Vavilovskii Zhurnal Genetiki i Selektsii
Vavilov Journal of Genetics and Breeding. 2025;29(1):15-25

doi 10.18699/vjgb-25-03

Generation and characterisation of seven induced pluripotent stem cell lines from two patients with Parkinson's disease carrying the pathological variant c.1087G>T of the *LGR4* gene

V.S. Podvysotskaya[1, 2 &], E.V. Grigor'eva [1, 3 &], A.A. Malakhova [1, 3], J.M. Minina [1], Y.V. Vyatkin[4], E.A. Khabarova[1, 5], J.A. Rzaev[5], S.P. Medvedev [1, 3], L.V. Kovalenko [6], S.M. Zakian [1, 3]

[1] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[2] Novosibirsk State University, Novosibirsk, Russia
[3] Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[4] NOVEL Ltd., Novosibirsk, Russia
[5] Federal Neurosurgical Center of the Ministry of Health of the Russian Federation, Novosibirsk, Russia
[6] Surgut State University, Surgut, Khanty-Mansiysk Autonomous Okrug – Ugra, Russia
evlena@bionet.nsc.ru

Abstract. Parkinson's disease is a neurodegenerative disorder affecting dopaminergic neurons of the substantia nigra pars compacta. The known pathological genetic variants may explain the cause of only 5 % of cases of the disease. In our study, we found two patients with a clinical diagnosis of Parkinson's disease with the genetic variant c.1087G>T (p.Gly363Cys) of the *LGR4* gene. The *LGR4* gene encodes the membrane receptor LGR4 (leucine rich repeat containing G protein-coupled receptor 4) associated with the G protein. We hypothesize that the *LGR4* gene may be either a direct cause or a risk factor for this disease, since it is one of the main participants of the WNT/β-catenin signalling pathway. This signalling pathway is necessary for the proliferation of neurons during their differentiation, which may lead to Parkinson's disease. To study the relationship between this genetic variant and Parkinson's disease, an ideal tool is a cellular model based on induced pluripotent stem cells (iPSCs) and their differentiated derivatives, dopaminergic neurons. We reprogrammed the peripheral blood mononuclear cells of the two patients with the c.1087G>T variant of the *LGR4* gene with non-integrating episomal vectors expressing OCT4, SOX2, KLF4, LIN28, L-MYC and mp53DD proteins. The obtained seven lines of induced pluripotent stem cells were characterised in detail. The iPSCs lines obtained meet all the requirements of pluripotent cells, namely, they stably proliferate, form colonies with a morphology characteristic of human pluripotent cells, have a normal diploid karyotype, express endogenous alkaline phosphatase and pluripotency markers (OCT4, NANOG, SSEA-4 and SOX2) and are capable to differentiate into derivatives of the three germ layers. The iPSC lines obtained in this work can be used as a tool to generate a relevant model to study the effect of the pathological variant c.1087G>T of the *LGR4* gene on dopaminergic neuron differentiation.

Key words: Parkinson's disease; reprogramming; induced pluripotent stem cells; *LGR4* gene.

For citation: Podvysotskaya V.S., Grigor'eva E.V., Malakhova A.A., Minina J.M., Vyatkin Y.V., Khabarova E.A., Rzaev J.A., Medvedev S.P., Kovalenko L.V., Zakian S.M. Generation and characterisation of seven induced pluripotent stem cell lines from two patients with Parkinson's disease carrying the pathological variant c.1087G>T of the *LGR4* gene. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov J Genet Breed*. 2025;29(1):15-25. doi 10.18699/vjgb-25-03

Funding. The study was carried out with the financial support of the Foundation for Scientific and Technological Development of Yugra within the framework of scientific project No. 2023-573-05.

Acknowledgements. Analysis of the results of immunofluorescence staining of preparations and differential staining of chromosomes was performed using the resources of the Center for Collective Use of Microscopic Analysis of Biological Objects of ICG SB RAS (https://ckp.icgen.ru/ckpmabo/), supported by the budget project of the Institute of Cytology and Genetics SB RAS (FWNR-2022-0015).

Создание и характеристика семи линий индуцированных плюрипотентных стволовых клеток от двух пациентов с болезнью Паркинсона, несущих вариант c.1087G>T гена *LGR4*

В.С. Подвысоцкая[1, 2 &], Е.В. Григорьева [1, 3 &], А.А. Малахова [1, 3], Ю.М. Минина [1], Ю.В. Вяткин[4], Е.А. Хабарова[1, 5], Дж.А. Рзаев[5], С.П. Медведев [1, 3], Л.В. Коваленко [6], С.М. Закиян [1, 3]

[1] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[2] Новосибирский национальный исследовательский государственный университет, Новосибирск, Россия

[&] These authors contributed equally to this work

[3] Институт химической биологии и фундаментальной медицины Сибирского отделения Российской академии наук, Новосибирск, Россия
[4] ООО «Новые Программные Системы», Новосибирск, Россия
[5] Федеральный центр нейрохирургии Министерства здравоохранения Российской Федерации, Новосибирск, Россия
[6] Сургутский государственный университет, Сургут, Ханты-Мансийский автономный округ – Югра, Россия
evlena@bionet.nsc.ru

Аннотация. Болезнь Паркинсона – тяжелое нейродегенеративное заболевание, поражающее дофаминергические нейроны компактной части черной субстанции головного мозга. Известные патологические генетические варианты, ассоциированные с болезнью Паркинсона, объясняют причину всего 5 % случаев заболевания, поэтому исследования в этой области актуальны и активно продолжаются. В данном исследовании мы обнаружили двух пациентов с клиническим диагнозом «болезнь Паркинсона» с генетическим вариантом *LGR4:c.1087G>T* (p.Gly363Cys, rs117543292). Этот ген кодирует мембранный рецептор LGR4 (leucine rich repeat containing G protein-coupled receptor 4), ассоциированный с G-белком, который участвует в регуляции функционирования сигнального пути WNT/β-катенин. Данный сигнальный путь необходим для пролиферации нейронов во время их дифференцировки, поэтому его дисфункция в результате гетерозиготной мутации c.1087G>T в гене *LGR4* нарушает дифференцировку дофаминергических нейронов, что может приводить к болезни Паркинсона. Идеальным инструментом для изучения связи этого генетического варианта с болезнью Паркинсона является клеточная модель на основе индуцированных плюрипотентных стволовых клеток (ИПСК) и их дифференцированных производных – дофаминергических нейронов. В результате репрограммирования эписомными векторами, экспрессирующими белки OCT4, SOX2, KLF4, LIN28, L-MYC и mp53DD, мононуклеарных клеток периферической крови двух пациентов с вариантом c.1087G>T гена *LGR4* нами были получены и детально охарактеризованы семь линий ИПСК. Данные ИПСК отвечают всем требованиям плюрипотентных клеток, а именно: стабильно пролиферируют, образуют колонии с характерной для плюрипотентных клеток человека морфологией, имеют нормальный диплоидный кариотип, экспрессируют щелочную фосфатазу и маркеры плюрипотентности (OCT4, NANOG, SSEA-4 и SOX2) и способны дифференцироваться в производные трех зародышевых листков – энто-, экто- и мезодерму. Полученные в работе линии ИПСК будут в дальнейшем использованы для создания релевантной модели, направленной на исследование эффекта варианта c.1087G>T гена *LGR4* на развитие патологического фенотипа дофаминергических нейронов.

