Lab resource: Stem Cell Line

Generation of human iPSC line (UCLi013-A) from a patient with microphthalmia and aniridia, carrying a heterozygous missense mutation c.372C>A p.(Asn124Lys) in PAX6

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ABSTRACT

A human induced pluripotent stem cell (hiPSC) line (UCLi013-A) was generated from fibroblast cells of a 34-year-old donor with multiple ocular conditions including severe microphthalmia and aniridia. The patient had a heterozygous missense mutation in PAX6 c.372C>A, p.(Asn124Lys), validated in the fibroblasts through Sanger sequencing. Fibroblasts derived from a skin biopsy were reprogrammed using integration free episomal reprogramming. The established iPSC line was found to express pluripotency markers, exhibit differentiation potential in vitro and display a normal karyotype. This cell line will act as a tool for disease modelling of microphthalmia and aniridia, identification of therapeutic targets and drug screening.

1. Resource table

| Unique stem cell line identifier | UCLi013-A |
| Alternative name(s) of stem cell line | PAX6 p.Asn124Lys |
| Institution | UCL Institute of Ophthalmology |
| Contact information of distributor | Mariya Moosajee (m.moosajee@ucl.ac.uk) |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info | Age: 34 |
| | Sex: Female |
| | Ethnicity if known: White – Caucasian |
| Cell Source | Dermal fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Episomal plasmid |
| Genetic Modification | Yes |
| Type of Modification | Congenital |
| Associated disease | Microphthalmia |
| | Aniridia/iris hypoplasia |
| | Cataracts |
| | Optic nerve coloboma |
| | Nystagmus |
| Type 2 diabetes |
| Gene/locus | Heterozygous PAX6 c.372C>A, p.(Asn124Lys)/11p13 |

(continued)

2. Resource utility

The iPSC line UCLi013-A was established after reprogramming of fibroblasts isolated from a female individual with severe microphthalmia, aniridia and other ocular disorders caused by a heterozygous missense mutation in PAX6, c.373C>A p.(Asn124Lys). This line provides a valuable resource for in vitro eye development studies, disease modelling and drug screening.

3. Resource details

PAX6 (OMIM:607108) is a highly conserved transcriptional regulator of oculogenesis (Lima Cunha et al., 2019). Switched on early in eye
development, PAX6 is expressed throughout the optic vesicle by 5 weeks development. Pathogenic heterozygous variants in PAX6 cause a variety of ocular disorders including microphthalmia (small eye), aniridia (absent iris), cataracts (clouded lens), nystagmus (uncontrolled eye movement) and coloboma (gap in eye structure).

An iPSC line was derived from fibroblasts of a 34-year-old female with severe microphthalmia, aniridia, cataracts, optic nerve coloboma and nystagmus, and genetically diagnosed with a heterozygous missense mutation in PAX6 c.372C> A p.(Asn124Lys) (Table 1). Missense mutations in DNA binding domains of PAX6, including p.(Asn124Lys), can result in reduced DNA binding ability (Williamson et al., 2019). Patients carrying this mutation exhibit severe microphthalmia, alongside complex ocular features phenocopying SOX2-associated microphthalmia syndrome, including iris defects, coloboma, congenital corneal opacification and lens defects.

hiPSCs provide a resource to investigate congenital human diseases, such as microphthalmia and aniridia, which affect early eye development so are otherwise inaccessible to study. Generation of patient-derived iPSCs with known PAX6 mutations may improve understanding of PAX6 function in eye development through in vitro human disease modelling. Consequently, researchers can clarify the molecular basis of aniridia (through modelling iris and optic nerve development), in addition to microphthalmia pathogenesis (by replicating early eye development). Additionally, these models may elucidate genotype/phenotype relationships observed in PAX6 patient cohorts, thereby improving diagnosis and management, and aiding development of novel treatments.

