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Lab resource: Stem Cell Line

Generation of the induced pluripotent stem cell line, ICAGi002-A, from unaffected carrier megabase scaled duplication involving the CNTN6 gene

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A B S T R A C T

The 3p26.3 microduplication involving the *CNTN6* gene cause developmental delay and the intellectual disability. However, the incomplete penetrance is described for this copy number variation (CNV). Here we describe ICAGi002-A line, which is supposed to use as a model for studying of the penetrance of the CNV in 3p26.3. The ICAGi002-A iPSCs line was obtained by the reprogramming of the skin fibroblasts from a healthy donor with 3p26.3 microduplication involving the *CNTN6* gene. The ICAGi002-A cells was pluripotent as it was shown by the expression of the pluripotency-associated markers and *in vitro* differentiation into the cells of three germ layers.

Resource utility

Some observations pointed at incomplete penetrance neurodevelopment disorders caused by the CNVs including *CNTN6* gene. ICAGi002-A cells, together with earlier obtained and published iPSCs from the patient with the same microduplication, are the good model for studying of the molecular mechanism of the disorders and incomplete penetrance of this CNV.

Resource details

TAF14dup, the culture of skin fibroblasts, was obtained from the skin punch biopsy from a 40-year-old healthy male with megabase scaled 3p26.3 microduplication. The chromosomal rearrangement was proved using aCGH (Fig. B) and included the only *CNTN6* gene. The microduplication was inherited from healthy mother. However, the donor has an affected son with neurodevelopmental and neuropsychiatric disorders and the same microduplication (Kashevarova et al., 2014).

iPSCs was obtained from the skin fibroblasts using the lentiviral delivery of the Yamanaka's cocktail (four reprogramming factors: OCT4, SOX2, KLF4, and C-MYC). After passage 5 the colony of ICAGi002-A cells had typical morphology of human iPSCs maintaining under feeder-dependent conditions (Fig. 1C). The cell line did not contaminate with mycoplasma (Fig. 1D). ICAGi002-A cells had 46,XY karyotype without any visible chromosomal abnormalities (Fig. 1A).

The RT-PCR showed the ICAGi002-A cells expressed pluripotency markers: *OCT4*, *NANOG* and SOX2 (Fig. 1E). Immunofluorescence staining showed the most cells was positive for the OCT4, NANOG, SSEA4 and TRA-1-60 as 97.5%, 96.9%, 98%, 97.7%, after 5 passages. The pluripotency of the ICAGi002-A cells was confirmed by the *in vitro* cell differentiation through embryoid body. RT-PCR showed the expression endodermal (*AFP*, *SOX17*, *HNF1*), mesodermal (*MSX1*, *FLK1*, *BRY*) and ectodermal (*SOX1*, *MAP2*, *PAX6*) genes (Fig. G) in the obtained embryoid body cells.

The STR profile of the ICAGi002-A cell line fully matched with that of the parental TAF14dup fibroblasts (loci analysed: D3S1358, TH01, D12S391, D1S1656, D10S1248, D22S1045, D2S441, D7S820, D13S317, FGA, TPOX, D18S51, D16S539, D8S1179, CSF1PO, D5S818, vWA, D21S11 and SE33).

Taken together, we described the ICAGi002-A iPSCs line, the cells showed expression of key markers of the pluripotency and can be differentiated into the cells of the three germ layers. ICAGi002-A cells had kariotype 46,XY and 3p26.3 microduplication. As the ICAGi002-A obtained from the fibroblast of the healthy donor, we strongly believe this cell line is suitable for studying of the 3p26.3 microduplication incomplete penetrance, especially together with previously described line of iPSCs (Gridina et al., 2018) from the patient with intellectual disability and exactly same microduplication.

