The generation of an induced pluripotent stem cell line (DCGi001-A) from an individual with FOXG1 syndrome carrying the c.460dupG (p.Glu154fs) variation in the FOXG1 gene

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ABSTRACT

FOXG1 syndrome is a neurodevelopmental disorder caused by mutations in the FOXG1 gene. Here, an induced pluripotent stem cell (iPSC) line was generated from human dermal fibroblasts of an individual with the c.490dupG (p.Glu154fs) mutation in the FOXG1 gene. Fibroblasts were reprogrammed using non-integrating episomal plasmids and pluripotency marker expression was confirmed by both immunocytochemistry and quantitative PCR in the resultant iPSC line. There were no karyotypic abnormalities and the cell line successfully differentiated into all three germ layers. This cell line may prove useful in the study of the pathogenic mechanisms that underpin FOXG1 syndrome.

1. Resource Table

| Unique stem cell line identifier | DCGi001-A |
|----------------------------------|-----------|
| Alternative names of stem cell line | FS_E154#3 |
| Institution | Division of Cancer and Genetics, Cardiff University School of Medicine, UK |
| Contact information of distributor | Professor Angus Clarke, ClarkeAJ@cardiff.ac.uk or Professor Andrew Teo, teoa@cardiff.ac.uk |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info | Age: 18, Sex: Female, Ethnicity: White European |
| Cell Source | Human dermal fibroblasts (HDF) |
| Clonality | Clonal |
| Method of reprogramming | Episomal plasmid-based iPSC reprogramming |
| Genetic modification | YES |
| Type of modification | Spontaneous heterozygous mutation |
| Associated disease | FOXG1 Syndrome (OMIM # 613454) |
| Gene/locus | FOXG1/14q12 |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | 06–2019 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/DCGi001-A |
| Ethical approval | South East Wales NHS Research Ethics Committee 10/WSE03/3 |

2. Resource utility

FOXG1 syndrome is a neurodevelopmental disorder caused by mutations in the FOXG1 gene. We generated iPSCs from fibroblasts of an individual with the c.460dupG FOXG1 mutation. This patient-derived cell line will be useful for modelling the pathogenic mechanisms that underpin FOXG1 syndrome.

3. Resource details

FOXG1 syndrome (originally called the congenital-onset variant of Rett syndrome) is a rare neurodevelopmental disorder associated with heterozygous mutations in the FOXG1 gene (located on chromosome 14q12). The range of variants associated with the disorder include chromosomal microdeletions, larger deletions and intragenic mutations (missense, nonsense and frameshift). Furthermore, a subset of cases are associated with structural variants occurring downstream of FOXG1 that may disrupt cis-regulation of FOXG1 expression. The clinical phenotype associated with FOXG1 syndrome includes severe intellectual disability, postnatal microcephaly, dyskinetic-hyperkinetic movement disorders, visual impairment, epilepsy, stereotypes, abnormal sleep patterns, and unexplained episodes of crying. Brain imaging of patients reveal structural abnormalities including hypoplasia of the corpus callosum and underdevelopment of the frontal cortex (Kortum et al., 2011; Vegas et al., 2018). The FOXG1 gene encodes the forkhead box G1 protein, a winged-helix transcriptional factor that is a master regulator of the development and regional specification of the ventral...
telencephalon (Wong et al., 2019). Through its array of protein and DNA interactions FOXG1 has pleiotropic and non-redundant roles in brain development, reflected in the complex genotype-phenotype presentations observed (Vegas et al., 2018; Mitter et al., 2018).

Fibroblasts were isolated from a skin biopsy taken from an 18-year old FOXG1 syndrome patient with a previously reported heterozygous pathogenic variant, c.460dupG (p.Glu154fs) (Vegas et al., 2018). iPSCs were generated by a non-integrating episomal plasmid-based method.

