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Lab resource: Stem Cell Line

Induced pluripotent stem cell line, ICAGi001-A, derived from human skin fibroblasts of a patient with 2p25.3 deletion and 2p25.3-p23.3 inverted duplication

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A B S T R A C T

Skin fibroblasts from a patient with developmental delay and chromosome 2p25.3 deletion syndrome were reprogrammed into induced pluripotent stem cells (iPSCs) and the clonal stem cell line ICAGi001-A (iTAF9-11) was established. ICAGi001-A pluripotency was demonstrated *in vitro* by three germ layer differentiation capacity. This line is a good model for studying of the developmental delay and brain disorder.

Resource table

Unique stem cell line identifier	ICAGi001-A
Alternative name(s) of stem cell line	iTAF9-11
Institution	Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia Research Institute of Medical Genetics, Tomsk NRMC, Tomsk, Russia
Contact information of distributor	Anna Khabarova, anya.khabarova@gmail.com
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 3 y Sex: Female Ethnicity: Caucasian
Cell source	Human skin fibroblasts Taf9
Clonality	Clonal
Method of reprogramming	Lentiviral reprogramming with four transcription factors (OCT4, SOX2, KLF4, C-MYC)
Genetic modification	Yes
Type of modification	spontaneous (*de novo*)
Associated disease	Developmental delay, autosomal dominant 39; MRD39; OMIM 616521 (chromosome 2p25.3 deletion syndrome)
Gene/locus	arr[GRCh38] 2p25.3(42444_2684871)x1 dn, 2p23.3-p25.3(2771354_24258056)x3 dn
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	May 2018
Cell line repository/bank	Collective Center of ICG SB RAS "Collection of Pluripotent Human and Mammalian Cell Cultures for Biological and Biomedical Research"; Bioresource collection of the Research Institute of Medical Genetics, Tomsk NRMC, "Biobank of the population of Northern Eurasia"
Ethical approval	Written informed consent was obtained from the patient's parents. The study was approved by the Scientific Ethics Committee of Research Institute of Medical Genetics, Tomsk NRMC (protocol number 106/2017)

Resource utility

The combination of 2p25.3 deletion and 2p25.3-p23.3 inverted duplication is a rarely described cytogenetic aberration in patients with intellectual disability. The use of hiPSC-derived neurons opens prospects in patient-specific studies to model human brain development *in*

