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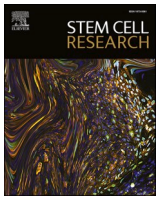

Lab Resource: Single Cell Line

Generation of *IGGi003-A* induced pluripotent stem cell line from a patient with Sotos Syndrome carrying c.1633delA NSD1 variant in exon 5

Giuseppina Conteduca [a], Chiara Baldo [b], Alessia Arado [b], Joana Soraia Martinheira da Silva [b], Barbara Testa [b], Simona Baldassari [c], Federico Zara [c], Gilberto Filaci [a,d], Domenico Coviello [b,*], Michela Malacarne [b]

[a] *Biotherapy Unit, IRCCS San Martino, Genoa, Italy*
[b] *Laboratory of Human Genetics, IRCCS Istituto Giannina Gaslini, Genova, Italy*
[c] *Unit of Medical Genetics, IRCCS Istituto Giannina Gaslini, Genova, Italy*
[d] *Department of internal Medicine (DIMI), University of Genoa, Genoa, Italy*

A B S T R A C T

Sotos syndrome (SoS) is a neurodevelopmental disorder that results from *NSD1* mutations that cause haploinsufficiency of NSD1. Here, we generated an induced pluripotent stem cell (iPSC) line from fibroblasts of a SoS patient *carrying the pathogenic variant (c.1633delA). The cell line shows typical iPSC morphology, high expression of pluripotent markers, normal karyotype, and it differentiates into three germ layers in vitro. This line is a valuable resource for studying pathological pathways involved in SoS.*

1. Resource Table:

Unique stem cell line identifier	*IGGi003-A*
	IGGi003-A
Alternative name(s) of stem cell line	*N/A*
Institution	*IRCCS Istituto Giannina Gaslini*
Contact information of distributor	*geneticbiobank@gaslini.org*
Type of cell line	*iPSC*
Origin	*human*
Additional origin info required for human ESC or iPSC	*Age: 17*
	Sex: F
	Ethnicity: Caucasian
Cell Source	*fibroblasts*
Clonality	*Clonal*
Method of reprogramming	*Non-integrating vector*
Genetic Modification	*yes*
Type of Genetic Modification	*De novo germline mutation*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*RT-/q-PCR*
Associated disease	*Sotos Syndrome*
Gene/locus	*chr5:177,131,830–177,300,213*
Date archived/stock date	*26/11/2023*
Cell line repository/bank	*https://hpscreg.eu/user/cellline/edit/IGGi003-A?source_platform = hescreg*
Ethical approval	This study was approved by the Ethics Committee of the Liguria Region, Italy (Approval #OG01IGG on 12/7/2021).

2. Resource utility

This iPSC line, generated from an individual carrying a pathogenic variant in NSD1 gene, provide an unlimited source for differentiating iPSC-derived neuronal cell *in vitro*. This is an excellent model to elucidate pathological pathways involved in SoS and to test a personalized and innovative therapeutic approach. Table 1..

3. Resource details

Sotos Syndrome (SoS) (OMIM #117550) is an autosomal dominant neurogenetic disorder caused by inactivating point mutations or deletion in NSD1 gene (NM 022455.4). Clinical features include intellectual disability, overgrowth, variable degrees of intellective disability, multiple congenital anomalies, and developmental delay (Tatton-Brown et al., 2005, Sotos et al., 1964, Kondoh et al., 2003, Testa et al., 2023). NSD1 encodes for a histone methyltransferase (nuclear-receptor-binding SET-domain-containing protein 1), and is implicated in chromatin structure regulation, histone modification, and cell cycle regulation by checkpoints (Conteduca et al., 2022a, Farhangdoost et al., 2021, Lucio-Eterovic et al.,2010, Conteduca et al., 2022b).

To establish a patient-derived cellular model of SoS, we

* Corresponding author at: Laboratory of Human Genetics, IRCCS Istituto Giannina Gaslini, via Gerolamo Gaslini, 5, 16147, Genoa, Italy.
E-mail address: domenicocoviello@gaslini.org (D. Coviello).

