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Establishment of the iPSC line CUIMCi005-A from a patient with Stargardt disease for retinal organoid culture

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Abstract

Pathogenic variation in the *ABCA4* gene is the underlying cause of Stargardt disease, the most common inherited retinal degeneration. We established an induced pluripotent stem cell line for retinal organoid research from a patient with mild disease features who is compound heterozygous for the frequent c.5882G>A (p. Gly1961Glu) missense variant and a c.4947delC (p.Glu1650Argfs*12) frameshift variant. Peripheral blood mononuclear cells were reprogrammed using a non-integrating Sendai virus approach. G-banded karyotyping was normal (46, XY) and mycoplasma testing was negative. Immunohistochemistry and RT-qPCR were performed to verify the expression of pluripotency and stemness markers (LIN28, NANOG, OCT4 and SOX2) and trilineage differentiation.

Resource Table

Unique stem cell line identifier	**CUIMCi005-A**
Alternative name(s) of stem cell line	9068
Institution	Columbia University Irving Medical Center (CUIMC)
Contact information of distributor	ps2931@cumc.columbia.edu; rla22@cumc.columbia.edu
Type of cell line	induced pluripotent stem cells (iPSCs)
Origin	human

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102973.

Unique stem cell line identifier	CUIMCi005-A
Additional origin info required for human ESC or iPSC	34-year-old Caucasian man
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Sendai Virus (CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific))
Genetic Modification	Yes
Type of Genetic Modification	Hereditary
Associated disease	Stargardt disease
Gene/locus	*ABCA4*/1p22.1
Date archived/stock date	8/21/2019
Cell line repository/bank	https://hpscreg.eu/cell-line/CUIMCi005-A
Ethics approval	All study procedures were defined under protocols #AAAI9906 and #AAAF1849 approved by the Institutional Review Board at Columbia University Irving Medical Center

1. Resource utility

This hiPSC line is derived from a 34-year-old Caucasian man with Stargardt disease. It was established for the purpose of generating a retinal organoid model system to investigate the cellular mechanism of action of c.5882G>A (p.Gly1961Glu), the major disease causing allele, when mutated in *ABCA4*.

2. Resource details

Autosomal recessive disease-causing variants in the *ABCA4* gene are the underlying cause of a large and complex group of predominantly juvenile-onset retinal dystrophies characterized by progressive loss of central vision.(Allikmets et al., 1997) Over 1,500 disease-causing variants have been identified in patients who exhibit a wide spectrum of clinical phenotypes. The most common disease allele, c.5882G>A (p. Gly1961Glu), is found in ~ 20 % of patients, and underlies a disproportionately mild clinical phenotype due in part to reduced penetrance. (Lee et al., 2021a; Lee et al., 2021b) In this study, a hiPSC line was established from PBMC isolated from the whole blood of a 34-year-old man with mild bilateral loss of central vision, beginning approximately 5 years prior to presentation. Fundus examination and retinal imaging revealed isolated bull's eye maculopathy lesions (Supplemental Fig. 1A) in the central macula that corresponded to degeneration of photoreceptor-attributable layers in the outer retina (Supplemental Fig. 1B). Peripheral blood (10 mL) was collected from the patient by venipuncture and temporarily stored in BD Vacutainer® lavender top tubes containing ethylenediaminetetraacetic acid (EDTA) for sequencing and hiPSC reprogramming. Whole exome sequencing was performed. The c.5882G>A (p.Gly1961Glu) and c.4947delC (p. Glu1650Argfs*12) variants were identified and confirmed by Sanger sequencing in the patient and his unaffected parent and validate the familial segregation of both variants (Supplemental Fig. 1C and 1D).

PBMC's were extracted by from peripheral blood by centrifugation and plated. After 12 days, 2×10^6 cells were collected and hiPSC reprogramming was performed using a non-integrating Sendai virus approach (Yang et al., 2008), while remaining cells were banked. After transduction, isolated colonies exhibiting morphology typical of hiPSC characteristics (Fig. 1A) were observed. Single clones were picked and expanded to generate the hiPSC line CUIMCi005-A for further characterization and organoid culture (Tables 1 and Table 2). G-banded karyotype analysis (Passage 6) was normal (46, XY) (Fig. 1B) and mycoplasma testing was negative (Fig. 1C). RT-qPCR was performed to assess the expression of pluripotency markers *LIN28, NANOG, OCT4* and *SOX2* relative to a previously published iPSC line (Patel et al., 2020) (Fig. 1D). Expression of intracellular (SOX2 and OCT4) and cell surface (SSEA4 and TRA-1-60) stemness/pluripotency markers (Fig. 1G) was also assessed by immunohistochemistry. Clearance of Sendai virus was confirmed (Fig. 1E) and pluripotency and trilineage differentiation potential was assessed by Scorecard (Fig. 1F).

