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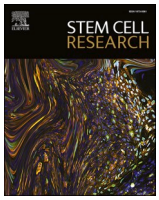

Lab Resource: Single Cell Line

Establishment and characterization of an iPSC line (UCLi023-A) derived from a Late-Onset Retinal Degeneration patient carrying a founder mutation in C1QTNF5

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A B S T R A C T

Late-Onset Retinal Degeneration (L-ORD) is a rare autosomal dominant macular disease, with most cases being caused by a founder mutation in C1QTNF5. Initial symptoms, which generally occur during or after the sixth decade, include abnormal dark adaptation and changes in peripheral vision. Over time, the build-up of sub-retinal pigment epithelium (RPE) deposits leads to macular atrophy and bilateral central vision loss[1]. Here, we describe the generation of a human induced pluripotent stem cell (iPSC) line from dermal fibroblasts of a 61-year-old L-ORD Caucasian male patient carrying the founder mutation (c.489C>G, p.Ser163Arg), using episomal reprogramming.

1. Resource table

Unique stem cell line identifier	UCLi023-A
Alternative name(s) of stem cell line	UCLIoO-041-A; LORD-S163R-01
Institution	UCL Institute of Ophthalmology, London UK
Contact information of distributor	Amanda-Jayne Francis Carr, a.carr@ucl.ac.uk
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 61 Sex: Male Ethnicity: Caucasian
Cell Source	Dermal Fibroblasts
Clonality	Clonal
Method of reprogramming	Episomal vectors (OCT3/4, SOX2, KLF4, L-MYC, LIN28A)
Genetic Modification	N/A
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	qPCR for episomal and endogenous transgene expression
Associated disease	Late-Onset Retinal Degeneration (L-ORD)
Gene/locus	C1QTNF5 c.489C > G Chr11:119339574 NM_001278431.2

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(continued)

Date archived/stock date	2023/01/30
Cell line repository/bank	https://hpscreg.eu/cell-line/UCLi023-A
Ethical approval	NRES Committee London – Riverside: 12/LO/0489

2. Resource utility

Late-Onset Retinal Degeneration (L-ORD) is an adult-onset retinal dystrophy caused mutations in *C1QTNF5* (Lando and Borooah, 2022). This iPSC line, derived from a patient carrying the founder autosomal dominant mutation (c.489C>G, p.Ser163Arg), serves as a powerful cell model to study molecular mechanisms behind macular disease, and as a platform to develop new therapeutics for L-ORD. Table 1.

3. Resource details

Human dermal fibroblasts were reprogrammed by nucleofection of pCXLE episomal vectors expressing the reprogramming factors OCT4, NANOG, L-MYC, LIN28A and KLF4 (Okita et al., 2011). gDNA sequencing from cells confirmed the presence of a c.489C>G

Abbreviations: L-ORD, Late-Onset Retinal Degeneration; iPSC, induced Pluripotent Stem Cell; RPE, Retinal Pigment Epithelium.

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast bright field microscopy	Normal	Fig. 1 panel B
Phenotype	Qualitative analysis by immunocytochemistry	Expression of pluripotency markers Oct3/4 and Sox2	Fig. 1 panel B
	Quantitative analysis	Immunocytochemistry counting: Sox2: 99.7 Oct3/4: 97.9	Fig. 1 panel C
Genotype	BACs on Beads (BOBS™) using KaryoLite Panel	No autosomal or sex chromosome aneuploidies were detected	Fig. 1 panel F
Identity	STR Analysis	Analysis with 27 STR markers confirms IPSC genotype matches that of original patient fibroblast cells.	Table 3
Mutation analysis (IF APPLICABLE)	Sanger Sequencing	Heterozygous c.489C > G autosomal dominant	Fig. 1 panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by MycoStrip Assay. Negative	Supplementary
Differentiation potential	Embryoid body formation	Confirmation of self-renewal genes in IPSC and upregulation of mesoderm, ectoderm and endoderm markers in EB after 1 week differentiation, using Taqman™ hPSC Scorecard™ Panel, Fast 96 well	Fig. 1 panel D and E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

