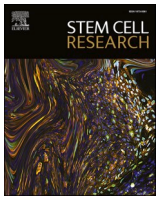
Stem Cell Research 86 (2025) 103741

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



Lab Resource: Multiple Cell Lines

Generation of two pairs of induced pluripotent stem cells from Angelman syndrome patients with class I 15q11.2-q13.1 deletions and their unaffected familial controls

João Camões dos Santos [a,b], Francisca Cazaux Mateus [a,b], Maria Arez [a,b], Evguenia P. Bekman [a,b,c], Simão T. da Rocha [a,b,*]

[a] Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal
[b] Associate Laboratory i4HB, Institute for Health and Bioeconomy, Lisbon, Portugal
[c] The Egas Moniz Center for Interdisciplinary Research, Caparica, Almada, Portugal

A B S T R A C T

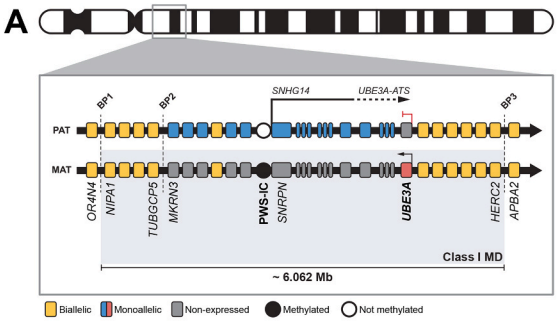
Angelman syndrome (AS) is a rare neurodevelopmental disorder characterized by severe developmental delay, speech impairment, ataxia and happy demeanor. AS is caused by loss-of-function of maternal *UBE3A* in neurons due to (epi)genetic abnormalities. Here, we report two new induced pluripotent stem cell (iPSC) lines from male and female patients carrying ~ 6 Mb deletions in chr15q11.2-q13.1, together with familial control iPSC lines. All lines express pluripotent stem cell markers, demonstrate trilineage differentiation, and maintain genetic and epigenetic integrity at the locus of interest. These iPSCs provide a platform to model class I deletions, the most severe AS cause, and accelerate therapy development.

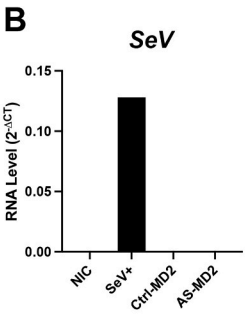
1. Resource Table:

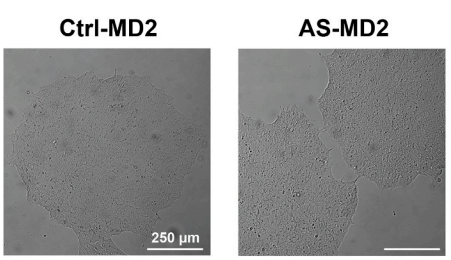
Unique stem cell lines identifier	1. IBBISTi014-A 2. IBBISTi015-A 3. IBBISTi016-A 4. IBBISTi017-A
Alternative name(s) of stem cell lines	1. Ctrl-MD1 [clone O] *(IBBISTi014-A)* 2. AS-MD1 [clone N] *(IBBISTi015-A)* 3. Ctrl-MD2 [clone 7] *(IBBISTi016-A)* 4. AS-MD2 [clone Q] *(IBBISTi017-A)*
Institution	Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico, University of Lisbon
Contact information of distributor	Dr. Simão T. da Rocha: simao.rocha@tecnico.ulisboa.pt
Type of cell lines	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Ctrl-MD1 Age: 41 Sex: Male AS-MD1 Age: 13 Sex: Male Ctrl-MD2 Age: 37 Sex: Female AS-MD2 Age: 6 Sex: Female
Cell Source	Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Non-integrating Sendai Virus (SeV)
Genetic Modification	N/A
Type of Genetic Modification	Congenital
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	qRT-PCR
Associated disease	Angelman Syndrome
Gene/locus	Class I deletion in chromosome 15q11.2-q13.1
Date archived/stock date	2024
Cell line repository/bank	https://hpsreg.eu/cell-line/IBBISTi014-A https://hpsreg.eu/cell-line/IBBISTi015-A https://hpsreg.eu/cell-line/IBBISTi016-A https://hpsreg.eu/cell-line/IBBISTi017-A
Ethical approval	The study was approved by the Ethical Committee of the Academic Medical Center of Lisbon (CAML), Lisbon, Portugal. Approval number: 300/22