3. Materials and methods

3.1. Human subjects

All study procedures were performed according to protocol #AAAI9906 and #AAAF1849 under the Institutional Review Board at Columbia University Irving Medical Center. The study was conducted according to the tenets outlined in the Declaration of Helsinki.

3.2. Molecular screening

Genomic DNA was extracted from patient's PBMC's and whole exome sequencing was performed at Baylor College of Medicine (Houston, TX) using the SureSelect Human All Exon V8 (Agilent, Santa Clara, CA). Sequence reads were aligned to the hg19 reference genome with BWA, and processed with GATK according to the best practices recommendations. After variant calling, we narrowed our analyses to variants in genes previously associated with retinal disease (https://sph.uth.edu/retnet/) at a minor allele frequency (MAF) ≤ 0.2 based on the gnomAD database (assessed December 2021). Pathogenicity of the c.5882G>A p.(Gly1961Glu) and c.4947delC variants were assessed by prediction algorithms and allele frequencies were obtained from the gnomAD database (https://gnomad.broadinstitute.org/gene/ENSG00000198691/dataset = gnomad_r2_1) (assessed January 2022). Both variants were confirmed in the proband by Sanger sequencing and segregation was verified in an unaffected parent.

3.3. Peripheral blood mononuclear cell isolation

To extract PBMC's, each tube of blood was centrifuged at 1,800 relative centrifugal force (RCF) for 30 min. Isolated cells were grown for several days in Expansion Medium (EM): QBSF-60 (cat# 160204101, Quality Biologicals), antibiotics (Primocin, Invivogen, #ant-pm-1; Pen/Strep, Life Technologies, #15140-155); L-Ascorbic Acid, (Sigma, # A4544-25G); growth factors (EPO, #287-TC-500; IL-3, #203-IL-010/CF; IGF-1, #291-G1-200; SCF, #255-SC-010/CF; R&D Systems) and Dexamethasone (Sigma, #D8893-1MG).

3.4. Reprogramming of peripheral blood mononuclear cells

Approximately 2×10^6 cells were collected, and reprogramming was performed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (cat# A16518, ThermoFisher Scientific), with multiplicity of infection (MOI) 5:5:3, KOS:c-myc: KLF4 according to the manufacturer's instructions. Two days later, infected PBMC's were transferred onto a 6-well plate coated with irradiated mouse embryonic fibroblast (MEF) (Life Technologies, cat #A34181) in DMEM/F12 (Corning) supplemented with 10 % FBS (Life Technologies, cat #10082147), 1x non-essential amino acids, 1x Penicillin-Streptomycin, 1x glutamine, 1x β-Mercaptoethanol (all Life Technologies) and 10 ng/ml FGF2 (R&D Systems). About 12–20 days later, colonies with typical iPSC morphology appeared, which were manually picked and expanded for further analysis.(Yang et al., 2008).

3.5. iPSC maintenance

iPSCs were grown on irradiated MEF (Life Technologies, cat #A34181) in DMEM/F12 (Corning) supplemented with 20 % KnockOut Serum Replacement (KO-SR), 1x non-essential amino acids, 1x Penicillin-Streptomycin, 1x glutamine, 1x β-Mercaptoethanol (all Life Technologies) and 10 ng/ml FGF2 (R&D Systems). Cells were fed daily and split 1:6 when they reached 80 % confluency using EDTA. Established iPSCs were maintained in feeder-free condition using Matrigel (Corning) -coated plates and mTeSR1 or mTeSR Plus medium (Stem Cell Technologies). All cells were kept in an incubator at 37°C, 5 % CO2, 20 % 02.

3.6. Embryoid bodies (EBs) differentiation

Individual EB were generated by dissociating iPSC colonies at passage 10 from one well of a 6-well plate into cell aggregates with a Gentle Cell Dissociation Reagent (Stem Cell Technologies #100–0485). 1×10^6 cells were seeded into one well of an AggreWell™400 24-well Plate (Stem Cell Technologies # 34411). EBs were fed with EB formation medium (Stem Cell Technologies #05893) until Day 7 and subsequently harvested. RNA was then isolated for Scorecard analysis.

3.7. Karyotyping

At passage 6, hiPSC's were examined by standard G-banding analysis (Cell Line Genetics, Madison, WI). Cytogenetic analysis was performed on 20 G-banded metaphase cells at 450–500 band resolution.

3.8. Short tandem repeat profiling

Cell Line ID BB9068, the donor PBMC line for the iPSC line CUIMCi005-A, and CUIMCi005-A at passage 11 were tested by STR profiling for these loci: Amelogenin, D18S51, vWA, Penta E, D8S1179, D5S818, TPOX, D13S317, FGA, D7S820, D3S1358, D16S539, THO1, CSF1PO, D21S11, Penta D (Cell Line Genetics, Madison, WI).