substitution in one of the C1QNTF5 alleles, corresponding to the S163R mutation (Fig. 1A, Table. 2). Reprogrammed cells presented typical iPSC morphology (phase positive compact colonies with distinct borders, large nucleus to cytoplasm ratio) which stained positive for pluripotency-associated transcription factors SOX2 and OCT4 (Fig. 1B and C and Table. 2). Pluripotency of iPSC was further confirmed using TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis. iPSC expressed self-renewal genes, and when embryoid bodies were generated, there was a downregulation of self-renewal gene expression and an increase on ectoderm, mesoderm and endoderm gene expression (Fig. 1D and E). Chromosomal analysis revealed no autosomal or sex chromosome aneuploidies of the sample following reprogramming (Fig. 1F). mRNA episomal vector expression was lost by passage 7 (Fig. 1G, Table. 2). STR analysis confirms the origin of the iPSC (Table 3).

4. Materials and methods

A skin biopsy was obtained from a 61-year old Caucasian male carrying a S163R mutation in C1QTNF5. Fibroblasts were grown in DMEM with 1X penicillin/streptomycin, 1X GlutaMAX, 1X Sodium Pyruvate (all GIBCO) and 20% FBS (Sigma-Aldrich). 1×10^6 fibroblasts were transfected in the Amaxa Nucleofector™ 2b System using P3 Primary Nucleofector Kit (Lonza) with episomal reprogramming plasmids (1ug each of pCXLE-hSK (#27078), pCXLE-hUL (#27080) and pCXLE-OCT3/4 (#27076): all Addgene) as previously described (Carter et al., 2016). Following transfection, culture medium, containing 0.5 mM Sodium Butyrate (Sigma-Aldrich), was changed daily. On day 7, 2.5×10^5 transfected cells were dissociated using TrypLE (Gibco), seeded onto a Matrigel-coated (Corning) 6-well plate (ThermoFisher) well in mTeSR (StemCell Technologies) with daily media replacement until iPSC colonies appeared. Individual iPSC colonies were isolated, clonally expanded and maintained at 37 °C, 5% CO_2, on Matrigel-coated plates with daily medium change. Cells were passaged at 70–80% confluency using 0.5 mM EDTA (Sigma-Aldrich) at 1:3 split ratio.

4.1. Mutation sequencing

Genomic DNA was extracted from cells using GenElute™ Mammalian Genomic DNA Kit (Sigma-Aldrich). A 398 bp region of C1QTNF5 was amplified (Table 2) using GoTaq® Green MasterMix (Promega) and resolved on a 2% Agarose gel. The amplicon was purified using PureLink Gel Extraction kit (ThermoFisher). Sanger sequencing was carried out using Source Bioscience services.

4.2. Immunocytochemistry

Passage 7 iPSC were fixed with 4% PFA for 30 min at 4 °C, and permeabilised using 0.3% Triton X-100 in PBS for 10 min at room temperature. Cells were incubated in blocking buffer (PBS with 5% Donkey Serum and 3% Bovine Serum Albumin (Sigma-Aldrich) for 30 min and incubated with primary antibodies (Table 2) overnight at 4 °C. After washing thrice with PBS, cells were incubated with secondary antibodies (Table 2) for 30 min at 4 °C. Cell nuclei were stained with DAPI, coverslipped and mounted using VECTASHIELD® (Vector Laboratories) and images were captured with EVOS FL Auto Imaging system (ThermoFisher). Scale bars: 100 μm.

4.3. In vitro differentiation

Embryoid bodies (EBs) were formed by transferring iPSC colonies, dissociated into small clumps, into ultra-low attachment plates in DMEM/F12 with GlutaMAX, 20% KnockOut Serum Replacement, 100 μM Non-Essential Amino acids, 50 μM β-mercaptoethanol (all GIBCO) and incubated at 37 °C in 5% CO_2. On day 7, EBs were collected for RNA extraction.

