Stem Cell Research 71 (2023) 103134

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Multiple Cell Lines

# Generation of TWO G51D SNCA missense mutation iPSC lines (CRICKi011-A, CRICKi012-A) from two individuals at risk of Parkinson's disease

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A B S T R A C T

Mutations or multiplications of the SNCA (Synuclein Alpha) gene cause rare autosomal dominant Parkinson's disease (PD). The SNCA G51D missense mutation is associated with a synucleinopathy that shares PD and multiple system atrophy (MSA) characteristics. We generated induced pluripotent stem cell (iPSC) lines from two individuals with SNCA G51D missense mutations at risk of PD. Dermal fibroblasts were reprogrammed to pluripotency using a non-integrating mRNA-based protocol. The resulting human iPSCs displayed normal morphology, expressed markers associated with pluripotency, and differentiated into the three germ layers. The iPSC lines could facilitate disease-modelling and therapy development studies for synucleinopathies.

## 1. Resource Table:

| | |
|---|---|
| Unique stem cell line identifier | *CRICKi011-A, CRICKi012-A* |
| Alternative name(s) of stem cell line | *iFCI016, iFCI017* |
| Institution | *THE FRANCIS CRICK INSTITUTE* |
| Contact information of distributor | *lyn.healy@crick.ac.uk liani.devito@crick.ac.uk* |
| Type of cell line | *iPSC* |
| Origin | *Human* |
| Additional origin info | *CRICKi011-A (iFCI016) Age at sampling: 53. Sex: Male CRICKi012-A (iFCI017) Age at sampling: 57. Sex: Female* |
| Cell Source | *Dermal Fibroblast* |
| Clonality | *Clonal* |
| Method of reprogramming | *mRNA* |
| Genetic Modification | *NO* |
| Associated disease | *Parkinson's disease (at risk)* |
| Gene/locus | *c.G152A mutation in Exon 3 of SNCA* |
| Date archived/stock date | *OCTOBER 2022* |
| Cell line repository/bank | *https://hpscreg.eu/cell-line/CRICKi011-A https://hpscreg.eu/cell-line/CRICKi012-A* |
| Ethical approval | This study was approved and reviewed by the National Health Services (NHS) Health Research Authority Research Ethics Committee reference no.19/LO/1796. |

## 2. Resource utility

We report two patient-derived iPSC lines for use as a disease-specific cellular model to further understand how SNCA G51D missense mutation influences pathology. The iPSC lines could facilitate disease-modelling and therapy development studies for synucleinopathies (see Table 1).

## 3. Resource details

Multiple missense mutations of the *SNCA* gene, which encodes the protein alpha-synuclein, have been identified in patients with autosomal dominant forms of Parkinson's disease (PD). The SNCA missense mutation G51D presents as a synucleinopathy which incorporates a mixture of PD and MSA pathological hallmarks, with both neuronal and oligodendroglial alpha-synuclein positive inclusions (Kiely et al., 2013,

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https://doi.org/10.1016/j.scr.2023.103134
Received 6 April 2023; Received in revised form 15 May 2023; Accepted 2 June 2023
Available online 6 June 2023
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**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Microscopic photography | Normal morphology at passage 7 | Fig. 1 panel A |
| Phenotype | Quantitative analysis Flow Cytometry | Staining of pluripotency markers: OCT4+, SSEA4+ and SSEA1− at passage 9 (*CRICKi011-A*) and 10 (*CRICKi012-A)* | Fig. 1 panel D |
| Genotype | CNV analysis: Karyostat assay (Thermo Scientific) with resolution >2 Mb for chromosomal gains and >1 Mb for chromosomal losses | *CRICKi011-A* Male individual *CRICKi012-A* Female individual both have no chromosome aberrations compared to the reference dataset | Fig. 1 panel C |
| | STR analysis | All 16 sites sites matched | submitted in archive with journal |
| Mutation analysis | Sanger Sequencing | *Heterozygous type of mutation* | Fig. 1 panel B |
| Microbiology and virology | Mycoplasma | Mycoplasma testing by RT-PCR Negative | not shown but available with author |
| Differentiation potential | *In vitro* differentiation | Direct differentiation to three germ layers confirmed by immunostaining at passage 11 (*CRICKi011-A*) and 12 (*CRICKi012-A)* | Fig. 1 panel F |
| List of recommended germ layer markers | Spontaneous *In vitro* differentiation: EB formation followed by TaqMan™ hPSC Scorecard™ Panel, Fast 96-well (Cat. N. A15876) | Expression of markers (qPCR) of Endoderm, Mesoderm and Ectoderm germ layer confirmed by Scorecard at passage 11 (*CRICKi011-A*) and 12 (*CRICKi012-A)* | Fig. 1 Panel E |

