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Lab Resource: Multiple Cell Lines

Generation of four iPSC lines from two siblings with a microdeletion at the *CNTN6* gene and intellectual disability

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A B S T R A C T

The human induced pluripotent stem cell (iPSC) lines, ICGi009-A, ICGi009-B, ICGi013-A and ICGi013-B, were generated from skin fibroblasts of two siblings with intellectual disability. Both patients were carriers of *CNTN6* gene microdeletion (Kashevarova et al., 2014). iPSC lines have normal karyotype, express pluripotency markers, are able to differentiate in vitro into derivatives of all three germ layers and represent a unique tool to study neurodevelopmental disorders.

Resource table

Unique stem cell lines identifier	ICGi009-A ICGi009-B ICGi013-A ICGi013-B
Alternative names of stem cell lines	iTAF3-17, iTAF3del17 (ICGi009-A) iTAF3-37, iTAF3del37 (ICGi009-B) iTAF13-26, iTAF13del26 (ICGi013-A) iTAF13-27, iTAF13del27 (ICGi013-B)
Institution	Institute of Cytology and Genetics Siberian Branch of the Russian Academy of Sciences (ICG SB RAS), Novosibirsk, Russia
Contact information of distributor	*Shnaider T.A., shnayder.t@yandex.ru*
Type of cell lines	iPSC
Origin	Human
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	lentivirus
Multiline rationale	Isogenic and same disease non-isogenic cell lines
Gene modification	Yes
Type of modification	Spontaneous mutation (*de novo*)
Associated disease	Intellectual disability
Gene/locus	arr[hg19]3p26.3(1197623 −1492721)×1.seq[hg19] del (3)(3p26.3)chr3:g.1158406-407T(19_22)_1568210del
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	September 2018
Cell line repository/bank	Bioresource collection of the Research Institute of Medical Genetics, Tomsk NRMC, "Biobank of the population of Northern Eurasia"; Collective Center of ICG SB RAS "Collection of Pluripotent Human and Mammalian Cell Cultures for Biological and Biomedical Research"
Ethical approval	Written informed consents were obtained from the patients. The study was approved by the Scientific Ethics Committee of Research Institute of Medical Genetics, Tomsk NRMC (protocol number 106/2017)

1. Resource utility

Neurodevelopmental disorders represent a large group of diseases characterized by significantly impaired intellectual and cognitive functions. Derived iPSC lines can provide a great opportunity to investigate cellular and molecular mechanisms of mental retardation caused by *CNTN6* gene mutation

2. Resource details

Human skin fibroblasts TAF3del and TAF13del were derived from two siblings: 20-years old male and 18-years old female with intellectual disability and microdeletion of *CNTN6* gene, previously described by Kashevarova et al. (2014). Cells were reprogrammed with