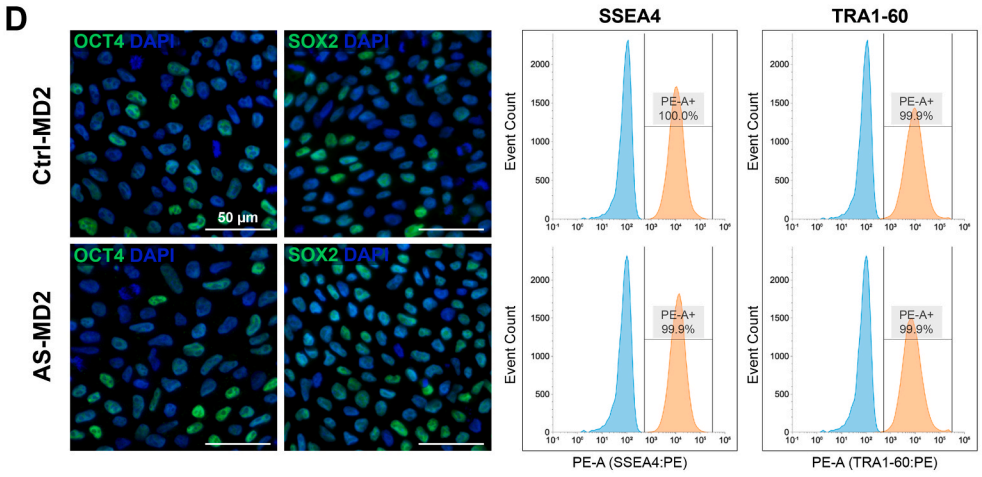
* Corresponding author.
E-mail address: simao.rocha@tecnico.ulisboa.pt (S.T. da Rocha).

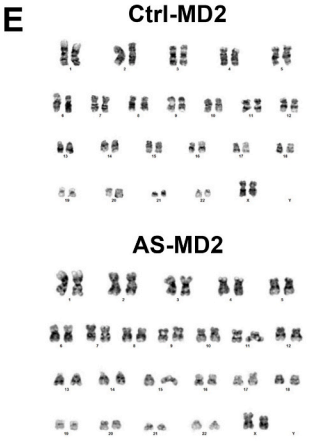
https://doi.org/10.1016/j.scr.2025.103741
Received 31 March 2025; Received in revised form 15 May 2025; Accepted 21 May 2025
Available online 22 May 2025