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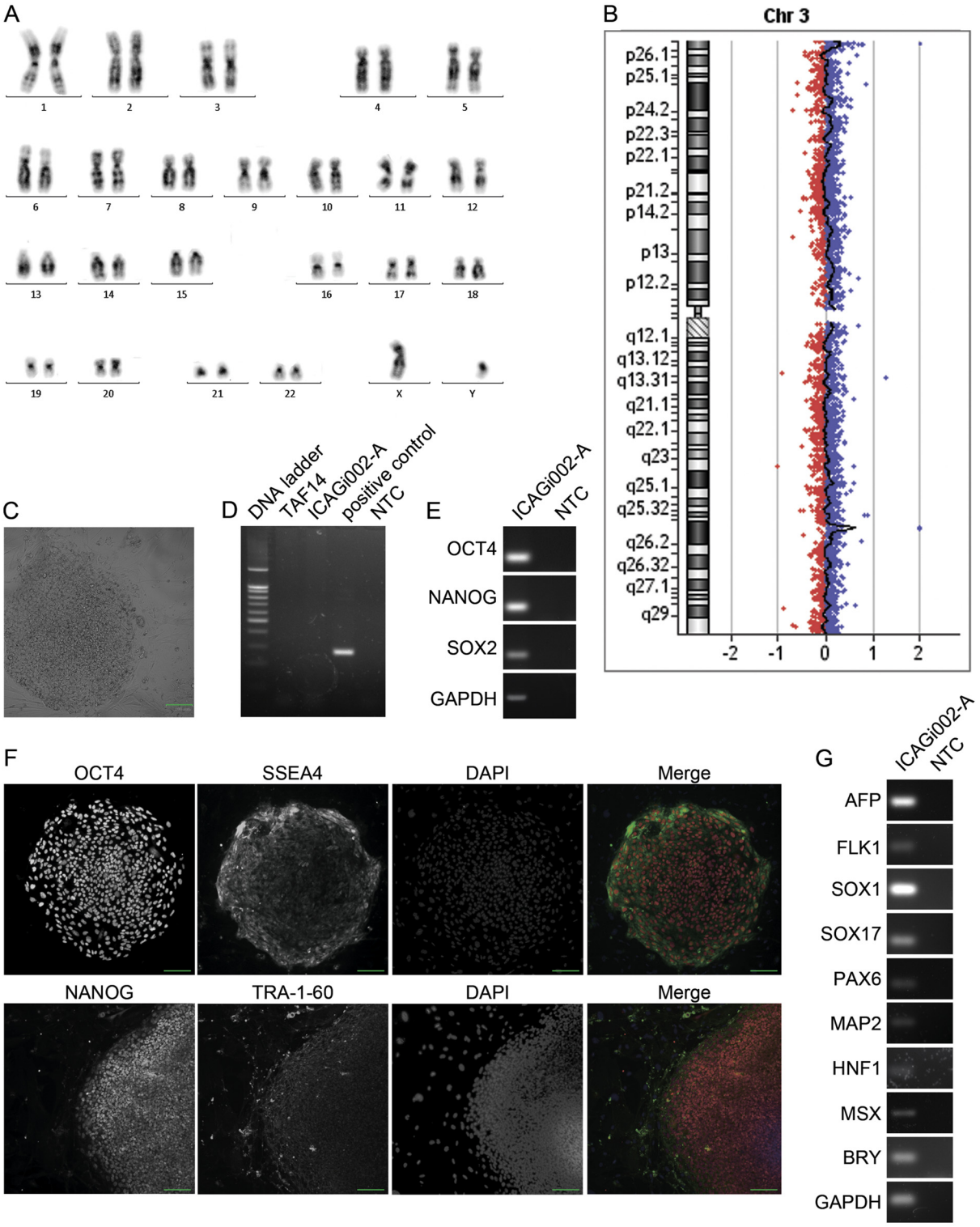

Fig. 1. Characterization of ICAGi002-A line. (A) Karyotypes. (B) aCGH analysis. (C) Morphology of the iPSC colonies. (D) Mycoplasma contamination test. (E) Expression of the pluripotency markers OCT4, NANOG, SOX2. (F) Immunofluorescence staining for the pluripotency markers NANOG, OCT4, SSEA4, TRA-1-60. Nucleus stained by DAPI (blue). (G) Expression of the endoderm (AFP, SOX17, HNF1), mesoderm (MSX1, FLK1, BRY) and ectoderm (SOX1, MAP2, PAX6) markers in the embryoid bodies and in ICAGi002-A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Materials and methods

Cell culture

The human fibroblasts were maintained in growth media (DMEM/F12 supplemented with 10% fetal bovine serum (FBS), 1% Pen Strep, 1% MEM Non-essential Amino Acid solution, 2 mM L-glutamine (all from Invitrogen)) at 37 °C in 5% CO_2.