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reprogrammed fibroblast cells from a female patient with clinical diagnosis of SoS, carrying a c.1633delA (p. (Thr545Argfs*10)) mutation located in exon 5 of NSD1 (Fig. 1, panel F). Fibroblasts were obtained from skin biopsies punch and fibroblasts at passage 4 (P4) were transfected with CytoTune-iPS 2.0 Sendai reprogramming kit (Baldassari et al., 2022). The generated iPSC lines presented the typical stem cell morphology with tightly packed colonies and high nuclear/cytoplasmic ratio (Fig. 1, panels A). iPSC colonies were picked approximately 20 days post virus transduction. Cytogenetic analysis confirmed a normal female karyotype (46, XX) without structural chromosomal abnormalities (Fig. 1, panels B). RT-PCR analysis confirmed the absence of exogenous Sendai vectors (SeV genome and the transgenes Klf4, KOS and c-Myc), in the IGGi003-A line (P15) (Supplementary Fig. 1A). Short tandem repeat (STR) analysis confirmed that parental fibroblasts and iPSC (P15) clone were both from the same patient. The absence of genomic rearrangement as deletion or duplication was confirmed by Comparative Genomic Hybridisation (CGH) (Table3). We verified the presence of pluripotency markers OCT4, SSEA4, TRA-1–60 and SOX2 by immunofluorescence staining (Fig. 1, panels C), and the gene expression of pluripotency markers, NANOG, SOX2, OCT4, DPPA2, DPPA4, KFLA4, FGF4, REX, RUNX1, and TGDF1 by Real-Time PCR, compared to patient's native fibroblasts (Fig. 1 panel E). All antibodies and primers are listed in Table 2. Furthermore, to confirm the iPSCs pluripotency, high quality cells were differentiated into the three embryonic germ layers. Specific markers for each germ layer were tested by Real Time PCR (Fig. 1, panel E) and immunofluorescence (Fig. 1, panel G). All antibodies and primers are listed in Table2. Finally, the established iPSC line was negative for Mycoplasma (Supplementary Fig. 1B). This cell line will serve as a valuable resource for both basic and translational studies in SoS.

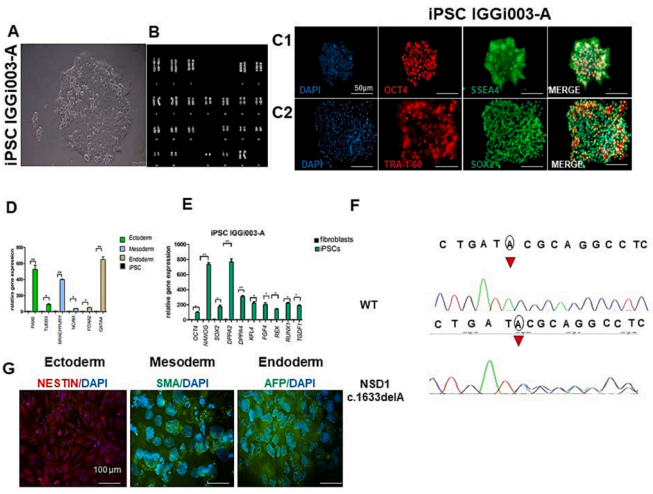

Fig. 1.

4. Materials and methods

4.1. Cell culture

Fibroblast cells were obtained from skin biopsies and cultured in Dulbecco's modified Eagles Medum (DMEM) supplemented with 10 % foetal bovine serum, 2 mM of L-glutamine and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin). We reprogrammed fibroblasts using CytoTune-iPS 2.0 Sendai kit (Thermo Fisher). iPSCs were maintained in Essential 8 media (Thermo Fisher) on plates pre-coated with Vitronectin (5ug/ml) (Thermo Fisher). iPSCs were harvested with Versene (Thermo Fisher), when they reached the 85–90 % confluence. ROCK inhibitor (10uM) was used for first passage. The cells were growth at 37 °C, with 5 % CO2, 20 % O2. RT-PCR was used to confirm if the iPSCs were free of exogenous factors (Primers in Table 2).

4.2. Immunostaining

We examined the pluripotency of iPSCs culture at passage 6 and 20, by Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Thermo Fisher). Briefly, cells were fixed with 4 % formaldehyde for 15 min at room temperature (RT), incubated with permeabilization solution for 15 min at RT, and sequentially with blocking solution for 30 min at RT. Specific primary antibodies for stemness or differentiation (Table 2) were incubated at 4 °C for 3 h. Secondary antibodies were subsequently stained for 1 h at RT. DAPI (0.1 µg/mL) was used to stain nuclei. Images were acquired using Leica SP8 confocal microscope.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis	Both iPSC lines express pluripotency markers: OCT4, SSEA4, TRA-1–60 and SOX2	Fig. 1 panel C
	Quantitative analysis	Both iPSC lines express pluripotency markers: OCT4, NANOG, SOX2, DPPA2, DPPA4, KFLA4, FGF4, REX, RUNX1, and TGDF1	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46 XX, Resolution 450–500	Fig. 1 panel B
Identity	STR analysis	10 markers tested-matched	submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous for c.1633delA (p.(Thr545Argfs*10)) frameshift mutation in *NSD1* gene	Fig. 1 panel F sequencing deposited at LOVD DATABASE: ID 00303612 (https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php? select_db = NSD1)
Microbiology and virology	Mycoplasma	Mycoplasma biochemical test Culture Negative	Supplementary Fig. 1 panel B
Differentiation potential	Directed differentiation	Three germ layers formation, ectoderm, mesoderm and endoderm	Fig. 1 panels G
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) and protein (IF) levels, at least 2 markers need to be shown per germ layer	Expression of germ layer specific markers: ectoderm (PAX6, TUBB3), mesoderm (BRACHYURY, NCAM, SMA) and endoderm (FOXA2, GATA4, AFP).	RT-PCR with reference gene(s): Fig. 1 panel D Immunostaining: Fig. 1 panel G
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