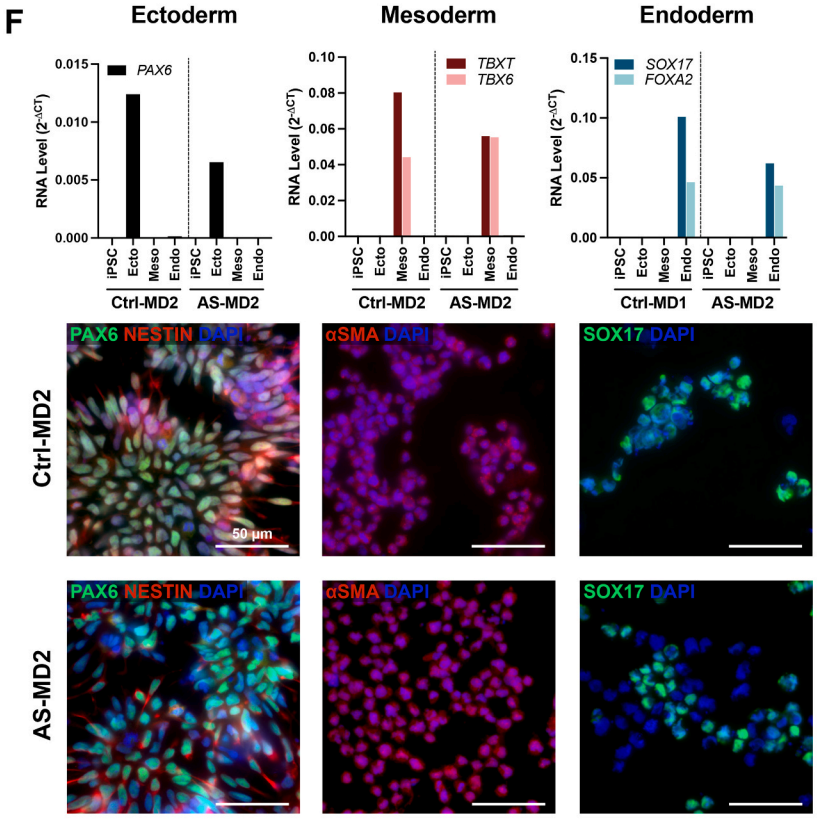


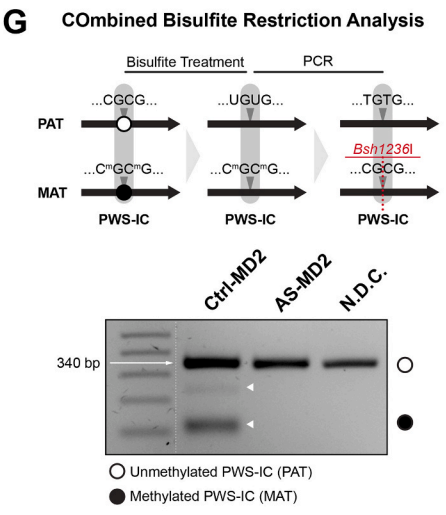


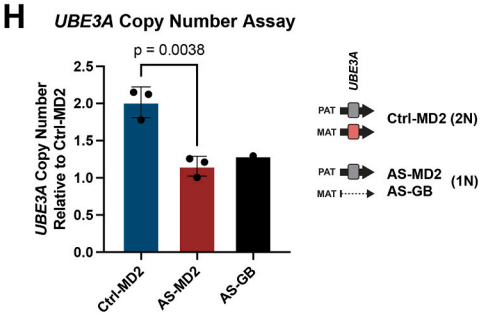












(caption on next page)

Fig. 1. Characterization of the new class I deletion AS cell line (IBBISTi017-A, AS-MD2) and the genetically matched familiar control (IBBISTi016-A, Ctrl-MD2). **(A)** Genomic map of chromosome 15q11.2–13 showing the imprinted genes and gene expression pattern in neurotypical individuals. The regions absent in this 6.062 Mb class I deletion is highlighted. **(B)** Sendai virus (SeV) RNA levels from PBMCs (non-infected control, NIC), infected samples from day 3 of reprogramming, and final iPSCs; RNA levels measured using *GAPDH* as a reference. **(C)** Brightfield microscopy images of the final iPSCs; Scale bar: 250 μm. **(D)** Expression of undifferentiated markers OCT4 and SOX2 by immunofluorescence (left) and SSEA4 and TRA1-60 by flow cytometry (right); Scale bar: 50 μm. **(E)** Karyotype of final iPSCs by G-banding. **(F)** Trilineage commitment evaluation by expression of PAX6/NESTIN (ectoderm), *TBXT/TBX6*/αSMA (mesoderm) and SOX17/*FOXA2* (endoderm) through qRT-PCR (top) and/or immunofluorescence microscopy (bottom); Scale bar: 50 μm; RNA levels measured using *GAPDH* as a reference. **(G)** Combined bisulfite restriction analysis (on top) detects both methylated and unmethylated PWS-IC in the Ctrl sample and only the unmethylated PWS-IC in the AS sample; N.D.C. is the non-digested control using the Ctrl sample. **(H)** *UBE3A* copy number assay shows MD cell lines (AS-MD2 and AS-GB) have only one copy of the *UBE3A* gene compared to the control; Unpaired *t*-test statistic for the *p*-value.