3.9. Mycoplasma detection

Absence of mycoplasma contamination was confirmed using e- Myco plus Mycoplasma PCR Detection Kit (Intron, Burlington, MA, #25234) according to manufacturer's instructions. iPSCs were tested at passage 6.

Real-time quantitative polymerase chain reaction (RT-qPCR).

Total RNA was isolated from 1×10^6 iPSCs at passage 10 using RNeasy Mini Kit (cat# 74104, Qiagen), and cDNA was generated by reverse transcription using the cDNA Synthesis Kit (cat# 11754050, Invitrogen™). The independently generated control iPSC line FA0000010 was used as positive control for the expression of exogenous genes (passage 32) (Patel et al., 2020). Gene expression level were normalized to the internal control Actin. Primer sequences are shown in (Table 2).

3.10. Scorecard analysis

Expression levels of marker genes responsible for self-renewal and trilineage (endoderm, mesoderm and ectoderm) potential were measured using the Taqman iPSC scorecard assay according to the manufacturer's protocol (ThermoFisher Scientific, Waltham, MA). Results were analyzed using the hPSC Scorecard Analysis Software (https://www.thermofisher.com/us/en/home/life-science/stem-cell-research/taqman-hpsc-scorecard-panel/scorecard-software.html).

3.11. Immunostaining

Human iPSCs at passage 10 were seeding on chamber slides (cat# 154534, ThermoFisher Scientific), then fixed with 4 % PFA (cat# 15710, Electron Microscopy Sciences) for 10 min at room temperature (RT). Cells were then permeabilized for 30 min at RT with 0.1 % Triton X-100 after washing three times with 1x PBS. Cells were incubated with 10 % donkey serum (cat# S30-M, MilliporeSigma) for 1 h at room temperature (25 °C), then incubated overnight at 4 °C with primary antibodies (Table 2) in 10 % donkey serum. Slides were washed twice with 1x PBS the next day, and then secondary antibody in 10 % donkey serum was applied (Table 2) for 1hr at RT. Nuclei were stained for 15 min with Hoechst 33,342 (cat# H1399, Thermo Scientific).

3.12. Sendai virus (SeV) detection

Total RNA isolation and cDNA synthesis were performed from iPSCs at passage 14. The RT-qPCR was performed on a QuantStudio 7 Flex system (Applied Biosystems). Cycle parameters were 30 cycles of denaturation at 95 °C for 60 s, annealing at 60 °C for 60 s and extension at 72 °C for 30 s.

4. Funding resources

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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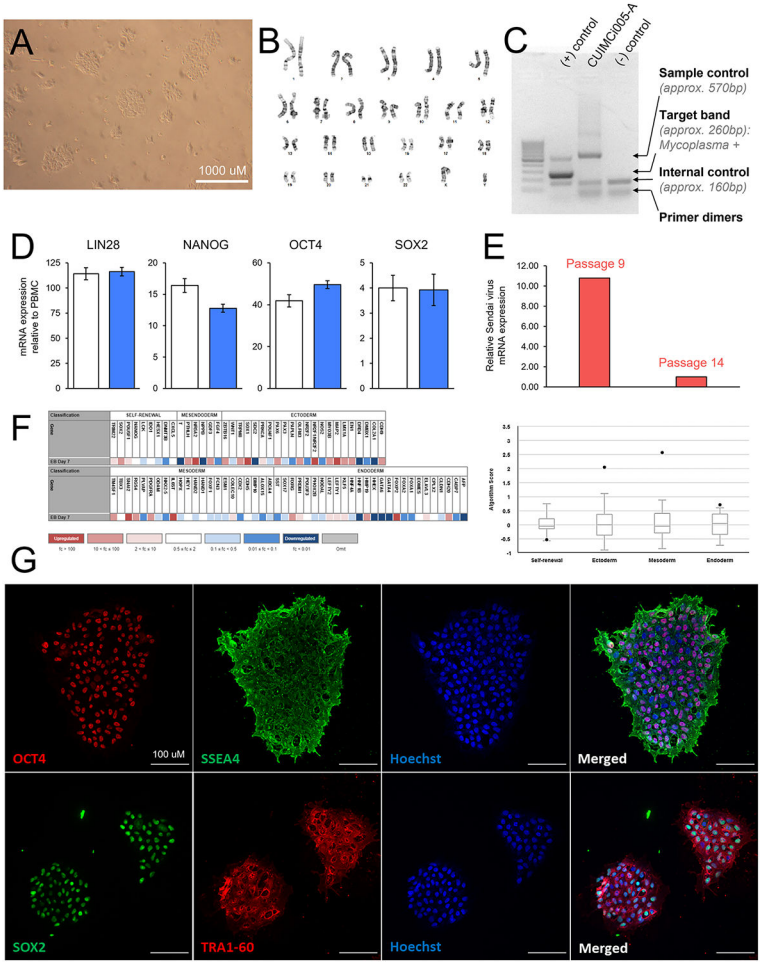

