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Lab resource: Stem Cell Line

Generation of the induced pluripotent stem cell line from a patient with autosomal recessive *ABCA4*-mediated Stargardt Macular Dystrophy

Johann N. Claassen[a,b], Dan Zhang[b], Shang-Chih Chen[b], Sang Yoon Moon[a,b], Tina Lamey[a,c], Jennifer A. Thompson[c], Terri McLaren[c], John N. De Roach[a,c], Samuel McLenachan[a,b,*], Fred K. Chen[a,b,c,d]

[a] Centre for Ophthalmology and Visual Science, The University of Western Australia, Perth, Western Australia, Australia
[b] Lions Eye Institute, Nedlands, Western Australia, Australia
[c] Australian Inherited Retinal Disease Registry and DNA Bank, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia
[d] Department of Ophthalmology, Royal Perth Hospital, Perth, Western Australia, Australia

A B S T R A C T

We report the generation of the human iPSC line LEIi007-A from a patient with autosomal recessive Stargardt disease caused by compound heterozygous mutations in the *ABCA4* gene (c.[5461-10 T > C];[4139C > T]). Reprogramming of patient dermal fibroblasts was performed using episomal plasmids containing OCT4, SOX2, KLF4, L-MYC, LIN28, shRNA for p53 and mir302/367 microRNA to establish the clonal iPSC line LEIl007-A. LEIl007-A displayed normal pluripotent stem cell colony morphology, expressed pluripotent stem cell markers, displayed a normal karyotype and differentiated into ectodermal, mesodermal and endodermal germ layer lineages.

Resource table

Unique stem cell line identifier	LEIi007-A
Alternative name(s) of stem cell line	1082ips10
Institution	The University of Western Australia
Contact information of distributor	Dr Samuel McLenachan smclenachan@lei.org.au, Dr. Fred K Chen fredchen@lei.org.au
Type of cell line	iPSC
Origin	Human
Additional origin info	15 years old Female Caucasian
Cell source	Dermal Fibroblasts
Clonality	Clonal
Method of reprogramming	Episomal Vectors
Genetic modification	NO
Type of modification	N/A
Associated disease	Stargardt Macular Dystrophy
Gene/locus	*ABCA4*/1p22.1
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	5/4/18
Cell line repository/bank	N/A
Ethical approval	Human Research Ethics Office, University of Western Australia (RA/4/1/7916)

Resource utility

Recessive mutations in the *ABCA4* gene cause Stargardt disease, the most common form of juvenile macular degeneration. The LEIi007-A iPSC line was generated from a patient with pathogenic compound heterozygous *ABCA4* variants with Stargardt disease phenotype. This iPSC line will aid in elucidating the pathophysiological mechanisms of *ABCA4* disease.

Resource details

ABCA4 gene mutations cause Stargardt disease (STGD1; MIM 248200), an early-onset autosomal recessive degenerative disease that has a prevalence of 1 in 10,000 (Tanna et al., 2017). It is the most common form of inherited retinal disease and varies greatly in clinical presentation and natural history due to the large and growing number of disease-causing *ABCA4* sequence variants identified (Cornelis et al.,

* Corresponding author at: Centre for Ophthalmology and Visual Science, The University of Western Australia, Perth, Western Australia, Australia.
E-mail address: smclenachan@lei.org.au (S. McLenachan).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 Panel A
Phenotype	Qualitative analysis: Immunocytochemistry	Positive for the pluripotency markers: OCT4, NANOG, SOX2, TRA-1-81, KLF4	Fig. 1 Panel C
	Quantitative analysis: RT-qPCR	Positive for the pluripotency markers: *OCT4*, *NANOG*, *SOX2*, *KLF4*	Fig. 1 Panel D
Genotype	Illumina Beadchip SNP Array with CNV Analysis	46XX 500 kbp resolution	Fig. 1 Panel F
Identity		DNA profiling performed	Results table archived with journal
	STR analysis	Matched at 16 loci	Supplementary Fig. S1
Mutation analysis	Sequencing	Compound heterozygous for *ABCA4* c.4139C > T and c.5461-10 T > C	Fig. 1 Panel B
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma – Lookout Mycoplasma PCR Detection Kit	Mycoplasma testing by PCR: Negative	Fig. 1 Panel G
Differentiation potential	Embryoid Body formation: qPCR	Positive for trilineage markers *PAX6*, *DCX*, *DCN*, *GATA2*, *BMP4*, *IGF2*, *AFP*	Fig. 1 Panel E

