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Lab Resource: Multiple Cell Lines

Generation of three isogenic induced Pluripotent Stem Cell lines (iPSCs) from fibroblasts of a patient with Aicardi Goutières Syndrome carrying a c.2471G > A dominant mutation in *IFIH1* gene

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A B S T R A C T

Aicardi-Goutières syndrome (AGS) is an early-onset monogenic encephalopathy characterized by intracranial calcification, leukodystrophy and cerebrospinal fluid lymphocytosis. To date, seven genes have been related to AGS. Among these, *IFIH1* encodes for MDA5, a cytosolic double-stranded RNA receptor, and is responsible for AGS type 7. We generated three isogenic iPSC clones, using a Sendai virus-based vector, starting from fibroblasts of a patient carrying a dominant mutation in *IFIH1*. All lines were characterized for genomic integrity, genetic uniqueness, pluripotency, and differentiation capability. Our clones might offer a good model to investigate AGS7 pathophysiological mechanism and to discover new biomarkers for this condition treatment.

Resource Table

Unique stem cell lines identifier	UNIBSi009-A UNIBSi009-B UNIBSi009-C
Alternative names of stem cell lines	AGS7-GS-C3.A (UNIBSi009-A) AGS7-GS-C6.4 (UNIBSi009-B) AGS7-GS-C7.4 (UNIBSi009-C)
Institution	"Angelo Nocivelli" Institute for Molecular Medicine, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy
Contact information of distributor	Stefania Masneri: s.masneri003@unibs.it
Type of cell lines	iPSC
Origin	Human
Additional origin info	Age: 14 years Sex: male Ethnicity: Caucasian
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*

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Multiline rationale	Isogenic clones
Gene modification	Congenital mutation
Type of modification	N/A
Associated disease	Aicardi Goutières Syndrome type 7 (AGS7)
Gene/locus	*IFIH1*/Cytogenetic band: 2q24.2
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	Jan 2016
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/UNIBSi009-A https://hpscreg.eu/user/cellline/edit/UNIBSi009-B https://hpscreg.eu/user/cellline/edit/UNIBSi009-C
Ethical approval	IRB Spedali Civili and University of Brescia, NP n.1603 -Studio AGS-CARIPLO

1. Resource utility

Aicardi-Goutières Syndrome type 7 (AGS7) is an autosomal dominant inflammatory disorder characterized by severe neurologic impairment, due to mutations in *IFIH1* gene, encoding for MDA5, a cytosolic double-stranded RNA receptor. The AGS7 specific iPSC lines could be a suitable tool for *in vitro* disease modelling, and early biomarkers discovering.

2. Resource details

Aicardi-Goutières Syndrome (AGS) is an early-onset genetically determined encephalopathy, characterized by intracranial calcification, leukodystrophy and cerebrospinal fluid lymphocytosis. To date, seven genes, encoding for proteins involved in nucleic acids metabolism and sensing (*TREX1, RNASEH2B, RNASEH2C, RNASEH2A, ADAR1, SAMHD1, IFIH1*) have been related with an AGS phenotype. Among these, *IFIH1* (interferon-induced helicase c domain-containing protein 1) encodes for MDA5, a cytosolic double-stranded RNA receptor mutated in AGS7 (OMIM 615846) (Crow et al., 2019; Garau et al., 2019; Amari et al., 2019). Only 3–5% of the AGS cases are AGS7, thus much remains to be discovered about its clinical course and features. *IFIH1* is essential in the mammalian immune system against viral infection, and heterozygous gain-of-function mutations lead to increased levels of type I interferons, causing the systemic manifestations of the disease (Amari et al., 2019; Oda et al., 2014; Rice et al., 2014).

