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Lab Resource: Multiple Cell Lines

Generation of induced pluripotent stem cells (iPSCs) by retroviral transduction of skin fibroblasts from four patients suffering Williams-Beuren syndrome (7q11.23 deletion)

B. Kuebler [a,b], B. Aran [a,b], R. Flores [c,d], L.A. Pérez-Jurado [c,d], A. Veiga [a,b,e], R. Corominas [c,d,f], I. Cuscó [c,d,g]

[a] Regenerative Medicine Programme, Institut d'Investigació Biomèdica de Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain
[b] Spanish National Stem Cell Bank-Barcelona Node, Plataforma de Proteómica, Genotipado y Lineas Celulares (PRB3), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[c] Department of Experimental and Health Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Spain
[d] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain
[e] Reproductive Medicine Service, Dexeus Mujer. Hospital Universitari Dexeus, Barcelona, Spain
[f] Department of Genetics, Microbiology and Statistics, Universitat de Barcelona, Barcelona, Spain
[g] Department of Clinical and Molecular Genetics, Vall d'Hebron University Hospital Medicine Genetics Group, Vall d'Hebron Research Institute, Barcelona, Spain

A B S T R A C T

Skin fibroblasts were obtained from four patients with Williams-Beuren syndrome (WBS) carrying the typical 1.5 Mb or 1.8 Mb deletion at the 7q11.23 genomic region. Induced pluripotent stem cells (iPSCs) were generated by retroviral infection of fibroblasts with polycystronic vectors. The generated iPSC clones ESi059A, ESi060B and ESi068A had the 1.5 Mb deletion of 7q11.23 and ESi069A the 1.8 Mb, with no novel additional genomic alterations, stable karyotype, expressed pluripotency markers and could differentiate towards the three germ layers *in vitro* via embryoid body formation and *in vivo* by teratoma formation. WBS patient's lines are a valuable resource for *in vitro* modelling of WBS.

Resource Table

Unique stem cell lines identifier	ESi059A ESi060B ESi068A ESi069A
Alternative names of stem cell lines	SWB FiPS-4F-1-1, FiPS-4F-1-1, 1.1, (ESi059A) SWB FiPS-4F-5-1, FiPS-4F-5-1, 5.1, (ESi060B) SWB FiPS159-R4F-4, 159-4 (ESi068A) SWB FiPS344-R4F-2, 344-2 (ESi069A)
Institution	Regenerative Medicine Programme, Institut d'Investigació Biomèdica de Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain Centro de Investigación Biomédica en Red (CIBERER and CIBER-BBN). Universitat Pompeu Fabra. Barcelona. Institució Catalana de Recerca I Estudis Avançats (ICREA)
Contact information of distributor	Anna Veiga: aveiga@idibell.cat Ivon Cuscó: icusco@vhebron.net
Type of cell lines	iPSC
Origin	Human (Table 1)
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Retrovirus
Multiline rationale	Same disease, different patients
Gene modification	NO
Type of modification	NO modifications
Associated disease	Williams-Beuren syndrome (WBS), OMIM #194050
Gene/locus	7q11.23 deletion [arr[hg18]7q11.23(72,338,350-73,816,391)x1]
Method of modification	No modification
Name of transgene or resistance	Not Applicable
Inducible/constitutive system	Not Applicable
Date archived/stock date	19.01.2017; 12.06.2018
Cell line repository/bank	https://eng.isciii.es/eng.isciii.es/QueHacemos/Servicios/BIOBANCOS/BNLC/Paginas/LineasiPS.html https://hpscreg.eu/search?q=SWB
Ethical approval	Patient's parents informed consent obtained/ Ethics Review Board-competent authority approval obtained (Comité de Ética e Investigación Clinica-CEIC-CMRB) and by the Catalan Authority for Stem Cell Research (Approval number: 05/2011 and 03/2015)

(*continued on next column*)

Resource Table (*continued*)

E-mail address: icusco@vhebron.net (I. Cuscó).

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1. Resource utility

Williams-Beuren Syndrome (WBS) is a multisystemic neurodevelopmental disorder caused by a 26–28 genes microdeletion at 7q11.23. Patients present craniofacial dysmorphic features, cardiovascular alterations and behavioral-cognitive defects. iPSC lines allow to study the functional mechanisms disrupted, specific alterations caused during development and overcome the inability to access the primary tissues affected.