2017). To provide material for further investigation into the pathophysiological mechanisms of *ABCA4* disease and drug screening, we have generated human induced pluripotent stem cells (iPSCs) from a 15-year-old female patient who developed vision loss and macular flecks at the age of 12 years due to autosomal recessive Stargardt disease (Table 1). Compound heterozygous mutations in *ABCA4* (NM_000350.2) including the common c.5461-10 T > C variant (Sangermano et al., 2016) located in intron 38 (inherited paternally) and the c.4139C > T (p.Pro1380Leu) variant (Fakin et al., 2016) located in exon 28 (inherited maternally) were identified in a Stargardt/Macular Dystrophy next generation sequencing panel (v3).

Dermal fibroblasts were cultured from a skin biopsy sample obtained from the patient. Reprogramming was performed on the patient fibroblasts using non-integrating and non-viral episomal plasmids expressing *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28*, *p53* shRNA, and the miR302/367 cluster. A clonal iPSC cell line (LEIl007-A) was established and further characterized to confirm pluripotency. LEIi007-A displayed typical pluripotent stem cell colony morphology (Fig. 1A). The c.4139C > T (Fig. 1B, top panel) and the c.5461-10 T > C (Fig. 1B, lower panel) mutations were confirmed in LEIi007-A iPSC by PCR and Sanger sequencing. Analysis of 16 microsatellite markers confirmed LEIi007-A was derived from the patient's fibroblasts (Supplementary Fig. S1). Expression of the pluripotency markers OCT4, NANOG, SOX2, KLF4 and TRA-1-81 was demonstrated by immunocytochemistry analyses (Fig. 1C, scale bars indicate 50 μm). Quantitative real time polymerase chain reaction (qPCR) analysis of *OCT4*, *NANOG*, *SOX2* and *KLF4* mRNA expression demonstrated similar levels of expression in LEIi007-A and a control human iPSC line (Fig. 1D). The capacity of LEIi007-A iPSC to differentiate into the three germ layers was demonstrated by the upregulation of ectoderm (*PAX6*, *DCX*), mesoderm (*DCN*, *GATA2*, *BMP4*, *IGF2*) and endoderm (*AFP*) lineage markers in differentiating embryoid body cultures, while *OCT4* expression was downregulated during EB differentiation (Fig. 1E). Genome-wide copy number variation profiling using the Illumina HumanCoreExome-24 Beadchip demonstrated that LEIi007-A had a normal diploid karyotype and was female (46, XX) (Fig. 1F). Analysis of the B allele frequencies (Fig. 1F, left panel) and LogR ratios (Fig. 1F, right panel) of 500,000 single nucleotide polymorphisms located across the genome demonstrated an absence of chromosomal rearrangements in LEIi007-A. The cell line was negative for mycoplasma as determined by using the Lookout Mycoplasma PCR Detection Kit. The 481 bp internal control band was amplified from the LEIi007-A and negative control samples, while the mycoplasma-specific 261 bp band was amplified from a positive control sample, but not the negative control or cell line LEIl007-A (Fig. 1G). Reprogramming episomes could be detected by PCR in passage 1 iPSCs (+) but were not detected in LEIi007 iPSCs after passage 3 (p3, p15, Fig. 1H).

Materials and methods

Ethics

Patient DNA was obtained and genetically analyzed by the Australian Inherited Retinal Disease Registry and DNA Bank, as approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee (2001–053). The collection of patient samples and subsequent iPSC generation was approved by the University of Western Australia Human Research Ethics Committee (RA/4/1/7916). Written consent was obtained from the patient and all procedures were carried out in accordance with the requirements of the National Health & Medical Research Council of Australia and the Declaration of Helsinki.