2015). Here, we report the generation and characterisation of two iPSC lines derived from two individuals from the same family with G51D *SNCA* mutation.

Patient dermal fibroblasts were obtained from the individuals at UCL Queen Square Institute of Neurology and reprogrammed using the non-integrating mRNA-based protocol (StemRNA™ 3rd Gen Reprogramming Kit, REPROCELL) that combines non-modified RNA (NM-RNA) and microRNA technology. It contains six reprogramming factors, Oct4, Sox2, Klf4, cMyc, Nanog, and Lin 28, together with three immune evasion factors E3, K3 and B18. Cells were reprogrammed in a feeder-free system according to the manufacturer's instructions. Colonies with a typical pluripotent stem cell morphology were individually and manually selected to establish clonal feeder-free iPSC lines.

Cells showed typical iPSC morphology after a few passages (Fig. 1A). Dideoxynucleotide sequencing (Sanger Sequencing) confirmed the presence of the pathogenic *c.G152A in Exon 3 of the SNCA gene* (Fig. 1B). Copy number variation analysis by chromosomal microarray confirmed the sex of the individuals (Fig. 1C). Stem cell identity of the *CRICKi011-A* and *CRICKi012-A* was confirmed by the expression of pluripotency markers OCT4 and SSEA4 on Flow Cytometry analyses (Fig. 1D).

*In vitro* differentiation (direct and spontaneous) confirmed the cell line's ability to differentiate into all three germ layers (Fig. 1E and F). The identical genetic identity of the donor of each iPSC was confirmed by short tandem repeat (STR) profiling. These results prove we have successfully produced iPSC lines from two patients with *G51D SNCA* mutation.

## 4. Materials and methods

### 4.1. iPSC cell generation and expansion

We thawed the dermal fibroblasts at passage 4 for *CRICKi011-A* and passage 5 for *CRICKi012-A* and seeded at a density of $5 \times 10^4$/well in 2 wells/ each of a 6-well plate coated with iMatrix (Stemgent). They were plated in Fibroblast Expansion Medium (DMEM(Gibco)/ Glutamax (Gibco)/10% Hyclone FBS (Thermo Scientific)) and cultured for 24 h in 37 °C, 5% $CO_2$ and 21% $O_2$. Then, on the first day of reprogramming, Day 0, the medium was switched to NutriStem medium (Stemgent), and cells were transferred to a hypoxic incubator at 37 °C, 5% $CO_2$ and 5% $O_2$.

Cells were reprogrammed 1-day post-seeding using the StemRNA™ 3rd Gen Reprogramming Kit (Stemgent) according to the manufacturer's instructions. Briefly, the NM-RNA cocktail was added to RNAi-MAX transfection reagent (Gibco) and transfected into the cells for four consecutive days with medium change 6 h post-transfection.

iPSC-like colonies started to show 9 to 12 days post the first day of transfections. We manually picked those with iPSC-like morphology and transferred them to Matrigel-coated 6-well plates with mTeSR1 medium (StemCell Technologies) containing 10 μM Y-27362. The medium was changed after 24 h. Colonies were expanded by splitting at a 1:3 to 1:6 ratio every 4–6 days and maintained in a hypoxic incubator at 37 °C, 5% CO2 and 5% O2.