4.4. Pluripotency gene expression analysis

RNA was purified from p7 iPSC and EBs, using RNAeasy mini kit (Qiagen). RT-PCR was performed using High-capacity cDNA Reverse Transcription Kit (ThermoFisher). cDNA was prepared for analysis in 2X TaqMan® Fast Advanced™ MasterMix (ThermoFisher) and assessed using TaqMan® hPSC Scorecard™ Panel 96w FAST plates (ThermoFisher) on a StepOne™ Real-Time PCR system. Data was analysed using hPSC Scorecard™ Analysis Software.

4.5. Karyotype analysis

DNA was isolated from cells using GenElute™ Mammalian Genomic DNA kit (Sigma-Aldrich). 1 μg of genomic DNA was used for karyotyping, following the BACs on Beads methodology (García-Herrero, 2014) (performed by TDL Genetics LTD), with no aneuploidies detected.

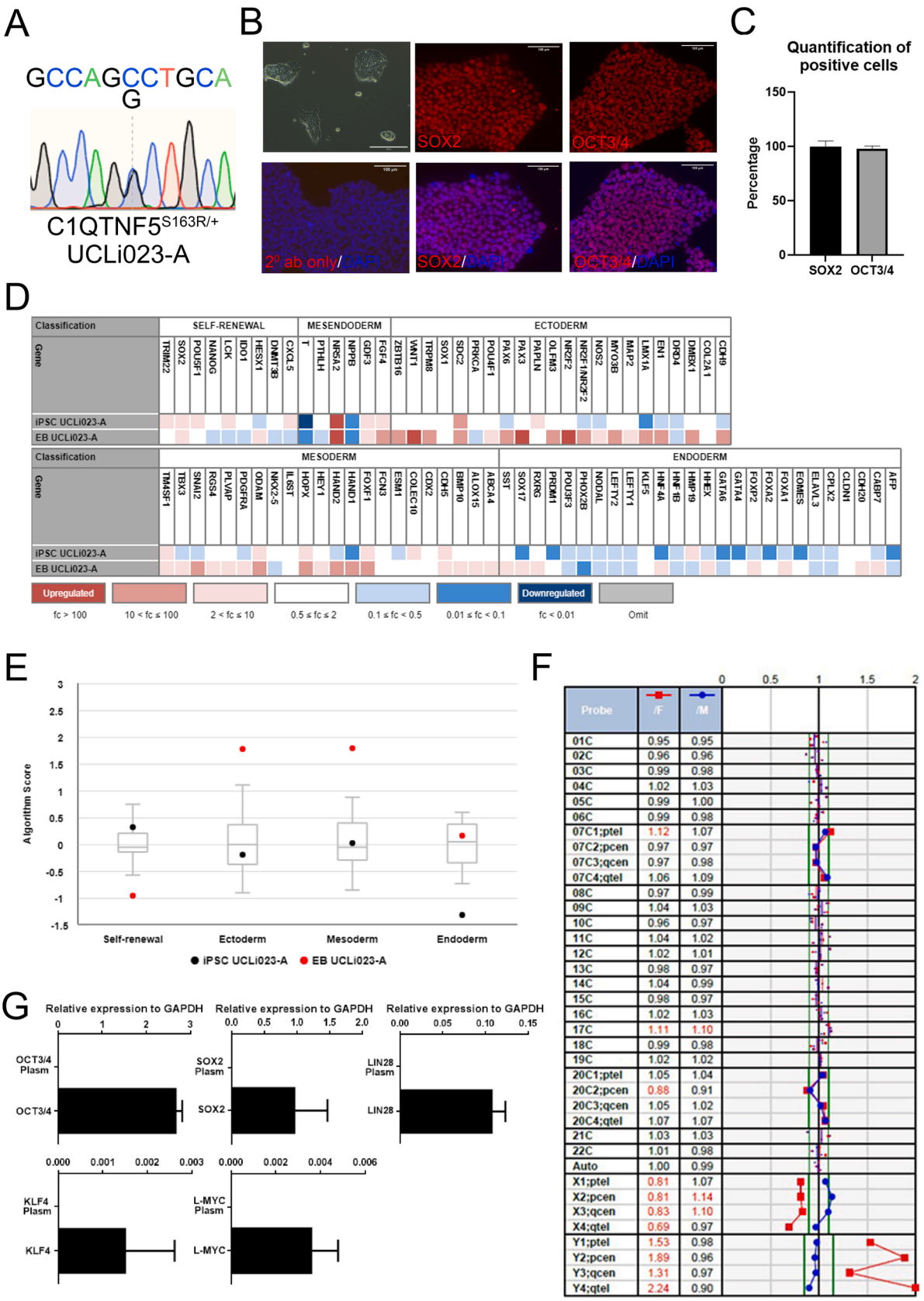

Fig. 1. Characterisation of L-ORD S163R iPSC.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit anti-Oct4	1:100	Merck, #AB5603	RRID: AB_445175
	Rabbit anti-Sox2			RRID: AB_2286686
Secondary antibodies	Goat Anti-Rabbit Alexa Fluor 555	1:2000	ThermoFisher #A27039	RRID: AB_2536100
	Primers Target	Amplicon	Sequence	
Episomal Plasmids (qPCR)	OCT4	124 bp	5′-CATTCAAACTGAGGTAAGGG-3′ 5′-TAGCGTAAAAGG AGCAACATAG-3′	
	KLF4	156 bp	5′-CCACCTCGCCTTACACATGAAGA-3′ 5′-TAGCGTAAAAGG AGCAACATAG-3′	
	SOX2	111 bp	5′-TTCACATGTCCCAGCACTACCAGA-3′ 5′-TTTGTTTGACAGGAGCGACAAT-3′	
	L-MYC	122 bp	5′-GGCTGAGAAGAGGATGGCTAC-3′ 5′-TTTGTTTGACAGGAGCGACAAT-3′	
	LIN28	251 bp	5′-AGCCATATGGTAGCCTCATGTCCGC-3′ 5′-TAGCGTAAAAGGAGCAACATAG-3′	
House-Keeping Genes (qPCR)	GAPDH	452 bp	5′-ACCACAGTCCATGCCATCAC-3′ 5′-TCCACCACCCTGTTGCTGTA-3′	