Cell culture

Patient fibroblasts were cultured in DMEM medium supplemented with 10% fetal calf serum (ThermoFisher). Patient iPSCs were cultured in feeder-free conditions, on geltrex (ThermoFisher) coated culture plates in TeSR-E8 medium (StemCell Technologies). A commercial human iPSC line was used for comparison with LEIi007-A (ThermoFisher, Cat#A18945, Fig. 1D).

Reprogramming

The patient fibroblasts were reprogrammed using the Episomal iPSC Reprogramming Plasmid kit (SC900A-1, System Biosciences), according to the manufacturer's instructions. 1 million patient fibroblast cells were electroporated using the NEON electroporation system (3 × 10ms pulses at 1650 V in a 100 μl tip), plated into 6 wells of a 6 well plate containing DMEM medium supplemented with 10% fetal calf serum and cultured for 7 days. On day 7, the media was changed to TeSR-E8. On day 25, individual iPSC colonies were picked for clonal expansion.

iPSC differentiation

For embryoid body (EB) differentiation, the iPSC were seeded into uncoated culture plates as cell clusters in TeSR-E8 media supplemented with 10 μm Y27632 (Abcam). On day 3, the media was changed to

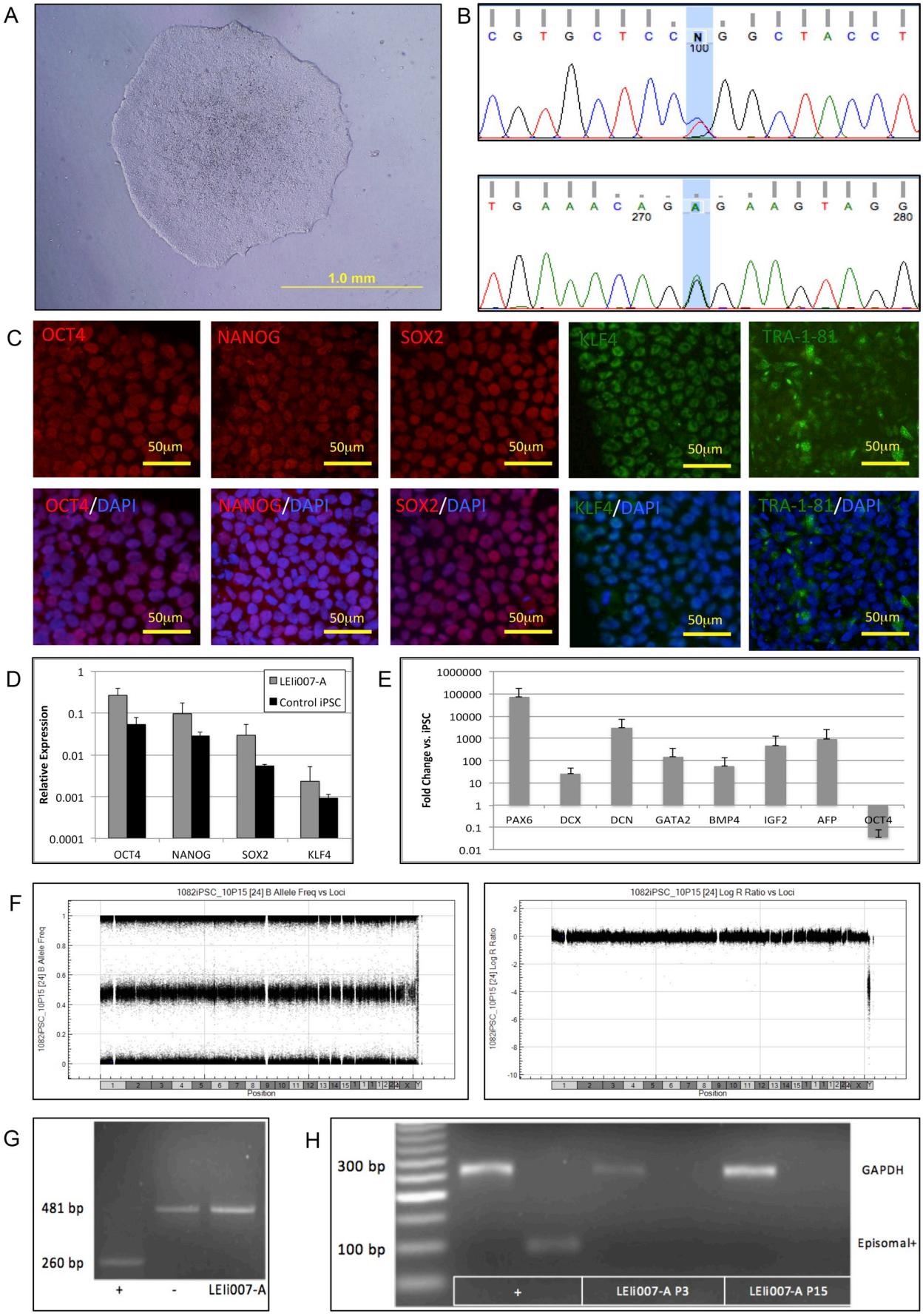

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Fig. 1. A: The LEIi007-A iPSC line displayed morphologies typical of pluripotent stem cell colonies. **B:** Sequencing of the *ABCA4* gene demonstrated the presence of the c.5461-10T > C (top panel) and c.4319C > T (lower panel) variants in LEIi007-A. **C:** The expression of pluripotency markers (OCT4, NANOG, SOX2, KLF4 and TRA-1-81) in LEIi007-A was demonstrated by immunocytochemistry. Nuclei were counterstained with DAPI. **D:** Quantitative RT-PCR demonstrated similar levels of expression of pluripotency genes (*OCT4*, *NANOG*, *SOX2* and *KLF4*) in LEIi007-A (grey