Generation of two human iPSC lines from patients with autosomal dominant retinitis pigmentosa (UCLi014-A) and autosomal recessive Leber congenital amaurosis (UCLi015-A), associated with RDH12 variants

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**Abstract**

Induced pluripotent stem cell (iPSC) lines were generated from two patients with RDH12 variants. UCLi014-A is from a patient with heterozygous frameshift mutation c.759del p.(Phe254Leufs*24), associated with autosomal dominant retinitis pigmentosa. UCLi015-A is from a patient with homozygous missense mutation c.619A > G p.(Asn207Asp), associated with Leber congenital amaurosis. Fibroblasts were derived from skin biopsies and reprogrammed using integration free episomal reprogramming plasmids. The iPSC lines expressed pluripotency markers, exhibited differentiation potential \textit{in vitro} and displayed normal karyotypes. These cell lines will act as a tool for disease modelling, enabling comparison of disease mechanisms, identification of therapeutic targets and drug screening.

**1. Resource table**

| Unique stem cell lines identifier | Unique cell line name 1 - UCLi014-A |
|---------------------------------|-------------------------------------|
| Alternative names of stem cell lines | Optional name from cell line 1 - RDH12 AD |
| Institution | UCL Institute of Ophthalmology |
| Contact information of distributor | Mariya Moosajee (m.moosajee@ucl.ac.uk) |
| Type of cell lines | iPSC |
| Origin | Human |
| Cell Source | Fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Episomal plasmid |
| Multiline rationale | Mutations in the same gene |
| Gene modification | No |
| Type of modification | N/A |
| Associated disease | UCLi014-A – Autosomal dominant retinitis pigmentosa |
| Gene/locus | Gene: RDH12 |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |

(continued on next column)

**2. Resource utility**

Autosomal dominant variants in RDH12 are associated with mild retinitis pigmentosa, and autosomal recessive variants are associated with Leber congenital amaurosis. The iPSC lines generated can be used to create disease models, enabling comparison of disease mechanisms between the two conditions and identification of therapeutics targets.

**3. Resource details**

Variants in the retinol dehydrogenase 12 (RDH12) gene are commonly associated with Leber congenital amaurosis (LCA), a severe retinal dystrophy characterised by night blindness, nystagmus and central loss of vision in early childhood, eventually leading to complete blindness in adulthood (Fahim et al., 2019). However, in rare cases,
heterozygous variants in \textit{RDH12} have been associated with an autosomal dominant late onset mild retinitis pigmentosa phenotype, characterised by nyctalopia and visual field loss, but relatively preserved central vision (Fingert et al., 2008, Sarkar et al., 2020). \textit{RDH12} is an NADPH-dependent retinal reductase, expressed in the inner segments of photoreceptors. Loss of functional \textit{RDH12} is thought to result in build-up of toxic retinoids, although the exact disease mechanisms are not yet fully understood (Sarkar and Moosajee, 2019). Induced pluripotent stem cells (iPSCs) provide a useful resource to investigate inherited retinal dystrophies in cell types that would otherwise be inaccessible for study. iPSCs derived from patients with \textit{RDH12} variants can be used to create retinal organoids to study the differences in disease mechanisms between autosomal dominant and autosomal recessive mutations. Understanding the molecular pathogenesis of \textit{RDH12}-related retinopathies will enable the identification of therapeutic targets and development of novel therapies.

