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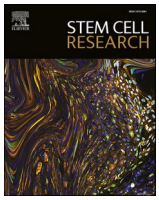

Lab Resource: Multiple Cell Lines

Generation of two human iPSC lines, HMGUi004-A and FINCBi004-A, from fibroblasts of MPAN patients carrying pathogenic recessive mutations in the gene *C19orf12*

Enrica Zanuttigh [a], Ejona Rusha [b], Camille Peron [c], Dario Brunetti [c], Giovanna Zorzi [c], Anna Pertek [b], Polyxeni Nteli [b], Juliane Winkelmann [a,d], Valeria Tiranti [c,*], Arcangela Iuso [a,d,*]

[a] *Institute of Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany*
[b] *iPSC Core Facility, Helmholtz Zentrum München, Neuherberg, Germany*
[c] *Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy*
[d] *Institute of Human Genetics, Technical University of Munich, Munich, Germany*

A B S T R A C T

Mitochondrial membrane Protein-Associated Neurodegeneration (MPAN) is a lethal neurodegenerative disorder caused by mutations in the human gene *C19orf12*. The molecular mechanisms underlying the disorder are still unclear, and no established therapy is available. Here, we describe the generation and characterization of two human induced pluripotent stem cell (iPSC) lines derived from skin fibroblasts of two MPAN patients carrying homozygous recessive mutations in *C19orf12*. These iPSC lines represent a useful resource for future investigations on the pathology of MPAN, as well as for the development of successful treatments.

Resource table

Unique stem cell lines identifier	HMGUi004-A https://hpscreg.eu/cell-line/HMGUi004-A FINCBi004-A https://hpscreg.eu/cell-line/FINCBi004-A
Alternative name(s) of stem cell lines	HMGUi004-A: 106300 FINCBi004-A: 140395
Institution	Helmholtz Zentrum München, Germany IRCCS Istituto Neurologico Carlo Besta, Italy
Contact information of distributor	Arcangela Iuso, arcangela.iuso@helmholtz-munich.de Valeria Tiranti, valeria.tiranti@istituto-besta.it
Type of cell lines	iPSC
Origin	Human
Additional origin info required *for human ESC or iPSC*	HMGUi004-A: female FINCBi004-A: female
Cell Source	Skin fibroblasts
Clonality	Clonal
Associated disease	Mitochondrial membrane Protein-Associated Neurodegeneration (MPAN)
Gene/locus	HMGUi004-A: NM_031448.6 c.[161G > T];[161G > T], p.[Gly54Val];[Gly54Val] FINCBi004-A: NM_031448.6 c.[139G > A];[139G > A], p.[Gly47Ser];[Gly47Ser]
Date archived/stock date	Date cell lines archived or deposited in repository HMGUi004-A, April 2023; FINCBi004-A, July 2015
Cell line repository/bank	https://hpscreg.eu/cell-line/HMGUi004-A https://hpscreg.eu/cell-line/FINCBi004-A
Ethical approval	Ethikkommission der Technischen Universität München. Approval no. 2022-674-S-SR

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1. Resource utility

The two iPSC lines generated in this study are an essential resource for the establishment of neuronal cellular models that will provide insight into the mechanisms underlying the pathology of MPAN, and that could be used as a tool to test potential pharmaceutical treatments. Table 1.

2. Resource details

Here we describe the generation and characterization of two iPSC lines from the skin fibroblasts of two MPAN patients carrying the homozygous recessive mutations c.[161G > T] (HMGUi004-A) and c.[139 G > A] (FINCBi004-A) in the gene *C19orf12* (Fig. 1A). We generated the two iPSC lines using two different approaches: the line HMGUi004-A was generated using StemRNA 3rd Gen reprogramming kit, and the