### 4.2. Pluripotency markers

We evaluated the pluripotency status of *CRICKi011-A* and *CRICKi012-A* line at passages 9 and 10, respectively, by Flow Cytometry using the BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis Kit (BD) as per the manufacturer's instruction. Briefly, we detached the cells using Accutase (Sigma-Aldrich) and passed through a 70 μM cell strainer to eliminate cell clumps. The cells were then washed with DPBS (without $Ca^2$ $Mg^2$) (Thermo Fisher Scientific) and resuspended at $1 \times 10^6$ cells/ml before adding the Live/ Dead staining (Thermo Fisher) for 30 min at room temperature. After another cell wash step with DPBS, cells were fixed in 4% paraformaldehyde (BD Stemflow Analyses kit component) for 20 min. For the permeabilization step, we used the 1X Perm/ Wash buffer (BD Stemflow Analyses kit component) for 10 min, followed by a wash in the same buffer. We incubated the cells with the antibodies (Table 2) for 30 min. We used DIVA software to analyse the cells and FlowJo to analyse the data.

### 4.3. Spontaneous differentiation into three germ layers

We tested the differentiation capacity of the *CRICKi011-A and CRICKi012-A* lines at passages 11 and 12, respectively, by spontaneous differentiation and embryoid body (EB) formation. Using the AggreWell™ 800 Microwell Plates, we seeded $5 \times 10^6$/well in APEL 2 medium (STEMCELL Technologies) and cultured the EBs for 14 days in a hypoxic incubator at 37 °C, 5% CO2 and 5% O2.

The expression of the lineage-specific markers was assessed by TaqMan hPSC Scorecard Assay (Thermo Scientific). Briefly, we isolated RNA using the QIAgen RNeasy mini kit and measured concentration with a Nanodrop. We used the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Thermo Fisher) for cDNA preparation. We analysed the data using the hPSC Scorecard™ – Analysis group (Thermo Fisher).

### 4.4. Direct differentiation into three germ layers

For direct differentiation, we used the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) as per the manufacturer's instructions. We evaluated the differentiation potential of *CRICKi011-A and CRICKi012-A* lines at passages 11 and 12, respectively, by