Two iPSC lines were generated from patients with mutations in \textit{RDH12}. The first (UCLi014-A) is from a 32-year old male with autosomal dominant retinitis pigmentosa, carrying a heterozygous frameshift mutation c.759del p.(Phe254Leufs*24). This variant is predicted to result in premature termination and expression of a truncated protein. The second (UCLi015-A) is from a 40 year old female with Leber congenital amaurosis, carrying a homozygous missense mutation c.619A > G p. (Asn207Asp). Fibroblasts were reprogrammed into iPSCs using non-integrating episomal plasmids encoding the reprogramming factors \textit{OCT4}, \textit{KLF4}, \textit{SOX2}, \textit{L-MYC} and \textit{LIN28}. Stem cell-like colonies were picked, and three iPSC clones were expanded and characterised for pluripotency. Mutations were confirmed in iPSCs by Sanger sequencing (Fig. 1D). The morphology of colonies were examined for characteristics of iPSCs, including flat, compact colonies with a cobblestone appearance and large nuclei to cytoplasmic ratio (Fig. 1A). Colonies stained red for alkaline phosphatase, indicating cells are undifferentiated (Fig. 1B). Colonies stained positive for pluripotency markers, \textit{OCT4} and \textit{SSEA3} (Fig. 1C). Expression of pluripotency markers \textit{OCT4} and \textit{SSEA3} were validated using qRT-PCR analysis, which showed upregulation of these markers compared to fibroblast controls (Fig. 1E). G-band karyotyping revealed a normal male 46,XY karyotype for UCLi014-A and low-pass whole genome sequencing analysis revealed normal female 46,XX karyotype for UCLi015-A (Fig. 1G). Random differentiation of embryoid bodies stained positive for markers of endoderm (AFP), mesoderm (Vimentin) and ectoderm (PAX6), confirming differentiation potential to the three germ layers (Fig. 1F). iPSC identity was confirmed by STR analysis (Table S2). Absence of mycoplasma was confirmed in iPSCs (Table S3).
In conclusion, two human iPSCs lines were generated from patients with RDH12-related retinopathies. These iPSC lines provide a valuable resource for disease modelling, comparison of disease mechanisms, therapeutic target identification and drug screening.

4. Materials and methods

4.1. Fibroblast derivation and culture

Skin biopsies were placed in 400 μL digestion media (DMEM high glucose, GlutaMAX Supplement, pyruvate, 20% FBS, 0.25% Collagenase 1, 0.05% DNase I, Pen/strep), incubated overnight at 37°C, 5% CO₂, then plated in derivation media (DMEM, 20% FBS and Pen/Strep). Fibroblasts were cultured in fibroblast media (DMEM, 10% FBS and Pen/Strep) and passaged with TrypLE Express (Gibco) (See Table 1).

4.2. Validation of mutation

DNA was extracted using QIAamp DNA Micro Kit (Qiagen). RDH12 was amplified using MyTaq PCR (Bioline) (Table 3). Mutations were confirmed by Sanger sequencing.

4.3. Fibroblast reprogramming and iPSC culture

1 × 10⁶ fibroblast cells were electroporated with 1 μg of each episomal plasmid (Table S1) using Neon Transfection System (1700 V, 20 ms, 1 pulse). Cells were plated into 1 well of a Matrigel-coated (Corning) 6-well plate in fibroblast media. On day 5, medium was changed to 3:1 fibroblast medium:mTeSR Plus (Stemcell). On day 7, medium was changed to 1:1 fibroblast medium:mTeSR Plus, from day 9 medium was changed daily with mTESR Plus. Colonies were expanded manually up to passage 4, then passaged using ReLeSR (Stemcell) at a 1:10 split ratio. iPSCs under passage 15 were used for all further characterisations (See Table 2).

### Table 1

| iPSC line names | Abbreviation in figures | Gender | Age | Ethnicity | Genotype of locus | Disease |
|-----------------|-------------------------|--------|-----|-----------|------------------|---------|
| RDH12 AD (UCLi014-A) | RDH12 AD | Male | 32 | Israeli Kurdistan and Tunisian | N/A | Retinitis pigmentosa |
| RDH12 AR (UCLi015-A) | RDH12 AR | Female | 40 | Pakistani | N/A | Leber congenital amaurosis |

### Table 2

| Classification | Test | Result | Data |
|----------------|------|--------|------|
| **Morphology** | **Photography** | Normal | Fig. 1 panel A |
| **Phenotype** | **Qualitative analysis:** Immunocytochemistry | Positive for pluripotency markers OCT4 and SSEA3 | Fig. 1 panel C |
| **Genotype** | **Karyotype (G-banding) and resolution:** qRT-PCR | Expression of OCT4, SOX2, L-MYC and LIN28 | Fig. 1 panel E |
| **Identity** | Microsatellite PCR (mPCR) | N/A | N/A |
| **Mutation analysis (IF APPLICABLE)** | Sequencing | RDH12 AD – Heterozygous frameshift mutation c.759del p.(Phe254Leufs*24) | Fig. 1 panel D |
| **Microbiology and virology** | Mycoplasma | N/A | N/A |
| **Differentiation potential** | e.g. Embryoid body formation | Positive for three germ layer markers: endoderm marker AFP, mesoderm marker Vimentin and ectoderm marker PAX6 | 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 |

