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Lab Resource: Multiple Stem Cell Lines

Establishment of three iPSC lines from fibroblasts of a patient with Aicardi Goutières syndrome mutated in *RNaseH2B*

Rosalba Monica Ferraro[a,b,*], Stefania Masneri[a,b], Gaetana Lanzi[a,b], Chiara Barisani[b], Giovanna Piovani[c], Giulia Savio[c], Marco Cattalini[d,e], Jessica Galli[d,f], Cristina Cereda[g], Marco Muzi-Falconi[h], Simona Orcesi[i,j], Elisa Fazzi[d,f], Silvia Giliani[a,b]

[a] *Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy*
[b] *Angelo Nocivelli Institute for Molecular Medicine, ASST Spedali Civili, Brescia, Italy*
[c] *Biology and Genetics Division, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy*
[d] *Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy*
[e] *Pediatric Clinic, ASST Spedali Civili, Brescia, Italy*
[f] *Child Neurology and Psychiatry Unit, ASST Spedali Civili, Brescia, Italy*
[g] *Center of Genomic and Post-Genomic, IRCCS Mondino Foundation, Pavia, Italy*
[h] *Department of Biosciences, University of Milano, Milano, Italy*
[i] *Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy*
[j] *Child Neurology and Psychiatry Unit, IRCCS Mondino Foundation, Pavia, Italy*

A B S T R A C T

We report the generation of three isogenic iPSC clones (UNIBSi007-A, UNIBSi007-B, and UNIBSi007-C) obtained from fibroblasts of a patient with Aicardi Goutières Syndrome (AGS) carrying a homozygous mutation in *RNaseH2B*. Cells were transduced using a Sendai virus based system, delivering the human *OCT4, SOX2, c-MYC* and *KLF4* transcription factors. The resulting transgene-free iPSC lines retained the disease-causing DNA mutation, showed normal karyotype, expressed pluripotent markers and could differentiate *in vitro* toward cells of the three embryonic germ layers.

Resource Table:

Unique stem cell lines identifier	UNIBSi007-A UNIBSi007-B UNIBSi007-C
Alternative names of stem cell lines	AGS2_MV_C3 (UNIBSi007-A) AGS2_MV_C4 (UNIBSi007-B) AGS2_MV_C6 (UNIBSi007-C)
Institution	"Angelo 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: 10 Sex: female 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
Gene modification	NO
Type of modification	N/A
Associated disease	Aicardi Goutières syndrome
Gene/locus	RNaseH2B/13q14.3
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/UNIBSi007-A https://hpscreg.eu/user/cellline/edit/UNIBSi007-B https://hpscreg.eu/user/cellline/edit/UNIBSi007-C
Ethical approval	IRB Spedali Civili and University of Brescia, NP n.1603 -Studio AGS-CARIPLO

* Corresponding author.
E-mail address: rosalba.ferraro@unibs.it (R.M. Ferraro).

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Table 1.
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UNIBSi007-A	UNIBSi007-A	Female	10 y	Caucasian	A/A	Aicardi Goutières Syndrome type 2 (AGS2).
UNIBSi007-B	UNIBSi007-B	Female	10 y	Caucasian	A/A	Aicardi Goutières Syndrome type 2 (AGS2).
UNIBSi007-C	UNIBSi007-C	Female	10 y	Caucasian	A/A	Aicardi Goutières Syndrome type 2 (AGS2).

Table 2.
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	normal	Supplementary Figure 1
Phenotype	Qualitative analysis: immunocytochemistry	Positive for OCT4, and TRA–1–60 expression	Fig. 1 panel B
	Quantitative analysis: TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis	Positive score for self-renewal gene expression and negative score for ectodermal, mesodermal, and endodermal gene expression	Fig. 1 panel C
Genotype	Karyotype (Q-banding) and resolution	46,XX Resolution 450–500 bands	Supplementary Figure 2
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	Homozygous mutation: c.[529G > *A*]	Fig. 1 panel A
	Southern Blot OR WGS	*N/A*	*N/A*
Microbiology and virology	Mycoplasma endpoint PCR	Negative	Supplementary Figure 3
Differentiation potential	Direct *in vitro* differentiation into three germ layers UNIBSi007-A: TaqMan® hPSC Scorecard™ analysis	UNIBSi007-A: negative score for self-renewal gene expression and positive score for trilineage gene expression	Fig. 1 panel D
	UNIBSi007-B and UNIBSi007-C: qPCR with TaqMan chemistry	UNIBSi007-B and UNIBSi007-C: induction of *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*

1. Resource utility

AGS is a severe monogenic inflammatory encephalopathy. To date, for disease studying, knockout mouse approach is not available because of embryonic lethality of RNaseH2–deficient mice (Rabe, 2013). Thus, iPSCs generation represents the best option to obtain patient-specific neuronal cells for *in vitro* modeling of the disease.