Ключевые слова: болезнь Паркинсона; репрограммирование; индуцированные плюрипотентные стволовые клетки; ген *LGR4*.

Introduction

Parkinson's disease is the second most prevalent neurodegenerative disorder after Alzheimer's disease (Wang et al., 2020). It is characterised by the degeneration of dopaminergic neurons in the substantia nigra, which are responsible for regulating movement. The resulting symptoms include tremor, bradykinesia and rigidity. The destruction of these neurons can be attributed to various factors. These include accumulation of alpha-synuclein protein and formation of Lewy bodies, dysfunction of mitochondria and lysosomes, problems of synaptic and vesicle transport. These factors, when combined, lead to an accelerated death of neurons. Some of the signs of Parkinson's disease at the cellular level are oxidative stress resulting from mitochondrial dysfunction (Niu et al., 2021) and endoplasmic reticulum (ER) stress resulting from the accumulation of large amounts of misfolded proteins (Fernandes et al., 2016; Marciniak et al., 2022).

It is currently understood that more than 20 genes are associated with Parkinson's disease, the most prevalent mutations of which are found in the *GBA1*, *LRRK2*, *PRKN*, and *PINK1* genes (Funayama et al., 2023). However, it has been determined that only approximately 5 % of cases of Parkinson's disease are attributable to the inheritance of one of the genes associated with the disease, with the remaining 16–36 % being manifested as a result of non-monogenic inheritance. It can thus be concluded that research into genetic variants associated with the onset of Parkinson's disease remains an active area of study.

Following a comprehensive analysis of the exomic sequencing results for a cohort of over 70 patients diagnosed with Parkinson's disease, who are under observation at the Federal Neurosurgical Center of the Ministry of Health of the Russian Federation (Novosibirsk), it was found that two patients of different genders, who are not related, carry the c.1087G>T variant of the *LGR4* gene (SRA databases, project PRJNA563295). There are currently 48 known single nucleotide substitutions in this gene, three of which are pathogenic missense mutations c.2531A>G (p.Asp844Gly), c.286A>G (p.Ile96Val) and c.1087G>T (p.Gly363Cys) (Mancini et al., 2023).

LGR4 is a G-coupled receptor that plays a role in the function of the WNT/β-catenin and cAMP/protein kinase A signalling pathways (Shi et al., 2021). Previously, it was shown that the c.1087G>T variant of the *LGR4* gene is pathogenic and leads to delayed puberty due to a violation of the WNT/β-catenin signalling pathway (Mancini et al., 2023), which is also involved in embryogenesis in the development of tissues and organs such as the gonads, kidneys, nervous system, liver and others. It should be noted that this pathway is also required for the development and differentiation of dopaminergic neurons (Marchetti et al., 2020). This correlation, together with the involvement of the LGR4 protein in the WNT/β-catenin signalling pathway, leads

us to speculate that the genetic variant *LGR4:c.1087G>T* may cause dysfunction or death of dopaminergic neurons, possibly leading to Parkinson's disease. To study the association of this genetic variant with pathogenic processes leading to the death of dopaminergic neurons, the first step is to create a cellular model based on induced pluripotent stem cells (iPSCs) and relevant cell types, dopaminergic neurons, differentiated from them.

The pluripotent state of iPSCs is maintained by adding basic fibroblast growth factor (FGF-basic/bFGF) to the culture medium. To confirm the characteristics of pluripotency and self-renewal of cells, various tests are performed (Grigor'eva et al., 2023), such as qualitative (immunofluorescence staining) and quantitative (real-time PCR) analyses of the expression levels of various pluripotency factors (NANOG, SSEA-4, OCT4, SOX2, TRA1-81, TRA1-60, etc.). It is known that various karyotype abnormalities (polyploidisation, translocations, deletions, aneuploidies, etc.) are possible during prolonged *in vitro* cell culture, so karyotype analysis is one of the key tests for characterising the obtained iPSC lines. The most important test of cell pluripotency is spontaneous differentiation, which is necessary to confirm the ability of iPSCs to give rise to cell derivatives of all three germ layers (ecto-, ento- and mesoderm). In addition, the following shall be performed to characterise the iPSC lines obtained: STR analysis to confirm the origin of the iPSC lines obtained from a peripheral blood mononuclear cell (PBMC) donor; tests to indicate the presence of genetic variants of the PBMC donor in the iPSCs or to confirm the presence of genetic modifications in the case of transgenesis of iPSCs; PCR to eliminate exogenous DNA/episomes that enabled somatic cell reprogramming; and routine tests to ensure the absence of intra-laboratory contamination.

The capacity of iPSCs to yield derivatives of a virtually limitless range of cell types, including neurons, cardiomyocytes, liver cells, endothelial cells and numerous others, renders them a promising tool for generating cell models and investigating the pathogenesis of various inherited diseases in relevant cell types. For instance, by reprogramming PBMCs, culturing the resulting iPSCs and further differentiating them into dopaminergic neurons (Grigor'eva et al., 2023) and astrocytes (Yarkova et al., 2023), it will be possible to obtain *in vitro* models of Parkinson's disease. This platform will facilitate the analysis of the contribution of the genetic variant *LGR4:c.1087G>T* to Parkinson's disease development, as well as the study of its molecular and genetic pathogenesis. It will also support the exploration of new drug targets within diverse signalling pathways, and the prevention of pathogenic processes that result in dopaminergic neuron death. Furthermore, it will enable the evaluation of potential medical drugs.

Materials and methods

Ethics Statement. The study was approved by the Research Ethics Committee of the Federal Neurosurgical Center of the Ministry of Health of the Russian Federation (Novosibirsk), Protocol No. 1, dated 14 March 2017. Peripheral blood samples from patients were provided by the Federal Neurosurgical Center. Patients signed a voluntary informed consent and an information sheet.