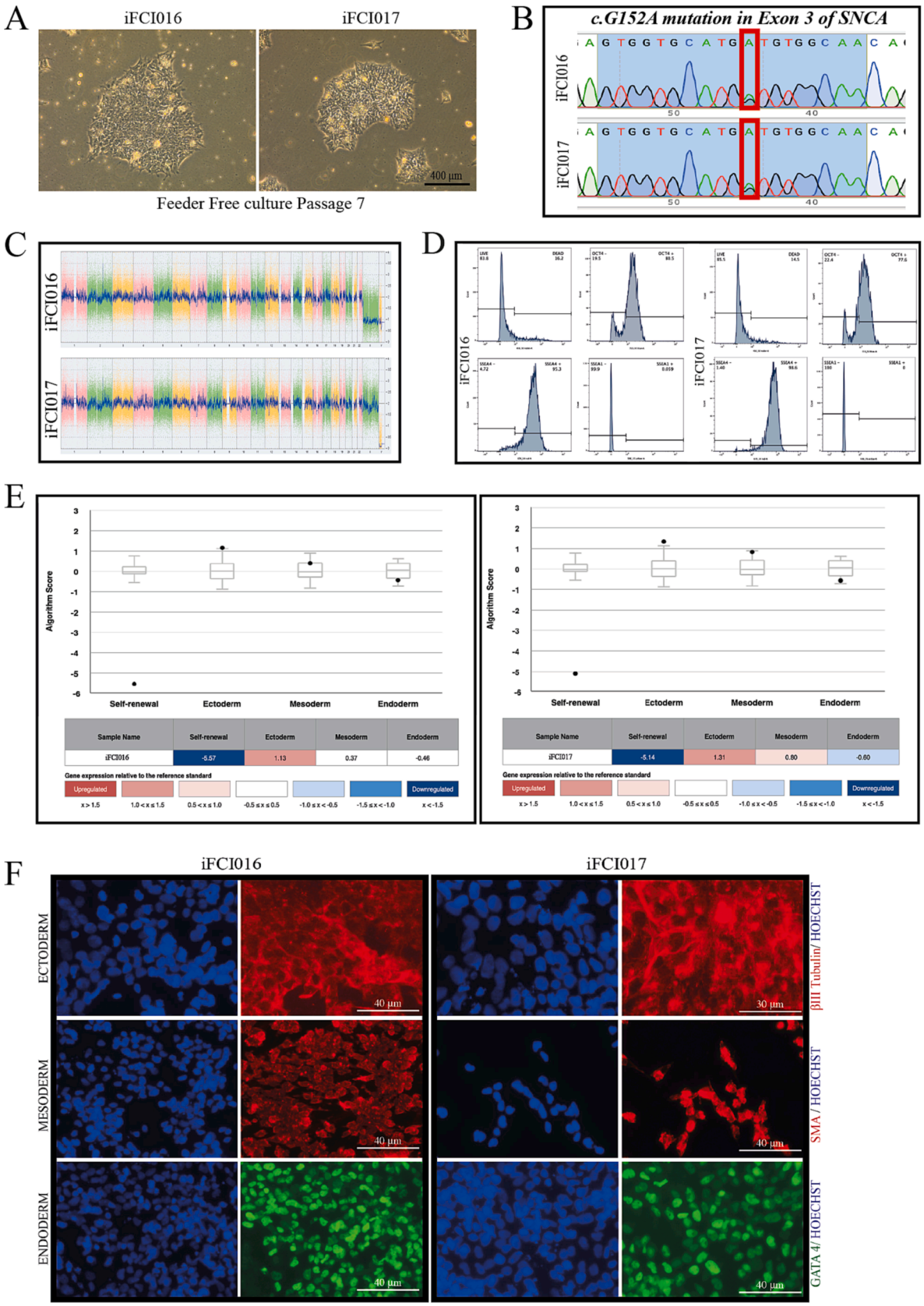
A
iFCI016
iFCI017
400 µm
Feeder Free culture Passage 7
B
c.G152A mutation in Exon 3 of SNCA
iFCI016
iFCI017
C
iFCI016
iFCI017
D
iFCI016
iFCI017
E
Algorithm Score
Self-renewal
Ectoderm
Mesoderm
Endoderm
Sample Name
iFCI016
-5.57
1.13
0.37
-0.46
Gene expression relative to the reference standard
Upregulated
Downregulated
iFCI017
-5.14
1.31
0.80
-0.60
F
iFCI016
iFCI017
ECTODERM
MESODERM
ENDODERM
40 µm
30 µm
βIII Tubulin/ HOECHST
SMA / HOECHST
GATA 4/ HOECHST

**Fig. 1.**

**Table 2**
Reagents details.

| Antibodies used for immunocytochemistry/flow-cytometry | | | | |
|---|---|---|---|---|
| | Antibody | Dilution/Amount of staining | Company Cat # | RRID |
| Pluripotency Markers | BD PharmingenTM Alexa Fluor® 647 Mouse anti-SSEA-4 | 20 μL per sample ($5 \times 10^5$ to $1 \times 0^6$ cells) | BD #560477 | AB_2869350 |
| | BD PharmingenTM PE Mouse anti-SSEA-1 | 20 μL per sample ($5 \times 10^5$ to $1 \times 0^6$ cells) | BD #560477 | AB_2869350 |
| | BD PharmingenTM PerCP-CyTM5.5 Mouse anti-Oct3/4 | 20 μL per sample ($5 \times 10^5$ to $1 \times 0^6$ cells) | BD #560477 | AB_2869350 |
| Differentiation Markers | Goat anti-GATA-4 IgG | 1:100 | R&D System #AF2606 | AB_2232177 |
| | Mouse anti-βIII-tubulin IgG | 1:100 | Sigma #T5076 | AB_532291 |
| | Mouse anti-smooth muscle actin SMA IgG | 1:100 | Sigma #A5228 | AB_262054 |
| Secondary antibodies | Donkey anti-mouse Rhodamine IgG | 1:100 | Jackson Immunoresearch #715-295-150 | AB_2340831 |
| | Donkey anti-goat FITC IgG | 1:100 | Jackson Immunoresearch #705-095-147 | AB_2340401 |

