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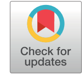
Lab Resource: Multiple Cell Lines

Generation of induced pluripotent stem cell (iPSC) lines from a Joubert syndrome patient with compound heterozygous mutations in *C5orf42* gene



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A B S T R A C T

We have generated new disease-specific induced pluripotent stem cell (iPSC) lines from skin fibroblasts obtained from a female patient with Joubert syndrome (JS) caused by compound heterozygous mutations in *C5orf42* gene. The generated iPSCs offer an unprecedented opportunity to obtain iPSC-derived neurons to investigate the pathogenesis of JS *in vitro* and to develop therapeutic strategies.

1. Resource Table.

Unique stem cell lines identifier	UNIBSi011-A UNIBSi011-B UNIBSi011-C
Alternative names of stem cell lines	JS_MA_C3 (UNIBSi011-A) JS_MA_C5 (UNIBSi011-B) JS_MA_C10 (UNIBSi011-C)
Institution	Angelo Nocivelli Institute for Molecular Medicine, ASST Spedali Civili di Brescia, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy
Contact information of distributor	Eltahir Ali, Email: e.ali@unibs.it
Type of cell lines	iPSCs
OriginAdditional origin information	Human Age: 27 yr Sex: Female Ethnicity: Caucasian
Cell source	Fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific). SeV-based vectors deliver and express the transcription genes *OCT4, SOX2, KLF4, and c-MYC.*
Multiline rationale	Isogenic clones with same gene mutations
Gene modification	Yes
Type of modification	Hereditary
Associated disease	Joubert syndrome
Gene/locus	*C5orf42*
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	November 2019
Cell line repository/bank	https://hpscreg.eu/cell-line/UNIBSi011-A https://hpscreg.eu/cell-line/UNIBSi011-B https://hpscreg.eu/cell-line/UNIBSi011-C
Ethical approval	The study was approved by the Scientific Committee and by the Board of the ASST Spedali Civili di Brescia (NP 3572 – Studio iPSCREP)

2. Resource utility

Joubert syndrome (JS) is a neurodevelopmental disorder characterized by ataxia, hypotonia, developmental delay and a neuroimaging finding known as the molar tooth sign (MTS). JS is caused by defects in more than 30 genes. The generated JS-derived iPSCs are a valuable source for *in vitro* modelling and therapeutics development.

3. Resource details

Joubert syndrome (JS) is a rare neurodevelopmental disorder characterized by congenital ataxia, developmental delay, intellectual disability, hypotonia and the molar tooth sign (MTS) in brain magnetic

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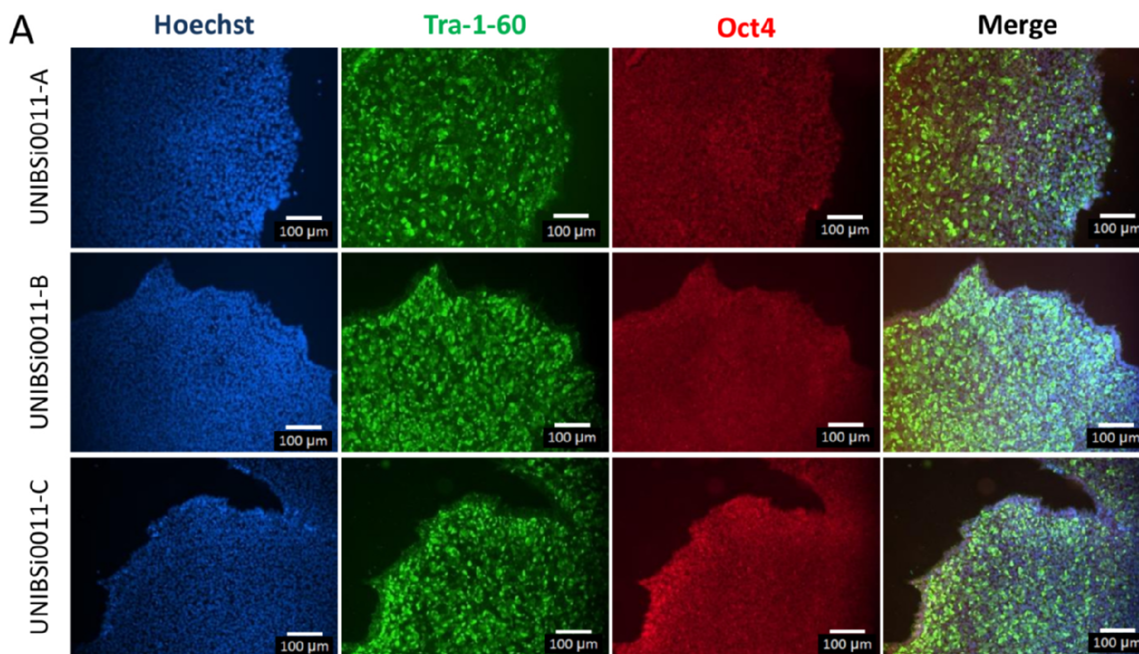

