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Lab Resource: Multiple Cell Lines

Generation of three iPSC lines from fibroblasts of a patient with Aicardi Goutières Syndrome mutated in *TREX1*

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A B S T R A C T

Fibroblasts from a patient with Aicardi Goutières Syndrome (AGS) carrying a compound heterozygous mutation in *TREX1,* were reprogrammed into induced pluripotent stem cells (iPSCs) to establish isogenic clonal stem cell lines: UNIBSi006-A, UNIBSi006-B, and UNIBSi006-C. Cells were transduced using the episomal Sendai viral vectors, containing human *OCT4, SOX2, c-MYC* and *KLF4* transcription factors. The transgene-free iPSC lines showed normal karyotype, expressed pluripotent markers and displayed *in vitro* differentiation potential toward cells of the three embryonic germ layers.

Resource utility

Aicardi Goutières syndrome (AGS) is a rare early-onset monogenic inflammatory encephalopathy. Considering the unavailability of patients' neuronal bioptic materials, the most suitable *in vitro* model is represented by iPSCs as a useful instrument to achieve patient-specific neuronal cells.

Resource details

AGS is a severe inflammatory encephalopathy, typically showing different degrees of neurological impairment, elevated cerebrospinal fluid (CSF) interferon-α (IFN-α) level and specific neuroradiologic features, with onset in early infancy (Fazzi et al., 2013). AGS is a genetically heterogeneous disorder, involving mutations in different antiviral genes related to nucleic acid processing. The first causative gene identified in AGS encodes for the Three-prime Repair Exonuclease 1 (TREX1) active against the single strand DNA and the nicked strand of double-stranded DNA. AGS type 1 (AGS1) is characterized by biallelic mutations in *TREX1* (Crow et al., 2006).

In this study we generated and characterized three isogenic iPSC clones derived from fibroblasts of a 5 years old male affected by AGS with the compound heterozygous mutation TREX1:NM_033629.6:c.[260insAG];[290G > A]:p.[S88 fs*22];[R97H] (Oliviero et al., 2013). This patient shows typical clinical features of AGS1 as microcephaly, chilblains-like lesions, severe tetraparesis, cerebral calcifications, leukodystrophy and raised CSF IFN-α (Oliviero et al., 2013).

Fibroblasts were reprogrammed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit, in feeder free condition. This kit utilizes a modified form of Sendai virus as episomal vector to introduce the Yamanaka's factors *OCT4, SOX2, KLF4*, and *c-MYC* into somatic cells. At day 20 post-transduction, several individual and isolate iPSC colonies were manually picked and expanded. After generation of a frozen stock for 10 different iPSC clones, 3 clones that best display an ESC-like morphology (Supplementary Fig. 1) were chosen for further expansion and characterization: UNIBSi006-A, UNIBSi006-B, and UNIBSi006-C (Tables 1 and 2).

We verified that these iPSC lines were mycoplasma-free (Supplementary Fig. 2) and we confirmed the presence of the patient mutations by Sanger sequencing (Fig. 1A). The iPSC lines were authenticated against the parental fibroblast lines *via* short tandem repeat (STR) profiling (available with the authors). The selected clones showed a normal karyotype (46,XY), assessed at different passages (passage 12, 25, and 42), confirming the cytogenetic stability in culture

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UNIBSi006-A	UNIBSi006-A	Male	5 y	Caucasian	−/AG G/A	Aicardi Goutières Syndrome type 1 (AGS1).
UNIBSi006-B	UNIBSi006-B	Male	5 y	Caucasian	−/AG G/A	Aicardi Goutières Syndrome type 1 (AGS1).
UNIBSi006-C	UNIBSi006-C	Male	5 y	Caucasian	−/AG G/A	Aicardi Goutières Syndrome type 1(AGS1).

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Supplementary Fig. 1
Phenotype	Qualitative analysis: immunocytochemistry	Positive for OCT4, and TRA-1-60 expression	Fig. 1 panel C
	Quantitative analysis: TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis	Positive score for self-renewal gene expression and a negative score for ectodermal, mesodermal, and endodermal gene expression.	Fig. 1 panel D
Genotype	Karyotype (Q-banding) and resolution	46,XY Resolution 450–500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR) OR STR analysis	*N/A* 16 distinct loci: all matched to parental cell line	*N/A* Available with the authors
Mutation analysis (If Applicable)	Sequencing	Compound heterozygous mutation: c.[260insAG];[290G > A].	Fig. 1 panel A
	Southern Blot OR WGS	*N/A*	*N/A*
Microbiology and virology	Mycoplasma	Negative	Supplementary Fig. 2
Differentiation potential	Direct differentiation into three germ layers	UNIBSi006-A: TaqMan® hPSC Scorecard™ analysis; negative score for self-renewal gene expression and positive score for trilineage gene expression	Fig. 1 panel E
		UNIBSi006-B and UNIBSi006-C: relative gene expression of PAX6-SOX1 (Ectoderm), NCAM1/CXCR4 –ACTA1 (Mesoderm), and GATA4-SOX17 (Endoderm).	Fig. 1 panel F
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*

(Fig. 1B).