2. Resource details

The iPSC lines were generated from fibroblasts derived from skin biopsies from patients with WBS, carrying the typical 1.5 Mb or 1.8 Mb hemizygous deletions at chromosome 7q11.23 (Table 1). WBS is a rare genetic neurodevelopmental disorder [OMIM #194050] caused by a heterozygous deletion of multiple contiguous genes at chromosome band 7q11.23, affecting approximately 28 genes (Pérez-Jurado et al., 1996; Morris and Mervis, 2000). The clinical characteristics of the patients include severe supravalvular aortic stenosis (SVAS), hypertension, hyperacusis, distinct facial appearance, and intellectual disability. The 1.5 Mb deletions had occurred *de novo* in the paternal (SWB FiPS-4F-5-1) or maternal (the other lines) chromosomes and were confirmed by molecular karyotype (Fig. 1A) and microsatellites marker typing (Bayés et al., 2003) (Table 2).

Skin derived fibroblasts were reprogrammed using two polycystronic retroviruses encoding the four factors (pMXs-OCT4-VP16-SOX2-mOrange; pMXs-KLF4-MYC-GFP). The morphology of the colonies was the typical of iPSCs. Two independent clones per patient were selected for characterization. Integration of retroviruses was determined by PCR (Supplementary Fig. S1C) and silencing of the transgenes was confirmed by quantitative RT-PCR (Supplementary Fig. S1E) using specific primers (Table 3). Expression of pluripotency markers was confirmed by immunocytochemistry with antibodies against human OCT4, SOX2, NANOG, TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4 (Fig. 1B, scale bars 50 μm). The mRNA expression of endogenous human factors OCT4, SOX2, KLF4 and c-MYC was confirmed by qRT-PCR (Fig. 1C) and all clones showed positive alkaline phosphatase (AP) activity (Supplementary Fig. S1F). The capacity of *in vitro* and *in vivo* differentiation towards the three germ layers was determined by embryoid body (EB) formation and differentiation and teratoma formation, respectively, followed by immunofluorescence analyses demonstrating the expression of definitive endoderm (AFP and FOXA2), ectoderm (TUJ1 and GFAP, or MAP2, PAX6, or Neurofilament) and mesoderm (ASMA and ASA) markers (Fig. 1D, E, scale bars 50 μm). Sections of iPSC derived teratomas were stained with Hematoxylin/Eosin (H/E). Representative pictures of differentiation to endoderm, ectoderm and mesoderm are shown (Supplementary Fig. S1D). The normal 46, XX, or 46, XY karyotype of the iPSC lines were confirmed by G-banding analysis (Supplementary Fig. S1A). The genomic integrity of the iPSC lines and the presence of the 1.5 Mb or 1.8 Mb 7q11.23 deletion was confirmed by SNP-array (Fig. 1A). Additionally, a 16p13.11 microduplication (chr16:15–16.2 Mb) in patient SWB FiPS159-R4F-4 was defined in iPSCs and fibroblasts. The iPSC identity was confirmed by short tandem repeat analysis and compared with the patients fibroblasts (Supplementary Table S1).

3. Materials and methods

Dermal fibroblasts at passage 4 were reprogrammed by retroviral infection. Retroviruses were produced in Phoenix Amphotrophic cells following transfection with pMX-OCT4_Flag-VP16-PTV-Sox2_HA-Orange or pMX-KLF4-cMYC-GFP polycistronic vectors. Retrovirus containing medium was collected 48 h post-transfection, 0.45 μm filtered, supplemented with Polybrene (4 μg/ml) and used to transduce $1x10^5$ cells by spin infection. After 3 days, transduced human fibroblasts were trypsinized and seeded onto irradiated human foreskin fibroblasts in hES medium (Knockout DMEM with 20% Knockout serum replacement, 2 mM Glutamax, 1% penicillin–streptomycin, 0.1 mM β-mercaptoethanol, 1% non-essential amino acids (NEAA) (all Gibco), and 10 ng/ml bFGF (Millipore)) until iPSC colonies appeared. Colonies were picked manually for expansion. From passage 5 on, colonies were adapted to feeder free conditions.

For integration analysis, genomic iPSC DNA was isolated using the DNeasy-blood-&-tissue kit (Qiagen). Standard PCR reaction was performed (BioTaq) with specific primers (Table 3). Products were visualized in 1.5% agarose gels. Fibroblasts were used as negative controls.

For silencing analysis, total mRNA was isolated from iPSCs following the Trizol-based procedure and treated with DNAase. cDNA was synthesized by SuperScript II reverse transcriptase protocol (Thermo Fisher Scientific). The qRT-PCR reactions were performed using the Power SYBR Green PCR Master Mix in an ABI-Prism7900 thermocycler (Applied Biosystems). Ct values were normalized with *GAPDH* as housekeeping gene and data were analyzed with the 2-ΔΔCt method. mRNA expression levels of virus derived trans *OCT4/SOX2* and trans *KLF4/cMyc* and endogenous pluripotency markers OCT4, *SOX2, KLF4* and c-*MYC* were analyzed in WBS iPSCs (Supplementary Fig. S1E and Fig. 1C).

