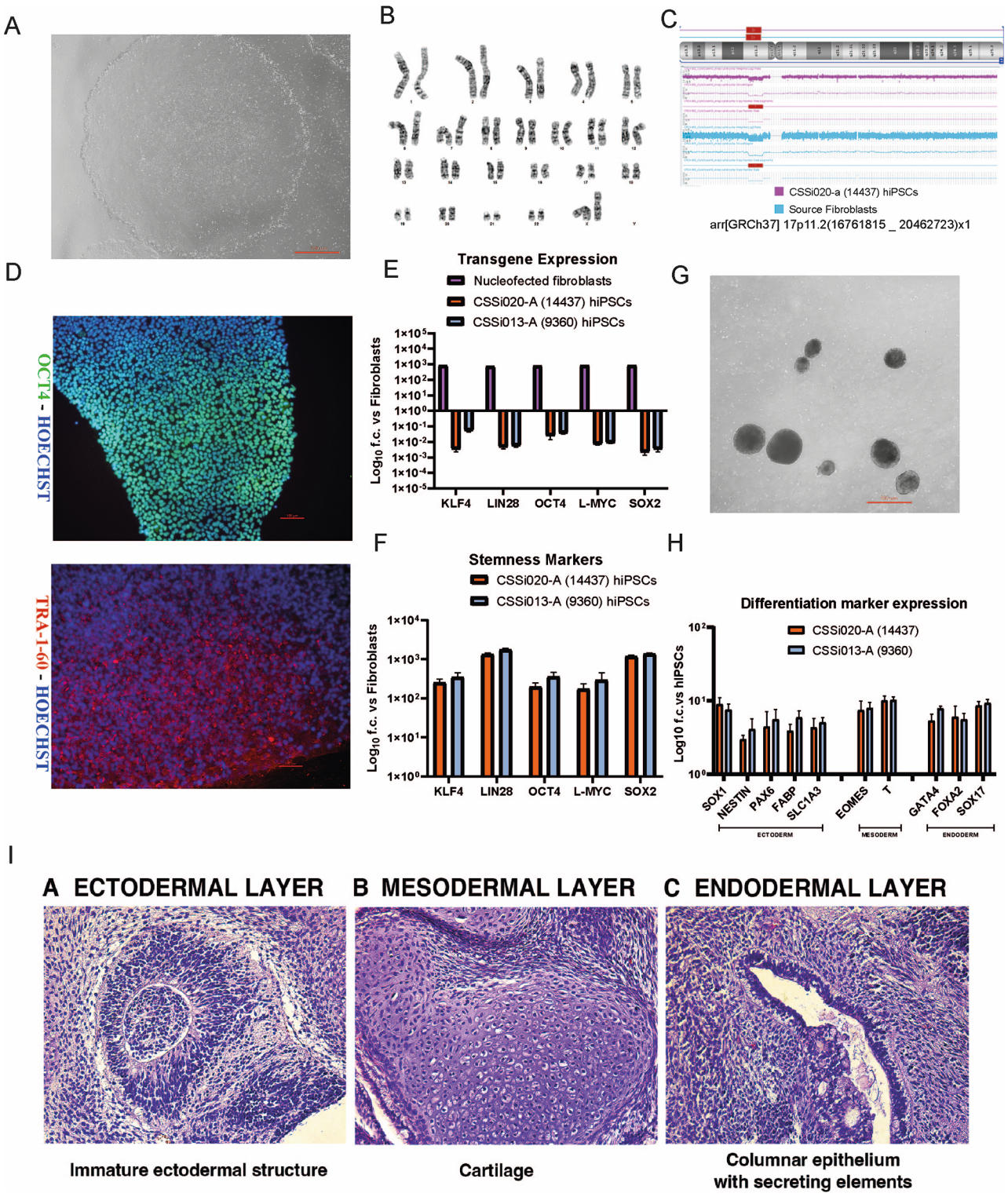

Fig. 1.

histological analysis.

3.6. STR analysis and CNV analysis

*Dneasy blood and tissue kit (QIAGEN) was used for DNA extraction. 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). CNV analysis was performed using a SNP-array platform (Cytoscan HD, ThermoFisher, USA) using 250 ng of genomic DNA hybridized for 16–18 h on the Cytoscan HD chip at 50 °C. The chips were washed, stained in GeneChip Fluidic Station 450 and scanned with Scanner 3000 7G. Data were analyzed with ChAS software (v4.3; ThermoFisher). Two hundred and seventy healthy controls of the International HapMap Project were used as a reference sample in the analysis (ThermoFisher).*

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*	*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 plasmid genes (qPCR)	*eOCT4*	*83 bp*	*Fwd: CAT TCA AAC TGA GGT AAG GG*	
		205 bp	*Rev: TAG CGT AAA AGG AGC AAC ATA G*	
	eLIN28			
	eL-MYC	*80 bp*	*Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C*	
	eSOX2	*66 bp*	*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*	
Stemness Markers (qPCR)	*OCT4*	*179 bp*	*Fwd: TTG CTG CAG AAG TGG GTG GA*	
	LIN28	*169 bp*	*Rev: TGG CTG ATC TGC TGC AGT GT*	
	L-MYC			
		142 bp	*Fwd: TGA GAG GCG GCC AAA AGG AA*	
	SOX2		*Rev: CAG CGG ACA TGA GGC TAC CA*	
		80 bp		
	KLF4		*Fwd: GCG AAC CCA AGA CCC AGG CCT GCT CC*	
		166 bp	*Rev: CAG GGG GTC TGC TCG CAC CGT GAT G*	
			Fwd: TTC ACA TGT CCC AGC ACT ACC AGA	
			Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC	
			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***	
Pluripotency 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*	
	β-ACTIN		*Hs 99999903_m1*	

CRediT authorship contribution statement

Angela Maria Giada Giovenale: Writing – original draft, Data curation. **Elisa Maria Turco:** Data curation. **Martina Mazzoni:** Data curation. **Ilaria Ferrone:** Data curation. **Barbara Torres:** Data curation. **Laura Bernardini:** Data curation. **Edvige Vulcano:** Data curation. **Daniela Ferrari:** Data curation. **Roberta Onesimo:** Data curation. **Stefano D'Arrigo:** Data curation. **Giuseppe Zampino:** Data curation. **Maria Pennuto:** Data curation. **Alessandro De Luca:** . **Angelo Luigi Vescovi:** Supervision. **Jessica Rosati:** Writing – review & editing, Supervision, Funding acquisition, Data curation.

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.2024.103544.

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