The expression of pluripotent markers was examined by immunostaining using antibodies against human Tra-1-60, properly localized on cell surface, and the transcriptional factor OCT4 expressed at nuclear level (Fig. 1C).

To deepen the pluripotency characterization, passage 10 UNIBSi006-A, passage 16 UNIBSi006-B, and passage 8 UNIBSi006-C iPSCs were subjected to TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis. Each line showed a positive score for self-renewal gene expression and a negative score for expression of genes involved in ectodermal, mesodermal, and endodermal formation. Furthermore, no residual Sendai virus was detected. Only UNIBSi004-B showed a borderline score for ectodermal gene expression that has been considered within the standard deviation range of acceptability to be a pluripotent iPSC line (Fig. 1D).

Finally, we tested the spontaneous capacity of iPSC clones to differentiate *in vitro* into three embryonic germ layers. In order to obtain a deeper analysis on a broad spectrum of genes, one clone, the UNIBSi006-A, was analysed through TaqMan® Human Pluripotent Stem Cell Scorecard™ showing the expected result (Fig. 1E). The remaining clones, UNIBSi006-B, and UNIBSi006-C, were evaluated by quantitative PCR (qPCR) for ectodermal, mesodermal and endodermal markers (PAX6-SOX1, NCAM1/CXCR4-ACTA2, GATA4-SOX17, respectively) (Fig. 1F).

Materials and methods

Fibroblasts reprogramming

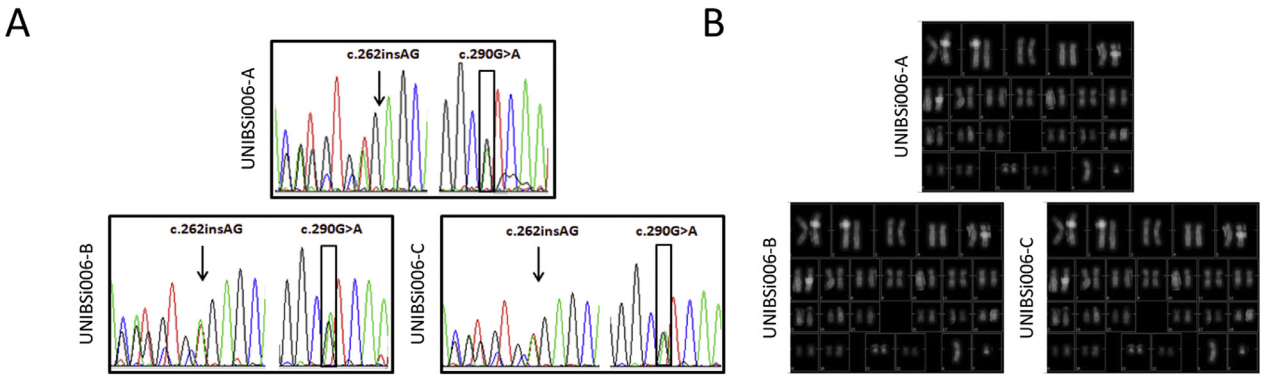
Primary fibroblasts, derived from AGS1 patient's skin biopsy, were cultured in DMEM with 10% Fetal Bovine Serum, 1% L-Glutamine, and 1% Penicillin/Streptomycin (Euroclone) at 37 °C in 5% CO_2. For iPSCs generation 100.000 fibroblasts at 60% of confluence were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific) following manufacturer's instructions. At day 8 post-transduction, cells were seeded onto a Matrigel (Corning) -coated culture dish and the next day medium was changed to Nutristem hPSC XF medium (Biological-Industries). After 20 days, colonies positive to Tra-1-60 staining, were manually picked to further expansion and characterization. iPSCs were fed daily with Nutristem hPSC-XF Medium, and manually picked every 5 days on new Matrigel-coated well plate with 1:2 ratio.

