



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

Philippa Harding<sup>a</sup>, Dulce Lima Cunha<sup>a</sup>, Cécile Méjécase<sup>a,b</sup>, Jonathan Eintracht<sup>a</sup>, Lyes Toualbi<sup>a,b</sup>, Hajrah Sarkar<sup>a,b</sup>, Mariya Moosajee<sup>a,b,c,d,\*</sup>

<sup>a</sup> UCL Institute of Ophthalmology, London, UK<sup>b</sup> The Francis Crick Institute, London, UK<sup>c</sup> Moorfields Eye Hospital NHS Foundation Trust, London, UK<sup>d</sup> Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

## 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<br>Sex: Female<br>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<br>Aniridia/iris hypoplasia<br>Cataracts<br>Optic nerve coloboma<br>Nystagmus<br>Type 2 diabetes |
| Gene/locus                            | Heterozygous <i>PAX6</i> c.372C>A, p.(Asn124Lys)/11p13  |

(continued on next column)

(continued)

|                                 |   |
|---------------------------------|---|
| Method of modification          | N/A   |
| Name of transgene or resistance | N/A   |
| Inducible/constitutive system   | N/A   |
| Date archived/stock date        | N/A   |
| Cell line repository/bank       | N/A   |
| Ethical approval                | 11/LO/243 NRES study of congenital eye diseases |

## 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

\* Corresponding author.

E-mail address: [m.moosajee@ucl.ac.uk](mailto:m.moosajee@ucl.ac.uk) (M. Moosajee).<https://doi.org/10.1016/j.scr.2021.102184>

Received 17 November 2020; Received in revised form 16 December 2020; Accepted 12 January 2021

Available online 18 January 2021

1873-5061/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

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

**Table 1**  
Characterization and validation.

| Classification            | Test  | Result   | Data                   |
|---------------------------|---|--|------------------------|
| Morphology                | Photography   | Normal   | Fig. 1 panel B         |
| Phenotype                 | Qualitative analysis: Immunocytochemistry           | Positive for pluripotency markers OCT4 and SSEA3   | Fig. 1 panel D         |
|                           | Qualitative analysis: Alkaline phosphatase activity | Visible activity   | Fig. 1 panel C         |
|                           | Quantitative analysis: qRT-PCR                      | Expression of <i>OCT4</i> , <i>SOX2</i> , <i>L-MYC</i> and <i>LIN28</i> in A101, E101 and F101 clones, and absence of expression in fibroblasts (FB) | Fig. 1 panel E         |
| Genotype                  | Low-pass whole genome                               | 46XX   | Fig. 1 panel G         |
| Identity                  | Microsatellite PCR (mPCR)                           | N/A  | N/A                    |
|                           | STR analysis  | 16 STR analyzed, all matched   | Submitted to journal   |
| Mutation analysis         | Sequencing  | Heterozygous missense mutation <i>PAX6</i> 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™ Mycoplasma Detection Kit (Lonza): Negative  | Supplementary Table S2 |
| Differentiation potential | Embryoid body formation                             | Positive for three germ layer markers: endoderm marker AFP, mesoderm marker Vimentin (VIM) and ectoderm marker <i>PAX6</i>                           | Fig. 1 panel F         |
| Donor screening           | HIV 1 + 2 Hepatitis B, Hepatitis C                  | N/A  | N/A                    |
| Genotype additional info  | Blood group genotyping                              | N/A  | N/A                    |
|                           | HLA tissue typing                                   | N/A  | N/A                    |

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. iPSC morphology was examined showing flat, compact colonies and cells with cobblestone appearance and large nuclei to cytoplasmic ratio (Fig. 1B). iPSCs were stained for alkaline phosphatase activity (Fig. 1C) and pluripotency markers OCT4 and SSEA3 (Fig. 1D). Gene expression of pluripotency markers *OCT4*, *SOX2*, *L-MYC* and *LIN28* was validated using qRT-PCR analysis, which showed upregulation of these markers compared to fibroblast controls (Fig. 1E). *In vitro* differentiation ability after embryoid body formation showed positive staining for all three germ layers, using endoderm marker AFP, mesoderm marker Vimentin (VIM) and ectoderm marker *PAX6* (Fig. 1F). Low-pass whole genome sequencing analysis of iPSCs revealed a normal female 46,XX karyotype (Fig. 1G). Genetic signature identity of fibroblasts and iPSCs was confirmed through STR analysis (submitted to journal). Absence of Mycoplasma was confirmed in iPSCs (Table S1).