With ethical approval, a skin biopsy was taken and fibroblasts derived. DNA was extracted from fibroblasts, and the variant c.372C>A in PAX6 exon 7 was confirmed by Sanger sequencing (Fig. 1A). Fibroblasts were reprogrammed into iPSCs using non-integrating episomal plasmids encoding the reprogramming factors OCT4, KLF4, SOX2, L-MYC and LIN28 as well as transient transcription enhancer EBNA (Table S1) (Parfitt et al., 2016). Embryonic stem cell-like colonies were picked, and three iPSC clones were expanded and characterised for pluripotency.

### Table 1

| Classification          | Test                        | Result          | Data |
|-------------------------|-----------------------------|-----------------|------|
| **Morphology**          | **Photography**             | Normal          | Fig. 1 panel B |
| **Phenotype**           | **Qualitative analysis:**   | Positive for   | Fig. 1 panel D |
|                         | **Immunocytochemistry**     | pluripotency    |                  |
|                         |                            | markers OCT4   |                  |
|                         |                            | and SSEA3       |                  |
| **Genotype**            | **Low-pass whole genome**  | 46XX            | Fig. 1 panel G  |
| **Identity**            | **Microsatellite PCR**      | N/A             | N/A |
|                         | (mPCR)                      |                 |                  |
|                         | **STR analysis**            | 16 STR analyzed, all matched | Submitted to journal |
| **Mutation analysis**   | **Sequencing**              | Heterozygous missense mutation PAX6 c.372C>A, p.(Asn124Lys) | Fig. 1 panel A |
| **Microbiology and virology** | **Southern Blot OR WGS** | N/A             | N/A |
|                         | **Mycoplasma**              | Mycoplasma testing by MycoAlert™ | Supplementary Table S2 |
|                         | **Mycoplasma testing**      |                 |                  |
|                         | **Detection Kit (Lonza):**  |                 |                  |
|                         | **Negative**                |                 |                  |
| **Differentiation potential** | **Embryoid body formation** | Positive for three germ layer markers: endoderm marker AFP, mesoderm marker Vimentin (VIM) and ectoderm marker PAX6 | Fig. 1 panel F |
| **Donor screening**     | **HIV 1 + 2 Hepatitis B, Hepatitis C** | N/A             | N/A |
| **Genotype additional info** | **HLA tissue typing** | N/A             | N/A |

4. Materials and methods

#### 4.1. Fibroblast derivation and culture

Skin biopsies were placed in 400 μL digestion media (DMEM pyruvate/high glucose, GlutaMAX, 20% fetal bovine serum (FBS), 1% penicillin/streptomycin, 0.25% Collagenase I and 0.05% DNase I), incubated overnight, then plated in derivation media (DMEM, 20% FBS, penicillin/streptomycin). Fibroblasts were cultured in fibroblast media (DMEM, 15% FBS, penicillin/streptomycin) and passaged with TrypLE Express (Gibco).

#### 4.2. Validation of mutation

DNA was extracted using QIAamp DNA Micro Kit (Qiagen). PAX6 exon 7 was amplified by MyTag PCR (Bioline) with designed primers (Sigma Aldrich) (Table 2) (Ye et al., 2012). Mutation was confirmed by Sanger sequencing.

#### 4.3. Fibroblast reprogramming and iPSC culture

1 x 10^6 fibroblast cells were electroporated (1700 V, 20 ms, 1 pulse) with 1 μg of each episomal plasmid (Table S1) using the Neon Transfection System (Parfitt et al., 2016). Transfected cells were plated in fibroblast media with 0.5 mM sodium butyrate on 0.1% gelatin-coated 100 mm dishes for 7 days. Cells were dissociated with TrypLE Express and 200,000 cells plated into each well of a Matrigel-coated (Corning) 6-well plate in mTeSR Plus (Stemcell). Colonies were picked manually for the first 4 passages, then passaged using ReLeSR (Stemcell) at 70% confluence.
4.4. Alkaline phosphatase staining

Cells were stained using StemAb Alkaline Phosphatase Staining Kit II (Reprocell).

4.5. Immunocytochemistry

Cells were fixed using 4% PFA for 20 min at 4°C, permeabilized and blocked for 1 h using 10% normal goat serum (NGS) and 0.1% Triton X-100 in PBS at RT. Cells were incubated for 1 h at RT with primary antibodies diluted in 1% NGS (Table 2). Secondary antibodies and DAPI were added for 1 h at RT (Table 2). Cells were imaged using the EVOS M7000 Imaging System.