2. Resource details

AGS is an early-onset monogenic type 1 interferonopathy, with severe neurologic injury. The syndrome is caused by altered nucleic acids metabolism due to defects in different nucleases or nucleotidases (Fazzi et al., 2013). Three of the six disease-causing genes identified to date encode components of the ribonuclease H2 (RNaseH2) complex (RNASEH2A, RNASEH2B, RNASE2HC). Among them, RNASEH2B mutations accounts for AGS type 2 (Crow et al., 2006).

RNaseH2 is an hydrolase able to process the RNA strand of RNA:DNA hybrids, and to recognize and cleave single ribonucleotides embedded in genomic DNA. The complex is composed by RNaseH2A, that contains the core of the catalytic domain, and by two accessory subunits RNaseH2B and RNaseH2C. Mutation in subunit A and B are known to have a destabilizing effect on the entire RNaseH2 complex, leading to an enzymatic function reduction (Pizzi et al., 2015).

In this study we generated and characterized three isogenic iPSC clones (UNIBSi007-A, UNIBSi007-B, and UNIBSi007-C) derived from fibroblasts of a 10 years old female affected by AGS with a homozygous mutation in RNaseH2B:NM_024570.3:c.[529G > *A*, 529G > *A*]:p.[A177T]; [A177T] (Table 1).

Reprogramming was performed in feeder free conditions using a modified form of Sendai virus as episomal vector expressing the Yamanaka's factors *OCT4, SOX2, KLF4*, and *c-MYC* (CytoTune-iPS 2.0 Sendai Reprogramming Kit).

The iPSC lines obtained displayed a round shape and growth behavior typical of embryonic stem cell (**Supplementary Fig.1**). Mycoplasma testing by PCR analysis was negative (**Supplementary Fig. 3**).

We confirmed by short tandem repeat (STR) profiling the identical profile between the iPSC lines and patient's fibroblasts (**Table 2**), and we also verified the presence of the homozygous mutation by Sanger sequencing (**Fig. 1A**). Karyotyping was performed at different passages, using standard QFQ-banding showing a normal 46, XX pattern for all selected clones (**Supplementary Fig. 2**).

Expression of key pluripotency markers, such as the cell membrane protein Tra-1-60, and the transcriptional factor OCT4, was demonstrated by immunostaining (**Fig. 1B**).

A deeper pluripotency characterization by TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis was performed at passage 15. Each line showed the expected result: a positive score for self-renewal gene expression and a negative score for expression of genes involved in ectodermal, mesodermal, and endodermal formation. Moreover, no residual Sendai virus was detected (**Fig. 1C**).

Finally, we tested the ability of iPSC clones to differentiate *in vitro* in the three germ embryonic layers. The UNIBSi007-A clone, was analyzed through TaqMan® Human Pluripotent Stem Cell Scorecard™ revealing increased gene expression in the three germ layers and reduced in self-renewal markers (**Fig. 1D**). The remaining clones, UNIBSi007-B and UNIBSi007-C, were evaluated by quantitative PCR (qPCR) for ectodermal, mesodermal and endodermal markers (*PAX6-SOX1, CXCR4-ACTA2, GATA4-SOX17*, respectively) showing the expected increased expression for all the genes (**Fig. 1E**).

In conclusion, we generated and characterized three isogenic iPSC clones carrying mutations in *RNaseH2B*, the most frequently mutated gene in AGS (Al Mutairi et al., 2018). These clones represents a useful tools for disease modelling, and a step forward to the data in literature, as to date, the description of AGS2 patient-derived-iPSCs has not been reported yet.