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, permeabilised and blocked for 1 h at room temperature (RT) in 10% normal goat serum (NGS), 0.1% X-100, PBS. Cells were incubated for 1 h with primary antibodies diluted in 1% NGS at RT (Table 3). Secondary antibodies and DAPI were added for 1 h at RT. Cells were washed and imaged using the EVOS M7000 Imaging System.

4.6. qRT-PCR

RNA was extracted using RNeasy Mini Kit (Qiagen). cDNA was synthesised from 1 μg of RNA using Superscript II First Strand cDNA synthesis kit (Invitrogen). Transcript levels were analysed using SYBR Green MasterMix on StepOne Plus RealTime PCR System (Table 3). Relative expression of each target gene was normalised to GAPDH and compared to fibroblast expression.

4.7. Embryoid body mediated spontaneous differentiation

Embryoid bodies (EBs) were formed by dissociation of cells using ReLeSR and culturing in Aggrewell media (Stemcell) supplemented with 10 μM Y27632 for 10 days. EBs were plated in 0.1% gelatin-coated plates in DMEM/20% FBS for 11 days, where EBs attached and spontaneously differentiated. Cells were fixed and immunostained for germ layer markers AFP (endoderm), Vimentin (mesoderm) and marker PAX6 (ectoderm) (Table 3).
4.8. Karyotyping

iPSCs were sent to Cell Guidance Systems for karyotyping and 20 metaphases were counted.

4.9. Low-pass whole genome sequencing and STR analysis

DNA was extracted using QiAamp DNA Micro Kit (Qiagen). For low-pass WGS, libraries were produced using Illumina DNA Prep library prep kit and sequenced on 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 was obtained for each cell line with Promega PowerPlex16HS system and was compared back to any available on commercial cell banks.

4.10. Mycoplasma testing

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

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.2021.102449.

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Table 3

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 1:250 | Santa Cruz Biotechnology Cat# sc-51506, RRID:AB_626514 |
|                                        | Mouse anti-Vimentin 1:100 | Santa Cruz Biotechnology Cat# sc-6260, RRID:AB_628437 |
|                                        | Rabbit anti-PAX6 1:100 | Covance Cat# PRB-278P, RRID:AB_291612 |
| Secondary antibodies                   | 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# A22731, RRID:AB_2633280 |
|                                       | Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 1:400 | Thermo Fisher Scientific Cat# A10011, RRID:AB_2534069 |
|                                       | Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 1:400 | Thermo Fisher Scientific Cat# A22731, RRID:AB_2534069 |

| Primers                                | Target | Forward/Reverse primer (5′-3′) |
|----------------------------------------|--------|--------------------------------|
| Pluripotency Markers (qRT-PCR)         | OCT4   | CCCAGGGGGCCCCCATTTTGTGACC/ACCTAGTGTAGATGGAGGAGAC |
|                                        | SOX2   | TTACATGGCCACACTCCAGAGCTAGG/TCCTGACAGTGGGGAGGAGG |
|                                        | LIN28  | AGGCATATGTTAGCCTTCTGCGGCC/TTAACCTGTGGTCAGCAGGAGGC |
|                                        | L-MYC  | GGGACACACCGACGGCTGCTCC/CAAGGGGCTCGCTGACAGGAGG |
| House-Keeping Genes (qRT-PCR)          | GAPDH  | ACATTGCCATGGTACCA/TTTCTGATGGCAGGAGGAGG |
| Targeted mutation sequencing (Sanger)   | RDH12 exon 8 | TGCCAGAGGTGTTCTG/CTGACACACATTCATCAGG |
|                                        | RDH12 exon 7 | GACCATAGATCTCTGCTG/CTGGCACAGGAGGAGGAGG |

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