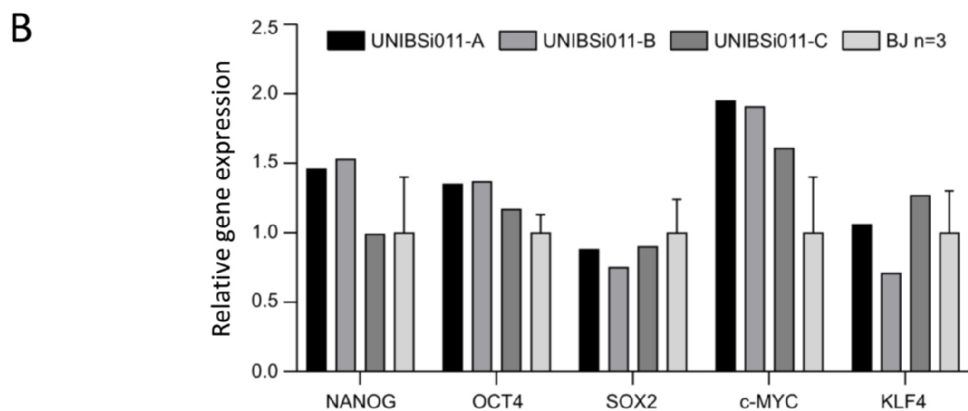

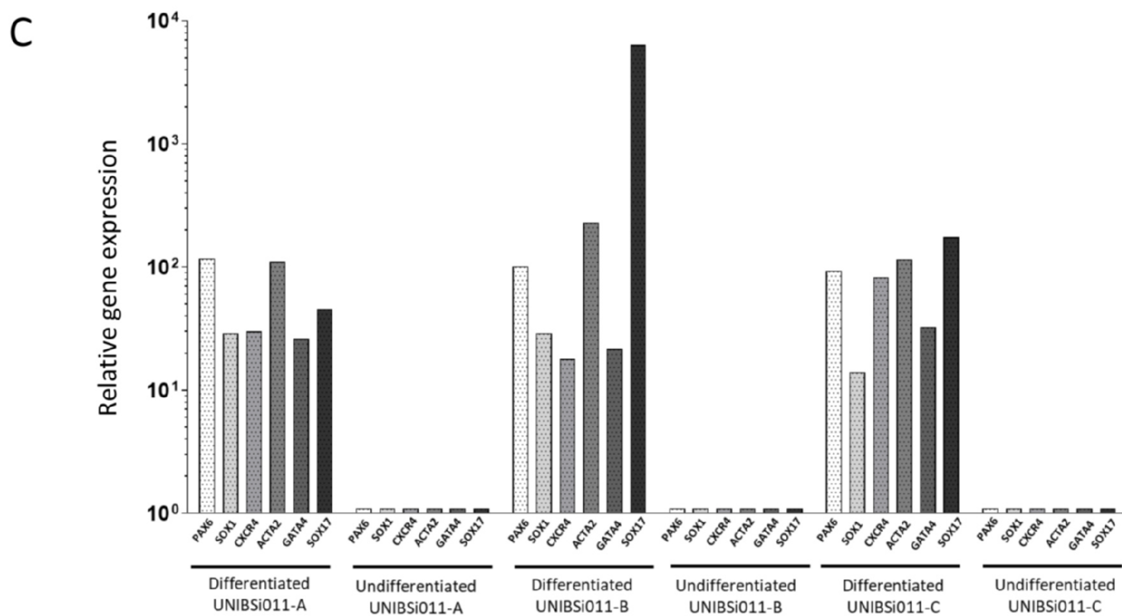

Fig. 1.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UNIBSi011-A UNIBSi011-B UNIBSi011-C	UNIBSi011-A UNIBSi011-B UNIBSi011-C	Female	27 Yr	Caucasian	c.T3868T > C / c.7477C > A	Joubert syndrome

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Supplementary Fig. 1A
Phenotype	Qualitative analysis (Immunocytochemistry)	Expression of pluripotency markers OCT4 and TRA-1–60	Fig. 1 panel A
	Quantitative analysis(SYBR Green RT-qPCR)	Expression of pluripotency markers *NANOG, OCT4, SOX2, c-MYC,* and *KLF4*	Fig. 1 panel B
Genotype	Karyotyping (Q-banding) and resolution	46, XX, Resolution 450–500	Supplementary Fig. 1B
Identity	STR analysis	16 distinct STRs and all are matched to the parental cell line	Available with the authors
Mutation analysis	Sequencing	Compound heterozygous mutations (c.T3868T > C / c.7477C > A)	Supplementary Fig. 1C
	Southern blot or WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Supplementary Fig. 1E
Differentiation potential	Direct differentiation into the three embryonic layers	Induction of selected genes expressed in the three germ layers (Ectoderm: *PAX6-SOX1*; Endoderm: *GATA4-SOX17*; and Mesoderm: *ACTA2- CXCR4*).	Fig. 1 panel C
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

resonance imaging (MRI). Additional features of JS may include unusual eye movements, abnormal neonatal respiratory patterns, liver and kidney disease and dysmorphic facial features (Valente et al., 2008; Joubert et al., 1969). JS is inherited as an autosomal recessive or X-linked disorder and caused by defects in several genes including *C5orf42* (also known as *CPLANE*). It is estimated to affect 1 in 80,000 to 100,000 new-borns. Pathogenesis of JS is associated with dysfunction of microtubule-based organelles known as primary cilia, that project from the surface of nearly all human cell types. Mutations in *C5orf42* gene lead to variable clinical phenotypes in JS patients (Srour et al., 2012), and defects in the primary cilia are associated with a wide spectrum of other neurological disorders known as ciliopathies (Romani et al., 2013). In human, neurons and other cell types express *C5orf42*, but little is