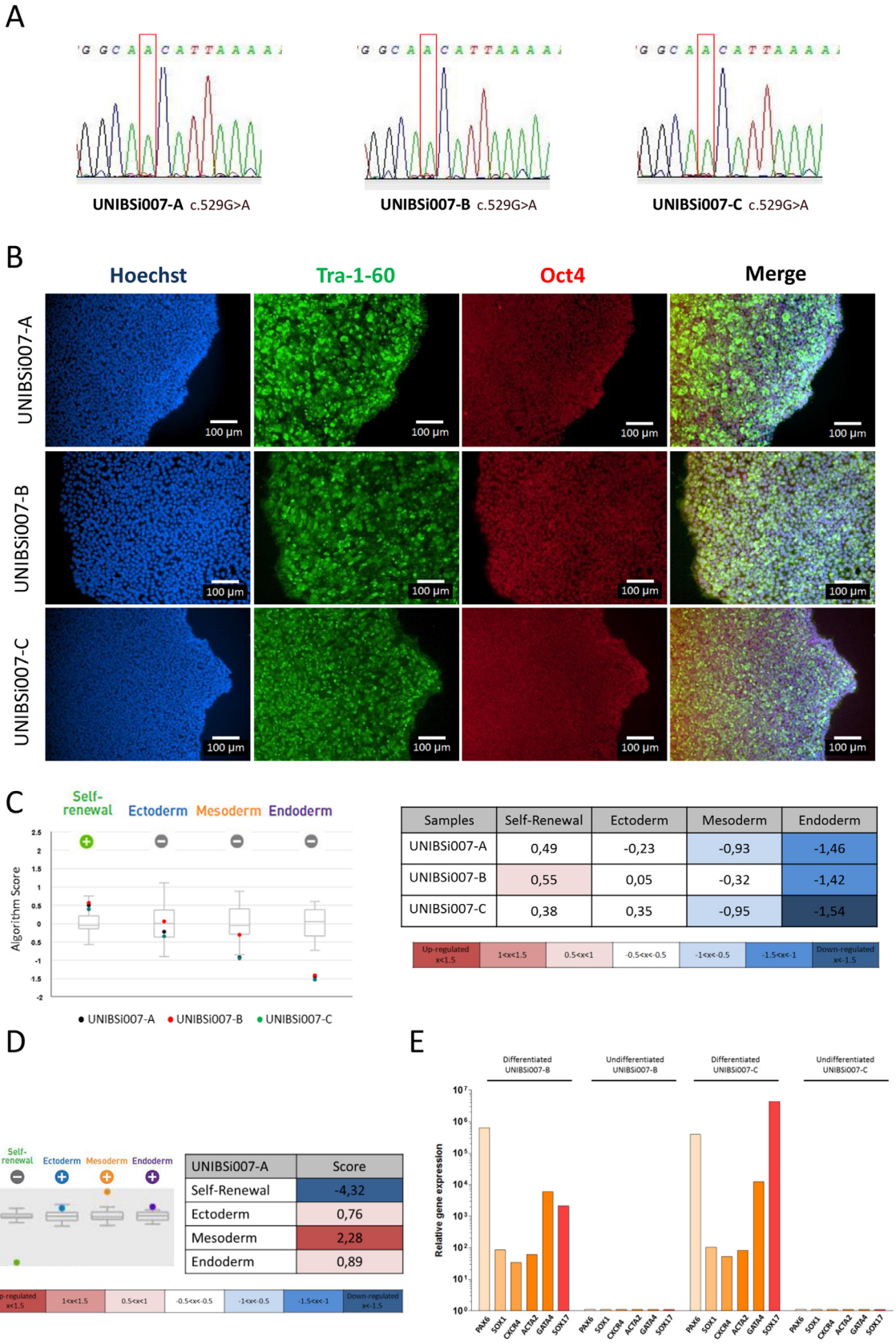

Fig. 1. Characterization of the iPSC lines UNIBSi007-A, UNIBSi007-B, and UNIBSi007-C. (A) Electropherograms showing the homozygous mutation in RNaseH2B. (B) Immunofluorescence staining for the stemness markers Tra-1-60 (green) and Oct4 (red). Nuclei were counterstained with Hoechst33342 (blue). (C) Pluripotency assessment using TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis. (D) Schematic summary of TaqMan® Human Pluripotent Stem Cell Scorecard™ panel depicting the in vitrotrilineage differentiation capability of UNIBSi007-A iPSC line. (E) Gene expression analysis of the three germ layers markers in UNIBSi007-B, and UNIBSi007-C iPSC clones.