bars) and a commercial human iPSC line (black bars). **E:** Expression of ectodermal (*PAX6, DCX*), mesodermal (*DCN, GATA2, BMP4, IGF2*) and endodermal (*AFP*) genes was increased in spontaneously differentiating embryoid bodies derived from LEIi007-A, compared with undifferentiated LEIi007-A iPSC. *OCT4* expression was downregulated in embryoid bodies compared with iPSC. **F:** B-allele frequencies (left panel) and LogR values (right panel) were plotted against genomic location for 500,000 human SNPs, demonstrating a normal, 46, XX karyotype in LEIi007-A. **G:** PCR screening demonstrated LEIi007-A was negative for mycoplasma. Positive (+) and negative (-) controls yielded bands of the expected sizes (260bp and 481bp, respectively). **H:** Reprogramming episomes were detected by PCR in passage 1 (+) iPSC, but were undetectable in the LEIi007-A iPSC line after passage 3 (LEIi007-A P3, LEIi007-A P15). A positive control band was amplified from all samples tested using primers targeting *GAPDH*.

DMEM/F12 supplemented with 20% knockout serum replacement, antibiotic-antimycotic (ThermoFisher) and MEM non essential amino acid solution (StemCell Technologies). Media was changed every other day. On day 14 RNA was harvested from EBs for analysis by qPCR.

Quantitative PCR analysis

Total RNA was isolated from cell culture samples using the TRIZOL reagent and cDNA was subsequently synthesized using the RT^2 First Strand Kit (Qiagen). qPCR was performed using the CFX Connect Real-Time System (BioRad) and data analyzed using the ΔΔCT method. Gene expression values were then normalized to the housekeeping gene GAPDH expression (Fig. 1D). For analysis of EBs, gene expression values were further normalized against undifferentiated LEIi007 iPSC (Fig. 1G). Primers used in the qPCR assays are listed in Table 2.