2. Resource utility

We generated new patient-derived iPSC lines carrying class I deletions of the chromosome 15q11.2-q13.1 (~6 Mb) together with genetically matched familial controls. These cells enable the generation of neuronal models of AS with minimal genetic variability. Additionally, they provide a novel platform to study the most severe genotype of AS, previously unavailable (Camões dos Santos et al., 2023).

3. Resource details

Angelman syndrome (AS, OMIM#105830) is a rare neurodevelopmental disorder affecting 1 in 20,000 live births caused by the loss of functional UBE3A protein in neurons. The *UBE3A* gene is located on chromosome 15q11-q13, a region regulated by genomic imprinting, an epigenetic mechanism driven by differential DNA methylation that restricts gene expression to a single parental allele (Fig. 1A). Whereas the maternal allele is methylated at the imprinting center of this region, known as PWS-IC, the paternal PWS-IC is not and serves as a promoter for the transcription of a large polycistronic unit, *SNHG14*, encompassing several genes and non-coding RNAs. In neurons, this transcript is extended and includes *UBE3A-ATS* that interferes with paternal *UBE3A* transcription, rendering *UBE3A* to be expressed exclusively from the maternal copy (Fig. 1A). AS can result from four distinct (epi)genetic mechanisms, with megabase-scale deletions (megadeletions, MDs) on the maternal chromosome being the most common (~70 %) and severe (Maranga et al., 2020; Yang et al., 2021). These deletions not only affect the imprinted region but also several other genes, including GABA receptor subunits and other neurodevelopmentally relevant genes (Fig. 1A). In patients, one of the largest deletions – class I (BP1-BP3) – spans approximately 6 Mb and encompasses 16 coding genes (Fig. 1A).

Here, we generated two sets of iPSCs from two unrelated AS individuals carrying MD, one male (AS-MD1) and one female (AS-MD2), together with genetically matched control iPSCs from the father (Ctrl-MD1) and mother (Ctrl-MD2), respectively. Comparative Genomic Hybridization (CGH) array analysis on the patients' DNA revealed approximately 6 Mb deletions of 15q11.2-q13.1 (AS-MD1, 6.175 Mb: chr15:22,582,283–28,757,592; AS-MD2, 6.062 Mb: chr15:22,681,309–28,743,714), both corresponding to class I MDs (Fig. 1A).

PBMCs were obtained from all individuals and reprogrammed into iPSCs using a non-integrating Sendai virus (SeV) method, transiently expressing OCT4, SOX2, KLF4 and c-MYC. Individual colonies, originating from a single cell, were isolated, expanded and screened for SeV clearance (Fig. 1B). The established SeV– iPSC lines exhibit typical PSC-like colony morphology (Fig. 1C) and express protein markers of the undifferentiated state including nuclear OCT4 and SOX2, and cell surface TRA1-60 and SSEA4, detected by immunofluorescence (IF) and flow cytometry, respectively (Fig. 1D). All cell lines have normal karyotype (MD1 Pair: 46, XY; MD2 Pair: 46, XX) with no abnormalities detectable by G-band karyotyping (Fig. 1E). To confirm the potency of the cell lines, a trilineage differentiation assay was performed to induce commitment into the three germ layers. At the endpoint of differentiation, expression of germ layer-specific markers was assessed by qRT-PCR and/or IF, including the markers PAX6 and NESTIN for ectoderm; *TBXT*, *TBX6* and αSMA for mesoderm; SOX17 and *FOXA2* for endoderm (Fig. 1F). All cell lines were able to commit to the three germ layers (Fig. 1F).