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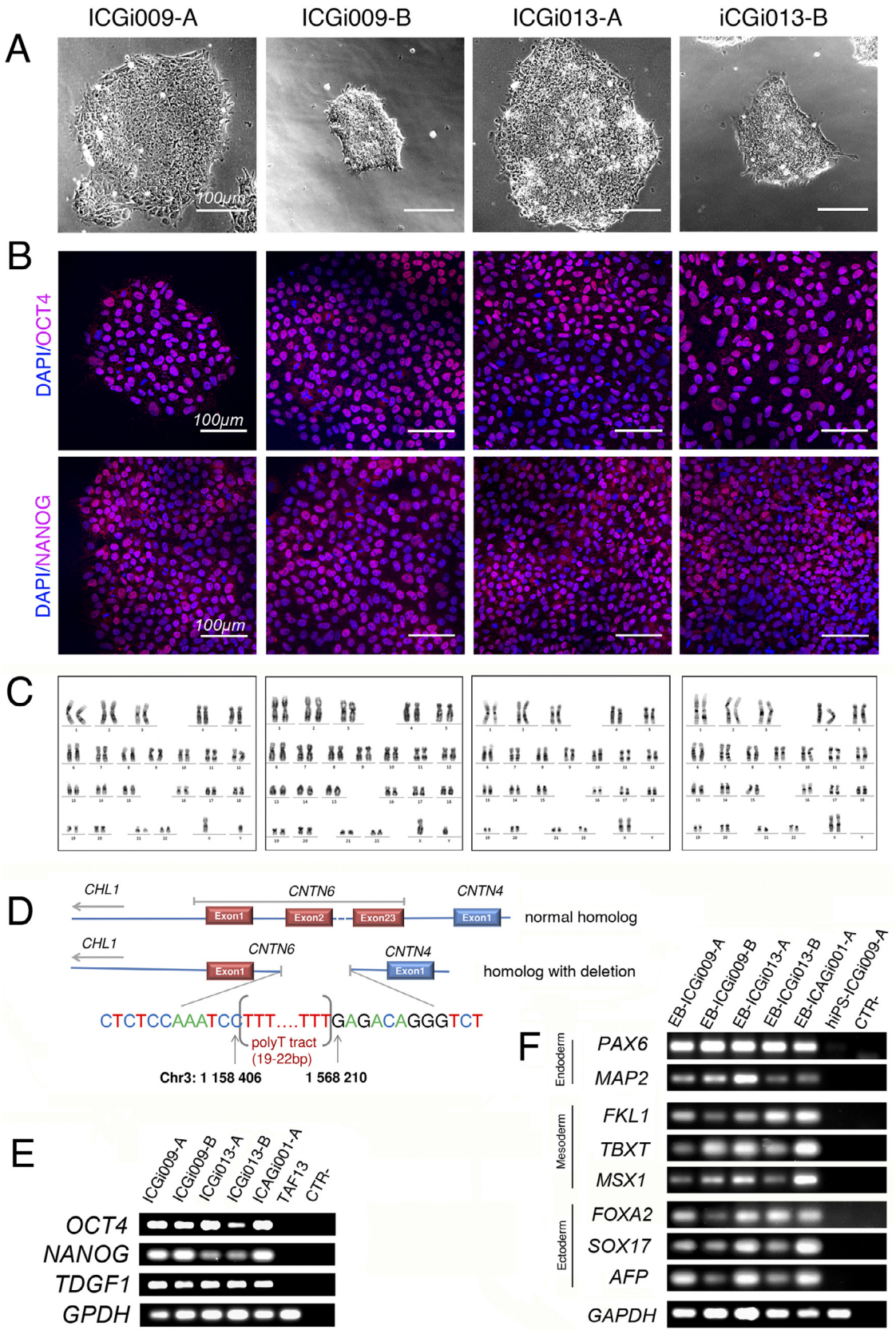

Fig. 1. Characterization of human iPSC lines ICGi009-A, ICGi009-B, ICGi013-A and ICGi013-B.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
ICGi009-A	*ICGi009-A*	*Male*	*20*	*Caucasian*	arr[hg19]3p26.3(1197623-1492721)×1.seq[hg19] del(3)(3p26.3) chr3:g.1158406-407T(19_22)_1568210del	*Intellectual disability*
ICGi009-B	*ICGi009-B*	*Male*	*20*	*Caucasian*	arr[hg19]3p26.3(1197623-1492721)×1.seq[hg19] del(3)(3p26.3) chr3:g.1158406-407T(19_22)_1568210del	*Intellectual disability*
ICGi013-A	*ICGi013-A*	*Female*	*18*	*Caucasian*	arr[hg19]3p26.3(1197623-1492721)×1.seq[hg19] del(3)(3p26.3)chr3:g.1158406–407T(19_22)_1568210del	*Intellectual disability*
ICGi013-B	*ICGi013-B*	*Female*	*18*	*Caucasian*	arr[hg19]3p26.3(1197623-1,492,721)×1.seq[hg19] del(3)(3p26.3)chr3:g.1158406-407T(19_22)_1568210del	*Intellectual disability*