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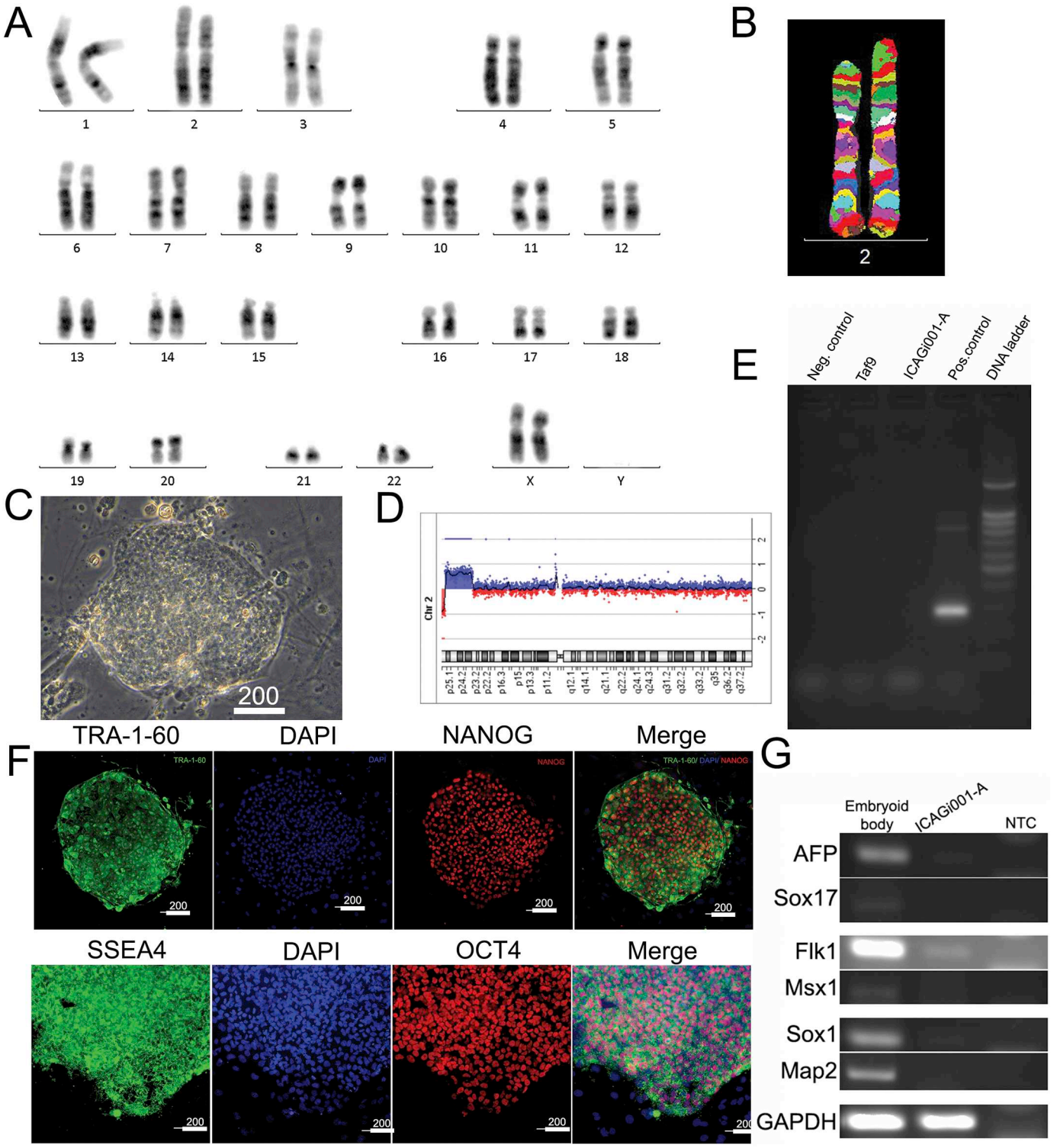

Fig. 1. Characterization of ICAGi001-A line. (A) Karyotypes. (B) The multicolor banding (MCB) analysis of chromosome 2. (C) Morphology of the iPSC colonies. (D) a CGH analysis. (E) Mycoplasma contamination test. (F) Immunofluorescence staining for the pluripotency markers NANOG, OCT4, SSEA4, TRA-1-60. (G) Expression of the endoderm (AFP, SOX17), mesoderm (MSX1, FLK1) and ectoderm (SOX1, MAP2) markers in the embryoid bodies and in ICAGi001-A.

vitro. ICAGi001-A hiPSC line is a good model for studying of the developmental delay *in vitro*.

Resource details

Human skin fibroblasts TAF9 were derived from a 3-year-old female with developmental delay and chromosome 2p25.3 deletion syndrome. The chromosomal rearrangement was investigated using aCGH, which detected the 2p25.3 deletion and 2p23.3-p25.3 duplication (Fig. 1D). The multicolor banding (MCB) analysis was applied to investigate the structural aberration of the der (2). The result showed an inverted duplication of distal 2p on the der (2) – dup (2) (p25.3p23.3) (Fig. 1B). Fibroblasts were reprogrammed into iPSCs through lentiviral delivery of four reprogramming factors (OCT4, SOX2, KLF4, and C-MYC)

