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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line from a patient with Stargardt disease caused by biallelic c.[5461–10T>C;5603A>T]; [6077T>C] mutations in the *ABCA4* gene

Di Huang [a,b], Dan Zhang [a], Shang-Chih Chen [a], May Thandar Aung-Htut [b,c], Tina M. Lamey [d,e], Jennifer A. Thompson [e], Terri L. McLaren [d,e], John N. De Roach [d,e], Sue Fletcher [b], Steve D. Wilton [b,c], Samuel McLenachan [a,d], Fred K. Chen [a,d,e,f,g]

[a] *Ocular Tissue Engineering Laboratory, Lions Eye Institute, Nedlands, Western Australia, Australia*
[b] *Centre for Molecular Medicine and Innovative Therapeutics, Health Futures Institute, Murdoch University, Western Australia, Australia*
[c] *Perron Institute for Neurological and Translational Science, Centre for Neuromuscular and Neurological Disorders, The University of Western, Australia*
[d] *Centre for Ophthalmology and Visual Sciences, The University of Western Australia, Nedlands, Western Australia, Australia*
[e] *Australian Inherited Retinal Disease Registry and DNA Bank, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia*
[f] *Department of Ophthalmology, Royal Perth Hospital, Perth, Western Australia, Australia*
[g] *Department of Ophthalmology, Perth Children's Hospital, Nedlands, Western Australia, Australia*

A B S T R A C T

Mutations in *ABCA4* gene are causative for autosomal recessive Stargardt disease (STGD1), the most common inherited retinal dystrophy. Here, we report the generation of an induced pluripotent stem cell (iPSC) line from a STGD1 patient carrying biallelic c.[5461–10T>C;5603A>T];[6077T>C] mutations in the *ABCA4* gene. Episomes carrying *OCT4, SOX2, KLF4, L-MYC, LIN28* and *mp53DD* were employed for the reprogramming of patient-derived fibroblasts. This iPSC line expressed comparable pluripotency markers as in a commercially available human iPSC line, displayed normal karyotype and potential for trilineage differentiation, and were negative for both reprogramming episomes and mycoplasma test.

Resource Table:

Unique stem cell line identifier	LEIi018-A
Alternative name(s) of stem cell line	1607ips6
Institution	Lions Eye Institute
Contact information of distributor	Samuel McLenachan: smclenachan@lei.org.au Fred K. Chen: fredchen@lei.org.au
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 21 Sex: Male Ethnicity if known: Caucasian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Associated disease	Stargardt disease
Gene/locus	*ABCA4*/1p22.1
Date archived/stock date	13/07/2020
Cell line repository/bank	https://hpscreg.eu/cell-line/LEIi018-A
Ethical approval	University of Western Australia Human Research Ethics Committee RA/4/1/7916

1. Resource utility

Mutations in the ATP-binding cassette transporter gene (*ABCA4)* cause Stargardt disease (STGD1) Allikmets et al. (1997), which exhibits a wide spectrum of retinal phenotypes. The iPSC line reported here was generated from a STGD1 patient carrying compound heterozygous mutations in the *ABCA4* gene and will provide a powerful resource for disease modelling studies.

2. Resource details

The functional effects of the majority of known *ABCA4* mutations are yet to be explored and novel mutations continue to emerge. The availability of *in silico* prediction algorithms, paralleled with the development of *in vitro* functional assays have contributed to our understanding of the consequences of certain *ABCA4* variants on splicing. However, given the high degree of alternative splicing utilized by human retinal cells, Pan et al. (2008), it is essential that the effects of mutations in retinal genes such as *ABCA4* are assessed in the affected retinal cell types. Here, we report the generation and characterization of an iPSC line from a STGD1 patient carrying biallelic *ABCA4* mutations, c.[5461–10T>C;5603A>T];

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[6077T>C].