Isolation of PBMCs. PBMCs were isolated in a Ficoll gradient and frozen as previously described (Grigor'eva et al., 2024b).

Reprogramming of patient-specific PBMCs. Reprogramming of PBMCs was performed by transfection with episomal vectors expressing SOX2, OCT4, KLF4, L-MYC, LIN28 and the dominant negative form of mouse p53 protein – mp53DD (Addgene ID No. 41813–14, 41855–57) according to the previously described method (Grigor'eva et al., 2023). Transfection was performed using a Neon Transfection System (Thermo Fisher Scientific), programme: 1,650 V, 10 ms, 3 times. Primary colonies of iPSCs were replated into 1 cm^2 wells previously coated with mouse embryonic fibroblasts (MEF) inactivated with mitomycin C (Sigma-Aldrich).

Cultivation of patient-specific iPSCs. iPSCs were cultured on MEF as previously described (Yarkova et al., 2024). Colonies of iPSCs were disaggregated using TrypLE Express (Thermo Fisher Scientific) and plated once every 3–4 days at a 1:10 ratio with the addition of a ROCK inhibitor, 2 μM thiazovivin (Sigma-Aldrich).

Histochemical detection of endogenous alkaline phosphatase. Alkaline phosphatase was detected using the SIGMAFAST™ BCIP®/NBT kit (Sigma-Aldrich). The result was analysed using a Nikon Eclipse Ti-E microscope (Nikon) with NIS Elements Advanced Research software version 4.30.

Immunofluorescence staining. Immunofluorescence staining was performed according to the previously described method (Grigor'eva et al., 2024a). The preparations were analysed on a Nikon Eclipse Ti-E microscope using NIS Elements Advanced Research version 4.30 software. The list of antibodies used is shown in Table 1.

Spontaneous differentiation of iPSCs. To determine the potential of the resulting iPSC lines, spontaneous differentiation was performed by the formation of embryoid bodies followed by immunofluorescence staining. iPSCs were grown on MEF in Petri dishes (20 cm^2) to a density of 80–90 %, after which the cells were incubated in 0.15 % collagenase type IV (Thermo Fisher Scientific) for 20–40 min. The iPSC colonies were then carefully pipetted to separate them from the feeder cells, centrifuged at 100 g for 5 min, the supernatant was decanted, suspended in iPSC growth medium without bFGF, and the colonies were transferred to a Petri dish (20 cm^2) coated with 1 % agarose. After 14 days, embryoid bodies obtained from the colonies were plated on 8-well Chambered Coverglass plates (Thermo Fisher Scientific) coated with Matrigel. After 7–9 days, they were fixed in 4 % paraformaldehyde (Sigma-Aldrich) for 10 min and immunofluorescence analysis was performed for markers of the three germ layers. The list of antibodies is given in Table 1.

Karyotyping of iPSC lines. Karyotype analysis was performed using a previously developed and described method (Yarkova et al., 2023).

Table 1. List of antibodies used in the work

Antibodies	Dilution	Company, Cat No.	RRID
Primary antibodies			
Markers of pluripotency			
Mouse IgG2b anti-OCT-3/4	1:100	Santa cruz Biotechnology, sc-5279	RRID:AB_628051
Mouse IgG3 anti-SSEA-4	1:25	Abcam, ab16287	RRID:AB_778073
Mouse IgG1 anti-NANOG	1:50	Santa cruz Biotechnology, sc-293121	RRID:AB_2665475
Rabbit IgG anti-SOX2	1:400	Cell Signaling, 3579	RRID:AB_2195767
Mesoderm markers			
Mouse IgG2a anti-αSMA	1:200	Dako, M0851	RRID:AB_2223500
Mouse IgG1 anti-CD29	1:100	Thermo Fisher Scientific, 14-0299-82	RRID:AB_1210468
Endoderm markers			
Mouse IgG2a anti-AFP	1:250	Sigma-Aldrich, A8452	RRID:AB_258392
Mouse IgG1 anti-Cytokeratin 18 (KRT18)	1:250	Abcam, ab668	RRID:AB_305647
Ectoderm markers			
Mouse IgG2a anti TUBB3	1:1,000	BioLegend, 801201	RRID:AB_2313773
Chicken IgY anti-MAP2	1:1,000	Abcam, ab5392	RRID:AB_2138153
Secondary antibodies			
Goat anti-Rabbit IgG (H + L) Alexa Fluor 568	1:400	Thermo Fisher Scientific, A11011	RRID:AB_143157
Goat anti-Mouse IgG2b Alexa Fluor 568	1:400	Thermo Fisher Scientific, A21144	RRID:AB_2535780
Goat anti-Mouse IgG3 Alexa Fluor 488	1:400	Thermo Fisher Scientific, A21151	RRID:AB_2535784
Goat anti-Mouse IgG (H + L) Alexa Fluor 488	1:400	Thermo Fisher Scientific, A11008	RRID:AB_143165
Goat anti-Mouse IgG2a Alexa Fluor 568	1:400	Thermo Fisher Scientific, A21134	RRID:AB_2535773
Goat anti-Mouse IgG1 Alexa Fluor 488	1:400	Thermo Fisher Scientific, A-21121	RRID:AB_2535764
Goat anti-Chicken IgY (H + L) Alexa Fluor 488	1:400	Abcam, ab150173	RRID:AB_2827653

Isolation of genomic DNA and RNA. DNA was isolated using QuickExtract™ DNA extraction solution (Lucigen) according to the manufacturer's protocol. For RNA isolation, cells were grown to an area of 8–12 cm^2, lysed in 0.5–1 ml TRIzol Reagent (Thermo Fisher Scientific), and RNA was isolated according to the manufacturer's protocol. RNA concentration was determined using an EzDrop 1000C spectrophotometer (Blue-Ray Biotech). cDNA synthesis was performed using M-MuLV–RH reverse transcriptase (Biolabmix).

Detection of genetic variants in PBMCs and iPSCs. Sequencing of the patients' exome was performed at Genoanalitika LLC, Moscow. Genomic DNA isolated from PBMCs was fragmented into 300 nucleotide pair fragments using ultrasound on a Covaris S2. Genomic DNA (800 ng) was used to prepare a library using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (New England Biolabs) and Sure Select AllExome V7 (Agilent). Library sequencing was performed on a HiSeq 2500 (Illumina) with paired reads of 150 nucleotides from both ends. Raw exome sequencing results are available in the SRA database (project PRJNA563295, samples SAMN42050732, https://www.ncbi.nlm.nih.gov/biosample/42050732 (PD58), SAMN42050755, https://www.ncbi.nlm.nih.gov/biosample/42050755 (PD69)).