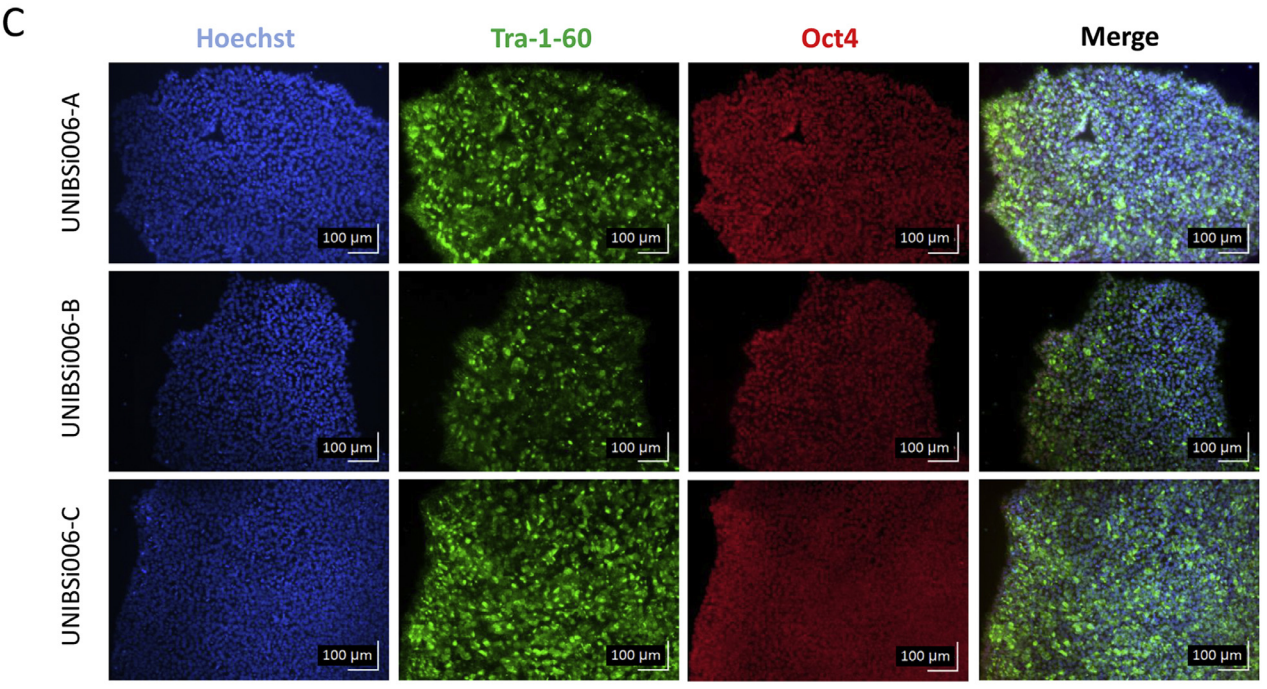
Sequencing

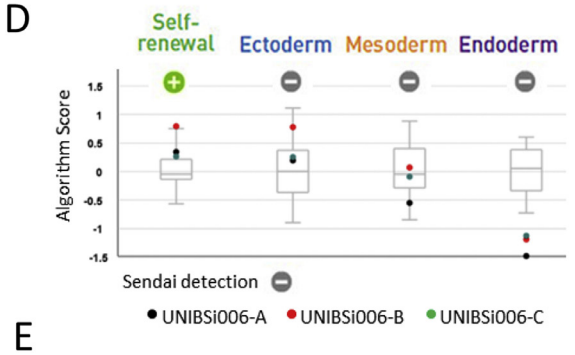
DNA was extracted using the QIAmp DNA Blood Mini Kit (Qiagen), and amplified by PCR using AmpliTaq Gold® DNA Polymerase (ThermoFisher Scientific) with *TREX1* primers (Table 3) using the GeneAmp PCR System 9700 (Applied Biosystem) following these PCR cycle parameters: initial denaturation at 95 °C for 12 min, denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, final extension at 72 °C for 7 min. Number of cycles: 38. Sequencing reactions were performed using BigDye Terminator v1.1 Cycle Sequencing Kit following manufacturer protocol: incubation at 94 °C for 5 min, denaturation at 94 °C for 10 s, annealing at 60 °C for 5 s, extension at 60 °C for 2 min and 30 s. Number of cycles: 25. The purified sequencing reactions were run using Prism 3130 Genetic Analyzer (ThermoFisher Scientific) and analysed using SeqScape v3.0 Software.