In conclusion, iPSCs were generated from a patient with a heterozygous c.372C>A p.(Asn124Lys) missense mutation in *PAX6*, associated with severe microphthalmia, aniridia and other ocular disorders. This iPSC line could be used as a resource for disease modelling, therapeutic target identification and drug screening for various ocular conditions.

## 4. Materials and methods

### 4.1. Fibroblast derivation and culture

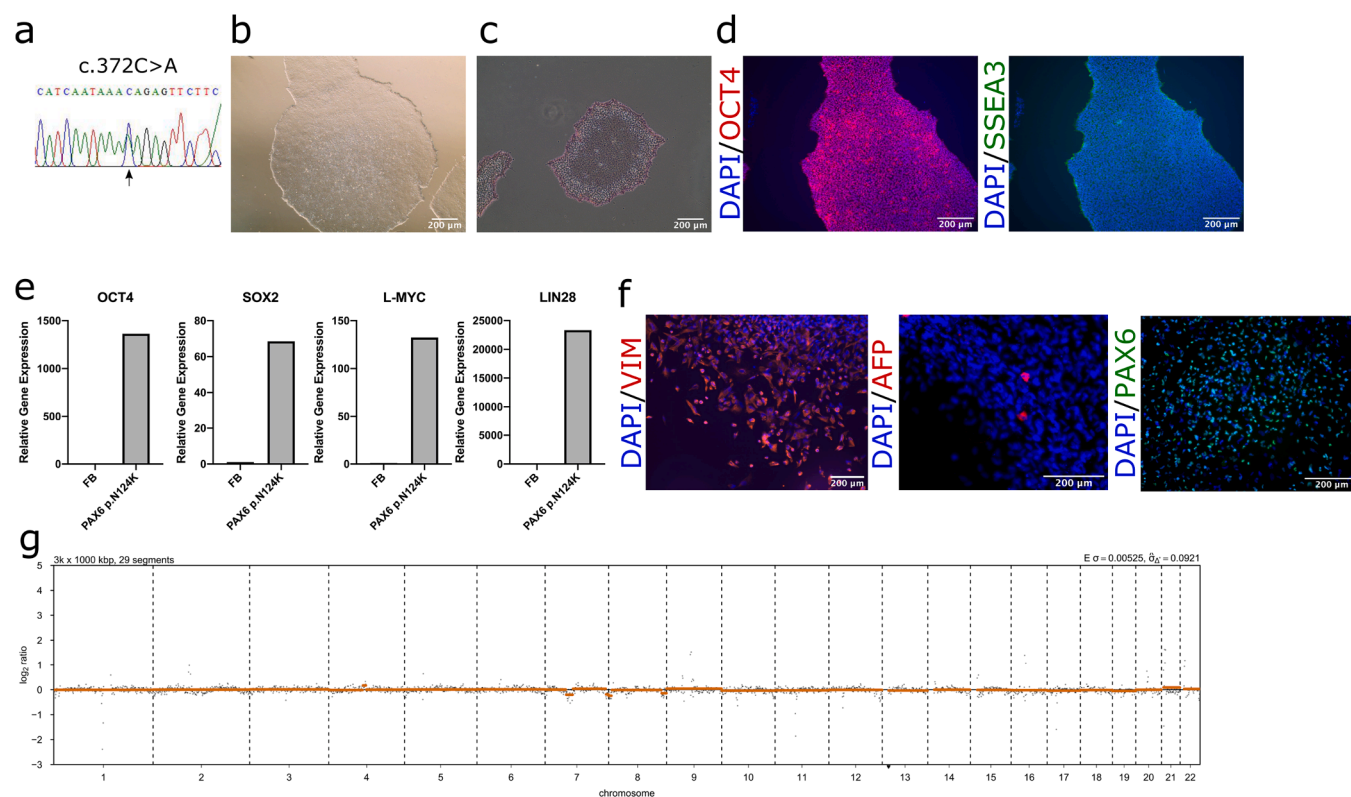
Skin biopsies were placed in 400 µL digestion media (DMEM pyruvate/high glucose, GlutaMAX, 20% fetal bovine serum (FBS), 1% penicillin/streptomycin, 0.25% Collagenase I and 0.05% DNase I), incubated overnight, then plated in derivation media (DMEM, 20% FBS, penicillin/streptomycin). Fibroblasts were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

$1 \times 10^6$  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% confluency.