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel C
Phenotype	Qualitative analysis Immunocytochemistry	Positive for pluripotency markers: OCT4, NANOG, SSEA4 and TRA-1-60	Fig. 1 panelF
	Quantitative analysis Immunocytochemistry counting (300 cells counted)	% of positive cells OCT4: 98.7% TRA1–60: 97.4% SSEA-4: 96.1% NANOG 98.55%	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46,XX,der (2) resolution 450	Fig. 1 panel A
Identity	STR analysis	20 sites tested, and 20/20 matched	available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	n/a	n/a
	aCGH and MCB analysis	arr[GRCh38] 2p25.3(42444_2684871)x1 dn, 2p23.3-p25.3(2771354_24258056)x3 dn	Fig. 1 Panel B and D
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Fig. 1 panel E
Differentiation potential	Embryoid body formation	Positive for expression of genes of the three germ layers in embryoid bodies (*AFP* and *SOX17* for endoderm; *MSX1* and *FLK1* for mesoderm; *SOX1* and *MAP2* for ectoderm)	Fig. 1 panel G
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

(Gridina et al., 2018) (Table 1). ICAGi001-A line display typical morphology of human pluripotent stem cells under feeder-dependent conditions in phase contrast microscopy (Fig. 1C), and expressed OCT4 and NANOG pluripotency markers (red) in the nucleus and SSEA4 and TRA-1-60 surface markers (green), as detected by immunofluorescence staining. Nucleus stained by DAPI (blue) (Fig. 1F). Immunocytochemistry counting allowed quantifying the percentage of ICAGi001-A cells positive for OCT4, NANOG, SSEA4 and TRA-1-60 as 98.7%, 98.55%, 96.1% and 97.4%, respectively. ICAGi001-A cell line had 46,XX,der(2p) karyotype (Fig. 1A). The STR profile of the ICAGi001-A cell line fully matched with that of the parental TAF9 fibroblasts (loci analyzed: *D1S1656, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D21S11, D22S1045, AMEL, CSF1PO, FGA, SE33, TH01, TPOX,* and *vWA*). The ICAGi001-A cell line was negative for *Mycoplasma* contamination (Fig. 1E). The ability of the ICAGi001-A cell line to differentiate into cells of the three germ layers following embryoid body formation was assessed by RT-PCR for endodermal (*AFP, SOX17*), mesodermal (*MSX1, FLK1*) and ectodermal (*SOX1, MAP2*) genes (Fig. 1G). These results clearly demonstrate that the ICAGi001-A cells are pluripotent.

Materials and methods

Cell culture

TAF9 human fibroblasts were derived from a patient with 2p25.3 - p23.3 inverted duplication and 2p25.3 deletion and cultured in growth media (DMEM/F12 (Gibco) supplemented with 10% fetal bovine serum (FBS) (HyClone), 1% Pen Strep (Gibco), 1% MEM Non-essential Amino Acid solution, 1% MEM Vitamin solution, 2 mM L-glutamine (all from Sigma)) at 37 °C in 5% CO_2.

Generation of iPSCs from patient's fibroblasts

To produce iPSC from the patient's fibroblasts we used LeGO lentiviral vectors (MOI = 19) containing the human reprogramming transcription factors OCT4, SOX2, C-MYC, and KLF4 (kindly provided to us by Dr. Sergei L. Kiselev (Moscow, Russia)). The lentiviruses were produced in the Phoenix cell line using Lipofectamine 3000 (Invitrogen) according to the manufacturer's recommendations. Fibroblasts plated on the previous day were transduced with viruses containing the four reprogramming transcription factors for two days (on the second day, the C-MYC lentivirus was omitted). From day 7 to 16 the culture medium was changed daily with addition of 1 mM valproic acid (Sigma) and Selleck Human iPSC Enhancer Kit (Selleckchem, K2010). On day 5, the transduced cells were seeded onto 10-cm culture dishes (2×10^3 cells per cm^2) containing mitomycin C-treated CD-1 mouse embryonic fibroblast feeder cells in iPSC medium (DMEM/F12 medium supplemented with 20% KnockOut Serum Replacement, 1% GlutaMAX™-I, 1% MEM NEAA, 1% Pen Strep (all from Gibco), 0.1 mM 2-mercaptoethanol, and 10 ng/ml bFGF (Invitrogen)). On day 16, colonies (near 46 iPSC colonies per 15×10^4 transformed fibroblasts) with iPSC morphology were picked up and expanded. All cell cultures were maintained at 37 °C in an atmosphere of 5% CO_2. iPSCs maintained on feeder cells were expanded mechanically with ratio of split 1:3.