The presence of the c.1087G>T variant (rs117543292) of the *LGR4* gene was determined by Sanger sequencing of the obtained iPSC lines and patient-derived PBMCs (PD58 and PD69) using primers for the *LGR4* gene (Table 2). DNA from a conditionally healthy donor was used as a control. Reac-

Table 2. List of primers used in the work

Application	Gene/locus	Product size, bp	Forward/reverse primer (5′–3′)
Detection of episomal vectors	*oriP*	544	TTCCACGAGGGTAGTGAACC/ TCGGGGGTGTTAGAGACAAC
Reference gene (RT-qPCR)	*B2M*	90	TAGCTGTGCTCGCGCTACT/ TCTCTGCTGGATGACGTGAG
Markers of pluripotency (RT-qPCR))	*NANOG*	116	TTTGTGGGCCTGAAGAAAACT/ AGGGCTGTCCTGAATAAGCAG
	OCT4	94	CTTCTGCTTCAGGAGCTTGG/ GAAGGAGAAGCTGGAGCAAA
	SOX2	100	GCTTAGCCTCGTCGATGAAC/ AACCCCAAGATGCACAACTC
Mycoplasma detection	Ribosomal *16S RNA* gene	280	GGGAGCAAACAGGATTAGATACCCT/ TGCACCATCTGTCACTCTGTTAACCTC
Confirmation of the mutation	*LGR4*	366	GCGTTTCATGGGATGCCTGATAG/ TCTTAGCTCTGCTTTCAACGCTTC

tions were performed on a T100 thermal cycler (Bio-Rad) using a BioMaster HS-Taq PCR-Color (2×) (Biolabmix) with the following program: 95 °C for 3 min; 35 cycles: 95 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s; and 72 °C for 5 min. Sequencing reactions were performed using the Big Dye Terminator V.3.1. Cycle Sequencing Kit (Applied Biosystems) and analysed on an ABI 3130XL Genetic Analyzer at the Genomics Core Facility SB RAS (http://www.niboch.nsc.ru/doku.php/sequest).

PCR analysis for detection of reprogramming episomes and mycoplasma contamination. PCR was performed using BioMaster HS-Taq PCR-Color (2×) (Biolabmix) on a T100 thermal cycler (Bio-Rad), programme: 95 °C for 5 min; 35 cycles: 95 °C for 15 s, 60 °C for 15 s, 72 °C for 20 s. DNA fragments of *Mycoplasma* spp. from the BioMaster Myco-visor kit for detection of mycoplasma by RT-PCR (Biolabmix) were used as a positive control. Negative control was H_2O. Primers are listed in Table 2. After electrophoresis, the result was visualised under ultraviolet light using the Gel Doc XR+ System (Bio-Rad).

Quantitative RT-PCR for pluripotency markers. Quantitative PCR (RT-qPCR) was performed on a LightCycler 480 II system (Roche) using BioMaster HS-qPCR SYBR Blue 2× (Biolabmix) with the following programme: 95 °C for 5 min; 40 cycles: 95 °C for 10 s, 60 °C for 1 min. The embryonic stem cell line HUES9 (HVRDe009-A) was used as a positive control for the expression of pluripotency markers (Cowan et al., 2004). The pluripotency marker primers used in this work are listed in Table 2. The results were processed using the ΔΔCT method (Livak, Schmittgen, 2001).

STR analysis. The genetic material of the obtained iPSC lines and PBMCs from patients PD58 and PD69 was sent to Genoanalitika LLC (Moscow) for STR analysis. The STR analysis was performed for 26 loci using the COrDIS EXPERT 26 reagent kit (Russia).

Results and discussion

Obtaining and characterisation of the cell lines

Exomic sequencing analysis of early onset Parkinson's disease patients at the Federal Neurosurgical Center of the Ministry of Health of the Russian Federation (Novosibirsk) revealed two individuals (a 37-year-old woman (PD58) and a 66-year-old man (PD69)) with the variant *LGR4:c.1087G>T* (rs117543292).

PBMCs were isolated from patients' peripheral blood and then reprogrammed to revert to a pluripotency state, using episomal vectors encoding the pluripotency factors OCT4, SOX2, KLF4, LIN28, L-MYC, and the dominant-negative form of the mouse p53 protein, mp53DD (Okita et al., 2013). In this study, 21 cell lines of PD58 and 10 cell lines of PD69 were generated. Primary analysis for the presence/absence of episomal vectors and karyotyping allowed us to select three lines from the first patient (ICGi053-A/PD58-4, ICGi053-B/PD58-7 and ICGi053-C/PD58-14) and four lines from the second one (ICGi054-A/PD69-1/1, ICGi054-B/PD69-2/1, ICGi054-C/PD69-4 and ICGi054-D/PD69-5). All the lines were registered in the Human Pluripotent Stem Cell Registry (hPSCreg, https://hpscreg.eu). Information on cell lines can be found in hPSCreg at the following links: https://hpscreg.eu/cell-line/ICGi053-A, https://hpscreg.eu/cell-line/ICGi053-B, https://hpscreg.eu/cell-line/ICGi053-C, https://hpscreg.eu/cell-line/ICGi054-A, https://hpscreg.eu/cell-line/ICGi054-B, https://hpscreg.eu/cell-line/ICGi054-C и https://hpscreg.eu/cell-line/ICGi054-D.

All lines from both patients have a morphology characteristic of pluripotent cells, flat monolayer dense colonies with a large nuclear to cytoplasmic ratio, expressing endogenous alkaline phosphatase (Fig. 1*a*, 2*a*).