lentiviral delivery of Yamanaka factors (*OCT3/4, SOX2, KLF4* and *c-MYC*). Generated iPSC lines (ICGi009-A, ICGi009-B, ICGi013-A and ICGi013-B) displayed typical morphology of human pluripotent stem cells both in feeder-dependent (data not shown) and feeder-free conditions (Fig. 1A) and expressed the typical pluripotent markers *OCT3/4, NANOG* and *TDGF* as shown by immunocytochemistry (Fig. 1B) and RT-PCR (Fig. 1D). All four iPSC lines demonstrated chromosomal stability and normal karyotype (Fig. 1C). One homologue of TAF13del chromosome 22 had prominent satellites (46, XX, 22ps+) as well as two iPSC lines which were produced from these fibroblasts. To prove pluripotency, ICGi009-A, ICGi009-B, ICGi013-A and ICGi013-B were spontaneously differentiated into embryoid bodies. RT-PCR revealed the presence of markers of all three germ layers: endoderm, mesoderm and ectoderm (Fig. 1E). Additionally, using the immunofluorescence staining of histone H3 tri-methylated at K27, enriched in transcriptionally silenced sex chromatin, we checked the status of X-chromosomes in TAF13del, ICGi013-A and ICGi013-B cell lines generated from female and showed the presence of two active X-chromosomes in both iPSC lines in comparison with one inactivated X-chromosome in fibroblasts (the red dot marked by the arrow on the Supplementary Fig. 1B). The STR profile of iPSC lines fully matched in pairs with that of the original TAF3del and TAF13del fibroblasts (loci analyzed: D1S1656, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D21S11, D22S1045, AMEL, CSF1PO, FGA, SE33, TH01, TPOX, and vWA). All iPSC lines were negative for Mycoplasma contamination (Supplementary Fig. 1C).

3. Materials and methods

3.1. Cell culture

Human fibroblasts TAF3del and TAF13del were derived from patients with 3p26.3 microdeletion and cultured in growth media (DMEM/F12 supplemented with 10% fetal bovine serum (FBS), 1% Pen Strep, 1% MEM NEAA(all from Thermo Fisher Scientific), 1% MEM Vitamin solution, 2 mM L-glutamine (both from Sigma-Aldrich) at 37 °C in 5% CO_2.

3.2. Reprogramming

To generate iPSCs lines from the patient's fibroblasts we used LeGO lentiviral vectors containing the human reprogramming transcription factors *OCT3/4, SOX2, C-MYC*, and *KLF4* (all vectors were kindly provided to us by Dr. Sergei L. Kiselev (Moscow, Russia)). The lentiviruses were produced in the Phoenix cell line using Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's recommendations. Multiplicity of infection was estimated as 3 using GFP lentiviral vector LeGO-G2 (https://www.addgene.org/25917/). Reprogramming factors were delivered using the same vector with eGFP substituted by transcription factor coding sequences. It was assumed that MOI for OKSM factors was close to LeGO-G2 MOI. Briefly, human fibroblasts plated on the previous day (15,000 cells per cm^2) were transduced with lentiviruses containing the four reprogramming transcription factors for two days (on the second day, the C-MYC lentivirus was omitted). Until day 10, the culture medium was changed daily with addition of 1 mM valproic acid (Sigma-Aldrich). On day 5, the transduced cells were seeded onto feeder cells (mitomycin C-treated CD-1 mouse embryonic fibroblasts) in iPSC medium (DMEM/F12 medium supplemented with 20% KnockOut Serum Replacement, 1% GlutaMAX™-I, 1% MEM NEAA, 1% Pen Strep, 10 ng/ml bFGF (all from Thermo Fisher Scientific) and 0.1 mM 2-mercaptoethanol (Helicon). On days 18–20, colonies with iPSC morphology were picked and expanded. All cell cultures were maintained at 37 °C in an atmosphere of 5% CO_2. iPSCs on feeder cells were expanded mechanically for several passages and then were adapted to feeder-free culture conditions: surface coating Matrigel®Matrix (Corning), enzymatic dissociation using Stem Pro