We reprogrammed fibroblasts of a 14 years old male carrying a heterozygous dominant missense variant, placed within Helicase C-terminal Domain (IFIH1:NM_022168.4:c.[2471G>*A*, =]:p.[R824K, =]), using a Sendai virus-based vector delivering the four Yamanaka factors *OCT4, SOX2, KLF4,* and *c-MYC* (CytoTune-iPS 2.0 Sendai Reprogramming Kit, Thermo-Fisher Scientific) in feeder free conditions. Three clones (UNIBSi009-A, UNIBSi009-B, and UNIBSi009-C, summarized in Table 1) displaying an ESC-like morphology (Fig. 1A) were

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UNIBSi009-A	C3.A	male	14	Caucasian	GA	AGS7
UNIBSi009-B	C6.4	male	14	Caucasian	GA	AGS7
UNIBSi009-C	C7.4	male	14	Caucasian	GA	AGS7

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis: Immunofluorescence	Positive for pluripotency markers: OCT4, and TRA–1–60	Fig. 1 panel B
	Quantitative analysis: TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis	All samples were found to be pluripotent and transgene free: scored positive for self-renewal markers and negative for all three trilineage markers	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46,XY. Resolution 450–500	Supplementary Figure 1
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A STR analysis: 16 distinct loci: all matched to parental cell line	N/A Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous: *IFIH1* gene: c.[2471G>A, =]:p.[R824K, =]	Fig. 1 panel C
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma endpoint PCR	negative	Supplementary Figure 2
Differentiation potential	Direct differentiation into three germ layers: qPCR with TaqMan chemistry	C3.A: positive for *PAX6-SOX1* (Ectoderm), *NCAM1-ACTA2* (Mesoderm), and *GATA4-SOX17* (Endoderm). C6.4 and C7.4: positive for *PAX6-SOX1* (Ectoderm), *CXCR4-ACTA2* (Mesoderm), and *GATA4-SOX17* (Endoderm).	Fig. 1 panel E
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