Genomic integrity of iPSCs was confirmed by G-banded metaphase karyotype analysis (Ambar, Barcelona). Briefly, 70% confluent feeder free iPSC colonies were treated with colcemid (KaryoMax, Gibco), trypsinized, incubated with hypotonic solution, fixed in Carnoy fixative (75% methanol, 25% acetic acid), and karyotypes performed following standard procedures.

To detect AP activity, iPSCs were fixed with 4% paraformaldehyde for 1 min, washed with PBS and incubated with AP staining solution (Sigma) until colonies turned blue.

To identify pluripotency markers, immunocytochemistry was performed (Marti et al., 2013). iPSCs were fixed with 4% paraformaldehyde (PFA), blocked and permeabilized with TBS + 0.5% Triton X-100 + 6% donkey serum. Primary antibodies (Table 3) were incubated overnight in TBS + 0.1% Triton X-100 + 6% donkey serum. Secondary antibodies (Table 3) were incubated for 2 h at 37 °C. Nuclei were stained with 4′,6-diamino-2-fenilindol (DAPI).

In vitro differentiation was promoted by EB formation. iPSC colonies were lifted manually and incubated in ultra-low attachment plates in mTeSR1 medium. After 24 h, medium was changed to differentiation medium for additional 24–48 h. Ectoderm medium: 50% Neurobasal medium, 50% DMEM/F12, 1% N2, 1% B27, 1% Glutamax and 1% Penicillin-Streptomycin; Endoderm medium: Knockout-DMEM, 10% FBS, 1% NEAA, 0.1% β-mercaptoethanol, 1% Glutamax and 1% Penicillin-Streptomycin (all Gibco); Mesoderm medium: Endoderm medium supplemented with 0.5 mM ascorbic acid. EBs were seeded on matrigel-coated slide flasks and cultured in differentiation media for

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
SWB FiPS-4F-1-1 (ESi059A)	1.1	Female	15	Caucasian	1.5 Mb deletion	Williams-Beuren Syndrome
SWB FiPS-4F-5-1 (ESi060B)	5.1	Female	15	Caucasian	1.5 Mb deletion	Williams-Beuren Syndrome
SWB FiPS159-R4F-4, 159-4 (ESi068A)	159.4	Male	14	Caucasian	1.5 Mb deletion	Williams-Beuren Syndrome
SWB FiPS344-R4F-2, 344-2 (ESi069A)	344.2	Male	7	Caucasian	1.8 Mb deletion	Williams-Beuren Syndrome