Immunostaining analysis

Cells were cultured on geltrex-coated coverslips for approximately 5 days, before being fixed with 4% paraformaldehyde. The cells were then washed and permeablized using phosphate buffered saline (PBS) with 0.1% Triton X-100 for 15 min. Cells were then incubated for 1 h at room temperature in blocking buffer (5% BSA in PBS). Primary antibodies were applied at 4 °C overnight. Secondary antibody and DAPI staining then took place the next day at room temperature in the dark for 2 h. The antibodies used and their respective dilutions are listed in Table 2.

Virtual karyotyping and microsatellite analysis

Virtual karyotyping was performed on the line using the Illumina HumanCoreExome-24 Beadchip SNP array. CNV analysis was performed using GenomeStudio 2.0 (Illumina). Microsatellite marker analysis was performed using the Promega PowerPlex 16H system (Australian Genome Research Facility) Supplementary Fig. S1.

Mycoplasma and episomal testing

For performing myocplasma testing the Lookout Mycoplasma PCR Detection Kit (Sigma-Aldrich), was used according to the

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company cat # and RRID
Pluripotency markers	Mouse anti-OCT4	1:200	Stem Cell Technologies Cat# 60093.1, RRID: AB_2561766
	Rabbit anti-NANOG	1:100	Abcam Cat# ab21624, RRID: AB_446437
	Rabbit anti-SOX2	1:200	Thermo Fisher Scientific Cat# 48–1400, RRID:AB_2533841
	Mouse anti-TRA-1-81-Alexa Fluor 488	1:100	Stem Cell Technologies Cat# 60065AD, RRID: AB_1089240
	Rabbit anti-KLF4	1:250	Abcam Cat# ab151733, RRID: AB2721027
Secondary antibodies	Alexa Fluor 546 goat anti-mouse	1:500	Molecular Probes Cat# A-11003, RRID: AB_141370
	Alexa Fluor 488 goat anti-rabbit	1:500	Molecular Probes Cat# A-11008, RRID: AB_143165

Primers		
	Target	Forward/reverse primer (5′-3′)
Episomal plasmid PCR	Reprogramming plasmids	AGGTCCCTCGAAGAGGTTCA/TTCCAACGCGAGAAGGTGTT
Pluripotency markers (qPCR)	*OCT4*	CCTGAAGCAGAAGAGGATCACC/AAAGCGGCAGATGGTCGTTTGG
	NANOG	CTCCAACATCCTGAACCTCAGC/CGTCACACCATTGCTATTCTTCG
	SOX2	GCTACAGCATGATGCAGGACCA/TCTGCGAGCTGGTCATGGAGTT
	KLF4	CATCTCAAGGCACACCTGCGAA/TCGGTCGCATTTTTGGCACTGG
House-keeping genes (qPCR)	*GAPDH*	GTCTCCTCTGACTTCAACAGCG/ACCACCCTGTTGCTGTAGCCAA
Trilineage markers (qPCR)	*PAX6*	CTGAGGAATCAGAGAAGACAGGC/ATGGAGCCAGATGTGAAGGAGG
	DCX	TGCCTCAGGGAGTGCGTTA/GAACAGACATAGCTTTCCCCTTC
	DCN	AGAGTACCTGGTGGGCTGG/GTGGGCAGAAGTCACTTGAT
	GATA2	CTGTCTGCAACGCCTGTG/GTTCCGAGTCTGGATCCCTT
	BMP4	GCACTGGTCTTGAGTATCCTG/TGCTGAGGTTAAAGAGGAAACG
	IGF2	AGACGTACTGTGCTACCCC/TGCTTCCAGGTGTCATATTGG
	AFP	TGAGCACTGTTGCAGAGGAG/TTGTTTGACAGAGTGTCTTGTTGA
Mutation sequencing	*ABCA4* c.4139C > T	CCAGGGGCTGATTAGGAGGC/TGAAGTGGGAAGGTCAGGGC
Mutation sequencing	*ABCA4* c.5461-10 T > C	CCCCAGTCCTTGATTGGTCAT/ACAGAGGCACCCTAATCCTCT

manufacturer's recommendations. Episomal testing was peformed by PCR analysis using primers to detect episomal plasmids within the LEIi007-A cell line (primers and sequences listed in Table 2).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.11.013.

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