Karyotyping

Passage 12, 25, and 42 iPSCs undergoing active cell division were blocked at metaphase by 10 µg/ml of colcemid for 3 h (KaryoMax, Gibco Co. BRL), detached from the growth surface by trypsin–EDTA,

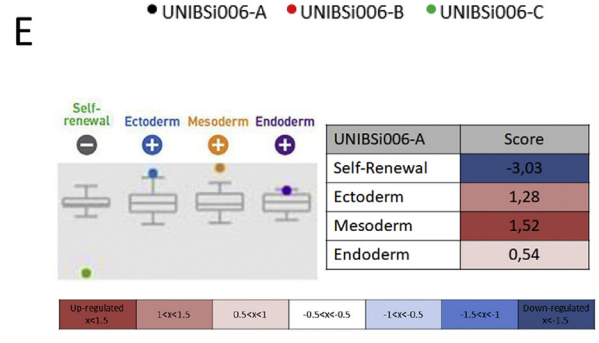






Samples	Self-Renewal	Ectoderm	Mesoderm	Endoderm
UNIBSi006-A	0,33	0,18	-0,56	-1,49
UNIBSi006-B	0,78	0,77	0,06	-1,20
UNIBSi006-C	0,25	0,24	-0,10	-1,14

Up-regulated x<1.5	1<x<1.5	0.5<x<1	-0.5<x<-0.5	-1<x<-0.5	-1.5<x<-1	Down-regulated x<-1.5



UNIBSi006-A	Score
Self-Renewal	-3,03
Ectoderm	1,28
Mesoderm	1,52
Endoderm	0,54

Up-regulated x<1.5	1<x<1.5	0.5<x<1	-0.5<x<-0.5	-1<x<-0.5	-1.5<x<-1	Down-regulated x<-1.5

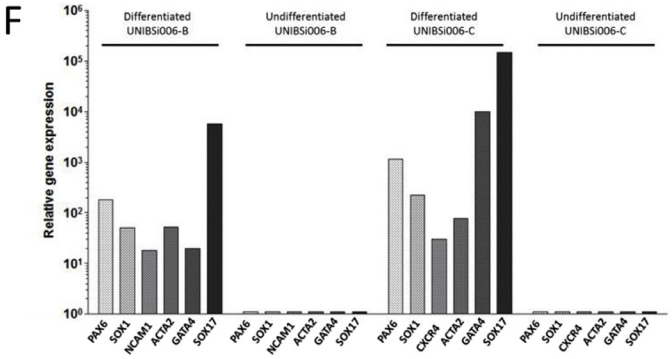


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Fig. 1. Characterization of the iPSC lines UNIBSi006-A, UNIBSi006-B, and UNIBSi006-C. (A) Electropherograms showing the compound heterozygous mutation in TREX1. (B) Karyotypes of representative metaphase displaying normal 46 chromosomes (XY). (C) Immunofluorescence staining for the stemness markers Tra-1-60 (green) and Oct4 (red). Nuclei were stained with Hoechst33342 (blue). (D) Pluripotency assessments using TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis. (E) Schematic summary of TaqMan® Human Pluripotent Stem Cell Scorecard™ panel depicting the in vitro trilineage differentiation capability of UNIBSi006-A iPSC line. (F) qPCR analysis of the three germ layers markers in UNIBSi006-B, and UNIBSi006-C iPSC clones.

and subsequently swollen by exposure to hypotonic KCl (0.075 M) solution for 7 min at 37 °C. Cells were fixed with methanol/glacial acetic acid (3:1) three times, and dropped onto glass slides. Cytogenetic analysis was performed using conventional QFQ-banding at 450 bands resolution according to the International System for Human Cytogenetic Nomenclature (ISCN 2016). A minimum of 20 metaphase spreads were analysed for each samples and karyotyped using a chromosome imaging analyzer software (Chromowin software, Tesi Imaging).

TaqMan hPSC scorecard assay

RNAs were extracted using NucleoSpin® RNA II kit (Macherey-Nagel) following instructions. 1 µg of RNA collected from each iPSCs were sent to ThermoFisher Scientific CellModel Service to perform TaqMan hPSC scorecard assay. This test was used to verify the loss of Sendai virus, and to evaluate the expression levels of genes involved in self-renewal, endoderm, mesoderm, and ectoderm development.

Immunofluorescence staining

iPSCs were fixed and permeabilized using Fix&Perm-Reagent kit (*SIC*). Then, blocking solution iBind™ Buffer (Invitrogen) was applied for 45 min. Primary and secondary antibodies, diluted in blocking solution, were added and incubated for 3 and 1 h respectively, at room temperature (RT). The antibodies used are summarized in Table 3. Cellular nuclei were counterstained with Hoechst 33342 (ThermoFisher Scientific). Cells were observed with an inverted fluorescence microscope (Olympus IX70), and images were analysed with the Image-Pro Plus software v7.0 (Media Cybernetics).