During iPSC reprogramming epigenetic defects at the level of imprinted regions may occur (Arez et al., 2022; Pólvora-Brandão et al., 2018). To check the imprinting fidelity of the new iPSC lines, we performed a COmbined Bisulfite Restriction Analysis (COBRA) for PWS-IC (Fig. 1G). In short, bisulfite-treated DNA was used to amplify this region by PCR followed by a *Bsh1236*I restriction assay which is only able to digest DNA with previously methylated cytosines (methylated allele). The samples from Ctrl cell lines show the expected digestion pattern, confirming the presence of both methylated and unmethylated PWS-IC (Fig. 1G). As for the AS samples, no digestion bands are present, confirming the absence of the maternal methylated PWS-IC (Fig. 1G). Finally, we performed a TaqMan™ probe-based copy number assay for *UBE3A* which confirmed both AS cell lines have approximately half the number of copies of their respective control, akin to the already established AS-GB (Maranga et al., 2022), carrying a ~5 Mb class II MD (Fig. 1H).

All iPSC lines were routinely monitored, confirmed negative for mycoplasma and STR profiling confirmed a match with the original PBMCs. All results are summarized in Fig. 1 for Ctrl-MD2 and AS-MD2, while results for Ctrl-MD1 and AS-MD1 are presented in Supplementary Fig. 1, with all cell lines summarized in Table 1. Besides the original lines, at least two additional independent clones were generated and biobanked, but not as extensively characterized.

4. Materials and methods

4.1. Generation and maintenance of iPSCs

Blood samples were collected with written informed consent and the guidelines of the Lisbon Academic Medical Centre Biobank (Biobanco-iMM). Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque and cryopreserved in 20 % dimethylsulfoxide (DMSO) in Fetal Bovine Serum (FBS, Gibco, A5670701), following Biobanco-iMM's standardized protocol.

For reprogramming, PBMCs were thawed and cultured in StemPro™-34 SFM medium (Gibco, 10639011) supplemented with IL-3, IL-6, FLT3 and SCF (PeproTech, 200–03, 200–06, 300–19, 300–07). After 4 days, cells were transduced with the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen, A16517) following manufacturer's instructions. Three days post-transduction, the cells were transferred to Matrigel® (Corning, 354230)-coated well plates and transitioned to mTeSR™Plus (Stem Cell Technologies, 05825) over the next two days. At around day 10, single cell-derived colonies emerged and were isolated, based on their morphology, into separate Matrigel®-coated well plates in mTeSR™Plus. After this, iPSCs were continuously passaged and checked for *SeV* clearance by qRT-PCR.

iPSCs were maintained in mTeSR™Plus medium, in a humidified incubator at 37 °C and 5 % CO_2, and were routinely passaged every 4 to 5 days at a ratio of 1:3–1:6 using 0.5 mM EDTA dissociating buffer

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal Ctrl-MD1 and AS-MD1 Normal Ctrl-MD2 and AS-MD2	Fig. 1, panel C Supplementary Fig. 1, panel C
Phenotype	Qualitative analysis	Expression of pluripotency markers OCT4 and SOX2: Ctrl-MD1 and AS-MD1 Ctrl-MD2 and AS-MD2	Fig. 1, panel D Supplementary Fig. 1, panel D
	Quantitative analysis	Ctrl-MD1, SSEA4: 94.6 % TRA1-60: 82.0 % AS-MD1, SSEA4: 99.8 % TRA1-60: 99.1 % Ctrl-MD2, SSEA4: 100 % TRA1-60: 99.9 % AS-MD2, SSEA4: 99.9 % TRA1-60: 99.9 %	Fig. 1, panel D Supplementary Fig. 1, panel D
Genotype	Karyotype (G-banding) and resolution	Ctrl-MD1: 46, XY [28 metaphases], resolution 300–500 AS-MD1: 46, XY [26 metaphases], resolution 300–500 Ctrl-MD2: 46, XX [20 metaphases], resolution 300–500 AS-MD2: 46, XX [22 metaphases], resolution 300–500	Fig. 1, panel E Supplementary Fig. 1, panel E
Identity	Microsatellite PCR (mPCR) OR STR analysis	Not Performed 16 STR loci tested 100 % matched for: Ctrl-MD1 iPSCs and PBMCs AS-MD1 iPSCs and PBMCs Ctrl-MD2 iPSCs and PBMCs AS-MD2 iPSCs and PBMCs	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing Southern Blot OR WGS	N/A N/A	N/A N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Submitted as Supplementary Data 2
Differentiation potential	Directed differentiation	All iPSC lines were differentiated into the three germ layers	Fig. 1, panel F Supplementary Fig. 1, panel F
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (qRTPCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm (PAX6, NESTIN); Mesoderm (TBXT, TBX6, αSMA); Endoderm (SOX17, FOXA2); Assessed by immunofluorescence or qRT-PCR	qRT-PCR IF
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