known about its role in the pathogenesis of JS. In this study, we generated iPSC lines by reprogramming fibroblasts from a 27-year female patient with JS. Fibroblasts were induced by introducing non-integrating Sendai virus (SeV) vectors containing a set of four genes known as the Yamanaka factors (*OCT4, SOX2, c-MYC*, and *KLF4)*. From the generated iPSCs, three isogenic clones (UNIBSi011-A, UNIBSi011-B, and UNIBSi011-C) were selected for further expansion based on their embryonic stem cell (ESC)-like colony morphology (Supplementary Fig. 1A) and positive staining for TRA-1–60 and OCT4 antibody (Fig. 1A). We used a short tandem repeat (STR) multiplex assay to verify that the iPSCs retained the same genetic profile of the parental fibroblasts. Elimination of SeV vectors was verified by PCR following the manufacturers' protocols (Supplementary Fig. 1D). We confirmed the presence of compound heterozygous mutations in *C5orf42* (c.T3868T > C;p.S1290P and c.7477C > A;p.R2493X) of the parental cells in the iPSCs by Sanger sequencing (Supplementary Fig. 1C). Cytogenetic analyses of the iPSCs at passages p5 and p24 for UNIBSi011-A and UNIBSi011-B, and p6 and p33 for UNIBSi011-C showed a normal 46, XX pattern for each selected clone (Supplementary Fig. 1B), and all the iPSCs were verified to be mycoplasma-free (Supplementary Fig. 1E). A quantitative polymerase chain reaction (qPCR) analysis has confirmed the expression of endogenous pluripotency genes *NANOG, OCT4, SOX2, c-MYC*, and *KLF4*, fully comparable to those of three control iPSC lines derived from BJ commercial line (ATCC® CRL-2522™) that we reprogrammed in our laboratory and checked with a deeper pluripotency characterization by TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis (Supplementary Fig. 1F). Moreover, all iPSC lines were investigated for their spontaneous ability to differentiate into the three germ layers by the expression of ectodermal, mesodermal and endodermal genes (*PAX6-SOX1, CXCR4-ACTA2, GATA4-SOX17* respectively) (Fig. 1C). Based on these results, the generated iPSC lines can be differentiated into neurons to investigate the pathogenesis of JS associated with mutations in *C5orf42* gene and to validate potential therapeutic agents.

