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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ABSTRACT

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 | (continued on next column) |

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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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$^\text{TM}$ 3rd Gen Reprogramming Kit (Stemgent) according to the manufacturer’s instructions. Briefly, the NM-RNA cocktail was added to RNAiMAX 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% CO$_2$ and 5% O$_2$.

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$^\text{TM}$ Human and Mouse Pluripotent Stem Cell Analysis Kit (BD) as per the manufacturer’s instructions. 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$^\text{TM}$ 800 Microwell Plates, we seeded $5 \times 10^4$/well in APEL 2 medium (STEMCELL Technologies) and cultured the EBs for 14 days in a hypoxic incubator at 37 °C, 5% CO$_2$ and 5% O$_2$.

The expression of the lineage-specific markers was assessed by TaqMan hPSC Scorecard Assay (Thermo Scientific). Briefly, we isolated RNA using the QIAgen RNasy 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$^\text{TM}$ – Analysis group (Thermo Fisher).

4.4. Direct differentiation into three germ layers

For direct differentiation, we used the STEMdiff$^\text{TM}$ 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
Fig. 1. L.G. Devito et al.
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 (Ca²⁺ Mg²⁺) (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.

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References