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	*Rabbit anti-OCT4*	1:200	Thermo Fisher Cat# A24867	RRID: AB_2650999
	Mouse anti-SSEA4	1:100	Thermo Fisher Cat# A24866	RRID: AB_2651001
	Mouse anti-TRA-1–60	1:100	Thermo Fisher Cat# A24868	RRID: AB_2651002
	Rat anti-SOX2	1:100	Thermo Fisher Cat# A24759	RRID: AB_2651000
Differentiation Markers	*Mouse anti- αSMA*	1:100	Thermo Fisher Cat# 14–9760-82	RRID: AB_2572996
	Mouse anti-AFP	1:500	Thermo Fisher Cat#MA514666	RRID: AB_10987005
	Mouse anti-NESTIN	1:100	Thermo Fisher Cat# 14–9843-82	RRID: AB_1548837
Secondary antibodies	Alexa Fluor® 594 conjugated donkey anti-rabbit IgG H&L	*1:500*	Thermo Fisher Scientific Cat# A24869	RRID: AB_2651006
	Alexa Fluor® 488 conjugated goat anti-mouse IgG H&L	*1:500*	Thermo Fisher Scientific Cat# A24877	RRID: AB_2651008
	Alexa Fluor®488 conjugated donkey anti-rat IgG H&L	*1:500*	Thermo Fisher Scientific Cat# A24876	RRID: AB_2651007
	Alexa Fluor® 594 conjugated goat anti-mouse IgM	*1:500*	Thermo Fisher Scientific Cat# A24872	Not available
Primers				
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Pluripotency Markers (qPCR)	NANOG	70 bp	Fw:TGTCTTCTGCTGAGATGCCT Rv:AATAAGCAGATCCATGGAGGA	
	OCT4	100 bp	Fw:GAGAAGGATGTGGTCCGAGT Rv:GTGCATAGTCGCTGCTTGAT	
	SOX2	90 bp	Fw:ACCAGCTCGCAGACCTACAT Rv:CCTGCTGCGAGTAGGACAT	
	TDGF1	115 bp	Fw:GGATACCTGGCCTTCAGAGA Rv:CAGGCAGCAGGTTCTGTTTA	
	FGF4	95 bp	Fw:CTCTATGGCTCGCCCTTCT Rv:TGTAGGACTCGTAGGCGTTG	
	REX	120 bp	Fw: GGCCTTCACTCTAGTAGTGCTCA Rv: CTCCAGGCAGTAGTGATCTGAGT	
	KFL4	98 bp	Fw: CGAACCCACACAGGTGAGAA Rv: GAGCGGGCGAATTTCCAT	
	DPPA2	103 bp	Fw: CATGCTTACCCTGAACAACG Rv: GAAGCCTTGCTCTCTTGGTC	
	DPPA4	110 bp	Fw: GAAGAGGATCAGCAGGCTTC Rv: GTTGTCAGTGTGCTCTGCCT	
	RUNX1	90 bp	Fw: GAGGATTTGGTCAGAATGCAG Rv: ACACTGTTCTGAAGTCCTGCTTT	
House-Keeping Genes (qPCR)	GAPDH	120 bp	Fw:AGCAAGAGCACAAGAGGAAGAG Rv:TAACTGGTTGAGCACAGGGTAC	
	PPIA	89 bp	Fw:GGAGGCTTTGAGGTTTTGCAA Rv:CCTGACATCTAACTGCCAGCA	
Differentiation Markers	PAX-6	88 bp	Fw:GATAACATACCAAGCGTGTCATCAATA Rv:TGCGCCCATCTGTTGCT	
	TUBB3	71 bp	Fw:GGCCAAGTTCTGGGAAGTCA Rv:CCGAGTCGCCCACGTAGTT	
	BRACHYURY	96 bp	Fw:GGGTCCACAGCGCATGAT Rv:ATTTTAAGAGCTGTGATCTCCTCGTT	
	NCAM	107 bp	Fw:TCCTGGGAACTGCAGTTTCTCT Rv:TTTGGCATCTCCTGCCACTT	
	FOXA2	60 bp	Fw:TTCAGGCCCGGCTAACTCT Rv:ACCCCCACTTGCTCTCTCACT	
	GATA4	69 bp	Fw:AGCTGGGTAGTTTAGCCAAACG Rv:TGTGTGACACGGTGAACGAA	
Sendai viral genome (PCR)	SeV	181 bp	Fw:GGATCACTAGGTGATATCGAGC Rv: ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528pb	Fw:ATGCACCGCTACGACGTGAGCGC Rv:ACCTTGACAATCCTGATGTGG	
	Klf4	410 bp	Fw:TTCCTGCATGCCAGAGGAGCCC Rv:AATGTATCGAAGGTGCTCAA	
	c-Myc	532 bp	Fw:TAACTGACTAGCAGGCTTGTCG Rv:TCCACATACAGTCCTGGA TGATGATG	
Genotyping	NSD1	569 bp	Fw:CAGAGAACCTTGGCCTAAAC Rv:TGCACTCTTAGTACAACCCA	