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-13,998. RRID: AB_2,534,182
Pluripotency Markers	Mouse anti–TRA–1–60	1:100	Thermo Fisher Scientific, Cat# 4,110,000. RRID: AB_2,533,494
Secondary antibodies	Goat anti rabbit IgG (*H + +L*) Alexa Fluor 568	1:300	Thermo Fisher Scientific, Cat# A-11,011. RRID: AB_143,157
Secondary antibodies	Goat anti mouse IgG (*H + +L*) Alexa Fluor 488	1:300	Thermo Fisher Scientific, Cat# A-11,001. RRID: AB_2,534,069

Primers for PCR assay		
	Target	**Forward/Reverse primer (5′ – 3′)**
Mutation sequencing	*RNaseH2B exon*	TAAATGGTCTGAAGGCCACC/ATGAGGCTTCTGTGATATTAAG (347 bp)
Mycoplasma detection	16 s *rRNA*	GGGAGCAAACAGGATTAGATACCCT/TGCACCATCTGTCACTCTGTTAACCTC (268 bp)

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
Endoderm	*GATA4*	Hs.PT.58.259457
	SOX17	Hs.PT.58.24876513
Housekeeping gene	*ACTB*	Hs.PT.39a.22214847

3. Materials and methods

3.1. Fibroblasts reprogramming

Primary fibroblasts, derived from AGS2 patient's skin biopsy, were cultured in DMEM with 10% Fetal Bovine Serum, 1% L-Glutamine, and 1% Penicillin/Streptomycin (Euroclone) in standard conditions. For iPSCs generation 10^5 fibroblasts were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific) under manufacturer's instructions. After 8 days, cells were transferred onto a Matrigel-coated culture dish and then cultured with Nutristem hPSC XF medium (Biological-Industries). Colonies positive for Tra-1-60 staining, appearing after 20 days were manually picked to further expansion and characterization.

3.2. Karyotyping

Conventional QFQ-banding at 450 bands resolution according to the International System for Human Cytogenetic Nomenclature (ISCN 2016) was used to perform karyotypes. A minimum of 20 metaphase for each sample were analysed.

3.3. Sequencing

Genomic DNA was extracted using the QIAmp DNA Blood Mini Kit (Qiagen), and amplified by PCR using AmpliTaq Gold® Taq Polymerase (ThermoFisher Scientific) with *RNaseH2B* specific primers (Table 3). Sequencing reactions were performed using BigDye Terminator v1.1 Cycle Sequencing Kit, and run on a ABI Prism 3130 Genetic Analyzer (ThermoFisher Scientific), then analysed using SeqScape v3.0 Software (ThermoFisher Scientific).

3.4. TaqMan hPSC scorecard assay

RNAs collected from iPSCs were sent to ThermoFisher Scientific CellModel Service to perform TaqMan hPSC scorecard assay. This test is designed to verify the loss of Sendai virus, and to evaluate the expression levels of genes involved in self-renewal, endodermal, mesodermal, and ectodermal development.

3.5. Immunofluorescence staining

iPSCs were fixed and permeabilized using Fix&Perm-Reagent kit (SIC), then, blocked for 45 min. with iBind™ Buffer solution (Invitrogen) Slides were incubated for 3 h at room temperature (RT) with primary antibodies and after washing, secondary antibodies were added for 1 h at RT. The antibodies used are summarized in Table 3. Cellular nuclei were counter stained with Hoechst 33,342 (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).

3.6. In vitro trilineage differentiation

iPSCs were dissociated into single-cell suspension and seeded on matrigel-coated 24-well plates (10^5, 8×10^4, 13×10^4 cells for ectoderm, mesoderm, and endoderm, respectively) in the specific medium according to the StemMACS™ Trilineage Differentiation Kit protocol (MACS Miltenyi Biotec). Cells were then collected 7 days later for RNA extraction and qPCR of lineage specific markers. Only for UNIBSi006-B, RNAs collected from each germinal layer were mixed in a 1:1:1 ratio to perform TaqMan hPSC scorecard assay.

3.7. RNA extraction and qPCR

Total RNA was extracted using NucleoSpin® RNA II kit (Macherey-Nagel). RNAs were retro-transcribed by ImPromII™ Reverse Transcription System (Promega). qPCR for iPSCs differentiation was assessed 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.

3.8. Mycoplasma detection

The absence of mycoplasma contamination was confirmed by PCR using primers listed in Table 3.

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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.2019.101620.

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