In the karyotyping of the obtained iPSC lines, 56 to 60 metaphases were analysed. From 6 to 12 chromosome

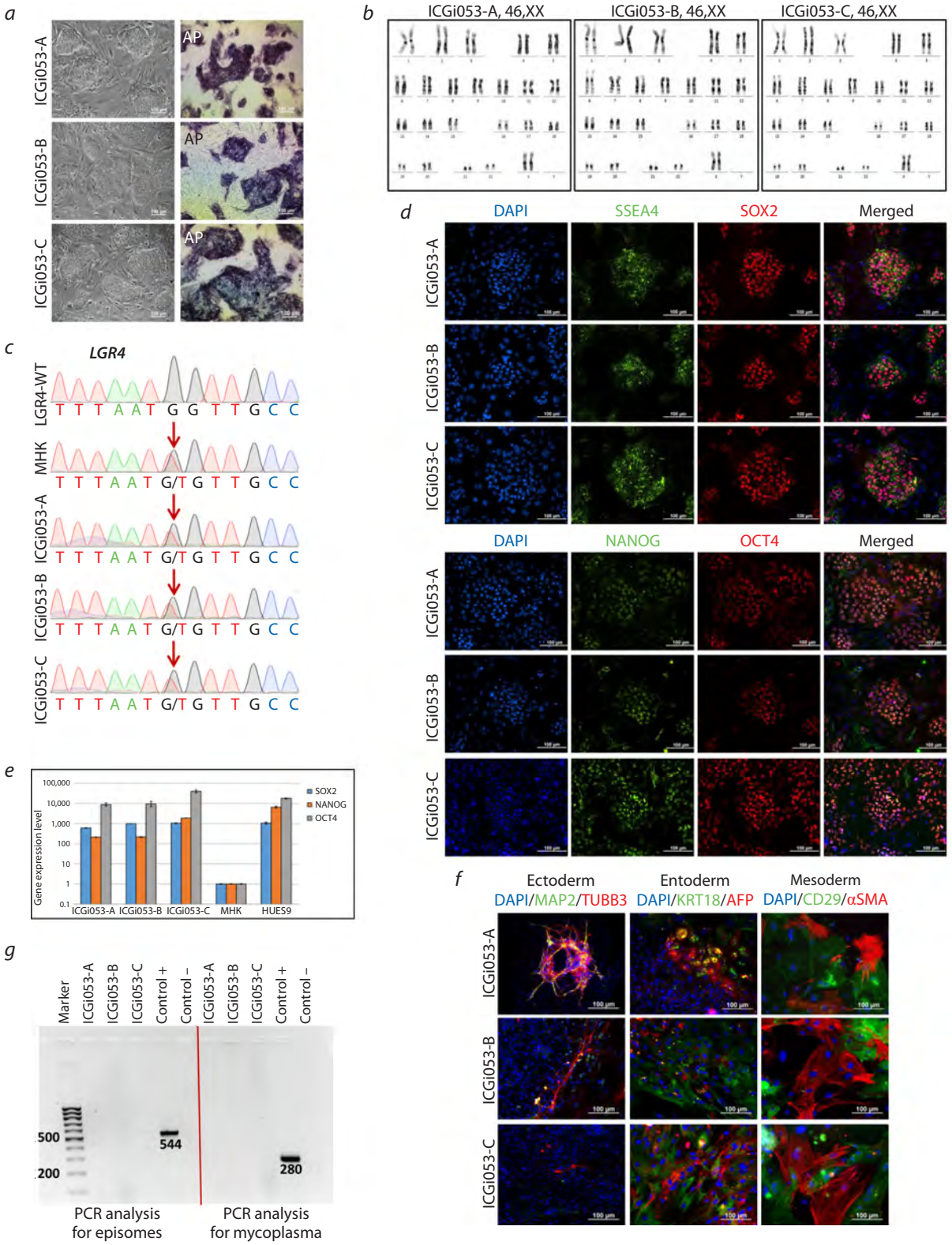

Fig. 1. Characterisation of three iPSC lines ICGi053-A, ICGi053-B and ICGi053-C.

a – colony morphology and detection of alkaline phosphatase (AP) in iPSCs; *b* – karyotyping of iPSC lines using DAPI banding; *c* – sequenograms of PCR products obtained from genomic DNA of a patient with early onset Parkinson's disease PD58, a healthy donor (LGR4-WT), iPSC lines and PBMCs corresponding to positions 27377174-27377186 on human chromosome 11 (GRCh38 genome assembly). The position of the gene variant studied is *LGR4* NC_000011.10:g.27377180C>A. The nucleotide substitution *LGR4:c.1087G>T* is indicated by the red arrow; *d* – immunofluorescence staining for markers of pluripotency: NANOG (green), OCT4 (red), SSEA-4 (green), SOX2 (red); nuclei stained with DAPI (blue); *e* – quantitative RT-PCR for pluripotency markers (OCT4, SOX2, NANOG) of derived iPSC lines, patient PBMCs and human embryonic stem cell line HUES9; *f* – immunofluorescence staining of spontaneously differentiated cells for markers of ectoderm (TUBB3 (red), MAP2 (green)), entoderm (keratin 18/KRT18 (green), AFP (red)) and mesoderm (αSMA (red), CD29 (green)); nuclei were stained with DAPI (blue); *g* – result of PCR assay for episomal vector elimination and mycoplasma contamination of the cells obtained. Scale bars are 100 μm.