4. Materials and methods

4.1. Reprogramming of fibroblasts into iPSCs

The patient's fibroblasts were transduced using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) and expressed the transcription genes *OCT4, SOX2, c-MYC*, and *KLF4*. The iPSC colonies were picked and expanded every 4–5 days under feeder-free conditions. The iPSCs were seeded on Matrigel-coated 12-well plates with NutriStem® hPSC XF Medium (Biological Industries) and incubated in a 37° C humidified cell culture incubator with 5% CO_2.

4.2. Mycoplasma detection

Mycoplasma contamination was tested at different passages and the Mycoplasma gene *16S rRNA* was amplified by PCR using AmpliTaq Gold™ DNA Polymerase (Thermo Fisher Scientific), following a standard protocol.

4.3. SeV genome and transgenes detection

Total RNA was isolated from the iPSCs using NucleoSpin® RNA Mini Kit (Macherey-Nagel) and was reverse-transcribed using ImProm-II™ Reverse Transcription System (Promega). PCR was performed using a standard protocol (Tables 1-3).

Table 3
Reagents details.

Antibodies used for immunofluorescence			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit anti-OCT4	1:400	Thermo Fisher Scientific, Cat# A-13998. RRID: AB_2534182
Pluripotency markers	Mouse anti–TRA–1–60	1:100	Thermo Fisher Scientific, Cat# 4110000. RRID: AB_2533494
Secondary antibodies	Goat anti rabbit IgG (H + L) Alexa Fluor 568	1:300	Thermo Fisher Scientific, Cat# A-11011. RRID: AB_143157
Secondary antibodies	Goat anti mouse IgG (H + L) Alexa Fluor 488	1:300	Thermo Fisher Scientific, Cat# A-11001. RRID: AB_2534069
Primer sets used for pluripotency gene expression by SYBR Green-based qPCR			
	Target	Forward/Reverse primer (5′-3′)	
Pluripotency markers	*NANOG*	TGAACCTCAGCTACAAACAG/TGGTGGTAGGAAGAGTAAAG	
	OCT4	CCTCACTTCACTGCACTGTA/CAGGTTTTCTTTCCCTAGCT	
	SOX2	CCCAGCAGACTTCACATGT/CCTCCCATTTCCCTCGTTTT	
	c-MYC	TGCCTCAAATTGGACTTTGG/GATTGAAATTCTGTGTAACTGC	
	KLF4	GATGAACTGACCAGGCACTA/GTGGGTCATATCCACTGTCT	
House-keeping gene	*βACTIN*	CGCCGCCAGCTCACCATG/CACGATGGAGGGGAAGACGG	
Primer sets used for SeV detection by qPCR			
Exogenous markers	*KOS*	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG (528 bp)	
	C-MYC	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG (532 bp)	
	KLF4	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA (410 bp)	
SeV genome sequence	*SeV*	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC (181 bp)	
Primer set for Mycoplasma detection			
	Target	Forward/Reverse primer (5′-3′)	
	16S rRNA	*GGGAGCAAACAGGATTAGATACCCT/ TGCACCATCTGTCACTCTGTTAACCTC (268 bp)*	
Primer sets for *C5orf42* mutations sequencing			
	Target	Forward/Reverse primer (5′-3′)	
	C5orf42 (Exon 22)	*AAATGATCAGCAGAGTTGGAG/AGATGGTAGACCAGAATGAGTTC*	
	C5orf42 (Exon 36)	*TGTATTTGCAGAATATCTTGCC / CAGCAATCAGACTAGATAAACC*	
Differentiation markers used for TaqMan-based qPCR			
	Target	Probe	
Ectoderm	*PAX6*	Hs.PT.58.25914558	
	SOX1	Hs.PT.58.28041414.g	
Mesoderm	*ACTA2*	Hs.PT.56a.2542642	
	CXCR4	Hs00607978_s1	
Endoderm	*GATA4*	Hs.PT.58.259457	
	SOX17	Hs.PT.58.24876513	
House-keeping gene	*βACTIN*	Hs.PT.39a.22214847	