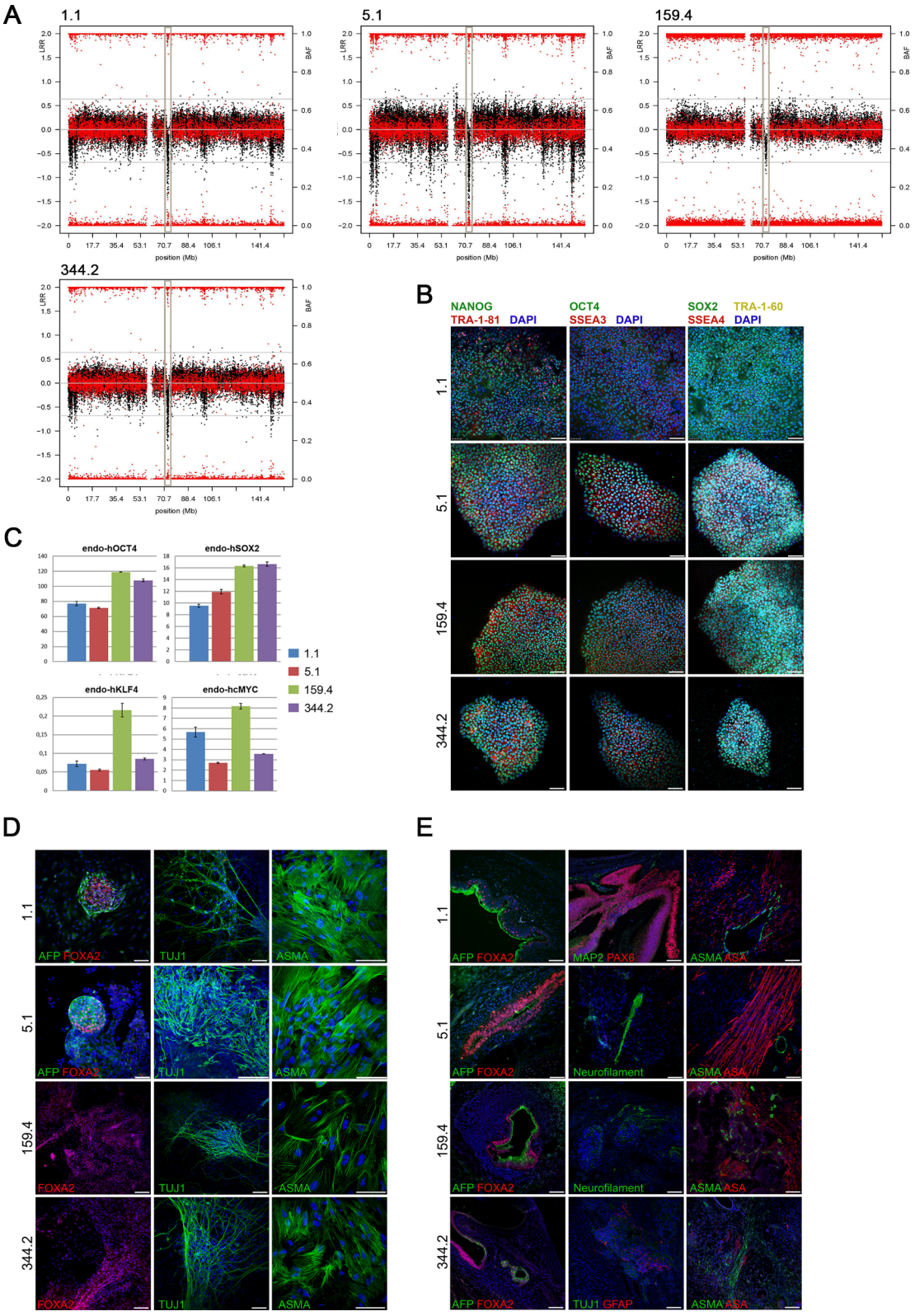

Fig. 1. Characterization of WBS iPSC lines. A. SNP-arrays of lines showing the presence of the typical 1.5 Mb or 1.8 Mb deletion at the 7q11.23 genomic region. B. Confocal images showing immunodetection of pluripotency markers. Scale bars: 50 mm. C. mRNA expression levels of endogenous pluripotency markers. 2-ΔΔCt values normalized to GAPDH. D. *In vitro* differentiation of embryoid bodies using specific antibodies against the endodermal markers a-fetoprotein (AFP) and forkhead box A2 (FOXA2), ectodermal markers paired box protein Pax-6 (PAX6), microtubule-associated protein 2 (MAP2), Neurofilament, bIII-tubulin (TUJ1) and Glial fibrillary acidic protein (GFAP) and mesodermal marker α-smooth muscle actin (ASMA) and α-sarcomeric actin (ASA). Nuclei were stained with DAPI. Scale bars: 50 mm. E. *In vivo* differentiation and teratoma formation using specific antibodies against the endodermal markers AFP and FOXA2, the ectodermal marker TUJ1 and the mesodermal marker ASMA. Nuclei were stained with DAPI. Scale bars: 50 mm.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Not shown but available with author
Phenotype	Immunocytochemisty	OCT4, SOX2, NANOG, TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4	Fig. 1 panel B
	Gene expression (qRT-PCR)	*endo*-hOCT4, *endo*-hSOX2, *endo*-KLF4, *endo*-c-MYC, *trans*-OCT4/SOX2, *trans*-KLF4/c-MYC	Fig. 1 panel C Supplementary Fig. S1 panel E
	Integration PCR	Integration of retroviral OCT4, SOX2, KLF4 and c-MYC	Supplementary Fig. S1 panel C
	AP staining	positive	Supplementary Fig. S1 panel F
Genotype	Karyotype (G-banding) and resolution	ESi059A, 46XX, resolution 500 ESi060B, 46XX, resolution 500 ESi068A, 46XY, resolution 500 ESi069A, 46XY, resolution 500	Supplementary Fig. S1 panel A
Identity	STR analysis (microsatellite Study)	Performed (10 markers tested, all matching)	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Molecular Karyotype	Molecular karyoype Illumina Infinium Human Core-24 BeadChip, IMPPC or CEGEN	Fig. 1 panel A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. S1 panel B
Differentiation potential	Embryoid body formation and Teratoma formation	(1) Embryoid body formation: AFP, FOXA2, TUJ1, GFAP, ASMA, ASA	Fig. 1 panel D Fig. 1 panel E
		(2) Teratoma formation: AFP, FOXA2, Neurofilament, MAP2, PAX6, TUJ1, GFAP, ASMA, ASA Hematoxylin/Eosin staining	Supplementary Fig. S1 panel D
Donor screening (OPTIONAL)	N/A	N/A	N/A
Genotype additional info (OPTIONAL)	N/A	N/A	N/A

15–20 days. Cells were analyzed by immunocytochemistry as described above. Confocal images were taken using Leica TSC SPE/SP5 microscopes.