The male patient presented with poor vision at age 7 years and was diagnosed with Stargardt disease at 9 years. Biallelic *ABCA4* c.[5461–10T>C;5603A>T];[6077T>C] mutations were identified by Sanger sequencing of patient and parental DNA. At age 21, his visual acuity was 20/200 in both eyes and fundus imaging showed extensive fleck lesions throughout the retina with central atrophy. Dermal fibroblast cultures were previously derived from a skin biopsy from the patient Huang et al. (2020), and transfected with iPSC reprogramming episomes carrying *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28* and *mp53DD*. After 4 weeks, a clonal iPSC line (LEIi018-A) was selected for expansion and characterization (Table 1). The LEIi018-A iPSC line displayed the typical morphology of pluripotent stem cell colonies (Fig. 1A). The expression of the pluripotency markers OCT4, NANOG, SOX2 and KLF4 was demonstrated by immunocytochemistry at passage 34 (Fig. 1A). Cell nuclei were labelled with DAPI (Fig. 1A). The *ABCA4* c.[5461–10T>C;5603A>T];[6077T>C] mutations were confirmed in the LEIi018-A iPSC line by Sanger sequencing (Fig. 1B). Quantitative polymerase chain reaction (qPCR) analysis demonstrated similar levels of *OCT4*, *NANOG*, *SOX2*, *KLF4* and *MYC* expression in LEIi018-A (passage 22) and in a commercial human iPSC line (HuiPSC, Cat#A18945, ThermoFisher, Fig. 1C). The capacity of the LEIi018-A (passage 35) to differentiate into three germ layers was demonstrated by the upregulation of markers of ectoderm (*PAX6*, *OTX1*), mesoderm (*TBXT*, *NKX2.5*) and endoderm (*AFP*, *SOX17*, *FOXA2*) lineages in differentiating embryoid body (EB) cultures (Fig. 1D). LEIi018-A was negative for both reprogramming episomes (Fig. 1E) and mycoplasma test at passage 16 (Fig. 1F), whilst episome specific products (544 bp and 666 bp) were detected in a positive control consisting of iPSC at passage 3 and mycoplasma specific product (504 bp) was detected in the positive control samples (Fig. 1E-F). The 130 bp *GAPDH* internal control product was amplified from LEIi018-A (Fig. 1E-F). Digital karyotyping of LEIi018-A was performed using the Infinium Human CoreExome-24 Beadchip SNP array (Illumina, San Diego, California, United States) with genome-wide copy number variation (CNV) profiling, demonstrating both lines were male and had a normal diploid karyotype (46, XY). Analysis of the B allele frequencies and LogR ratios of ≈500,000 single nucleotide polymorphisms located across the genome demonstrated an absence of reprogramming-induced chromosomal rearrangements in LEIi018-A (passage 16, Fig. 1G). Analysis of 16 microsatellite markers confirmed LEIi018-A was derived from the patient's fibroblasts (FB) (data not shown).

3. Materials and methods

Ethics: This work was approved by the University of Western Australia Human Research Ethics Committee (RA/4/1/7916) with written consent from the patient, and performed in accordance with the National Health & Medical Research Council of Australia National Statement on Ethical Conduct in Human Research (2007, updated 2018) and the Declaration of Helsinki.

Cell culture: Fibroblasts were cultured in DMEM (11995065, Gbico) supplemented with 10% fetal bovine serum (FBS, 26140079, Gibco) and Antibiotic-Antimycotic (15240062, Gibco). The Epi5™ Episomal iPSC Reprogramming Kit (A15960, Invitrogen) was used for reprogramming. Fibroblasts (1×10^5 cells) were electroporated using the NEON electroporation system (Invitrogen) (three 10 ms 1650 V pulses in a 10 μl tip) and seeded into a geltrex (A1413302, Gibco)-coated well containing fibroblast culture medium. At Day 4, medium was changed to DMEM/F12 (11320082, Gibco) supplemented with N2 (17502001, Gibco), B27 (17504001, Gibco) and 100 ng/mL basic Fibroblast Growth Factor (bFGF, 78134, StemCell Technologies) for 2 weeks, then to TeSR-E8 medium (05990, StemCell Technologies). IPSC colonies were picked for clonal expansion after 4 weeks. IPSC were passaged onto geltrex-coated plates using TrypLE Express Enzyme (12604021, Gibco) every 4–5 days using a split ratio of 1:3–1:6. All cell cultures were incubated at 37 °C with 5% CO_2.