| **Primers** | **Target** | **Forward/Reverse primer (5′-3′)** | **Size of Band** |
|---|---|---|---|
| Targeted mutation analysis | SNCA | *GTGGTGGTTACTGGAGTTCC* | 292 |
| | SNCA | *ACTGGGCCACACTAATCACT* | |

immunostaining for lineage-specific markers on Day 5 (Mesoderm and Endoderm) and Day 7 (Ectoderm) as described (Devito et al., 2021). Differentiated cells were washed twice in DPBS ($Ca^2$ $Mg^2$) (Thermo Fisher Scientific) before fixation with 3.7% paraformaldehyde (Sigma-Aldrich) for 20 min at room temperature. Cells were permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) for 5 min at room temperature, then incubated with primary antibodies (Table 2) overnight at 4 °C. The following day, cells were washed twice with DPBS and incubated with secondary antibodies (Table 2) for 30 min at room temperature.

### 4.5. Dideoxynucleotide sequencing

We performed PCR amplification (primers listed in Table 2) using the Q5 High-Fidelity 2X Master-Mix (BioLabs) on both cell lines at passage 7. The genomic DNA was extracted using the QIAamp DNA micro Kit (Qiagen). Then we purified the PCR product using the Monarch PCR and DNA cleanup Kit (BioLabs). Finally, we sent the samples for Sanger sequencing to Source Biosciences (UK) and analysed the data using SnapGene software.

### 4.6. Chromosomal microarray

Using the genomic DNA of each iPSC line, Thermo Scientific (USA) performed the KaryoStat assay (Thermo Scientific, USA), an array comparative genomic hybridization (CGH).

### 4.7. Short tandem repeat (STR) profiling

The Cell Services, a Science Technology Platform (STP) within the Francis Crick Institute, performed the STR profiling on DNAs from the parental sample and iPSC line using the Powerplex 16 HS System (Promega). Since reprogramming started, all lines were sent regularly for STR profiling (every 3 passages).

### 4.8. Mycoplasma detection test

The Cell Services (STP) confirmed the absence of mycoplasma contamination using PCR amplification using the Universal Mycoplasma Detection Kit (ATCC 30–1012 K) for PCR amplification. Cells were regularly sent for Mycoplasma testing (every 3 passages) since reprogramming started.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We thank the Francis Crick Institute Genomics Equipment Park, Cell Services STPs. This work was supported by: The Francis Crick Institute, which receives its funding from Cancer Research UK (CC0199), the UK Medical Research Council (CC0199) and the Wellcome Trust (CC0199). S.G. is an MRC Senior Clinical Fellow [MR/T008199/1]. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

## References

Devito, L.G., Healy, L., Mohammed, S., Guillemot, F., Dias, C., 2021. Generation of an iPSC line (CRICKi001-A) from an individual with a germline SMARCA4 missense mutation and autism spectrum disorder. Stem Cell Res. 53, 102304.

Kiely, A.P., Asi, Y.T., Kara, E., Limousin, P., Ling, H., Lewis, P., Proukakis, C., Quinn, N., Lees, A.J., Hardy, J., Revesz, T., Houlden, H., Holton, J.L., 2013. α-Synucleinopathy associated with G51D SNCA mutation: a link between Parkinson's disease and multiple system atrophy? Acta Neuropathol. 125 (5), 753–769.

Kiely, A.P., Ling, H., Asi, Y.T., Kara, E., Proukakis, C., Schapira, A.H., Morris, H.R., Roberts, H.C., Lubbe, S., Limousin, P., Lewis, P.A., Lees, A.J., Quinn, N., Hardy, J., Love, S., Revesz, T., Houlden, H., Holton, J.L., 2015. Distinct clinical and neuropathological features of G51D SNCA mutation cases compared with SNCA duplication and H50Q mutation. Mol. Neurodegener. 10, 41.