Citometría pluripotencia ESi063-A
Marcador TRA1-60 (ISOTYPO como control negativo)
|                | Fibroblasts | ARSFiPS4F1-Clon 1 | ARSFiPS4F1-Clon 10 |
|----------------|-------------|-------------------|-------------------|
| D19S572        | 119/129     | 119/129           | 119/129           |
| D2S159         | 174/178     | 174/178           | 174/178           |
| D14S972        | 199/199     | 199/199           | 199/199           |
| D8S601         | 223/225     | 223/225           | 223/225           |
| D9S1853        | 252/252     | 252/252           | 252/252           |
| Gene    | HDF11 FIBROS | ARS-FiPS4F1 |
|---------|--------------|-------------|
| AMEL    | X, Y         | X, Y        |
| CSF1PO  | 11, 13       | 11, 13      |
| D13S317 | 11, 12       | 11, 12      |
| D16S539 | 11, 13       | 11, 13      |
| D21S11  | 28, 29       | 28, 29      |
| D5S818  | 11           | 11          |
| D7S820  | 10, 12       | 10, 12      |
| TH01    | 6, 7         | 6, 7        |
| TPOX    | 8, 11        | 8, 11       |
| vWA     | 17, 19       | 17, 19      |
Lab Resource: Stem Cell Line

Generation of a human iPSC line from a patient with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) caused by mutation in SACSIN gene

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ABSTRACT

The human iPSC cell line, ARS-FiPS4F1 (ESi063-A), derived from dermal fibroblast from the patient autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) caused by mutations on the gene SACSIN, was generated by non-integrative reprogramming technology using OCT3/4, SOX2, CMYC and KLF4 reprogramming factors. The pluripotency was assessed by immunocytochemistry and RT-PCR. Differentiation capacity was verified in vitro. This iPSC line can be further differentiated toward affected cells to better understand molecular mechanisms of disease and pathophysiology.

Resource table.

| Unique stem cell line identifier | ESi063-A |
|---------------------------------|----------|
| Alternative name(s) of stem cell line | ARS-FiPS4F1 |
| Institution | Research Center Principe Felipe, Eduardo Primo Yúfera 3, Valencia, Spain |
| Contact information of distributor | Slaven Erceg, serceg@cipf.es |
| Type of cell line | iPSC |
| Origin | Human |
| Sex: male Age: 14 | |
| Additional origin info | Dermal fibroblasts |
| Cell Source | Sendai virus |
| Clonality | Clonal |
| Reprogramming | n/a |
| Genetic Modification | No |
| Type of Modification | n/a |
| Associated disease | Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) |
| Gene/locus | Gene: SACSIN gene (SACS) |
| Locus: 13q12.12 Mutations: c.9938delC (p.G3313Qfs*11) and c.11374C > T (p.R3792*) mutation in compound heterozygosity |
| Method of modification | n/a |
| Name of transgene or resistance | n/a |
| Inducible/constitutive system | n/a |
| Date archived/stock date | |
| Cell line repository/bank | http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros/ |

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Resource utility

The generation of human induced pluripotent stem cells (hiPSC) from the ARSACS patients permits the development of disease specific stem cells that can be further differentiated toward affected cells to better understand molecular mechanisms of disease and pathophysiology.