4.6. qRT-PCR

RNA was extracted from cell pellets using RNeasy Mini Kit (Qiagen) and 1 μg of cDNA synthesised using SuperScript III First-Strand Synthesis kit (Invitrogen). qRT-PCR was performed using SYBR green mastermix (Applied Biosystems), run on the StepOne Plus RealTime PCR System (Thermo Fisher) using standard cycle conditions (Table 2). The relative expression of each target gene was normalised to housekeeper GAPDH and compared to fibroblast expression using the comparative CT method.

4.7. Embryoid body mediated spontaneous in vitro differentiation

Embryoid bodies were formed by cell dissociation with ReLeSR and culturing in Aggrewell media (Stemcell) supplemented with 10 μM Y27632 for 7–10 days. Embryoid bodies were plated in 0.1% gelatin-coated plates for 11–15 days, where embryoid bodies attached and spontaneously differentiated. Cells were fixed and immunostained for AFP, Vimentin and PAX6 (Table 2).

4.8. Low-pass whole genome sequencing and STR analysis

DNA was extracted using QIAamp DNA Micro Kit (Qiagen). Low-pass WGS libraries were produced using the Illumina DNA Prep library prep kit and sequenced on the Illumina HiSeq 4000 with paired 100 bp reads. After alignment, copy number estimation was performed using the QDNASeq package (Scheinin et al., 2014). Short Tandem Repeat (STR) profiling of 16 sites was obtained for iPSC and fibroblast lines with the Promega PowerPlex16HS system and compared to any commercial cell banks (such as ATCC).

4.9. Mycoplasma testing

Absence of Mycoplasma contamination was confirmed using MycoAlert™ Mycoplasma Detection Kit (Lonza).

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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.

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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.2021.102184.

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Table 2

Reagents details.

| Antibody Type | Antibody | Dilution | Company Cat # and RRID |
|--------------|----------|----------|------------------------|
| Pluripotency Markers | Mouse anti-OCT4 | 1:100 | Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051 |
| | Rat anti-SSEA3 | 1:50 | Millipore Cat# MAB4303, RRID: AB_177628 |
| Differentiation Markers | Mouse anti-AFP | 1:300 | Santa Cruz Biotechnology Cat# sc-51506, RRID: AB_626514 |
| | Mouse anti-VIM | 1:250 | Santa Cruz Biotechnology Cat# sc-6260, RRID: AB_626437 |
| | Rabbit anti-PAX6 | 1:100 | Covance Cat# PRB-278P, RRID: AB_291612 |
| Secondary antibodies | Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | 1:400 | Thermo Fisher Scientific Cat# A-21235, RRID: AB_2535804 |
| | Goat anti-Rat IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | 1:400 | Thermo Fisher Scientific Cat# A-11066, RRID: AB_2534074 |
| | Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | 1:400 | Thermo Fisher Scientific Cat# A23731, RRID: AB_2635280 |
| | Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | 1:400 | Thermo Fisher Scientific Cat# A-10011, RRID: AB_2534069 |

Primer details.

| Target | Forward/Reverse primer (5′-3′) |
|--------|--------------------------------|
| OCT4   | CCCAGGGCCCCATTTTGTGACC/ACCTCAGTTTGGAATGCTGAGGAGGC |
| SOX2   | TCTCACATGCCAGACACTTACGACA/TCACATGTTGAGAGGGCAGTGGC |
| LIN28  | AGGATATGGAGGTCCAGAGGAGGAGGTGGC/ACGATATGGAGCTCTAGTTGGG |
| L-MYC  | GGGGGGAGACCAAGGGCGGTGTCGCC/CAAGGGGGTAGCTGGCGGTTG |

Primer details.

| Target | Forward/Reverse primer (5′-3′) |
|--------|--------------------------------|
| GAPDH  | ACAGTTGGCATGTGACAC/TTTTGGTTGAGGAGCAG |
| PAX6   | TTACCTGGTAGGTTGCC/GCTGGGAGCTTTTAACGGG |