



Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line from a patient with Angelman syndrome carrying UBE3A mutation

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A B S T R A C T

Angelman syndrome (AS) is a neurodevelopmental disorder caused by abnormal expression or function defects of the UBE3A gene in the maternal chromosome region 15q11-13. In order to study the pathogenesis of Angelman syndrome and further search for its effective treatment, we established a human induced pluripotent stem cells (iPSCs) from an AS patient carrying the mutation p.Asp563Gly of UBE3A gene at maternal 15q11.2-q13. The established patient-derived iPSC showed normal karyotype, expressed pluripotency markers, and had the capacity to differentiate into three germ layers.

Resource Table:

Unique stem cell line identifier	WMUi032-A
Alternative name(s) of stem cell line	N/A
Institution	Wenzhou Medical University
Contact information of distributor	Qian Yang, aslemon@126.com
Type of cell line	Induced pluripotent stem cell (iPSC)
Origin	Human
Additional origin info	Sex: female
Cell Source	blood
Clonality	Clonal
Method of reprogramming	Non-integrating plasmids
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Angelman syndrome
Gene/locus	chromosome 15q11.2-q13, p.Asp563Gly, c.1688A > G
Method of modification	Not applicable
Name of transgene or resistance	Not applicable
Inducible/constitutive system	Not applicable
Date archived/stock date	March 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/WMUi032-A
Ethical approval	The study was approved by the Ethical Committee of the Wenzhou Medical University (2021-238 K-20801)

1. Resource utility

The expression or functional deficiency of maternal UBE3A gene leads to Angelman syndrome (AS). As a potential source of regenerative medicine, the construction of this iPSCs will be a powerful tool for both *in vivo* and *in vitro* studies to clarify the pathological mechanism and to discover and develop effective therapeutic drugs for AS.

2. Resource details

AS is a neurodevelopmental disorder caused by disruption of the maternally expressed and paternally imprinted UBE3A, which encodes an E3 ubiquitin ligase (Buiting et al., 2016). Clinically, it is characterized by severe cognitive disability, absent speech, motor dysfunction, hyperactivity, and frequent seizures. Currently, the molecular genetic mechanisms leading to the loss of function of UBE3A gene have been clarified, including the deletion of maternal 15q11-13 region including UBE3A gene, the paternal uniparental disomy of chromosome 15, the imprinting defect of maternal 15q11.2-13, and the pathogenic mutation of maternal UBE3A gene. Among them, the deletion of maternal 15q11-13 was the most common (Margolis et al., 2015). Here, we generated human iPSCs from peripheral blood mononuclear cells (PBMCs) of an AS patient with UBE3A mutation p.Asp563Gly (c.1688A > G).

To provide patient-derived AS-specific iPSC lines for further study, we collected whole blood from an AS patient with UBE3A gene