Resource details

Skin punch biopsy was taken from a 14-year-old patient who was diagnosed with autosomal recessive spinal ataxia of Charlevoix-Saguenay (ARSACS) harbouring the c.9938delC (p.G3313Qfs*11) and c.11374C > T (p.R3792*) mutations in compound heterozygosis in the SACS gene (NM_014363.5) and primary fibroblast cell line was established. The generation of the human induced pluripotent stem cell (hiPSC) line, ARS-FiPS4F1 (registered as ES063-A at www.hPSCreg.com), was carried out using non-integrative Sendai virus containing the human reprogramming factors, Oct3/4, Sox2, C-Myc, and Klf4 (Takahashi et al., 2007), following instructions by manufacturer. After 30 days generated colonies displayed a typical ES-like morphology (polygonal shape; refractive edges, high nuclear/cytoplasmic ratio) and growth behaviour. DNA sequencing analysis of ARS-FiPS4F1 confirmed the SACS mutations in each allele (Fig. 1A). The clearance of the virus and the exogenous reprogramming factor genes were confirmed by RT-PCR after twelve cell culture passages (Fig. 1B). The genetic fingerprinting was performed with ARS-FiPS4F1 hiPSC line and proved its genetic identity to parental fibroblasts (available with the author). The selected line showed normal karyotype (46, XY) at low passages (passage 9) (Fig. 1C) and medium passage number (passage 30). Genetic and functional assays were performed to determine the quality of the ARS-FiPS4F1 line. Pluripotency was assessed by immunocytochemistry to pluripotency markers OCT-4, SOX2, NANOG and SSEA-4 and flow cytometry for SSEA-4 pluripotency marker (Fig. 1D). The alkaline phosphatase is known to be more active in hiPSCs and the colorimetric fingerprinting was performed with ARS-FiPS4F1 hiPSC line and proved its genetic identity to parental fibroblasts and hiPSCs was isolated using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany). To detect the SACS c.9938delC variant, the used primers were: Forward: 5'- GCAGACACATCTTCCTCAGGA -3' and Reverse: 5'- CCGCTATGTAAGCATTGG AAA-3', and to investigate the SACS c.11374C > T change, the used primers were: Forward: 5'- TGGTTAACCTGGATCCTCCTC -3' and Reverse: 5'- GAACAATGGTGAAATGTGC -3'.

Detection of pluripotency markers by RT-PCR

Total RNA was isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany), and treated with DNase I to remove any genomic DNA contamination. QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) was used to carry out cDNA synthesis from 1 μg of total RNA according to the manufacturer's instructions. The PCR reaction was performed with MyTaq DNA Polymerase (Bioline GmbH, Germany) and used Applied Biosystems Veriti Thermal Cycler. The expression level of pluripotency markers was analyzed using the primers described in Table 2. Fibroblasts and hESC H9 (WiCell) were used as negative and positive control, respectively.

Immunocytochemistry

Cells were washed in PBS and fixed in 4% PFA for 15 min at room temperature (RT). Fixed cells were washed twice in PBS and placed in blocking solution (3% serum, 0,5% Triton-X100 in PBS) for 1 h at RT. Cells were then incubated overnight at 4 °C with primary antibodies. The following day, cells were washed three times in PBS and incubated with an appropriate secondary antibody at RT for 1 h. Thereafter, cells were stained with DAPI (1:1000) at RT during 5 min, washed three times in PBS and visualized on Leica DM6000 fluorescent microscope equipped with Leica DCS500 camera. Samples grown on coverslips were mounted using Vectashield.

In vitro differentiation assay

For in vitro differentiation assay the colonies from a fully confluent 6-well plate were cut mechanically and cultured in suspension to form embryoid bodies in hiPSCs media without bFGF. After 7 days in suspension, embryoid bodies were transferred into 0.1% gelatin-coated plates and cultured for additional 7–10 days to allow spontaneous differentiation. Then, the cells were fixed and immunostained to detect cells from the three germ layers.

Karyotype analysis

The hiPSCs were adapted to feeder-free cell culture on Matrigel (BD, #354277) coated plates using mTeSR1 medium. Passages were performed using Dispase (STEMCELL Technologies, #07913), every 5–7 days. The karyotype was analyzed by G-bandaging at 400–550 band resolution, 30 metaphases analyzed (Service of Biobanco de Sistema Sanitario Público, Granada, Spain).

Fingerprinting

gDNA from fibroblasts and hiPSCs was extracted using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) in the presence of RNase (Roche). Fingerprinting analyses was performed using 5 microsatellite markers (D19S572, D2S159, D14S972, DBS601, D9S1853) and analyzed on Abi PRISM 3130 using GeneMapper (Thermo Fisher) by Biobanco de Sistema Sanitario Público, Granada, Spain.