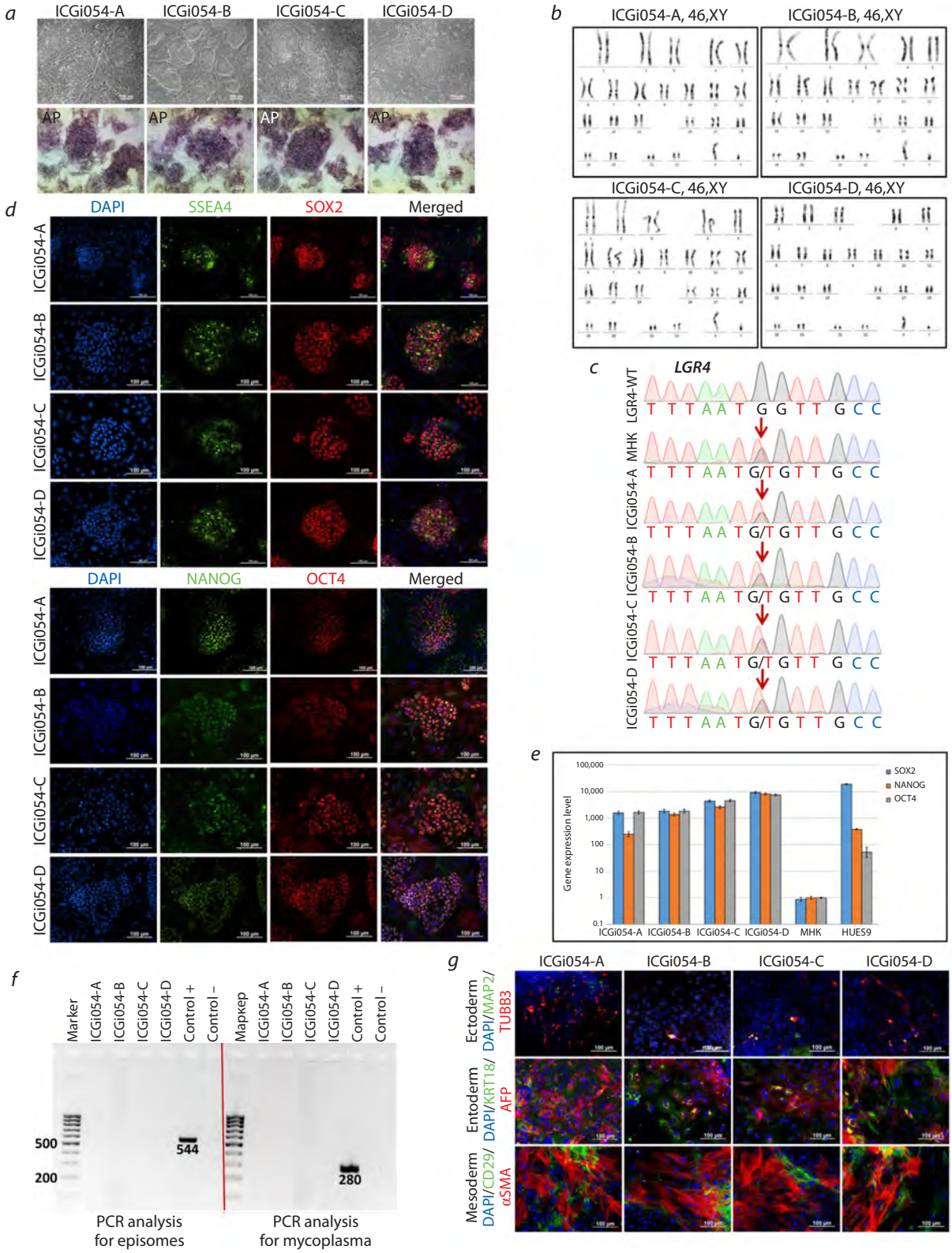

Fig. 2. Characterisation of four patient-specific ICGi054-A, ICGi054-B, ICGi054-C, and ICGi054-D iPSC lines.

a – colony morphology and detection of alkaline phosphatase (AP) in iPSCs; *b* – karyotyping of iPSC lines using DAPI banding; *c* – sequenograms of PCR products obtained from genomic DNA from a PD69 Parkinson's disease patient, a healthy donor (LGR4-WT), iPSC lines, and PBMCs corresponding to positions 27377174-27377186 on human chromosome 11 (GRCh38 genome assembly). The position of the gene variant studied is *LGR4* NC_000011.10:g.27377180C>A. The nucleotide substitution *LGR4:c.1087G>T* is indicated by the red arrow; *d* – immunofluorescence staining for markers of pluripotency: NANOG (green), OCT4 (red), SSEA-4 (green), SOX2 (red); nuclei stained with DAPI (blue); *e* – quantitative RT-PCR for pluripotency markers (OCT4, SOX2, NANOG) of derived iPSC lines, patient PBMCs and human embryonic stem cell line HUES9; *f* – immunofluorescence staining of spontaneously differentiated cells for markers of ectoderm (TUBB3 (red), MAP2 (green)), entoderm (keratin 18/KRT18 (green), AFP (red)) and mesoderm (αSMA (red), CD29 (green)); nuclei were stained with DAPI (blue); *g* – result of PCR assay for episomal vector elimination and mycoplasma contamination of the cells obtained. Scale bars are 100 μm.

Table 3. Detailed analysis of the chromosomal composition of the metaphase spreads of the resulting iPSC lines

iPSC line	Number of metaphases with different numbers of chromosomes						Number of metaphases analysed	Karyotype of diploid cells
	42–44	45	46	47	48–50	80–92		
ICGi053-A	4	3	40	8	2	4	58	46,XX
ICGi053-B	9	3	37	4	0	5	58	46,XX
ICGi053-C	5	4	37	3	1	8	58	46,XX
ICGi054-A	4	8	37	5	2	4	60	46,XY
ICGi054-B	7	4	41	3	1	1	57	46,XY
ICGi054-C	15	5	37	6	1	2	60	46,XY
ICGi054-D	8	10	31	4	1	1	56	46,XY

spreads were obtained for each cell line, in accordance with the recommendations based on the European standards for cytogenetic and molecular cytogenetic studies of constitutive and acquired chromosomal abnormalities (Hastings et al., 2012; ISCN, 2020). The appearance of cells with the number of chromosomes ranging from 42 to 45 is the result of the loss of individual chromosomes during preparation. Since the number of such cells was low and they showed an absence of different chromosomes, we did not consider them as the formation of new subclones. It is known that cultured stem cells are characterised by aneuploidy (Menzorov et al., 2016), but monosomal cells are prone to apoptosis or less intensive proliferation, so in cell culture it is common to consider lines with more than 50 % of metaphases with a full set of chromosomes as normal. Detailed analysis showed that all lines have more than 55 % of cells with a normal diploid karyotype – 46,XX for ICGi053 (Fig. 1*b*) and 46,XY for ICGi054 (Fig. 2*b*). It should be noted that the analysis of the seven iPSC lines obtained did not reveal any metaphase spreads with structural rearrangements. Detailed information on the composition of the analysed metaphase spreads can be found in Table 3.

Sanger sequencing demonstrated the presence of the c.1087G>T (rs117543292) variant of the *LGR4* gene in a heterozygous state in the obtained cell lines as well as in PBMCs (Fig. 1*c*, 2*c*, the genetic variant is indicated by a red arrow). Immunofluorescence analysis of lines ICGi053-A (passage 27), ICGi053-B (passage 26), ICGi053-C (passage 27), ICGi054-A (passage 26), ICGi054-B (passage 22), ICGi054-C (passage 25) and ICGi054-D (passage 24) for pluripotency markers revealed the presence of the SSEA-4 surface antigen and the transcription factors NANOG, SOX2 and OCT4 (Fig. 1*d*, 2*d*). Quantitative real-time PCR (RT-PCR) of patient PBMCs, patient-derived iPSC lines, and the control embryonic stem cell line HUES9 showed increased gene expression levels of *OCT4*, *NANOG*, and *SOX2* in the iPSC lines, which was comparable to the expression level in HUES9 (Fig. 1*e*, 2*e*). The test for the ability to spontaneously differentiate into derivatives of three germ layers, which is the main confirmation of the pluripotency status of the obtained cell lines, and further immunofluorescence analysis showed the expression of markers of ectoderm (tubulin β3 (TUBB3/TUJ1), microtubule-associated protein 2 (MAP2)), entoderm (alpha-fetoprotein (AFP)), keratin 18 (KRT18)) and mesoderm (α-smooth muscle actin (αSMA), surface marker CD29) (Fig. 1*f*, 2*f*). During cultivation, all lines underwent PCR testing for mycoplasma contamination and elimination of episomal vectors (Fig. 1*g*, 2*g*). STR analysis at 26 loci showed the identity of the iPSC lines derived from patient PBMCs (data available on request from the authors). The passports of the cell lines derived from PD58 and PD69 patients are shown in Tables 4 and 5, respectively.