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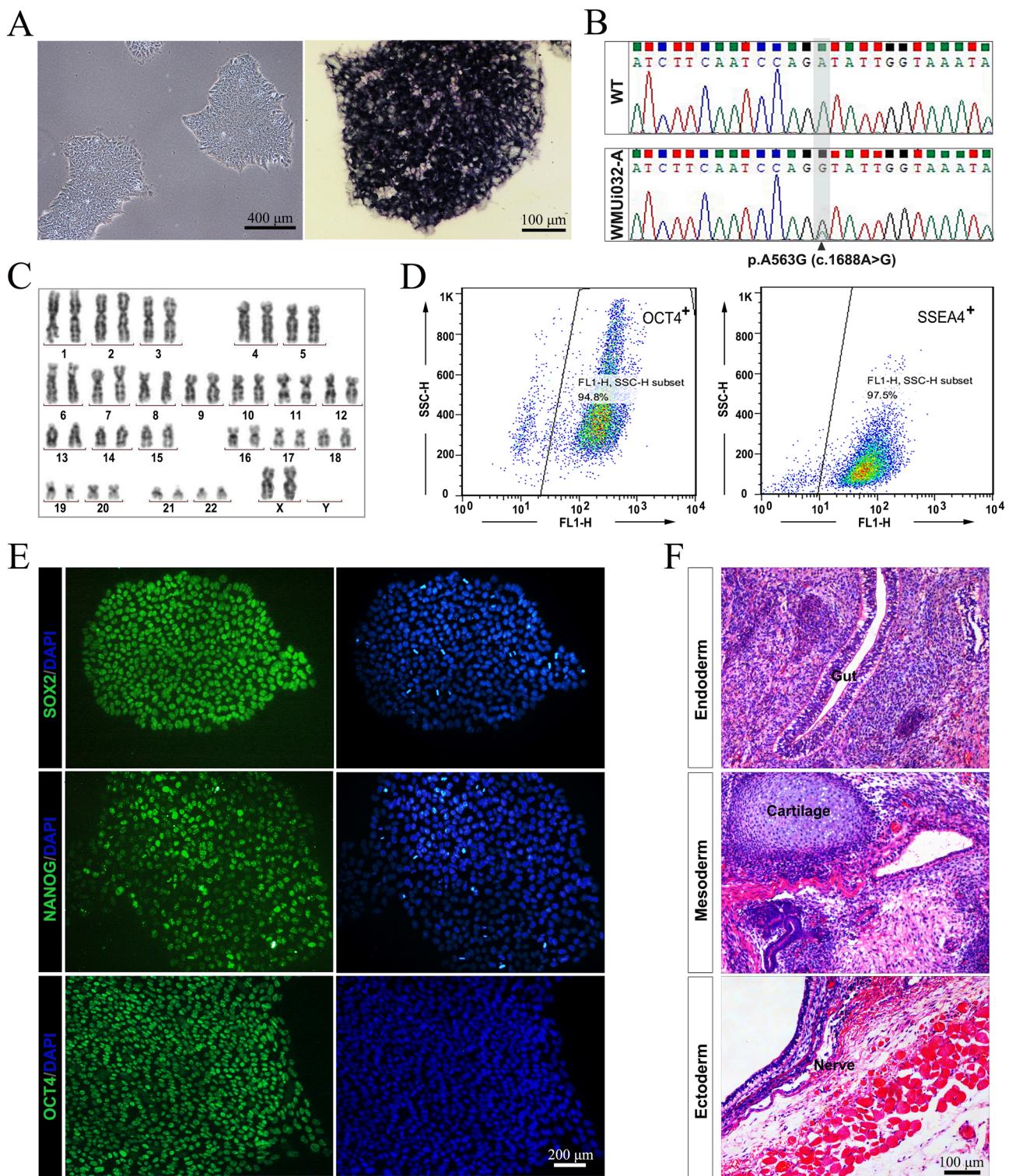


Fig. 1.

mutation. PBMCs were isolated from whole blood and passage 3 (P3) PBMCs were transfected with episomal vectors carrying reprogramming factors OCT4, SOX2, MYC, NANOG, LIN28 and KLF4. A normal human embryonic stem cell-like phenotype (Fig. 1A, left panel) with a 46, XX normal karyotype (Fig. 1C) was detected in the reprogrammed iPSC line. iPSC (P5) colonies were picked about 20 days after 5 passages. RT-PCR analysis confirmed the absence of episomal plasmids in the WMU032-A line (P16) (Supplementary Fig. 1A). Short tandem repeat (STR) analysis confirmed that iPSCs were derived from the patient-donated PBMCs.

The patient's cell line was confirmed to harbor pathogenic variant compound mutation of chromosome 15q11.2-q13 region with c.1688 A > G: p.Asp563Gly substitution in UBE3A by Sanger sequencing of DNA isolated from the iPSC cultures (Fig. 1B). Then, we examined the expression of the pluripotent markers OCT4 and SSEA4 by flow cytometry (Fig. 1D), while we measured expression of the pluripotent markers OCT4, SOX2 and NANOG via immunofluorescence staining (Fig. 1E). The pluripotency status was also characterized by positive alkaline phosphatase activity (Fig. 1A, right panel). In addition, we

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A, left panel
Phenotype	Qualitative analysis Immunocytochemistry Quantitative analysis Flow cytometry	Positive for OCT4, SOX2, NANOG OCT4: 94.8% SSEA4: 97.5%	Fig. 1E Fig. 1D
Genotype	Karyotype (G-banding) and resolution	46XX	Fig. 1C
Identity	Microsatellite PCR (mPCR) OR STR analysis	Resolution: 450–500 N/A	N/A
Mutation analysis (IF APPLICABLE)	Sequencing Southern Blot OR WGS	21 sites tested, matched Heterozygous, p.Asp563Gly N/A	available with the authors Fig. 1B N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR. Negative	Supplementary Fig. 1
Differentiation potential	Teratoma formation	Three germ layers formation, ectoderm, mesoderm and endoderm	Fig. 1F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A N/A	N/A

confirmed pluripotency via teratoma formation assay, which showed this iPSC line could successfully differentiate *in vivo* into all three germ layers (endoderm, mesoderm and ectoderm) (Fig. 1F). Finally, the established iPSC line was negative for Mycoplasma (Supplementary Fig. 1B). Characterizations of the iPSC line were summarized in Table 1.