Fig. 1.
Characterization of CUIMCi005-A hiPSC line iPSC line.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field microscopy	Typical hiPSC morphology	Fig. 1A
Genotype	Karyotype (G-banding resolution 450–500)	46, XY (*P*6)	Fig. 1B
Mutarion analysis	Sequencing	*ABCA4* c.5882G>A (p. Gly1961Glu); c.4947delC (p.Glu1650Argfs*12)	submitted in archive with journal and available with authors
Microbiology and virology	Mycoplasma	PCR, Negative	Fig. 1C
Identity	STR analysis	DNA Profiling Performed	submitted in archive with journal and available with authors
Phenotype	Quantitative analysis (RT-qPCR)	Assess relative mRNA expression of pluripotency/ stemness markers: LIN28, NANOG, OCT4 and SOX2	Fig. 1D
	Qualitative analysis (Immunostaining)	Assess staining of intracellular and surface expression of pluripotency/stemness markers: OCT4, SSEA4, SOX2, and TRA-1-60	Fig. 1G
	Quantitative analysis (RT-qPCR)	Sendai clearance assessment	Fig. 1E
Differentiation potential	Three germ layer differentiations	TaqMan hPSC Scorecard and algorithm scores	Fig. 1F

Table 2

Reagent details.

Antibodies used for immunocytochemistry			
	Antibody	***Dilution***	***Company Cat # and RR1D***
Pluripotent Marker	Rabbit anti-OCT4	1:1000	ThermoFisher Scientific Cat# PA5-27438 and AB_2544914
	Rabbit anti-SOX2	1:500	ThermoFisher Scientific Cat# PA1-094 and AB_2539862
	Mouse anti-SSEA4	1:500	ThermoFisher Scientific Cat# 41–4000 and AB_2533506
	Mouse anti-TRA-1–60	1:200	ThermoFisher Scientific Cat# MA1-023 and AB_2536699
Secondary antibody	Goat anti-Rabbit IgG Alexa Fluor 488	1:1000	ThermoFisher Scientific Cat# A-11070 and AB_2534114
	Goat anti mouse IgM Alexa Fluor 488	1:1000	ThermoFisher Scientific Cat# A-11017 and AB_2534084
	Goat anti-Rabbit IgG Alexa Fluor 555	1:1000	ThermoFisher Scientific Cat# A-21430 and AB_2535851
	Goat anti mouse IgM Alexa Fluor 555	1:1000	ThermoFisher Scientific Cat# A-21425 and AB_2535846
Primers			
Primers (RT-qPCR)	***Target***	***Product size***	***Sequence***
Transgene detection	LIN28 F’	203 bp	5′- AGT GGT TCA ACG TGC GCA TGG G −3′
	LIN28 R’		5′- AGG TCC GGT GAC ACG GAT GGA T −3′
	NANOG F’	158 bp	5′- CAA AGG CAA ACA ACC CAC TT −3′
	NANOG R’		5′- TCT GCT GGA GGC TGA GGT AT −3′
	OCT4 F’	324 bp	5′- CGA GCA ATT TGC CAA GCT CCT GAA −3′
	OCT4 R’		5′- TTC GGG CAC TGC AGG AAC AAA TTC −3′
	SOX2 F’	448 bp	5’- CCC CCG GCG GCA ATA GCA −3′
	SOX2 R’		5′- TCG GCG CCG GGG AGA TAC AT −3′
Transgene housekeeping	Human β-actin F’	184 bp	5′- AGA GCT ACG AGC TGC CTG AC −3′
	Human β-actin R’		5′-AGC ACT GTG TTG GCG TAC AG −3′
Sendai virus detection	SeV F’	181 bp	5′-GGATCACTAGGTGATATCGAGC-3′
	SeV R’		S′-ACCAGACAAGAGTTTAAGAGATATGTATC-3′
Targeted mutation sequencing	ABCA4 exon 35F’	336 bp	5′-CCTTTCCTTCACTGATTTCTGC 3′
	ABCA4 exon 35 R’		5′-AATCAGCACTTCGCGGTG-3′
	ABCA4 exon 42F’	214 bp	5′-CTCCTAAACCATCCTTTGCTC-3′
	ABCA4 exon 42 R’		5′-AGGCAGGCACAAGAGCTG-3′