The iPSCs were maintained on mitomycin C-treated CD-1 mouse embryonic fibroblast feeder cells in following medium: DMEM/F12 medium supplemented with 20% KnockOut Serum Replacement, 1% GlutaMAX™-I, 1% MEM NEAA, 1% Pen Strep, 0.1 mM 2-

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	normal	Fig. 1 panel C
Phenotype	Qualitative analysis	Assess staining of pluripotency markers: OCT4, NANOG, SOX2, SSEA4, TRA-1-60;	Fig. 1 panel F.
		PR-PCR analysis of the pluripotency markers	Fig. 1 panel E.
	Quantitative analysis	% of positive cells	Fig. 1 panel F
		Oct3/4: 97.5%, NANOG: 96.9%, Tra 1–97.7%, SSEA-4: 98%	
Genotype	Karyotype (G-banding) and resolution	46XY, arr[hg19] 3p26.3(838934_1558564) × 3 mat	Fig. 1 panel A
		Resolution 450–500	
Identity	STR analysis	20 sites tested, 20/20 sites completely matched	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR Negative	Fig. 1 panel D
Differentiation potential	Embryoid body formation	Expression of three germ layers marker-genes in the embryoid bodies' cells: endodermal (AFP, SOX17, HNF1), mesodermal (MSX1, FLK1, BRY) and ectodermal (SOX1, MAP2, PAX6)	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

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 -SOX2	1:400	RSE National center for Biotechnology, Astana, Cat# NCB 1601
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

Primers

	Target	Forward/Reverse primer (5′-3′)
House-Keeping Genes	*GAPDH*	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT Expected product size: 153 bp
Pluripotency Marker	*OCT4*	CTGGGTTGATCCTCGGACCT/CACAGAACTCATACGGCGGG Expected product size: 128 bp
Pluripotency Marker	*NANOG*	AAAGAATCTTCACCTATGCC/GAAGGAAGAGGAGAGACAGT Expected product size: 110 bp
Pluripotency Marker	*SOX2*	GCATCGCAGCTTGGATACAC/GCTTCAGCTCCGTCTCCAT Expected product size: 119 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
Differentiation Markers	*TBXT* (BRACHYURY)	AATTGGTCCAGCCTTGGAAT/CGTTGCTCACAGACCACA Expected product size: 112 bp
Differentiation Marker	*PAX6*	GTCCATCTTTGCTTGGGAAA/TAGCCAGGTTGCGAAGAACT Expected product size: 110 bp
Differentiation Marker	*HNF-3B*	GGAGCGGTGAAGATGGAA/TACGTGTTCATGCCGTTCAT Expected product size: 122 bp

mercaptoethanol, and 10 ng/ml bFGF (all from Invitrogen) at 37 °C in an atmosphere of 5% CO2. iPSCs were passed mechanically with ratio of split 1:3.

Reprogramming donor's fibroblasts

The lentiviruses for reprogramming were produced in the Phoenix cell line using Lipofectamine 3000 (Invitrogen) according to the manufacturer's recommendations. Plated on the previous day human fibroblasts were transduced with the lentiviral vectors (MOI = 19)

containing four human reprogramming transcription factors: OCT4, SOX2, C-MYC, and KLF4 (kindly provided to us by Dr. S.L. Kiselev (Moscow, Russia)). The second round of the transduction was without the C-MYC containing lentivirus. From the day 7 to 16 the culture medium was changed daily with addition of 1 mM valproic acid, 5 μM PD0325901, 1 μM CHIR-99021, 2 μM SB 431542 (all from Sigma) and 10 μM Y-27632 (Abcam). On day 5, the transduced cells were seeded in iPSC medium on feeder cells. On day 16, colonies with iPSC morphology were picked up and expanded.

Immunocytochemistry and immunocytochemistry counting

The iPSCs were fixed with 3% formaldehyde for 20 min, permeabilized with 0.1% Triton X-100 in PBS for 3 min, 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). 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

Undifferentiated iPSCs were harvested using TrypLE Reagents (Thermo Fisher Scientific) and plated in the iPSCs culture media without bFGF on the plates covered with 1% agarose. Cell aggregates were growing during 16 days, the media was change every second day.