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry and RT-PCR	Positive for *OCT3/4, NANOG* and expression of *OCT3/4, NANOG* and *TDGF1*	Fig. 1 panel B and E
	Quantitative analysis Immunocytochemistry	Assessed% of positive cells: ICGi009-A: NANOG – 94.3% (n = 282), OCT3/4 – 95.5% (n = 356); ICGi009-B: NANOG – 98.4% (n = 252), OCT3/4 – 94.1% (n = 102); ICGi013-A: NANOG – 97.4% (n = 193), OCT3/4 – 96.8% (n = 249); ICGi013-B: NANOG – 96.6% (n = 262), OCT3/4 – 98.5% (n = 204)	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	ICGi009-A: 46,XY ICGi009-B: 46,XY ICGi013-A: 46,XX, 22ps+; ICGi013-B: 46,XX, 22ps+; Resolution 450	Fig. 1 panel C
Identity	STR analysis	Performed	Available with the authors
		20 sites were tested, 20/20 matched	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	*Male*: arr[hg19]3p26.3(1197623-1492721)×1.seq[hg19] del(3)(3p26.3) chr3:g.1158406–407T(19_22)_1568210del *Female*: arr[hg19]3p26.3(1197623-1492721)×1.seq[hg19] del(3)(3p26.3)chr3:g.1158406-407T(19_22)_1568210del	Fig. 1 panel D; Supplementary, Fig. 1 panel A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR. Negative	Supplementary, Fig. 1 panel C
Differentiation potential	Embryoid body formation	Expression of the three germ layer markers in embryoid bodies: Endoderm (*SOX17, FOXA2* and *AFP*) Mesoderm (*FLK1, MSX1* and *TBXT*) Ectoderm (*PAX6 and MAP2*)	Fig. 1 panel F
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

Accutase (Thermo Fisher Scientific) and cultivation in mTeSR™1 medium (STEMCELL Technologies). iPSCs were split every 7–10 days in ratio 1:3–1:4 under feeder-dependent conditions and every 6–10 days in ratio 1:4–1:6 under feeder-free condition. After splitting iPSCs either mechanically or enzymatically ROCK inhibitor Y27632 (Abcam) was added in concentration 10 μM to increase cell viability.

3.3. Immunocytochemistry

The iPSCs were fixed in 3% paraformaldehyde for 20 min at room temperature (RT), blocking of unspecific sites was achieved by incubation with blocking buffer: 5% FBS (Thermo Fisher Scientific) and 2% BSA (Sigma-Aldrich) in the presence of 0.1% Triton-X100 (Sigma-Aldrich) for 20 min at RT. Primary antibodies were diluted in blocking buffer and incubated overnight at 4 °C. Secondary antibodies were diluted in PBS and incubated for 2 h at RT (Table 3). Nuclei were stained with DAPI (Sigma-Aldrich) and immunofluorescence was visualized under confocal fluorescence microscope LSM 780 NLO (Zeiss) with ZEN software (Zeiss).

3.4. RNA isolation and RT-PCR

To confirm expression of pluripotency markers in iPSCs and differentiation markers in generated embryoid bodies, RT-PCR with

Table 3
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	*Rabbit anti-OCT4*	*1:200*	Abcam Cat# 19857, RRID: AB_445175
Pluripotency markers	*Rabbit anti-NANOG*	*1:100*	Abcam Cat# 21624, RRID: AB_446437
X-inactivation marker	*Mouse anti-Histone H3 (tri methyl K27)*	*1: 200*	Abcam Cat# 6002, RRID:AB_305237
Secondary antibodies	*Alexa Fluor 488 Goat Anti-Mouse IgG*	*1:500*	Life technologies Cat# A-11029, RRID:AB_138404
Secondary antibodies	*Alexa Fluor 546 Goat Anti- Rabbit IgG*	*1:500*	Life technologies Cat# A-11010, RRID:AB_143156