Amplification of mutation region for sequencing (PCR)	C1QTNF5	398 bp	5′-CGCACCCTTGCCCTTCGACC-3′ 5′-AGCATGAGCTCACTTTGCAG-3′	
Mutation sequencing (Sanger)	C1QTNF5	N/A	5′-CGCACCCTTGCCCTTCGACC-3′	

4.6. Mycoplasma analysis

Conditioned media from p8 iPSC was negative for mycoplasma using MycoStrip Detection Assay (InvivoGen).

4.7. STR analysis

50 ng of genomic DNA from fibroblasts and reprogrammed iPSC was analysed using a set of 27 STR markers using King's College London DNA analysis services, confirming single origin.

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.

Table 3
STR analysis as Excel table.

AMEL	Fibroblast genomic DNA		iPSC genomic DNA	
	X	Y	X	Y
CSF1PO	12	13	12	13
D10S1248	12	15	12	15
D12S391	15	18	15	18
D13S317	11		11	
D16S539	10	12	10	12
D18S51	18	19	18	19
D19S433	14		14	
D1S1656	12	116	12	116
D21S11	29	30	29	30
D22S1045	11	15	11	15
D2S1338	17	25	17	25
D2S441	11	14	11	14
D3S1358	15	17	15	17
D5S818	11	13	11	13
D7S820	8	10	8	10
D8S1179	13	14	13	14
DYS391	9		9	
DYS570	20		20	
DYS576	16		16	
FGA	19	20	19	20
Penta D	11	12	11	12
Penta E	12	14	12	14
SE33	18.3	20	18.3	20
TH01	8	9.3	8	9.3
TPOX	8		8	
vWA	14	17	14	17

Cell line identity testing – confirmation of patient mutation in derived fibroblast and IPSCs.
Abnormal karyotype – no autosomal or sex chromosome aneuploidies were detected (in text).

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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.2023.103110.

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