Conclusion

As a result of reprogramming PBMCs from two Parkinson's disease patients carrying the c.1087G>T (rs117543292) variant of the *LGR4* gene, seven iPSC lines – ICGi053-A, ICGi053-B, ICGi053-C, ICGi054-A, ICGi054-B, ICGi054-C and ICGi054-D – were obtained and characterised. The cell lines obtained correspond to human pluripotent cells: they have a characteristic morphology, a normal karyotype, express pluripotency factors, are able to differentiate spontaneously into derivatives of three germ layers and have the same genetic variant *LGR4:c.1087G>T* as donor PBMCs. This work is the first and necessary step to study the effect of the *LGR4:c.1087G>T* genetic variant on the manifestation of Parkinson's disease. The iPSC lines obtained and their differentiated derivatives represent a unique cell platform on which it will be possible in the future to study at the molecular genetic level the early pathological processes that trigger the cascade of signalling pathways leading to neuronal death, to search for targets that modulate these signalling pathways and to test new potential drugs.

Table 4. Passport of cell lines ICGi053-A, ICGi053-B, ICGi053-C

Unique identifier	ICGi053-A, ICGi053-B, ICGi053-C
Alternative cell line names	PD58-4, PD58-7, PD58-14
Institution	Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
Ethical approval	The study was approved by the Research Ethics Committee of the Federal Neurosurgical Center of the Ministry of Health of the Russian Federation, Novosibirsk, Russia, Protocol No. 1 dated 14 March 2017
Cell type	iPSCs
Origin	Human
Additional information on the origin of the cell line	Age: 37 Gender: F Ethnicity: Caucasian race
Original cell type	Peripheral blood mononuclear cells (PBMCs)
Date of biomaterial collection	2022
Reprogramming method	Non-integrating episomal vectors
Reprogramming factors	OCT4, SOX2, KLF4, LIN28, L-MYC and mp53DD
Clonality	Clonal
Genetic modification	No
Type of genetic modification	N/A
Confirmation of elimination/ silencing of reprogramming transgenes	PCR, not detected
Disease	Parkinson's disease
Age of disease onset	36
Gene/locus	*LGR4:c.1087G>T*, rs117543292
Morphology	Human pluripotent cell-like monolayer colonies
Pluripotency	Confirmed in tests for the formation of embryoid bodies
Karyotype	46,XX
Check for contamination	Bacteria, fungi and mycoplasma not detected
Scope of application	*In vitro* model of Parkinson's disease
Cultivation method	On the feeder layer of mitotically inactivated mouse embryonic fibroblasts
Growth medium	85 % KnockOut DMEM, 15 % KnockOut Serum Replacement, 0.1 mM NEAA, 0.1 mM 2-mercaptoethanol, 1 % Penicillin-Streptomycin, GlutaMAX-I (all Thermo Fisher Scientific), 10 ng/ml bFGF (SCI Store)
Temperature, °C	37
CO_2 concentration, %	5
O_2 concentration, %	20
Passage method	TrypLE Express
Split ratio	1:8–1:10
Cryopreservation	90 % FBS, 10 % DMSO
Storage conditions	Liquid nitrogen
Registry ID	https://hpscreg.eu/cell-line/ICGi053-A https://hpscreg.eu/cell-line/ICGi053-B https://hpscreg.eu/cell-line/ICGi053-C
Date of certification/registration	09.07.2024 (ICGi053-A) 18.07.2024 (ICGi053-B) 22.07.2024 (ICGi053-C)

Table 5. Passport of cell lines ICGi054-A, ICGi054-B, ICGi054-C, ICGi054-D

Unique identifier	ICGi054-A, ICGi054-B, ICGi054-C, ICGi054-D
Alternative cell line names	PD69-1/1, PD69-2/1, PD69-4, PD69-5
Institution	Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
Ethical approval	The study was approved by the Research Ethics Committee of the Federal Neurosurgical Center of the Ministry of Health of the Russian Federation, Novosibirsk, Russia, Protocol No. 1 dated 14 March 2017
Cell type	iPSCs
Origin	Human
Additional information on the origin of the cell line	Age: 66 Gender: M Ethnicity: Caucasian race
Original cell type	Peripheral blood mononuclear cells (PBMCs)
Date of biomaterial collection	2022
Reprogramming method	Non-integrating episomal vectors
Reprogramming factors	OCT4, SOX2, KLF4, LIN28, L-MYC и mp53DD
Clonality	Clonal
Genetic modification	No
Type of genetic modification	N/A
Confirmation of elimination/ silencing of reprogramming transgenes	PCR, not detected
Disease	Parkinson's disease
Age of disease onset	41
Gene/locus	*LGR4:c.1087G>T*, rs117543292
Morphology	Human pluripotent cell-like monolayer colonies
Pluripotency	Confirmed in tests for the formation of embryoid bodies
Karyotype	46,XY
Check for contamination	Bacteria, fungi and mycoplasma not detected
Scope of application	*In vitro* model of Parkinson's disease
Cultivation method	On the feeder layer of mitotically inactivated mouse embryonic fibroblasts
Growth medium	85 % KnockOut DMEM, 15 % KnockOut Serum Replacement, 0.1 mM NEAA, 0.1 mM 2-mercaptoethanol, 1 % Penicillin-Streptomycin, GlutaMAX-I (all Thermo Fisher Scientific), 10 ng/ml bFGF (SCI Store)
Temperature, °C	37
CO_2 concentration, %	5
O_2 concentration, %	20
Passage method	TrypLE Express
Split ratio	1:8–1:10
Cryopreservation	90 % FBS, 10 % DMSO
Storage conditions	Liquid nitrogen
Registry ID	https://hpscreg.eu/cell-line/ICGi054-A https://hpscreg.eu/cell-line/ICGi054-B https://hpscreg.eu/cell-line/ICGi054-C https://hpscreg.eu/cell-line/ICGi054-D
Date of certification/registration	26.07.2024 (ICGi054-A) 26.07.2024 (ICGi054-B) 29.07.2024 (ICGi054-C) 09.08.2024 (ICGi054-D)