Fig. 1. Figure content described in Resource Details.
through expression of the reprogramming factors OCT4, SOX2, KLF4, L-MYC, LIN28 and p53 shRNA. A panel of iPSC-like colonies were manually picked after 20 days post-electroporation and expanded in culture over several passages for establishment as iPSC clones for further characterisation. Amongst the panel of clones was FS_E154#3 that is described herein. Polymerase chain reaction (PCR) analysis performed at the 10th passage using validated primers against the EBNA1 backbone, common to all the episomal plasmids, confirmed their elimination from the iPSC line (Supplementary Fig. 1A). The established FS_E154#3 iPSC line showed typical human embryonic stem cell-like morphology as judged by brightfield microscopy (Fig. 1A, scale bar is 100 μm). The line expressed the pluripotency markers OCT4 and SSEA4 as shown by immunocytochemistry (Fig. 1A). Quantitative real-time PCR analysis (qPCR) demonstrated the expression of pluripotency markers NANOG, OCT4 and SOX2 in the FS_E154#3 line comparable to the control iPSC line (sex-matched) previously generated in house (Fig. 1B). FS_E154#3 displayed a normal diploid 46 XX karyotype (passage 9, Fig. 1C), and sequencing analysis confirmed the presence of OCT4, SOX2 and NANOG. 46XX, Resolution (=5–10 Mb/=450-hand) (Addgene; Supplementary Fig. 1C). Patient-derived fibroblasts (5 × 10^5 cells) were electroporated with all three vectors (1 ug of each) using the Amaxa Human Dermal Fibroblast Nucleofector Kit and Nucleofector 2b (Lonza) on program U023. Nucleofected fibroblasts were plated onto Matrigel-coated dishes with culture conditions and iPSC colony isolation according to the Essential 6 (E6) media instructions (MAN0007568). iPSCs were propagated in StemFlex medium and generally passaged at a 1:6 ratio every 4 days using ReLeSR (STEMCELL Technologies).

4.3. In vitro differentiation

EBs were formed as previously described (Ungrin et al., 2008) with modifications. iPSCs were dissociated using Accutase, seeded into v-bottom plates (Greiner; 2000 cells/well) and cultured for 24 h in StemFlex medium with 10 μM Y-27632 (Hello Bio) and 0.4% (w/v) PVA (Sigma). EBs were washed in D-PBS, resuspended in E6 media and transferred to low-attachment 96-well plates (Greiner). EBs were cultured for a further 7 days before seeding onto Matrigel-coated 24-well plates and cultured in E6 media for an additional 7 days prior to analysis.

4.4. RNA isolation, PCR and qPCR

RNA was isolated using the RNeasy Mini Kit (QIAGEN) followed by DNase treatment (DNAnfree kit). Complementary DNA was reverse transcribed from 250 ng total RNA using SuperScript IV. qPCRs were performed using Power SYBR Green PCR master mix and an ABI 7300 Real Time PCR System, with data analysed by the ΔΔCt method using ACTNB for normalisation. PCR and qPCR parameters are available on request and Table 1.

4.5. Immunocytochemistry

Cells were fixed in 4% paraformaldehyde for 20 mins at room temperature (RT), permeabilised in 0.1% (v/v) Triton X-100 for 15 mins at 4 °C and incubated in blocking solution (10% FBS in PBS) for 30 mins at RT. Fixed cells were incubated with primary antibodies (Table 2) in blocking solution (1–3 h at RT). Following three PBS washes, cells were incubated with Alexa Fluor-conjugated secondary antibodies (Table 2) in blocking solution (1 h at RT), washed three times and examined using a Delta Vision OMX SR (Applied Imaging) microscope.

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Table 1
Characterization and validation.

| Classification | Test | Result | Data |
|---------------|------|--------|------|
| Morphology    | Brightfield microscopy | Normal hiESC-like morphology | Fig. 1 panel A |
| Phenotype     | Qualitative analysis (Immunocytochemistry) | Expression of OCT4 and SSEA4. | Fig. 1 panel A |
| Genotype      | Quantitative analysis (RT-qPCR) | Expression of OCT4, SOX2 and NANOG. | Fig. 1 panel B |
| Identity      | Karyotype (G-banding), passage 9 | 46XX, Resolution (=5–10 Mb/=450-hand) | Fig. 1 panel C |
| STR analysis  | Not performed | Available with the authors |
| Mutation analysis | Sequencing | Heterozygous FOXG1 c.460dupG | Fig. 1 panel D |
| Microbiology and virology | Southern Blot OR WGS | Not performed | Supplementary Fig. 1 |
| Differentiation potential | Embryoid body formation | Expression of germ layer markers (qPCR): endoderm (GATA4, SOX17, FOXA2), mesoderm (RUNX1, MSX1, MYH6, NKX2.5), ectoderm (NGCAM, PAX6, TUBB3, SOX1). | Fig. 1 panel E and F |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | Not performed | Not done |
| Genotype additional info (OPTIONAL) | Blood group genotyping | Not performed | Not done |
| Phenotype Qualitative analysis | HLA tissue typing | Not performed | Not done |
with PBS and mounted in AntiFading Mounting Medium containing DAPI counterstain (Dianova). Images were acquired using a Leica DM IL LED Microscope with Leica DMC3000 G CCD camera.