In vitro trilineage differentiation

iPSCs were dissociated into single-cell suspension and seeded on Matrigel-coated 24-well plates (10^5, 0.8×10^5, 1.3×10^5 cells for ectoderm, mesoderm, and endoderm, respectively) in the specific medium according to the StemMACSTM Trilineage Differentiation Kit protocol (MACS Miltenyi Biotec). Seven days later, cells were collected for RNA extraction and qPCR of lineage specific markers was performed. Only for UNIBSi006-A, RNA collected from each germinal layers differentiation was mixed in a 1:1:1 ratio to perform TaqMan hPSC scorecard assay.

RNA extraction and qPCR

Total RNAs were extracted using NucleoSpin® RNA II kit (Macherey-Nagel) and quantified by a Spectrofluorometer. RNAs were retro-transcribed by ImPromII™ Reverse Transcription System (Promega), following the protocol. qPCR for iPSCs differentiation capacity was performed using iQ MPLX powermix and TaqMan Probe based assays. Probes are listed in Table 3. Assays were performed on CFX96 C1000 Touch™ Real-Time PCR Detection System, and analysed with CFX manager software v.3.1 (BioRad). The relative quantification of target genes was calculated by the $2^{-\Delta\Delta Ct}$ method, using *βACTIN* as housekeeping gene.

Mycoplasma detection

The absence of mycoplasma contamination was confirmed using a standard PCR to amplify the 16Sr RNA of the genus Mycoplasma from the supernatant of confluent cell culture and positive controls. The amplification was performed with AmpliTaq Gold™ DNA Polymerase

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

	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

Primers for PCR assay

	Target	Forward/Reverse primer (5′-3′)
Mutation sequencing	*TREX1* (200 bp)	ACAAGCTCTCCCTGTGTGTG/GAAGTCGTAGCGGTCACCAT
Mycoplasma detection	*16 s rRNA* (268 bp)	GGGAGCAAACAGGATTAGATACCCT/TGCACCATCTGTCACTCTGTTAACCTC

Differentiation RT-qPCR assays with TaqMan chemistry

	Target	Probe
Ectoderm	*PAX6*	Hs.PT.58.25914558
	SOX1	Hs.PT.58.28041414.g
Mesoderm	*ACTA2*	Hs.PT.56a.2542642
	NCAM1	Hs.PT.58.39694135
	CXCR4	Hs00607978_s1
Endoderm	*GATA4*	Hs.PT.58.259457
	SOX17	Hs.PT.58.24876513
Housekeeping gene	*ACTB*	Hs.PT.39a.22214847

(ThermoFisher Scientific) using the GeneAmp PCR System 9700 (Applied Biosystem) with PCR cycle parameters described as above. Primers used are listed in Table 3.

STR analysis

DNAs from parental fibroblasts and iPSC clones were extracted as above, and amplified with AmpFlSTR® Identifiler® Plus (LifeTechnologies) following instructions.

Key resource table

Unique stem cell lines identifier	UNIBSi006-A UNIBSi006-B UNIBSi006-C
Alternative names of stem cell lines	AGS1_MM_C12 (UNIBSi006-A) AGS1_MM_C13 (UNIBSi006-B) AGS1_MM_C14 (UNIBSi006-C)
Institution	A. Nocivelli Institute for Molecular Medicine, Department of Molecular and Translational Medicine, University of Brescia, 25,123 Brescia, Italy
Contact information of distributor	Rosalba Monica Ferraro: rosalbamonica.ferraro@gmail.com
Type of cell lines	iPSCs
Origin	Human
Additional origin info	Age: 5 Sex: male Ethnicity: Caucasian
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific). The episomal reprogramming vectors include the four Yamanaka factors *OCT4, SOX2, KLF4*, and *C-MYC*
Multiline rationale	Isogenic clones of same disease mutation
Gene modification	YES
Type of modification	Hereditary
Associated disease	Aicardi Goutières Syndrome
Gene/locus	TREX1
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	Jan-2017
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/UNIBSi006-A https://hpscreg.eu/user/cellline/edit/UNIBSi006-B https://hpscreg.eu/user/cellline/edit/UNIBSi006-C
Ethical approval	IRB Spedali Civili and University of Brescia, NP n.1603 -Studio AGS-CARIPLO

Declaration of Competing Interest

None.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.scr.2019.101580.

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