(Invitrogen, 15575020). All analyses were performed below passage 15 for all clones.

4.2. Quantitative real time PCR (qRT-PCR)

For gene expression analysis, total RNA was extracted from cultured cells using NZYol (NZYTech, MB18501), treated with DNase I (Roche, 04716728001) and used to synthesize cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, 4368814). cDNA was amplified by qRT-PCR with the NZYSupreme qPCR Green Master Mix (NZYTech, MB44002) using the specific primer pairs shown in Table 2. Expression levels of genes were normalised to the *GAPDH* housekeeping gene.

For copy number assay, DNA from iPSCs and PBMCs, as well for the AS-GB line with a class II deletion (Maranga et al., 2022), was extracted with the DNA Miniprep Plus kit (Zymo Research, D4068). Three independent biological replicates from each cell line were used in the TaqMan™ Copy Number Assay for *UBE3A* (ThermoFisher Scientific, Hs02713225_cn), with a *RNase P* probe as internal reference and the TaqMan™ Genotyping Master Mix (Applied Biosystems, 4371353). All PCR reactions were run in triplicates using the StepOne Plus Real Time PCR System (Applied Biosystems, 4376592) for 40 cycles.

4.3. Immunofluorescence

Cells grown on Matrigel®-coated glass coverslips were fixed with 3 %(w/v) paraformaldehyde in PBS for 10 min at room temperature, treated with 10 mM Glycine (Sigma-Aldrich, G8898) for 10 min, permeabilized with 0.5 %(v/v) Triton X-100 (Sigma-Aldrich, T9284) and blocked in 1 % FBS for 20 min at room temperature. Coverslips were incubated with the specific primary antibodies (Table 2) diluted in blocking solution overnight at 4 °C, with the secondary antibodies (Table 2) diluted in blocking solution for 1 h at room temperature and counterstained with 0.2 mg/mL DAPI (Sigma-Aldrich, D9542) for 5 min. Microscopy experiments were performed using Zeiss Cell Observer with a 63x oil immersion objective and analyzed using FiJi software.

4.4. Flow cytometry

iPSCs at 70 % confluency were dissociated to single cells with Accutase® Solution (Sigma-Aldrich, A6964), resuspended in PBS and divided into three bins: unstained and two single stains. Single stains were then incubated with the specific fluorochrome conjugated antibodies in Table 2, diluted in 4 % FBS in PBS, for 15 min at room temperature in the dark. After three washes with PBS, the cells were resuspended in 4 % FBS and analyzed using FACS Canto II (BD Biosciences). Data analysis was performed using the platform Floreada.io.

4.5. G-band karyotyping

iPSCs at 50 % confluency were treated with 0.1 μg/mL KaryoMAX™ Colcemide™ Solution (Gibco, 15212012) diluted in mTeSR™Plus at 37 °C for 5 h to arrest cells in metaphase. iPSCs were dissociated to single cells with Accutase® Solution, incubated with 75 mM KCl solution at 37 °C for 30 min and fixed with a 1:3 solution of acetic acid: methanol. Karyotype analyses were performed by Genomed SA (Lisbon, Portugal).