* Corresponding authors at: Institute of Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany (A. Iuso).
E-mail addresses: valeria.tiranti@istituto-besta.it (V. Tiranti), arcangela.iuso@helmholtz-munich.de (A. Iuso).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1, panel B
Phenotype	Qualitative analysis: immunocytochemistry	All clones express the pluripotency markers OCT4, NANOG, SOX2 and LIN28. Passage number at which the cells were tested: HMGUi004-A P11, FINCBi004-A P12-20	Fig. 1, panel C
	Quantitative analysis: flow cytometry	HMGUi004-A: 94.25% and 99.6% of positive cells for cell surface markers Tra 1–60 and SSEA- 4. FINCBi004-A: 91.6% and 90.95% of positive cells for cell surface markers Tra 1–60 and SSEA- 4. Passage number at which the cells were tested: HMGUi004-A P12, FINCBi004-A P21-23	Fig. 1, panel D
Genotype	Karyotype (G-banding) and resolution	46XX, resolution 400–600. Passage number at which the cells were tested: HMGUi004-A P12-18, FINCBi004-A P8-16	Supplementary Fig. 1, panel B
Identity	STR analysis	16 sites tested, all matching	Data are available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	HMGUi004-A carries the homozygous recessive mutation c.[161G > T]. FINCBi004-A carries the homozygous recessive mutation c.[139 G > A].	Fig. 1, panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. All clones were negative. Passage number at which the cells were tested: HMGUi004-A P15-16, FINCBi004-A P6-10	Supplementary Fig. 1, panel C
Differentiation potential	Directed differentiation	Proof of the three germ layers formation via quantification of the expression of germ-layer specific markers. Passage number at which the cells were tested: HMGUi004-A P10-11, FINCBi004-A P18-20	Fig. 1, panel E
List of recommended germ layer markers		Ectoderm: PAX6, NCAD, SOX1 Mesoderm: MESP1, TBXT, MIXL Endoderm: SOX17, CXCR4, FOXA2	RT-qPCR
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

line FINCBi004-A by using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit. The absence of the Sendai virus was confirmed by RT-qPCR (Fig. S1A). The newly generated iPSC lines showed typical iPSC morphology (Fig. 1B), and the expression of the pluripotency markers OCT4, NANOG, SOX2, and LIN28 was verified via immunofluorescence (Fig. 1C). The pluripotent state was further assessed by the presence of cell surface markers via flow cytometry. More than 90% of the cells resulted positive for the pluripotency markers Tra 1–60 and SSEA- 4 (Fig. 1D). The potency of the iPSC clones was investigated via directed differentiation into the three germ layers. The expression of germ-layer specific markers (PAX6, NCAD, and SOX1 for the ectoderm; MESP1, T, and MIXL for the mesoderm; SOX17, CXCR4, and FOXA2 for the endoderm) was quantified via RT-qPCR (Fig. 1E). All the iPSC lines generated in this study show normal karyotype (Fig. S1B) and tested negative for mycoplasma contamination (Fig. S1C).

3. Materials and methods

3.1. iPSCs generation and culturing

HMGUi004-A was generated from fibroblasts using NM-RNA kit (Reprocell, #00-0076). Fibroblasts were cultured in NutriStem® XF (Sartorius, #05-100-1A). Four daily transfections were performed. On day 10, cells were switched to iPS-Brew XF (Miltenyi Biotec, #130-104-368). FINCBi004-A was reprogrammed from fibroblasts using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher, #A16517). Clones emerged from day 20, were picked and grown in feeder-free conditions (Corning, #354277) with Essential 8™ Flex (Thermo Fisher, #A2858501).

iPSCs were cultured on Matrigel-coated (Corning, #354234) cultureware in mTeSR™ Plus (STEMCELL Technologies, #100-0276) at 37 °C and 5% CO_2, and passaged as clumps using StemMACS™ Passaging Solution XF (Miltenyi Biotec, #130-104-388).