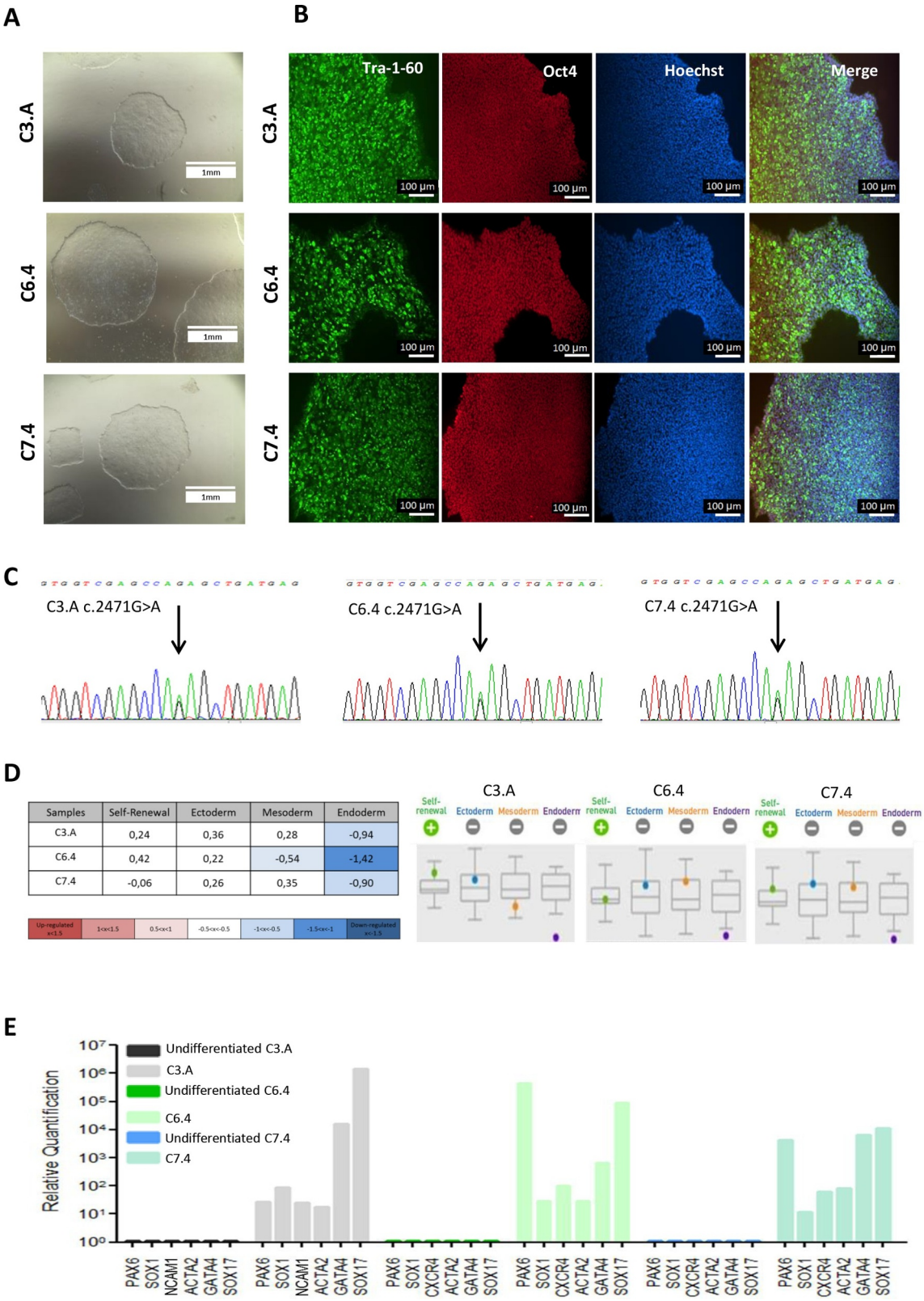

Fig. 1. A. iPSC clones morphology; scale bar = 1 mm. B. Immunofluorescence staining of iPSCs with the pluripotency markers Tra-1-60 (green) and Oct4 (red). Cell nuclei were stained with Hoechst 33342 (blue), and the three channels were merged; scale bar = 100 µm. C. iPSCs genomic sequence, showing the missense variation c.2471 G > A, marked with a black arrow. D. TaqMan hPSC Scorecard Report, displaying iPSCs pluripotency, using numerical values and the "Plus" "Minus" graphical symbols. E. Gene expression analysis of three germ layers markers.

Table 3
Reagents details.

Antibodies used for immunocytochemistry			
	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

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
	CXCR4	Hs00607978_s1
	NCAM1	Hs.PT.58.39694135
Endoderm	*GATA4*	Hs.PT.58.259457
	SOX17	Hs.PT.58.24876513
Housekeeping gene	*ACTB*	Hs.PT.39a.22214847

Primers		
	Target	Forward/Reverse primer (5′ – 3′)
Genus Mycoplasma (GSO/MGSO)	*16S rRNA*	GGGAGCAAACAGGATTAGATACCCT/TGCACCATCTGTCACTCTGTTAACCTC (268 nt)
Targeted mutation analysis	*IFIH1* (exon 13)	TGAAGACTGGCATGCTGAACAA/CAGAGATATCAATGGCAACCA (360 nt)

chosen for characterization.

These selected iPSC lines were checked for genetic uniqueness by short tandem repeat (STR) profiling against the parental fibroblasts (Table 2) and for private mutation by targeted Sanger Sequencing (Fig. 1C). Furthermore, iPSC lines were demonstrated to be mycoplasma-free (Supplementary Fig.2) and retained a normal karyotype (46,XY) (Supplementary Fig.1).

Pluripotency was assessed by immunofluorescence staining using Tra-1–60 and OCT4, markers that are expressed on the cell surface, and at nuclear level, respectively (Fig. 1B). Pluripotency characterization was performed using TaqMan hPSC Scorecard Panel (Thermo-Fisher Scientific). Scorecard analysis compares the expression of 94 genes of an unknown sample with that of 13 ESC (Embryonic Stem Cell) and iPSC control lines. The comparative analysis generates a reference value called Pluripotency Score. In order that a cell line to be called pluripotent, the score must be ≥ -0.5 for the self-renewal genes, and ≤ 0.5 for the genes of the three embryonic germ layers. The UNIBSi009-A, UNIBSi009-B, and UNIBSi009-C clones respect this condition and therefore are to be considered pluripotent as shown in Fig. 1D, that displays both the numerical values and the “Plus” “Minus” graphical symbols, referring to the self-renewal and Ectoderm, Mesoderm, Endoderm genes.

Finally, we verified the capability of iPSC clones to differentiate *in vitro* into ectoderm, mesoderm and endoderm layers, using the commercial StemMACS Trilineage Differentiation Kit (Miltenyi). We evaluated the gene expression of *PAX6-SOX1, NCAM1-CXCR4-ACTA2, GATA4-SOX17*, specific for each germ layers, by TaqMan quantitative PCR (Fig. 1E).