3. Materials and methods

3.1. Establishment and maintenance of the iPSC lines

PBMCs were isolated from 5 mL peripheral blood of patient by using SepMate-50 (STEMCELL Technologies) according to manufacturer's instructions. Isolated PBMCs were cultured in StemSpan SFEM II supplemented with StemSpan Erythroid Expansion Medium (STEMCELL Technologies) a days for erythroid cells enrichment and expansion. After a week of cell culture, human cDNAs for reprogramming factors (Oct4, Sox2, Lin28, L-Myc, Klf4) and additional improvements were transduced in PBMCs (P3) with episomal vectors pCE-hOCT3/4, pCE-hSK, pCE-hUL, pCE-mP53DD and pCXB-EBNA1 (Thermo Fisher Scientific, A15960) using an Amaxa P3 Primary Cell 4D Nucleofector X Kit (LONZA) and program E0-100. After culture with StemSpan SFEM II and ReproTeSR medium, iPSC colonies (P5) were picked and maintained on Matrigel (1:100 dilution, Growth Factor Reduced, Corning 354277)-coated 6-well plates in Essential 8 medium (STEMCELL Technologies) with the CO₂ condition of 5% and the temperature of 37°C. The fresh medium was replaced daily, and when reaching 90% confluence, cells were passaged at the ratio of 1:6–1: 10 using 0.5 mM EDTA every 3–5 days.

3.2. Immunofluorescence staining

Firstly, iPSCs (P10) were fixed with 4% paraformaldehyde for 15 min at room temperature (RT). The cells were then washed twice with phosphate buffered saline (PBS) and incubated with 0.3% Triton X-100 for 10 min, and then blocked with 1% bovine serum albumin (BSA) for 30 min at RT. After blocking, cells were stained overnight at 4 °C using primary antibodies (Table 2). The next day, after washed with PBS for 10 min three times, they were incubated with corresponding secondary antibodies for 2 h at RT in the dark and the nuclei were stained with DAPI (1:1000, Invitrogen) for 10 min (Liu et al., 2020). Images were captured using a Nikon Eclipse TE2000-S inverted microscope (Nikon Instruments).

3.3. Flow cytometry analysis

iPSCs (P10) were dissociated into single cells using Accutase (Millipore, SCR005) for 4–6 min, 2 × 10⁷ digested cells were collected and incubated with conjugated antibodies (Table 2) diluted in PBS containing 0.2% BSA at RT in the dark for 30 min. FACS analysis was performed with a FACS Calibur (BD Biosciences).

3.4. Alkaline phosphatase activity

AP staining was performed using a SIGMAFAST BCIP/NBT tablet (Sigma) according to the manufacturer's instructions. iPSCs at P10 were used.

3.5. Karyotype analysis

G-banding karyotype was used to confirm genomic stability of the iPSC (P10). The G-banding karyotyping was performed at Biocytogen Laboratory Services.

3.6. STR analysis

Genomic DNA was extracted from whole blood and iPSC line (P10) using Chelex method. Short tandem repeat (STR) analysis of 21oci (D5S818, D13S317, D7S820, D16S539, VWA, TH01, AMEL, TPOX, CSF1PO, D3S1358, D18S51, PENTAD, D6S1043, D2S1338, D21S11, D19S433, D12S391, FGA, D1S1656, D8S1179, PENTAE) was performed for PBMCs and iPSCs using Promega PowerPlex 21 System.

3.7. Mycoplasma detection

Mycoplasma detection was performed according to the EZ-PCR Mycoplasma Test Kit (BI, 20-700-20). iPSCs at P16 were used.