4.6. Sequencing

The FOXG1 c.460dupG variant was confirmed in iPSCs and parental fibroblasts by Sanger Sequencing of PCR-amplified sequence from genomic DNA using specific primers (Table 2). DNA was isolated using the QIAamp DNA mini kit (QIAGEN). Sequencing reactions were performed using Big Dye v3.1 chemistry and analysed by the All Wales Medical Genomics Service (Cardiff, UK).

4.7. STR analysis

STR analysis of 16 loci was performed using the Promega Powerplex 16HS kit with amplicon detection using an Applied Biosystems 3730xl (undertaken by Source BioScience, Nottingham, UK).

4.8. Karyotyping

G-banding analysis of 20 metaphase spreads was performed by Cell Guidance Systems (Cambridge, UK). Fixed iPSCs for analysis were prepared according to service provider instructions (http://cellgs.fund.ac.uk/Downloads/Karyotype_FixedSamples.pdf).

4.9. Mycoplasma test

Mycoplasma contamination was tested using the PCR-based Venor®GeM Classic detection kit (Minerva Biolabs) according to manufacturer’s instructions.

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Appendix A. Supplementary data

Supplementary Fig. 1 can be found online at https://doi.org/10.1016/j.scr.2020.102018.

References

Kortum, F., Das, S., Flindt, M., Morris-Rosendahl, D.J., Stefanova, I., Goldstein, A., Horn, D., Klopeci, E., Kluger, G., Martin, P., Rauch, A., Roumer, A., Saitta, S., Walsh, L.E., Wiesneth, D., Yianou, G., Kutsche, K., Dobyns, W.B., 2008. Reproducible, ultra-high throughput formation of multicellular organization from single cell suspension-derived human embryonic stem cell aggregates. PLoS One 3, e1565.

Vegas, N., Cavallin, M., Maillard, C., Rodda, N., Toulouse, J., Schaefer, E., Lerman-

Table 2

Reagents details.

| Antibodies used for immunocytochemistry | Antibody | Dilution | Company Cat # | RRID |
|----------------------------------------|----------|----------|---------------|------|
| Pluripotency Markers | Rabbit anti-OCT4 | 1:500 | Abcam Cat# Ab19857, RRID:AB_445175 |
| | Mouse anti-SSEA4 | 1:50 | DSBB Cat# MC 813-70, RRID:AB_528477 |
| Differentiation Markers | Mouse anti-AFP | 1:20 | R and D Systems Cat# MAB1368-SP, RRID:AB_357658 |
| | Mouse anti-nSMA | 1:50 | R and D Systems Cat# MAB1420-SP, RRID:AB_262054 |
| | Mouse anti-Nestin | 1:50 | R and D Systems Cat# MAB1259-SP, RRID:AB_2251304 |
| | Rabbit anti-PAX6 | 1:100 | Proteintech Cat# 12323-1-AP, RRID: AB_2159695 |
| Secondary antibodies | Alexa Fluor 488 Donkey anti-Rabbit IgG | 1:1500 | Thermo Fisher Scientific Cat# A-21206, RRID:AB_2535792 |
| | Alexa Fluor 568 Goat anti-Mouse IgG | 1:1500 | Thermo Fisher Scientific Cat# A-11031, RRID: AB_144696 |

References
Sagie, T., Lev, D., Magalie, B., Moutton, S., Haan, E., Isidor, B., Heron, D., Milh, M., Rondeau, S., Michot, C., Valence, S., Wagner, S., Hully, M., Mignot, C., Masurel, A., Datta, A., Odent, S., Nizon, M., Lazaro, L., Vincent, M., Cogne, B., Guerrot, A.M., Arpin, S., Pedespan, J.M., Caubel, I., Pontier, B., Troude, B., Rivier, F., Philippe, C., Bienvenu, T., Spitz, M.A., Bery, A., Bahi-Buisson, N., 2018. Delineating FOXG1 syndrome: From congenital microcephaly to hyperkinetic encephalopathy. Neurol. Genet 4, e281.

Wong, L.C., Singh, S., Wang, H.P., Hsu, C.J., Hu, S.C., Lee, W.T., 2019. FOXG1-related syndrome: from clinical to molecular genetics and pathogenic mechanisms. Int. J. Mol. Sci. 20.