Trilineage differentiation: EBs were differentiated by seeding iPSC clusters into suspension culture plates containing mTesR1 medium (85850, StemCell Technologies) and 10 μM Y27632 (ab120129, Abcam). Media was changed after 48 h to DMEM/F12 supplemented with 20% knockout serum replacement (KSR, 10828028, Gibco), MEM-non essential amino acids (MEM-NEAA, 11140050, Gibco) and antibiotic–antimycotic. Half media changes were performed daily. EBs were harvested at Day 14.

RNA Analysis: RNA was isolated using MagMAX TM -96 Total RNA Isolation kit (AM1830, Applied Biosystems). qPCR was performed with RT^{2}SYBR Green qPCR Mastermix (330503, Qiagen) on the BioRad CFX Connect Real-Time System (45 cycles, 95 °C for 30 s, 60 °C for 60 s). Gene expression was normalized to *GAPDH* expression. Primers used are listed in Table 2.

Immunocytochemistry: iPSCs were fixed with 4% paraformaldehyde for 15 min at 37 °C, washed with PBS and incubated with blocking buffer (5% normal goat serum in PBS containing 0.3% Triton X100) for 1 h at room temperature, followed by incubation with primary antibodies at 4 °C overnight. Secondary antibodies and DAPI (1 μg/mL)

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1, panels A
Phenotype	Qualitative analysis Immunocytochemistry	Positive for the pluripotency markers: OCT4, NANOG, SOX2, KLF4	Fig. 1, panels A
	Quantitative analysis RT-qPCR	Positive for the pluripotency markers *OCT4, NANOG, SOX2, KLF4, C-MYC*	Fig. 1, panel C
Genotype	Karyotype (the Infinium Human CoreExome-24 Beadchip SNP array) and resolution	46XX, Resolution 500 kb	Fig. 1, panels F
Identity		STR analysis performed	
	STR analysis	Matched at 16 loci	Archived with journal
Mutation analysis (IF APPLICABLE)	Sequencing	*ABCA4* c.[5461–10T>C;5603A>T];[6077T>C]	Fig. 1, panels B
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Fig. 1, panel E
Differentiation potential	Embryoid body formation	Upregulation of ectoderm (*PAX6*, *OTX1*), mesoderm (*TBXT*, *NKX2.5*) and endoderm (*AFP*, *SOX17*, *FOXA2*) markers	Fig. 1, panel D
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