3.2. Immunocytochemistry

Cells were fixed with 4% PFA (Thermo Fisher, #28906) for 10 min and permeabilized with 0.2% Triton™X-100 (Merck, #X100-500ML) for 15 min at room temperature (RT). Antibodies, listed in Table 2, were diluted in 0.2% Triton™X-100 with 10% FBS (Cytiva, #SH30071.03IR25-40) and incubated overnight at 4 °C (primary) or 1 h at RT (secondary). Slides were mounted using ProLong™ Glass Antifade Mountant with NucBlue™ Stain (Thermo Fisher, #P36985) and imaged using a Zeiss Axio Imager M.2 with Colibri 7 and an EVOS™ M500 microscope.

3.3. Flow cytometry

Cells were harvested as single-cell suspensions using TrypLE™ Express Enzyme (Thermo Fisher, #12605010) and incubated with primary antibodies for 45 min on ice. Tra 1–60 stained samples were further incubated with a secondary antibody for 30 min. Antibodies, listed in Table 2, were diluted in 1% KnockOut™ Serum Replacement (Thermo Fisher, #10828028). Cells were washed twice with PBS (Thermo Fisher, #10010023), collected via centrifugation and measured using BD FACSAria™ III Cell Sorter. Data were analyzed with the FlowJo software.

3.4. Potency assay

Upon reaching 90% confluence, iPSC clones from two passages were differentiated for 5 days as previously described (Shi et al., 2012; Borchin et al., 2013; Ori et al., 2021). Cultures were washed three times with DPBS (Thermo Fisher, #14190144) and harvested using RLT buffer (Qiagen, #74106). Previously published ISFi001-A hiPSC line was used as a positive control (Kunze et al., 2018).

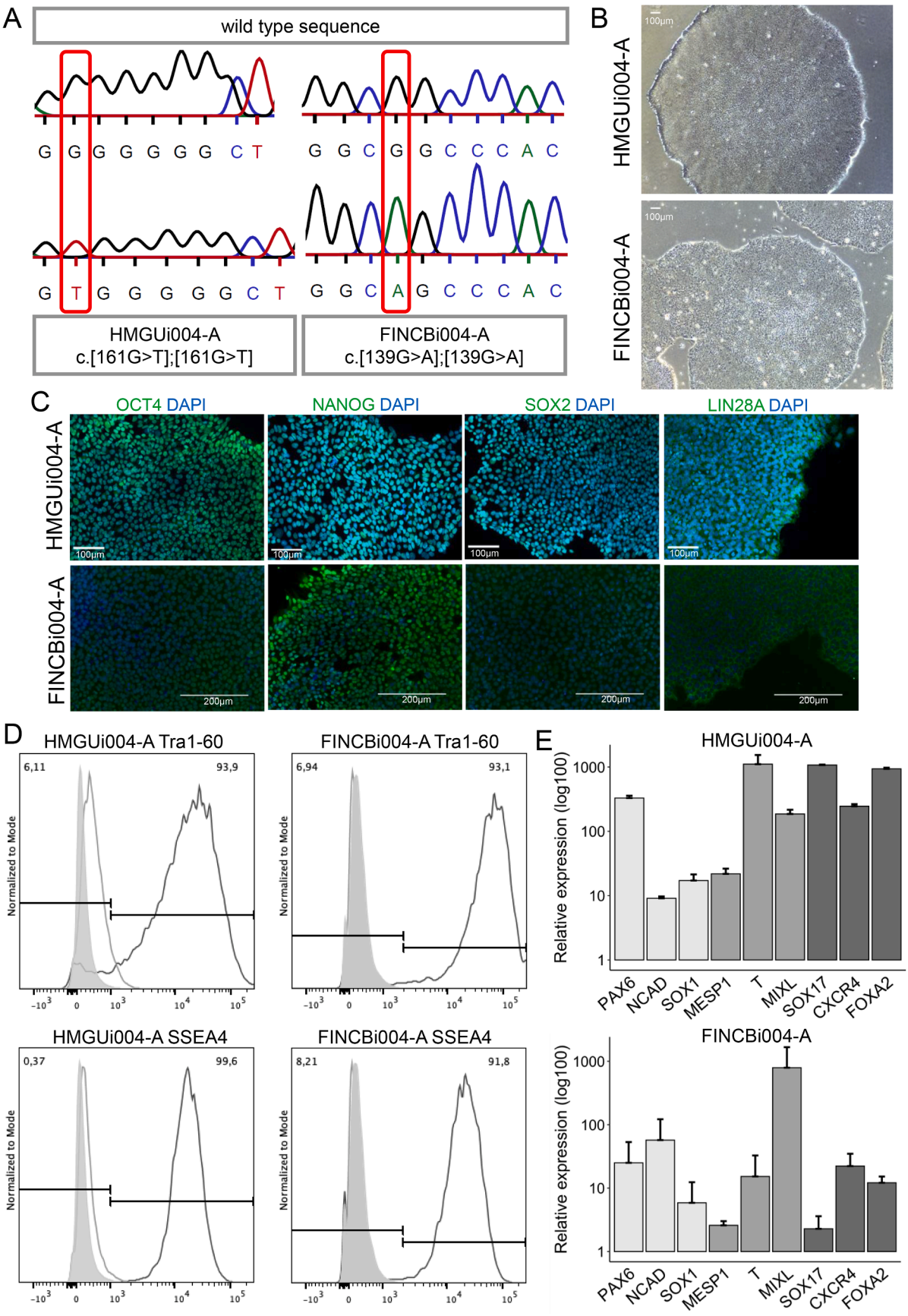
A
wild type sequence
G G G G G G G C T
G G C G G C C C A C
G T G G G G G C T
G G C A G C C C A C
HMGUi004-A
c.[161G>T];[161G>T]
FINCBi004-A
c.[139G>A];[139G>A]
B
HMGUi004-A
FINCBi004-A
100µm
C
OCT4 DAPI
NANOG DAPI
SOX2 DAPI
LIN28A DAPI
HMGUi004-A
FINCBi004-A
100µm
200µm
D
HMGUi004-A Tra1-60
FINCBi004-A Tra1-60
6,11
93,9
6,94
93,1
Normalized to Mode
HMGUi004-A SSEA4
FINCBi004-A SSEA4
0,37
99,6
8,21
91,8
E
HMGUi004-A
FINCBi004-A
Relative expression (log100)
1000
100
10
1
PAX6
NCAD
SOX1
MESP1
T
MIXL
SOX17
CXCR4
FOXA2