Primers		
	Target, size	Forward/Reverse primer (5′ – 3′)
House-keeping genes	GAPDH, 153 bp	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT
Pluripotency markers	*NANOG,* 110 bp	AAAGAATCTTCACCTATGCC/GAAGGAAGAGGAGAGACAGT
Pluripotency markers	*OCT3/4,* 127 bp	CTGGGTTGATCCTCGGACCT/CACAGAACTCATACGGCGGG
Pluripotency markers	*TDGF1,* 139 bp	TCCTTCTACGGACGGAACTG/AGAAATGCCTGAGGAAAGCA
Differentiation markers	*AFP,* 136 bp	AAATGCGTTTCTCGTTGCTT/GCCACAGGCCAATAGTTTGT
Differentiation markers	*FKL1,* 131 bp	TGATCGGAAATGACACTGGA/CACGACTCCATGTTGGTCAC
Differentiation markers	*FOXA2,* 122 bp	GGAGCGGTGAAGATGGAA/TACGTGTTCATGCCGTTCAT
Differentiation markers	*MAP2,* 212 bp	CAGGTGGCGGACGTGTGAAAATTGAGAGTG/CACGCTGGATCTGCCTGGGGACTGTG
Differentiation markers	*MSX1,* 307 bp	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAGCTTCTCCAG
Differentiation markers	*PAX6,* 100 bp	GTCCATCTTTGCTTGGGAAA/TAGCCAGGTTGCGAAGAACT
Differentiation markers	*SOX17,* 102 bp	CTCTGCCTCCTCCACGAA/CAGAATCCAGACCTGCACAA
Differentiation markers	*TBXT,* 112 bp	AATTGGGTCCAGCCTTGGAAT/CGTTGCTCACAGACCACA
Primers for sequencing	*Region contained deletion,* ~850 bp	TCAGTGGACAACATTTGTATAGGC/TGTGAAGTTGGCAAAACCACAT

primers specific for pluripotency genes (Table 3) was performed. Total RNA was isolated with TRI Reagent® according to the manufactures protocol (Sigma Aldrich). To eliminate possible sample contamination with genomic DNA RNA was treated with DNase I (Thermo Fisher Scientific) according to manufacturer's instructions under following conditions: incubation for 30 min at 37 °C and inactivation for 10 min at 65 °C. RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was used for reverse transcription with random hexamer primers according to the manufacturer' protocol. PCR was performed with T100 ThermoCycler (BIO-RAD) and Taq MasterMix (IMCB SB RAS, Russia) under the following conditions: 95 °C for 5 min followed by 35 cycles of 95 °C for 1 s, 55 °C for 15 s and 72 °C for 30 s, then 72 °C for 5 min and 12 °C for holding.

3.5. In vitro differentiation

Embryoid bodies were produced according to previously published protocol (Bock et al., 2011). Primers for RT-PCR were taken from Huangfu et al. (2008).

3.6. Karyotyping

iPSCs were karyotyped at passage 7, 8. Karyotype analysis was performed using conventional GTG banding techniques according to standard cytogenetic protocols (Prokhorovich et al., 2007) based on the International System for Human Cytogenetic Nomenclature (2016). We have analyzed at least 50 metaphases for each iPSC line (450-band resolution).

3.7. Mycoplasma contamination detection

The absence of mycoplasma contamination was confirmed by PCR using primers from Pisal et al. (2016). Genomic DNA from iPSCs was isolated by phenol/chloroform extraction. PCR was performed with T100 ThermoCycler (BIO-RAD) and Taq MasterMix (IMCB SB RAS, Russia) under the following conditions: 95 °C for 5 min followed by 35 cycles of 95 °C for 20 s, 55 °C for 15 s and 72 °C for 30 s, then 72 °C for 5 min and 12 °C for holding.

3.8. STR analysis

Genomic DNA extracted by phenol/chloroform from TAF3del and TAF13del fibroblast and their derivative iPSC lines ICGi009-A, ICGi009-B, ICGi013-A and ICGi013-B were authenticated by STR analysis by biotechnological company Gordiz (http://gordiz.ru/)

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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.2019.101591.

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