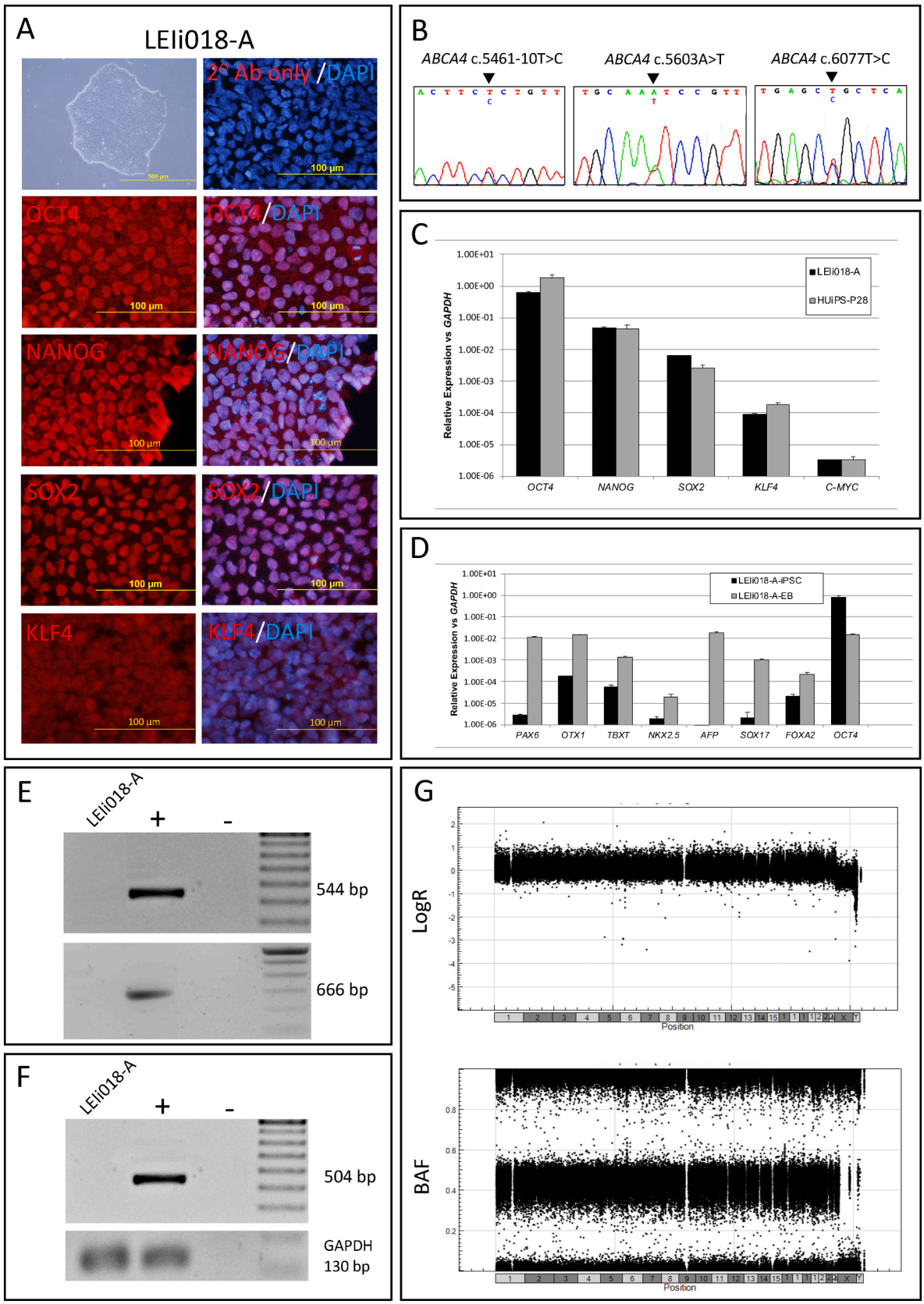

Fig. 1. STR analysis.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit anti-OCT4	1:1000	Sino biological 101282-T02	AB_2810309
	Rabbit anti-NANOG	1:1000	Sino biological 101286-T34	AB_2810308
	Rabbit anti-SOX2	1:1000	Sino Biological 101284-T42	AB_2810307
	Rabbit anti-KLF4	1:100	Invitrogen 710,659	AB_2532749
Secondary antibodies	Goat anti-Rabbit AlexaFluor 546	1:250	Invitrogen A11035	AB_2534093
	Primers			
	Target	**Size of amplicon**	**Forward/Reverse primer (5′-3′)**	
Episomal Plasmids (PCR)	Episomal vectors (*OriP*)	544 bp	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGAGACAAC	
	Episomal vectors (*EBNA1*)	666 bp	ATCGTCAAAGCTGCACACAG/CCCAGGAGTCCCAGTAGTCA	
Mycoplasma Primers (PCR)	Mycoplasma DNA	504 bp	YGCCTGVGTAGTAYRYWCGC/GCGGTGTGTACAARMCCCGA	
Pluripotency Markers (qPCR)	*OCT4*	105 bp	CCTGAAGCAGAAGAGGATCACC/AAAGCGGCAGATGGTCGTTTGG	
	NANOG	114 bp	CTCCAACATCCTGAACCTCAGC/CGTCACACCATTGCTATTCTTCG	
	SOX2	134 bp	GCTACAGCATGATGCAGGACCA/TCTGCGAGCTGGTCATGGAGTT	
	KLF4	110 bp	CATCTCAAGGCACACCTGCGAA/TCGGTCGCATTTTTGGCACTGG	
	C-MYC	128 bp	CCTGGTGCTCCATGAGGAGAC/CAGACTCTGACCTTTTGCCAGG	
Trilineage Markers (qPCR)	*PAX6*	130 bp	CTGAGGAATCAGAGAAGACAGGC/ATGGAGCCAGATGTGAAGGAGG	
	OTX1	159 bp	CTACCCTGACATCTTCATGCGG/GGAGAGGACTTCTTCTTGGCTG	
	TBXT	152 bp	CCTTCAGCAAAGTCAAGCTCACC/TGAACTGGGTCTCAGGGAAGCA	
	NKX2.5	146 bp	AAGTGTGCGTCTGCCTTTCCCG/TTGTCCGCCTCTGTCTTCTCCA	
	AFP	96 bp	TGAGCACTGTTGCAGAGGAG/TTGTTTGACAGAGTGTCTTGTTGA	
	SOX17	111 bp	ACGCTTTCATGGTGTGGGCTAAG/GTCAGCGCCTTCCACGACTTG	
	FOXA2	133 bp	GGAACACCACTACGCCTTCAAC/AGTGCATCACCTGTTCGTAGGC	
House-Keeping Genes (qPCR)	*GAPDH*	130 bp	GTCTCCTCTGACTTCAACAGCG/ACCACCCTGTTGCTGTAGCCAA	
Targeted mutation sequencing	*ABCA4*	765 bp	CCTTGAGGCACTGCTTGTAAG/GGTCAGGAGGAAGTACACCAC	
	ABCA4	490 bp	CAGGATTCAGTTTCTAACCG/CTAGAACAGTACTTGGCACA	