Fig. 1.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency markers, immunocytochemistry	Rabbit anti-OCT-4a	1:400	CST #2840S	RRID: AB_2167691
Pluripotency markers, immunocytochemistry	Rabbit anti-SOX2	1:200	CST #2748S	RRID: AB_823640
Pluripotency markers, immunocytochemistry	Rabbit anti-NANOG	1:200	CST #4903S	RRID: AB_10559205
Pluripotency markers, immunocytochemistry	Rabbit anti-LIN28A	1:800	CST #3978S	RRID: AB_2297060 RRID: AB_2576217
Secondary antibodies, immunocytochemistry	Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed, Alexa Fluor 488	1:1000	Thermo Fisher # A-11034	RRID: AB_2576217
Nuclear stain	ProLong Glass Antifade Mountant with NucBlue Stain (Hoechst 33342)	N/A	Thermo Fisher # P36985	
Flow cytometry	TRA-1–60 (R) antibody [TRA-1–60] - Stem Cell Marker	1:100	Abcam #ab16288	RRID: AB_778563
Flow cytometry	FITC anti-human SSEA-4 Antibody	5 μl per million cells in 100 μl staining volume	BioLegend #330410	RRID: AB_1089204
Flow cytometry	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488	1:1000	Thermo Fisher #A32790	RRID: AB_2762833