In vivo differentiation was induced by intratesticular injection of iPSCs into Severe Combined immunodeficient mice. PFA-fixed teratomas were paraffin embedded and analyzed by immunocytochemistry as described above and sections stained with Hematoxylin and Eosin (H/E).

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT4	1:25	Santa Cruz, sc-5279, RRID: AB_628051
	Goat anti-NANOG	1:25	R&D Systems, AF1997, RRID:AB_355097
	Rabbit anti-SOX2	1:100	ABR, PA1-16968, RRID: AB_2195781
	Rat anti-SSEA3	1:2	Hybridoma Bank, MC-631, RRID:AB_528476
	Mouse anti-SSEA4	1:2	Hybridoma Bank, MC-813-70,RRID:AB_528477
	Mouse anti-TRA-1-60	1:100	Millipore, MAB4360, RRID: AB_2119183
	Mouse anti-TRA-1-81	1:100	Millipore, MAB4381, RRID: AB_177638
Differentiation Markers	Mouse anti-TUJ1	1:40	Covance, MMS-435P, RRID: AB_2313773
	Rabbit anti-GFAP	1:1000	Dako, Z0334, RRID: AB_10013382
	Rabbit anti-Neurofilament	1:100	Sigma N4142, RRID: AB_477272
	Mouse anti-Map2	1:25	Santa Cruz 32791, RRID: AB_627948
	Rabbit anti-Pax6	1:100	Covance PRB-278P, RRID: AB_291612
	Mouse anti-ASMA	1:400	Sigma, A5228, RRID: AB_262054
	Mouse anti-ASA	1:400	Sigma, A2172, RRID: AB_476695
	Rabbit anti-AFP	1:200	Agilent, A0008, RRID: AB_2650473
	Goat anti-FOXA2	1:50	R&D Systems, AF2400, RRID:AB_2294104
Secondary antibodies	A488 Goat anti-mouse	1:500	Jackson, 715-545-151, RRID:AB_2341099 and 115-546-071, RRID: AB_2338865
	A488 Donkey anti-Rabbit	1:500	Jackson, 711-545-152, RRID:AB_2313584
	CY2, Goat anti-rat	1:200	Jackson, 112-225-075, RRID:AB_2338276
	Cy3, Donkey anti-rabbit DyLight	1:200	Jackson, 711-475-152, RRID:AB_2340616
	Cy3, Donkey anti-mouse	1:200	Jackson, 715-165-140, RRID:AB_2340812
	Cy3, Donkey anti-Goat		Jackson, 705-165-147, RRID:AB_2307351

Primers

	Target	**Forward/Reverse primer (5'-3')**
Integration	pMXs-OS-Orange	GAGCAAGGGCGAGGAGAATAAC/ AAGTAGTCGGGGATGTCGGC
	pMX-KM-GFP	GCACCATCTTCTTCAAGGACGAC/ TCTTTCGCTCAGGGCGGACTG
Silencing	Tg-mcMYC	GCTTCGAAACTCTGGTGCAT/ CCTACAGGTGGGGTCTTTCA
	Tg-mSOX2	GGCCATTAACGGCACACT/ CCTACAGGTGGGGTCTTTCA
Pluripotency Markers (qPCR)	endo-hOCT4	GGGTTTTTGGGATTAAGTTCTTCA/ GCCCCCACCCTTTGTGTT
	endo-hSOX2	CAAAAATGGCCATGCAGGTT/ AGTTGGGATCGAACAAAAGCTATT
House-Keeping Gene (qPCR)	GAPDH	GCACCGTCAAGGCTGAGAAC/ AGGGATCTCGCTCCTGGAA

The deletions at 7q11.23 and the absence of novel rearrangements were confirmed by SNP-array (Illumina Infinium Human Core-24 BeadChip, IMPPC or CEGEN) using 0.5 μg of iPSC genomic DNA. Line identities were corroborated by microsatellites genotyping (STRs-study).

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.

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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.2020.102087.

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