4.3. Real Time PCR

Total RNA was extracted using Trizol (Thermo Fisher) at passage 6 and 20. RNA was reverse transcribed into cDNA using the Advantage RT cDNA Kit (Clontech). Real Time PCR was performed using the Light-Cycler 480 SYBR Green I Master (Roche) and specific primers, listed in Table 2. Data were normalized to the housekeeping gene GAPDH using the $2^{-\Delta\Delta Ct}$ method and we considered $p < 0.05$ as statistically significant and $p < 0.001$ as statistically highly significant.

4.4. Karyotyping

Quinacrine (Q) -banding was performed on iPSCs cultures (between passages 10–15) and a minimum of 15 metaphase-state nuclei were analysed with Cytovision 3.93.2 analytical system.

4.5. Comparative genomic Hybridisation (CGH)

CGH was used to screen targeted regions of the genome for losses associated with chromosomal imbalances such as aneuploidy, deletions

Table 3
CGH analysis summary; IGGi003-A (passage 11) reporting a female cell line with no detectable abnormalities.

CGH summary	
Sample name	*IGGi003-A*
Date reported	23th September 2023
Hybridisation	Balanced hybridization was observed for all chromosomes
Balance	relative to reference DNA
Copy number change	No copy number changes above 400 kb were detected
Interpretation	Female cell line — no abnormalities detected

and duplications. CGH was performed using Sureprint G3 Human CGH Microarray Kit (8 × 60 K format) which were scanned with the Agilent Scanner C and analysed using CytoGenomics software (Agilent Technologies).

4.6. DNA profiling

DNA from iPSCs and parental fibroblasts was extracted using the QIAamp DNA Mini Kit (Qiagen) and analysed using the Geneprint 10 system (Promega).

4.7. 4.7.Sanger sequencing

Genomic DNA was amplified by PCR using specific primers for *NSD1* gene (Conteduca et al, 2023). Purified PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher). Primers are listed in Table 2.

4.8. Trilineage differentiation

Differentiation of iPSCs to all three germ layers, (ectoderm, mesoderm and endoderm) was performed using STEMdiffTM Trilineage Differentiation Kit (Stem Cell Technologies), following manufactuer's instructions/guidelines. Real Time PCR was performed with various sets of primers as described above.

4.9. Mycoplasma detection

Supernatant of cell culture (between passages 8–15) was used for mycoplasma assay following manufacturer's instructions/guidelines (Lonza's MycoAlert ® Mycoplasma Detection Kit.

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Italian Ministry of Health, Ricerca Corrente IRCCS Istituto Giannina Gaslini 2023, "Sviluppo di un modello cellulare *in vitro* per l'identificazione di target neuronali in pazienti con Sindrome di Sotos in prospettiva di terapie specifiche per l'aploinsufficienza". Italian Ministry of Health, 5M-2020-23682542 5xmille IRCCS Istituto Giannina Gaslini.

CRediT authorship contribution statement

Giuseppina Conteduca: . **Chiara Baldo:** Formal analysis, Methodology. **Alessia Arado:** Data curation, Formal analysis. **Joana Soraia Martinheira da Silva:** . **Barbara Testa:** Software, Visualization. **Simona Baldassari:** Conceptualization, Validation. **Federico Zara:** Supervision, Validation, Visualization. **Gilberto Filaci:** Supervision, Validation, Writing – review & editing. **Domenico Coviello:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Michela Malacarne:** .

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.2024.103324.

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