RT-PCR

Total RNA were isolated by TRI Reagent (Sigma) according to manufacturer's recommendations. To exclude contamination with genome DNA 1 mkg RNA was treated with DNAseI (Thermo Fisher Scientific). cDNA were obtained by RevertAid RT kit (Thermo Fisher Scientific) and RT-PCR were performed with HP-Taq DNA polymerase.

Karyotyping

Preparation of metaphase chromosomes from iPSC was performed on passages 10–12 as previously described (Prokhorovich et al., 2007). Sixty metaphase spreads were analysed using a Carl Zeiss Axioplan 2 imaging microscope, digital images were analysed using ISIS 3 (*In Situ* Imaging System, MetaSystems GmbH) software. Multicolor banding (MCB) was carried out using XCyte 2 mBAND probe (MetaSystems GmbH) (Table 1).

Mycoplasma contamination detection

Genome DNA was isolated from the cells using phenol-chloroform extraction method. PCR was set up with 50 ng DNA as template using primers from Choppa et al., 1998.

STR analysis

STR analysis for parent TAF14dup fibroblasts and the ICAGi002-A (iTAF14dup10) was perform by Gordiz (http://gordiz.ru/).

Key resources table

Unique stem cell line identifier	ICAGi002-A
Alternative name(s) of stem cell line	TAF14dup10
Institution	THE FEDERAL RESEARCH CENTER INSTITUTE OF CYTOLOGY AND GENETICS
Contact information of distributor	Maria Gridina gridinam@gmail.com
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 40 Sex: male Ethnicity if known: Caucasian
Cell Source	skin fibroblast cell line
Method of reprogramming	Lentiviral reprogramming with four transcription factors (OCT4, SOX2, KLF4, C-MYC)
Associated disease	N/A
Gene/locus	N/A
Method of modification	N/A
Gene correction	YES
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	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	Scientific Ethics Committee of Research Institute of Medical Genetics, Tomsk NRM: 106/2017

Declaration of Competing Interest

None.

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References

Choppa, P.C., Vojdani, A., Tagle, C., Andrin, R., Magtoto, L., 1998. Multiplex PCR for the detection of Mycoplasma fermentans, M. hominis and M. penetrans in cell cultures and blood samples of patients with chronic fatigue syndrome. Mol. Cell. Probes 12, 301–308. https://doi.org/10.1006/mcpr.1998.0186.

Gridina, M.M., Matveeva, N.M., Fishman, V.S., Menzorov, A.G., Kizilova, H.A., Beregovoy, N.A., Kovrigin, I.I., Pristyazhnyuk, I.E., Oscorbin, I.P., Filipenko, M.L., Kashevarova, A.A., Skryabin, N.A., Nikitina, T.V., Sazhenova, E.A., Nazarenko, L.P., Lebedev, I.N., Serov, O.L., 2018. Allele-specific biased expression of the CNTN6 gene in iPS cell-derived neurons from a patient with intellectual disability and 3p26.3 microduplication involving the CNTN6 gene. Mol. Neurobiol. 55, 6533–6546. https://doi.org/10.1007/s12035-017-0851-5.

Kashevarova, A.A., Nazarenko, L.P., Schultz-Pedersen, S., Skryabin, N.A., Salyukova, O.A., Chechetkina, N.N., Tolmacheva, E.N., Rudko, A.A., Magini, P., Graziano, C., Romeo, G., Joss, S., Tümer, Z., Lebedev, I.N., 2014. Single gene microdeletions and microduplication of 3p26.3 in three unrelated families: CNTN6 as a new candidate gene for intellectual disability. Mol. Cytogenet. 7 (1), 97. https://doi.org/10.1186/s13039-014-0097-0.

Prokhorovich, M.A., Lagar'kova, M.A., Shilov, A.G., Karamysheva, T.V., Kiselyov, S.L., Rubtsov, N.B., 2007. Cultures of hESM human embryonic stem cells: chromosomal aberrations and karyotype stability. Bull. Exp. Biol. Med. 144 (1), 126–129. https://doi.org/10.1007/s10517-007-0271-z.