Immunocytochemistry and immunocytochemistry counting

The iPSCs fixed with 3% formaldehyde for 20 min at room temperature. After fixation, the cells were permeabilized with 0.1% Triton X-100 in PBS for 3 min at room temperature, blocked with 3% bovine serum albumin in PBS for 25 min and incubated overnight at 4 °C with primary antibodies. Secondary antibodies were incubated for 1 h at room temperature (Table 2) and further counterstained with DAPI. All antibodies were diluted in PBS with 1.5% BSA. Immunofluorescence and immunofluorescence counting (300 cells counted) were examined with a fluorescence microscope AxioObserver Z1 (Zeiss) using ZEN software in collective Microscopic Center of ICG SB RAS, Novosibirsk and Fiji soft (ImageJ).

In vitro differentiation and RT-PCR

Embryoid bodies were produced according to previously published protocol (Bock et al., 2011). RNA were isolated by TRI Reagent (Sigma), cDNA were obtained by RevertAid RT kit (Thermofisher) and RT-PCR were performed with HP-Taq DNA polymerase in following conditions: 15 s–95 °C, 15 s–62 °C and 30 s–72, 34 cycles (BioRad T100 Thermal Cycler).

Karyotyping

Preparation of metaphase chromosomes from iPS cells was performed on passages 10–12 as previously described with minor modifications (Prokhorovich et al., 2007). Fifty eight metaphase spreads were analyzed using a Carl Zeiss Axioplan 2 imaging microscope, digital images were analyzed using ISIS 3 (*In Situ* Imaging System, MetaSystems GmbH) software at the Center for Microscopy of the Institute of Cytology and Genetics. Multicolor banding (MCB) was carried out using XCyte 2 mBAND probe (MetaSystems GmbH).

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-NANOG	1:100	Abcam Cat# 21624, RRID: AB_446437
Pluripotency Markers	Rabbit anti-OCT4	1:200	Abcam Cat# 19857, RRID: AB_445175
Pluripotency Markers	Mouse anti-SSEA4	1:600	Abcam Cat# 16287, RRID:AB_778073
Pluripotency Markers	Mouse anti-TRA-1-60	1:600	Abcam Cat# 16288, RRID:AB_778563
Secondary antibodies	Alexa Fluor 546 Goat Anti- Rabbit IgG	1:400	Life technologies Cat# A-11010, RRID:AB_143156
Secondary antibodies	Alexa Fluor 488 Goat Anti-Mouse IgG	1:400	Life technologies Cat# A-11029, RRID:AB_138404

Primers		
	Target	Forward/Reverse primer (5′-3′)
House-Keeping Genes	*GAPDH*	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT Expected product size: 153 bp
Differentiation Markers	*AFP*	AAATGCGTTTCTCGTTGCTT/GCCACAGGCCAATAGTTTGT Expected product size: 136 bp
Differentiation Markers	*SOX1*	CACAACTCGGAGATCAGCAA/GGTACTTGTAATCCGGGTGC Expected product size: 133 bp
Differentiation Markers	*MAP2*	CAGGTGGCGGACGTGTGAAAATTGAGAGTG/CACGCTGGATCTGCCTGGGGACTGTG Expected product size: 212 bp
Differentiation Markers	*SOX17*	CTCTGCCTCCTCCACGAA/CAGAATCCAGACCTGCACAA Expected product size: 102 bp
Differentiation Markers	*MSX1*	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAGCTTCTCCAG Expected product size: 307 bp
Differentiation Markers	*FLK1*	TGATCGGAAATGACACTGGA/CACGACTCCATGTTGGTCAC Expected product size: 131 bp

Mycoplasma contamination detection

The absence of *Mycoplasma* contamination was confirmed by PCR using primers from Choppa et al., 1998.

STR analysis

Parent TAF9 fibroblasts and their derivative iPSC line ICAGi001-A (iTAF9-11) were authenticated by STR analysis by Gordiz (http://gordiz.ru/).

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