In conclusion, we generated three isogenic iPSC clones from a *IFIH1* mutated patient which represent the first description to our knowledge and can be considered an advanced *in vitro* model for AGS7. The iPSCs differentiation towards neurons could help in proceed to a better understanding of this syndrome.

3. Materials and methods

3.1. Fibroblasts reprogramming

Fibroblasts of a 14 years-old male patient with AGS7 were cultured in DMEM with 10% Fetal Bovine Serum, 1% L-Glutamine, and 1% Penicillin/Streptomycin (Euroclone) at 37 °C in 5% CO_2. Fibroblasts at 60% of confluence were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo-Fisher Scientific) following manufacturer's instructions. At day 8, cells were seeded onto a Matrigel-coated plate (Corning), the next day medium was changed to Nutristem hPSC XF medium (Biological-Industries) and after 20 days, colonies displaying an ESC-like morphology and positive for Tra-1–60 staining were manually picked and seeded on Matrigel-coated plates with daily renewal of the medium, to further expansion and characterization. iPSCs were manually picked every 5 days and incubated at 37 °C- 5% CO_2.

3.2. Sequencing

Genomic DNA was isolated with QIAmp DNA Bood Mini Kit (Qiagen). Mutation sequencing was performed by PCR amplification, using AmpliTaq Gold DNA Polymerase (Thermo-Fisher Scientific) and *IFIH1* primers listed in Table 3.

3.3. Karyotyping

Cells in active cell division were blocked in metaphase adding 10 μg/ml of colcemid to culture medium for 3 h at 37 °C (KaryoMax, Gibco Co. BRL), detached by trypsin–EDTA, swollen by exposure to hypotonic KCl solution (0.075 M) for 7 min at 37 °C, fixed in methanol/glacial acetic acid (3:1), and dropped on glass slides. Conventional QFQ-banding at 450–500 bands resolution was performed, according to the International System for Human Cytogenetic Nomenclature (ISCN 2016). A minimum of 20 metaphase spreads were evaluated for each sample.

3.4. RNA extraction

Total RNAs was extracted using NucleoSpin RNA II kit (Macherey-Nagel) and quantified by a spectrophotometer.

3.5. Pluripotency assays: immunofluorescence staining and TaqMan hPSC scorecard panel

iPSCs were fixed, permeabilized (Fix&Perm-Reagent kit, SIC), blocked (iBind Buffer, Invitrogen), and incubated with primary antibodies for 3 h at room temperature (RT). Then, cells were washed and

incubated with secondary antibodies for 1 h at RT (Table 3). Nuclei were stained with Hoechst 33342 (Thermo-Fisher Scientific). Cells were visualized under inverted fluorescence microscope (Olympus IX70), with the Image-Pro-Plus software v7.0 (Media Cybernetics).

For a deeper characterization, RNAs were sent to CellModel Services (Thermo-Fisher Scientific) that allows verification of pluripotency using The TaqMan hPSC Scorecard Panel.

3.6. Differentiation capability

Clones were differentiated *in vitro* according to the StemMACS Trilineage Differentiation Kit protocol (MACS Miltenyi Biotec). Briefly, iPSCs were dissociated into single cells and seeded on Matrigel-coated 24-well plates (10^5, 8×10^4, $1{,}3 \times 10^5$ cells for ectoderm, mesoderm, and endoderm, respectively) in specific media. At day 7, cells were collected for RNA extraction and cDNA synthesis with ImPromII Reverse Transcription System (Promega). Differentiation capability was evaluated by qPCR with lineage specific markers (Table 3), using TaqMan Probes (Thermo-Fisher Scientific) and iQ-MPLX powermix on CFX96 C1000 Touch Real-Time PCR Detection System, with CFX manager software v.3.1 (Bio-Rad). The relative quantification of target genes was calculated by the $2^{-}\Delta\Delta Ct$ method, using *ßACTIN* as housekeeping gene.

3.7. Mycoplasma detection

The absence of mycoplasma contamination was confirmed by PCR amplification (Table 3).

3.8. STR analysis

Fibroblasts and iPSCs were authenticated using AmpFlSTR Identifiler Plus (Life-Technologies) following the manufacturer's instructions.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101623.

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