References

Cowan C.A., Klimanskaya I., McMahon J., Atienza J., Witmyer J., Zucker J.P., Wang S., Morton C.C., McMahon A.P., Powers D., Melton D.A. Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med.* 2004;350(13):1353-1356. doi 10.1056/NEJMsr040330

Fernandes H.J.R., Hartfield E.M., Christian H.C., Emmanoulidou E., Zheng Y., Booth H., Bogetofte H., Lang C., Ryan B.J., Sardi S.P., Badger J., Vowles J., Evetts S., Tofaris G.K., Vekrellis K., Talbot K., Hu M.T., James W., Cowley S.A., Wade-Martins R. ER stress and autophagic perturbations lead to elevated extracellular α-synuclein in GBA-N370S Parkinson's iPSC-derived dopamine neurons. *Stem Cell Rep.* 2016;6(3):342-356. doi 10.1016/j.stemcr.2016.01.013

Funayama M., Nishioka K., Li Y., Hattori N. Molecular genetics of Parkinson's disease: contributions and global trends. *J Hum Genet.* 2023;68(3):125-130. doi 10.1038/s10038-022-01058-5

Grigor'eva E.V., Kopytova A.E., Yarkova E.S., Pavlova S.V., Sorogina D.A., Malakhova A.A., Malankhanova T.B., Baydakova G.V., Zakharova E.Y., Medvedev S.P., Pchelina S.N., Zakian S.M. Biochemical characteristics of iPSC-derived dopaminergic neurons from N370S GBA variant carriers with and without Parkinson's disease. *Int J Mol Sci.* 2023;24:4437. doi 10.3390/ijms24054437

Grigor'eva E.V., Karapetyan L.V., Malakhova A.A., Medvedev S.P., Minina J.M., Hayrapetyan V.H., Vardanyan V.S., Zakian S.M., Arakelyan A., Zakharyan R. Generation of iPSCs from a patient with the M694V mutation in the *MEFV* gene associated with Familial Mediterranean fever and their differentiation into macrophages. *Int J Mol Sci.* 2024a;25:6102. doi 10.3390/ijms25116102

Grigor'eva E.V., Malakhova A.A., Yarkova E.S., Minina J.M., Vyatkin Y.V., Nadtochy J.A., Khabarova E.A., Rzaev J.A., Medvedev S.P., Zakian S.M. Generation and characterization of two induced pluripotent stem cell lines (ICGi052-A and ICGi052-B) from a patient with frontotemporal dementia with parkinsonism-17 associated with the pathological variant c.2013T>G in the *MAPT* gene. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov J Genet Breed.* 2024b;28(7):679-687. doi 10.18699/vjgb-24-76

Hastings R., Howell R., Bricarelli F.D., Kristoffersson U., Cavani S. General guidelines and quality assurance for cytogenetics. *Eur Cytogenet Assoc Newsl.* 2012;29:11-25

ISCN 2020: An International System for Human Cytogenomic Nomenclature. S. Karger AG, 2020. doi 10.1159/isbn.978-3-318-06867-2

Livak K.J., Schmittgen T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods.* 2001;25(4):402-408. doi 10.1006/meth.2001.1262

Mancini A., Howard S.R., Marelli F., Cabrera C.P., Barnes M.R., Sternberg M.J.E., Leprovots M., Hadjidemetriou I., Monti E., David A., Wehkalampi K., Oleari R., Lettieri A., Vezzoli V., Vassart G., Cariboni A., Bonomi M., Garcia M.I., Guasti L., Dunkel L. LGR4 deficiency results in delayed puberty through impaired Wnt/β-catenin signaling. *JCI Insight.* 2023;5(11):e133434. doi 10.1172/jci.insight.133434

Marchetti B., Tirolo C., L'Episcopo F., Caniglia S., Testa N., Smith J.A., Pluchino S., Serapide M.F. Parkinson's disease, aging and adult neurogenesis: Wnt/β-catenin signalling as the key to unlock the mystery of endogenous brain repair. *Aging Cell.* 2020;19(3):e13101. doi 10.1111/acel.13101

Marciniak S.J., Chambers J.E., Ron D. Pharmacological targeting of endoplasmic reticulum stress in disease. *Nat Rev Drug Discov.* 2022;21(2):115-140. doi 10.1038/s41573-021-00320-3

Menzorov A., Pristyazhnyuk I., Kizilova H., Yunusova A., Battulin N., Zhelezova A., Golubitsa A., Serov O.L. Cytogenetic analysis and *Dlk1-Dio3* locus epigenetic status of mouse embryonic stem cells during early passages. *Cytotechnology.* 2016;68(1):61-71. doi 10.1007/s10616-014-9751-y

Niu Y., Zhang J., Dong M. Nrf2 as a potential target for Parkinson's disease therapy. *J Mol Med* (*Berl*). 2021;99(7):917-931. doi 10.1007/s00109-021-02071-5

Okita K., Yamakawa T., Matsumura Y., Sato Y., Amano N., Watanabe A., Goshima N., Yamanaka S. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. *Stem Cells.* 2013;31(3):458-466. doi 10.1002/stem.1293

Shi S., Li S., Zhang X., Wei Z., Fu W., He J., Hu Y., Li M., Zheng L., Zhang Z. *LGR4* gene polymorphisms are associated with bone and obesity phenotypes in Chinese female nuclear families. *Front Endocrinol.* 2021;12:656077. doi 10.3389/fendo.2021.656077

Wang M., Ling K.-H., Tan J.J., Lu C.-B. Development and differentiation of midbrain dopaminergic neuron: from bench to bedside. *Cells.* 2020;9(6):1489. doi 10.3390/cells9061489

Yarkova E.S., Grigor'eva E.V., Medvedev S.P., Pavlova S.V., Zakian S.M., Malakhova A.A. IPSC-derived astrocytes contribute to in vitro modeling of Parkinson's disease caused by the *GBA1* N370S mutation. *Int J Mol Sci.* 2023;25(1):327. doi 10.3390/ijms25010327

Yarkova E.S., Grigor'eva E.V., Medvedev S.P., Tarasevich D.A., Pavlova S.V., Valetdinova K.R., Minina J.M., Zakian S.M., Malakhova A.A. Detection of ER stress in iPSC-derived neurons carrying the p.N370S mutation in the *GBA1* gene. *Biomedicines.* 2024;12:744. doi 10.3390/biomedicines12040744

Conflict of interest. The authors declare no conflict of interest.

Received September 16, 2024. Revised November 20, 2024. Accepted November 21, 2024.