4.4. In vitro Trilineage Differentiation

Following StemMACS Trilineage Differentiation Kit protocol (Miltenyi Biotec), the iPSC colonies were detached and re-plated as single cells (100,000, 80,000, 130,000 cells for ectoderm, mesoderm, and endoderm, respectively) on Matrigel-coated 24-well plates containing lineage-specific medium. The media were changed from day 1–6. On day 7, cells were collected for RNA purification.

4.5. qPCR analysis

Total RNA was purified and reverse-transcribed. Using BioRad CFX96 RT-PCR system and BioRad CFX Manager™ Software, SYBR Green- and TaqMan-based qPCR assays were performed to test the expression of pluripotency and the embryonic layers markers, respectively. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative quantification of the target genes, using *βACTIN* as a reference gene.

4.6. Immunofluorescence staining

Immunofluorescence staining of the iPSCs was performed using anti-TRA-1–60 and anti-OCT4 antibodies (Thermo Fisher Scientific). Cells were fixed and permeabilized on glass slides at room temperature (RT) using Fix & Perm-Reagents Kit (SIC). iBind™ Buffer (Invitrogen) was used for blocking incubation for 45 min at RT. The iPSC nuclei were stained with Hoechst 33342 (Thermo Fisher Scientific). An inverted fluorescence microscope (Olympus IX70) was used to examine the slides and the images were analysed using Image-Pro Plus software v7.0 (Media Cybernetics).

4.7. Genomic DNA sequencing and STR analysis

Genomic DNA was extracted from the parental fibroblasts and the iPSCs using QIAamp® DNA Mini Kit (Qiagen) protocol. Sanger sequencing was performed to confirm that the iPSCs retain *C5orf42* mutations of the parental fibroblasts. 16 distinct STRs were amplified using AmpFlSTR® Identifiler® Plus PCR Amplification Kit (Applied

Biosystems) and analysed by Applied Biosystems® 3130 Genetic Analyzer and GeneMapper® ID software.

4.8. Karyotyping

The iPSCs were treated with 10 µg/ml of Colcemid (KaryoMax, Gibco Co. BRL) for 3 hrs to be blocked at metaphase stage. The cells were detached by trypsin–EDTA and exposed to a hypotonic solution 0.075 M KCl for 7 min at 37 °C. Methanol/glacial acetic acid (3:1) solution was used to fix the cells three times. Slides were prepared with a drop of the metaphase preparations, then stained with quinacrine dihydrochloride at 400–450 bands resolution. For each clone preparation, a minimum of 20 metaphase spreads were analysed and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN 2016).

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.

Acknowledgment

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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.2020.102007.

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