	Primers		
	Target	Size of band	Forward/Reverse primer (5′-3′)
Trilineage differentiation, ectoderm marker (RT-qPCR)	PAX6	95 bp	GCGGAGTTATGATACCTACACC/ GAAATGAGTCCTGTTGAAGTGG
Trilineage differentiation, ectoderm marker (RT-qPCR)	NCAD	51	CCCACACCCTGGAGACATTG/ GCCGCTTTAAGGCCCTCA
Trilineage differentiation, ectoderm marker (RT-qPCR)	SOX1	70	GAGAACCCCAAGATGCACAA/ CCTCGGACATGACCTTCCA
Trilineage differentiation, mesoderm marker (RT-qPCR)	MESP1	102 bp	CTGCCTGAGGAGCCCAAGT/ GCAGTCTGCCAAGGAACCA
Trilineage differentiation, mesoderm marker (RT-qPCR)	T	101 bp	CAACCTCACTGACGGTGAAAAA/ ACAAATTCTGGTGTGCCAAAGTT
Trilineage differentiation, mesoderm marker (RT-qPCR)	MIXL	58 bp	CCGAGTCCAGGATCCAGGTA/ CTCTGACGCCGAGACTTGG
Trilineage differentiation, endoderm marker (RT-qPCR)	SOX17	61 bp	GGCGCAGCAGAATCCAGA/ CCACGACTTGCCCAGCAT
Trilineage differentiation, endoderm marker (RT-qPCR)	CXCR4	79 bp	CACCGCATCTGGAGAACCA/ GCCCATTTCCTCGGTGTAGTT
Trilineage differentiation, endoderm marker (RT-qPCR)	FOXA2	89 bp	GGGAGCGGTGAAGATGGA/ TCATGTTGCTCACGGAGGAGTA
Trilineage differentiation, House -Keeping (RT-qPCR)	GAPDH	184 bp	GCTCATTTCCTGGTATGACAACG/ GAGATTCAGTGTGGTGGGGG
C19orf12, exon 2 (Sanger sequencing)	*C19orf12* mutation c.[139 G > A]	305 bp	AACTAGAGTGGCATTGTGATGG/ GTTTCAACGGCCCTTTTATG
C19orf12, exon 3 (Sanger sequencing)	*C19orf12* mutation c.[161G > T]	394 bp	AAGTAAGGCCATCGCTTATG/ AACTCCCAAGCCACCTCTTC

RRID Requirement for antibodies: use https://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

3.5. RT-qPCR

RNA was extracted using the RNeasy Mini Kit. cDNA was synthesized from 500 ng RNA using SuperScript™ III Reverse Transcriptase (Thermo Fisher, #18080085). The RT-qPCR was run using the default program from the QuantStudio™ 12 K Flex Real-Time PCR System (Thermo Fisher, #4471081) and Power SYBR™ Green PCR Master Mix (Thermo Fisher, #4367659) with primers (Merck) listed in Table 2. For the Sendai clearance assay, TaqMan™ Gene Expression Master Mix (Thermo Fisher, #4369514) and Taqman probes (Thermo Fisher, #351370 assay IDs: SeV:Mr04269880_mr/ KOS:Mr04421257_mr/ Klf4: Mr04421256_mr cMyc:Mr04269876_mr) were used. Relative expression levels were calculated using the ΔΔCt method normalized to *GAPDH*.

3.6. Sequencing

DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, #69504). The region of interested was amplified via PCR using the Thermo-Start Taq DNA Polymerase (Thermo Fisher, #AB-1057/B), and sequenced via Sanger sequencing (Eurofins Genomics) with primers (Eurofins Genomics) listed in Table 2.

3.7. Karyotyping

Karyotyping was performed by the Institute of Human Genetics of the Technical University of Munich via metaphase preparation and G-banding (≥20 metaphases counted).

3.8. Mycoplasma testing

Mycoplasma was measured using the MycoAlert® Mycoplasma detection kit (Lonza, #LT07-118).

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

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Cell lines identity testing

Cell line identity was validated by the confirmation of the respective mutations in the *C19orf12* gene, and by STR analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103197.

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