Mutation screening

Genomic DNA from fibroblasts and hiPSCs was isolated using the QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany). To detect the SACS c.9938delC variant, the used primers were: Forward: 5'- CGACAACATCTTCCTCAGGA -3' and Reverse: 5'- CCGCTATGTAAGCATTGG AAA-3', and to investigate the SACS c.11374C > T change, the used primers were: Forward: 5'-TGGTTAACCTGGATCCTCCTC -3' and Reverse: 5'-GAACAATGGTGAAATGTGC -3'.
Table 1: Characterization and validation.

| Classification          | Test                        | Result                                                                 | Data                                      |
|-------------------------|-----------------------------|------------------------------------------------------------------------|--------------------------------------------|
| Morphology              | Photography                 | Normal                                                                 | Shown by immunocytochemistry              |
|                         | Immunocytochemistry         | Positive staining/expression of pluripotency markers: Oct4, Nanog, Sox2, SSEA4 | Fig. 1 panel D                            |
| Phenotype               | Cytometry                   | SSEA4 99%                                                               | Fig. 1 panel D                            |
| Genotype                | Karyotype (G-banding) and  | 46XY, Resolution 450-500                                               | Fig. 1 panel D                            |
|                         | resolution                  |                                                                        | Fig. 1 panel D                            |
| Identity                | Microsatellite PCR (mPCR)   | N/A                                                                    | Available with authors                     |
|                         | STR analysis                | 10 loci analyzed, all matching                                        | N/A                                        |
| Mutation analysis (IF   | Sequencing                  | Compound heterozygous                                                  | N/A                                        |
| APPLICABLE)             |                             |                                                                        | Supplementary Fig.1                        |
| Microbiology and virology| Mycoplasma                  | Mycoplasma testing by luminescence. Negative                          | N/A                                        |
|                         | Embryoid body formation     | Positive TUJ1 and TUBB ectodermal staining, positive SMA mesodermal     | N/A                                        |
|                         |                             | staining and positive AFP endodermal staining.                         |                                             |
| Donor screening (OPTIONAL)| N/A                        | N/A                                                                    |                                             |
| Genotype additional     | N/A                         | N/A                                                                    |                                             |
| info (OPTIONAL)         | N/A                         | N/A                                                                    |                                             |
hiPSCs were dissociated using Accutase (Innovative Cell Technologies) for 2–4 min at RT, centrifuged at 300 rcf for 5 min and resuspended in PBS + 2% FBS. Anti-human SSEA-4 antibody was added and incubated for 20 min at RT. IgG3, kappa isotype (STEMCELL technologies # 60073PE.1) was used as negative control. The cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter) and data analyzed by CytExpert 2.0 software (Table 1).

Alkaline phosphatase staining

Alkaline phosphatase staining was carried out using Alkaline Phosphatase Staining Kit II (Stemgent, Cambridge, MA, USA) according to manufacturer's instructions.

Mycoplasma detection

The presence of mycoplasma was tested regularly measuring enzyme activity via luciferase (MycoAlert™ PLUS Mycoplasma Detection Kit, Lonza).

hiPSC nomenclature

The generated hiPSC line was named following Spanish National Stem Cell Bank recommendations. The line is registered on https://hpscreg.eu/ as ESi043-A line.

Flow cytometry

hiPSCs were dissociated using Accutase (Innovative Cell Technologies) for 2–4 min at RT, centrifuged at 300 rcf for 5 min and resuspended in PBS + 2% FBS. Anti-human SSEA-4 antibody was added and incubated for 20 min at RT. IgG3, kappa isotype (STEMCELL technologies # 60073PE.1) was used as negative control. The cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter) and data analyzed by CytExpert 2.0 software (Table 1).

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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.2018.07.012.

References

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| Sample           | Read B/Read A |
|------------------|---------------|
| Positive control | 3,537         |
| Negative control | 0,347         |
| ARS-FiPS4F1      | 0,582         |