3.8. Teratoma formation assay

1 × 10⁷ iPSC cells (P12) were digested and collected, and then a total of 200 μL cell suspension mixture containing 50% Matrigel (Corning, 354277) was prepared and injected into the hindlimb muscles of the NSG mice. 6–8 weeks after injection, detectable teratoma can be generated. Teratoma tissue was removed and fixed in 10% neutral

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry		Dilution	Company Cat # and RRID
	Antibody		
Pluripotency Markers	Rabbit anti-SOX2	1:300	Millipore Cat# AB5603, RRID: AB_2286686
Pluripotency Markers	Rabbit anti-Nanog	1:500	Millipore Cat# AB9220, RRID: AB_11213156
Pluripotency Markers	Rabbit anti-OCT4	1:50	Proteintech Cat# 11263-1-AP, RRID: AB_2167545
Flow Cytometry Antibodies	Mouse anti-Oct34	1:250	BD Biosciences Cat# 560253, RRID: AB_1645304
Flow Cytometry Antibodies	Mouse anti-SSEA4	1:50	Thermo Fisher Scientific Cat# MA1-021-D488, RRID: AB_2536688
Fluorescent Secondary Antibodies	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21206, RRID: AB_2535792
Primers			
RT-PCR primers	Target	Size of band (bp)	Forward/Reverse primer (5'-3')
Exogenous reprogramming of transgene (RT-PCR)	oriP (pCE-hOCT3/4, pCE-hSK, pCE-hUL, pCE-mP53DD)	544	Fw: TTCCACGAGGGTAGTGAACC Rv: TCGGGGGTTAGAGACAAC
Exogenous reprogramming of transgene (RT-PCR)	EBNA-1 (pCE-hOCT3/4, pCE-hSK, pCE-hUL, pCE-mP53DD, pCXB-EBNA1)	666	Fw: ATCGTCAAAGCTGCACACAG Rv: CCCAGGAGTCCCAGTAGTC
House-Keeping Genes (RT-PCR)	GAPDH	112	Fw: AACCATGAGAAAGTATGACAAC Rv: CTTCCACGATAACCAAGT
Plasmid specific primers (PCR) UBE3A-1	UBE3A	365	Fw: CTTAAAATAAATGTGTTATGCC Rv: TGTCCTATCTCCATTAACTGC
Plasmid specific primers (PCR) UBE3A-2	UBE3A	620	Fw: AGTCAGGAGTTGGTAAATAGTGC Rv: AGTTATTATTCTGTCCGTTACCC
Plasmid specific primers (PCR) UBE3A-3	UBE3A	1518	Fw: GAATGTTGGCTGTTTACTTTAG Rv: CACATCTAAACTTGACAGGAAC
Plasmid specific primers (PCR) UBE3A-4	UBE3A	452	Fw: TTTATCAGTTGCTTCTGTTCC Rv: CCTAAACCTACAAATTCACTGATG
Plasmid specific primers (PCR) UBE3A-5	UBE3A	621	Fw: ACCATTACCCCCAATTATAGC Rv: TACAAAAGCTTGTGCTGACAG
Plasmid specific primers (PCR) UBE3A-6	UBE3A	494	Fw: GGACTGGAGGGATACTGTCTACAC Rv: GTGCCAACACTGTGCTTTC
U Plasmid specific primers (PCR) BE3A-7-8	UBE3A	654	Fw: TGAAAAAATTAAAAGCTATGCAG Rv: CAAATGCAGGAGATTACATGAGAC
Plasmid specific primers (PCR) UBE3A-9	UBE3A	469	Fw: TTGGAAATTAAATCACCGAG Rv: AAGTTCTCACACAATGACAGC
Plasmid specific primers (PCR) UBE3A-10-1	UBE3A	1275	Fw: TATAGTTGCATTGCAAGACCGTC Rv: TGATTCAACAAGATGATGCCAAC
Plasmid specific primers (PCR) UBE3A-10-2	UBE3A	1404	Fw: AGCCCTCAGAACTTAGTAACACG Rv: TAAGGGTAGGTTACCTGACTCTG

formalin buffer, embedded in paraffin and stained with hematoxylin. Then the formation of endoderm, mesoderm and ectoderm tissues was examined under microscope.

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.

Acknowledgments

This work was supported by the Wenzhou Technology Bureau Project (Y20190103).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2022.102791>.

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