diluted in blocking buffer were applied for 1 h at room temperature. Antibodies are listed in Table 2.

DNA Analyses: DNA was extracted using the FlexiGene DNA kit (51206, Qiagen). Karyotyping was performed by the Australian Genome Research Facility (AGRF), using the Infinium HumanCoreExome-24 Beadchip SNP array (Illumina, San Diego, California, United States), followed by CNV analysis using GenomeStudio 2.0 software with the CNVpartition 2.0 plugin (Illumina). Microsatellite analysis was performed by AGRF using the Promega Powerplex 16H system (Promega, Madison, Wisconsin, United States). PCR for Sanger sequencing was performed on a GeneTouch Thermal Cycler (35 cycles, 94 °C for 30 s, 60 °C for 60 s, 72 °C for 120 s). Mycoplasma testing was performed using the Mycoplasma Testing Kit (#091, Media and Monoclonal Laboratories, Harry Perkins Institute of Medical Research, Fig. 1G). Primers used are listed in Table 2.

Acknowledgements

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References

Allikmets, R., Singh, N., Sun, H., Shroyer, N.F., Hutchinson, A., Chidambaram, A., Gerrard, B., Baird, L., Stauffer, D., Peiffer, A., Rattner, A., Smallwood, P., Li, Y., Anderson, K.L., Lewis, R.A., Nathans, J., Leppert, M., Dean, M., Lupski, J.R., 1997. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat. Genet. 15, 236–246.

Huang, D.i., Thompson, J.A., Charng, J., Chelva, E., McLenachan, S., Chen, S.-C., Zhang, D., McLaren, T.L., Lamey, T.M., Constable, I.J., De Roach, J.N., Aung-Htut, M.T., Adams, A., Fletcher, S., Wilton, S.D., Chen, F.K., 2020. Phenotype-genotype correlations in a pseudodominant Stargardt disease pedigree due to a novel ABCA4 deletion-insertion variant causing a splicing defect. Mol. Genet. Genomic Med. 8 (7), e1259.

Pan, Q., Shai, O., Lee, L.J., Frey, B.J., Blencowe, B.J., 2008. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat. Genet. 40, 1413–1415.