4.6. Trilineage differentiation potential

Trilineage differentiation potential was assessed using the STEMdiff™ Trilineage Differentiation Kit (Stem Cell Technologies, 05230) following manufacturer's instructions. In brief, iPSCs at 70 % confluency were dissociated to single cells with Accutase® Solution at 37 °C for 6 min and counted with Trypan Blue. The required number of cells was plated onto Matrigel®-coated 12 well plates, with or without 13 mm

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers (IF)	Rabbit anti-OCT4	1:500	Abcam, ab19857	AB_445175
	Rabbit anti-SOX2	1:500	Abcam, ab97959	AB_2341193
Pluripotency Markers (FC)	SSEA4 antibody, anti-human (PE); TRA1-60 antibody, anti-human (PE)	1:20 1:10	Miltenyi Biotec, 130–122-914 Miltenyi Biotec, 130–122-921	AB_2811404 AB_2801969
Differentiation Markers (IF)	Rabbit anti-PAX6	1:400	ThermoFisher Scientific, 42–6600	AB_10375178 AB_2282664
	Mouse anti-NESTIN	1:400		
	Mouse anti-αSMA	1:100	R&D Systems, MAB2736	AB_476701
	Rabbit anti-SOX17	1:200	Sigma, A2547 Abcam, ab224637	AB_2801385
Secondary Antibodies (IF)	Goat anti-Rabbit IgG, Alexa Fluor™ 488	1:500	ThermoFisher Scientific, A-11034	AB_2576217
	Goat anti-Mouse IgG, Alexa Fluor™ 546	1:500	ThermoFisher Scientific, A-11003	AB_2534071
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Sendai reprogramming vector (qRT-PCR)	*SeV*	181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
House-Keeping Genes (qRT-PCR)	*GAPDH*	238 bp	GAGTCAACGGATTTGGTCGT/ TTGATTTTGGAGGGATCTCG	
Differentiation Genes (qRT-PCR)	*PAX6*	303 bp	GAATCAGAGAAGACAGGCCA/	
	TBXT	102 bp	GTGTAGGTATCATAACTCCG	
	TBX6	75 bp	TCAGCAAAGTCAAGCTCACCA/	
	SOX17	94 bp	CCCCAACTCTCACTATGTGGATT	
	FOXA2	96 bp	ACCGTGTCTACATTCACCCC/ CACGATGGAAAGACACAGGC GTGGACCGCACGGAATTTG/ GGAGATTCACACCGGAGTCA GGTGATTGCTGGTCGTTTGT/ CTCGTGCCCTTCCATCTTCA	
PWS-IC (PCR)	*SNRPN* DMR outer *SNRPN* DMR inner	340 bp	GGTTTTTTTTTATTGTAATAGTGTTGTGGGG/ CTCCAAAACAAAAAACTTTAAAACCCAAA GGTTTTAGGGGTTTAGTAGTTTTTTTTTTTTAG/ CAATACTCCAAATCCTAAAAACTTAAAATATCTA	
Copy Number Assay (TaqMan® probe)	*UBE3A*	80 bp	Assay ID: Hs02713225_cn	

round glass coverslips, in mTeSR®Plus supplemented with 10 μM Y-27632 (Stem Cell Technologies, 72302). The next day, for each well, culture medium was changed to the corresponding medium for ectoderm, mesoderm and endoderm. Medium was replaced daily, and cells were collected for RNA and fixed for IF at day 5 for mesoderm and endoderm, and at day 7 for ectoderm.

4.7. Combined bisulfite restriction analysis (CoBRA)

DNA from iPSCs was treated with bisulfite using the EZ DNA Methylation Gold kit (Zymo Research, D5006), following manufacturer's instructions. Bisulfite-treated DNA was then used to amplify the PWS-IC locus by nested PCR using the specific primers in Table 2 with KAPA HiFi HotStart Uracil + ReadyMix (Roche, KK2801). The PCR product was purified using NZYGelpure (NZYTech, MB01102), hydrolised by FastDigest *Bsh1236*I (Thermo Scientific, FD0924) for 15 min at 37 °C and analysed by electrophoresis in 2 % agarose gel.

4.8. CGH Array analysis

CGH array analysis of DNA from the original PBMCs was performed on a Cytoscan 750 K by CGC Genetics, Unilabs (Porto, Portugal).