**Fig. 1.** Characterisation of iPSC line PAX6 p.Asn124Lys (UCLi013-A) generated from dermal fibroblasts of a female patient with severe microphthalmia, aniridia and other ocular disorders. (a) Sanger sequencing trace of PAX6 DNA showing heterozygous c.372C>A mutation. (b) Brightfield images of healthy iPSC colonies. (c) Alkaline phosphatase activity in healthy iPSCs. (d) Immunofluorescent staining of cells expressing pluripotency markers SSEA3 (green) and OCT4 (red), with nuclear DAPI stain (blue). (e) Quantitative PCR analysis of stem cell markers OCT4, SOX2, L-MYC and LIN28. CT values normalised to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), housekeeping gene. (f) Immunofluorescent staining of cells derived from embryoid body differentiation expressing markers of three germ layers: endoderm marker AFP (red), mesoderm marker Vimentin (VIM) (red) and ectoderm marker PAX6 (green). (g) Low-pass whole genome sequencing displaying normal karyotype.

#### 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).

#### Funding

This research was funded by The Wellcome Trust, grant number 205174/Z/16/Z; and Moorfields Eye Charity.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial

**Table 2**  
Reagents details.

| Antibodies used for immunocytochemistry |  |  |   |
|---|--|--|---|
|   | 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_628437  |
|   | 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-11006, RRID: AB_2534074 |
|   | Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | 1:400  | Thermo Fisher Scientific Cat# A32731, RRID: AB_2633280  |
|   | Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488         | 1:400  | Thermo Fisher Scientific Cat# A-10011, RRID: AB_2534069 |
|   |  |  |   |
| Primers                                 |  |  |   |
|   | Target   | Forward/Reverse primer (5'-3')                               |   |
| Pluripotency Markers (qRT-PCR)          | OCT4   | CCCCAGGGCCCCATTTTGGTACC/<br>ACCTCAGTTTGAATGCATGGGAGAGC       |   |
|   | SOX2   | TTCACATGTGCCAGCACTACCGA/<br>TCACATGTGTGAGAGGGGCGAGTGTGC      |   |
|   | LIN28  | AGCCATATGGTAGCCTCATGTCCGC/<br>TCAATTCTGTGCCTCCGGGAGCAGGGTAGG |   |
|   | L-MYC  | GCGAACCAAGACCCAGGCTGCTCC/<br>CAGGGGGTCTGCTCGCACCGTGATG       |   |
|   |  |  |   |
| House-Keeping Genes (qRT-PCR)           | GAPDH  | ACAGTTGCCATGTAGACC/<br>TTTTTGGTTGAGCACAGG                    |   |
| Targeted mutation sequencing            | PAX6   | TTACCTTGCGTAGGTTGCCC/<br>GCTGGGAGCTTTTAAACGGG                |   |

interests or personal relationships that could have appeared to influence

the work reported in this paper.

## Acknowledgments

Mariya Moosajee gratefully acknowledges the support of the Wellcome Trust, Moorfields Eye Charity and National Institute for Health Research (NIHR) Biomedical Research Centre based at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology. The authors also wish to thank the patients for their contribution and support of this study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2021.102184>.

## References

- Lma Cunha, Duluze, Arno, Gavin, Corton, Marta, Moosajee, Mariya, 2019. The spectrum of PAX6 mutations and genotype-phenotype correlations in the eye. *Genes* 10 (12), 1050. <https://doi.org/10.3390/genes10121050>.
- Parfitt, David A., Lane, Amelia, Ramsden, Conor M., Carr, Amanda-Jayne, Munro, Peter M., Jovanovic, Katarina, Schwarz, Nele, et al., 2016. Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. *Cell Stem Cell* 18 (6), 769–781. <https://doi.org/10.1016/j.stem.2016.03.021>.
- Scheinin, Ilari, Sie, Daoud, Bengtsson, Henrik, Van De Wiel, Mark A., Olshen, Adam B., Van Thuijl, Hinke F., Van Essen, Hendrik F., et al., 2014. DNA copy number analysis of fresh and formalin-fixed specimens by shallow whole-genome sequencing with identification and exclusion of problematic regions in the genome assembly. *Genome Res.* 24 (12), 2022–2032. <https://doi.org/10.1101/gr.175141.114>.
- Williamson, Kathleen A., Nikki Hall, H., Owen, Liusaidh J., Livesey, Benjamin J., Hanson, Isabel M., Adams, G.G.W., Bodek, Simon, et al., 2019. Recurrent heterozygous PAX6 missense variants cause severe bilateral microphthalmia via predictable effects on DNA–protein interaction. *Genet. Med.* <https://doi.org/10.1038/s41436-019-0685-9>.
- Ye, Jian, Coulouris, George, Zaretskaya, Irena, Cutcutache, Ioana, Rozen, Steve, Madden, Thomas L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13 (1), 134. <https://doi.org/10.1186/1471-2105-13-134>.