4.9. Short Tandem Repeats (STR) analysis

To confirm cell line clonality, DNA from iPSCs and PBMCs was sent to Genomed SA (Lisbon, Portugal), where STR DNA analyses were performed. Briefly, AmpFLSTR® Indentifiler® Plus PCR Amplification Kit was used in multiplex PCR to amplify fifteen STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA) plus a gender determining marker, Amelogenin.

4.10. Mycoplasma detection

iPSC cultures were confirmed to be mycoplasma free using qPCR Mycoplasma Test (Mycoplasmacheck, Eurofins Genomics) following manufacturer's instructions.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve language and readability, with caution. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

João Camões dos Santos: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Francisca Cazaux Mateus:** Writing – review & editing, Methodology, Investigation. **Maria Arez:** Writing – review & editing, Supervision, Methodology. **Evguenia P. Bekman:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Simão T. da Rocha:** Writing – review & editing,

Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Simao T. da Rocha reports financial support was provided by Angelman Syndrome Alliance. The other 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.

Acknowledgements

We sincerely thank the two families for their generous blood donation. Our gratitude also goes to ANGEL – Associação de Síndrome de Angelman Portugal for assistance with recruitment, Centro de Medicina Laboratorial Germano de Sousa for blood collection, and Ângela Afonso and her team at Biobanco-iMM, now known as GIMM Biobank, for PBMC processing. This research was funded by an Angelman Syndrome Alliance grant granted in 2021 for the project "Stem cell toolkit for modelling cerebellar dysfunction in Angelman syndrome". Additional support came from the 2022.01532.PTDC research grant from Fundação para a Ciência e Tecnologia (FCT). João Camões dos Santos, Francisca Cazaux Mateus, and Maria Arez are funded by FCT PhD fellowships (2023.01932.BD, 2024.04039.BDANA, and SFRH/BD/151251/2021, respectively).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103741.

References

Arez, M., Eckersley-Maslin, M., Klobučar, T., von Gilsa Lopes, J., Krueger, F., Mupo, A., Raposo, A.C., Oxley, D., Mancino, S., Gendrel, A.-V., Bernardes de Jesus, B., da Rocha, S.T., 2022. Imprinting fidelity in mouse iPSCs depends on sex of donor cell and medium formulation. Nat. Commun. 13, 5432. https://doi.org/10.1038/s41467-022-33013-5.

Camões dos Santos, J., Appleton, C., Cazaux Mateus, F., Covas, R., Bekman, E.P., da Rocha, S.T., 2023. Stem cell models of Angelman syndrome. Front. Cell Dev. Biol. 11. https://doi.org/10.3389/fcell.2023.1274040.

Maranga, C., Fernandes, T.G., Bekman, E., da Rocha, S.T., 2020. Angelman syndrome: a journey through the brain. FEBS J. 287, 2154–2175. https://doi.org/10.1111/febs.15258.

Maranga, C., Pereira, C., Raposo, A.C., Vieira, A., Duarte, S., Bekman, E.P., Milagre, I., da Rocha, S.T., 2022. Generation and characterization of induced pluripotent stem cell line (IBBISTi004-A) from an Angelman syndrome patient carrying a class II deletion of the maternal chromosome 15q11.2-q13. Stem Cell Res. 61, 102757. https://doi.org/10.1016/j.scr.2022.102757.

Pólvora-Brandão, D., Joaquim, M., Godinho, I., Aprile, D., Álvaro, A.R., Onofre, I., Raposo, A.C., Pereira de Almeida, L., Duarte, S.T., da Rocha, S.T., 2018. Loss of hierarchical imprinting regulation at the Prader–Willi/Angelman syndrome locus in human iPSCs. Hum. Mol. Genet. 27, 3999–4011. https://doi.org/10.1093/hmg/ddy274.

Yang, L., Shu, X., Mao, S., Wang, Y., Du, X., Zou, C., 2021. Genotype-Phenotype Correlations in Angelman Syndrome. Genes 12, 987. https://doi.org/10.3390/genes12070987.