Generation of induced pluripotent stem cells (UCLi024-A) from a patient with argininosuccinate lyase deficiency carrying a homozygous c.437G > A (p.Arg146Gln) mutation

Claire Duff [a,*], Madeha Islam [a], Onelia Gagliano [b,c,d], Hema Pramod [a], Hassan Rashidi [a], Manju Kurian [a,e,f], Paul Gissen [a,e,f], Julien Baruteau [a,e,f]

[a] Great Ormond Street Institute of Child Health, University College London, London WC1N 1EH, UK
[b] Onyel Biotech S.r.l., Padova, PD, Italy
[c] Department of Industrial Engineering, University of Padova, Padova, Italy
[d] Veneto Institute of Molecular Medicine, Padova, Italy
[e] National Institute of Health Research, Great Ormond Street Biomedical Research Centre, London WC1N 1EH, UK
[f] Metabolic Medicine Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London WC1N 3JH, UK

A B S T R A C T

Argininosuccinic aciduria (ASA) is a rare inherited metabolic disease caused by argininosuccinate lyase (ASL) deficiency. Patients with ASA present with hyperammonaemia due to an impaired urea cycle pathway in the liver, and systemic disease with epileptic encephalopathy, chronic liver disease, and arterial hypertension. A human induced pluripotent stem cell (iPSC) line from the fibroblasts of a patient with ASA with homozygous pathogenic c.437G > A mutation of *hASL* was generated. Characterization of the cell line demonstrated pluripotency, differentiation potential and normal karyotype. This cell line, called UCLi024-A, can be utilized for *in vitro* disease modelling of ASA, and design of novel therapeutics.

1. Resource utility

Patients with ASA present with hyperammonaemia and a chronic systemic disease with nitric oxide deficiency. We generated an iPSC line from a patient with *hASL* genotype c.437G > A/c.437G > A. This cell line can help investigate the pathophysiology of ASL deficiency *in vitro* by differentiation into relevant cell subtypes and therapeutic screening.

2. Resource Table

Unique stem cell line identifier	UCLi024-A
Alternative name(s) of stem cell line	SIMD277
Institution	University College London
Contact information of distributor	Dr Julien Baruteau; j.baruteau@ucl.ac.uk
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 11 years
	Sex: Male
	Ethnicity if known: White British
Cell Source	Fibroblasts
Clonality	Clonal

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Unique stem cell line identifier	UCLi024-A
Method of reprogramming	Microfluidic RNA technology
Genetic Modification	Yes
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
Associated disease	Argininosuccinic aciduria or Argininosuccinate lyase deficiency
Gene/locus	c.437G > A/c.437G > A; R146Q/R146Q
Date archived/stock date	December 2023
Cell line repository/bank	https://hpscreg.eu/cell-line/UCLi024-A
Ethical approval	The study was approved by UK Health Research Authority. Ethics number: 13/LO/0171

3. Resource details

The iPSC line UCLi024-A was generated from fibroblasts from an 11-year-old male patient with ASA. Fibroblasts were reprogrammed using microfluidic RNA technology. The resultant iPSC line displayed the

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expected stem cell-like morphology (Fig. 1A). The iPSC line was expanded for further characterisation. The cell line displayed a normal karyotype, as assessed by SNP analysis (Fig. 1B). Pluripotency was confirmed by immunofluorescence against NANOG, OCT4 and TRA-1–60 (Fig. 1C). In addition, quantitative reverse transcription real-time PCR (qRT-PCR) similarly demonstrated expression of pluripotency genes *LIN28*, *NANOG* and *OCT4* (Fig. 1D). The genotype of the iPSC line (c.437G > A) was confirmed via Sanger Sequencing (Fig. 1E). The iPSC line underwent trilineage differentiation and showed expression of mesoderm marker *HAND1*, ectoderm marker *SOX17*, and endoderm marker *PAX6* (Fig. 1F). The iPSC line also underwent directed differentiation into cortical neurons with expression of cortical neuronal markers *FOXG1*, *OTX1*, *EMX1*, *SOX2* and *PAX6* confirmed by qRT-PCR (Fig. 1G). Short Tandem Repeat (STR) analysis demonstrated that 16 loci of the iPSC line matched with the patient's fibroblast line (data available with the authors). The iPSC line was free from mycoplasma and grew well in culture. Therefore, the UCLi024-A provides a valuable resource as a potential model to study ASL deficiency (Table 1).

4. Materials and methods

4.1. Generation of iPSCs

A written consent was obtained from the patient's legal representative under the ethics 13/LO/171 approved by the UK Health Research Authority. Primary fibroblasts were cultured in DMEM high glucose medium (Gibco) supplemented with 10 % foetal bovine serum and incubated at 37 °C in 5 % CO_2 and 20 % O_2. Cells were reprogrammed to iPSCs using microfluidic RNA technology, as previously outlined (Gagliano et al., 2019). Briefly, 1500 cells per microfluidic chamber were seeded, with an RNA mix of commercially available non-modified mRNAs (reprogramming factors *OCT4*, *SOX2*, *KLF4*, *C-MYC*, *NANOG*, *LIN28*) combined with immune evasion non-modified RNAs encoding E3, K3 and B18R protein, and two microRNAs from the 302/367 cluster. This RNA mix was transfected daily for 8 days with iPSC colonies emerging at day 8 but able to be positively identified at day 14 onwards.

iPSCs were subsequently cultured in Essential 8™ media (STEMCELL Technologies) on geltrex-coated 6-well plates. All cells were incubated at 37 °C in 5 % CO_2. Cells were passaged every 3–5 days using either EDTA (Sigma) or Accutase (Thermo Fisher) and split at a 1:3–1:6 ratio, both with and without survival promoter ROCK inhibitor (Bio-Techne).

4.2. Immunofluorescence staining

iPSCs were fixed at passage 15 in 4 % paraformaldehyde and permeabilised with 0.1 % Triton X-100. Cells were then blocked with 5–15 % goat serum (Sigma) for 1 h and incubated with primary antibodies (Table 2) diluted at 1:200 at 4 °C overnight. Samples were then washed three times in PBS before incubation with secondary antibodies diluted in goat serum for 2 h in the dark at room temperature, with Hoescht used as a nuclear stain. Cells were then further washed three times in PBS and mounted with Vectashield (Vectorlabs) before obtaining fluorescence images using an Olympus IX71 inverted microscope.

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

RNA was isolated from iPSCs at passage 16 with RNeasy mini kit (Qiagen) according to manufacturer guidelines. cDNA was reverse transcribed using the High-Capacity RNA-to-cDNA™ Kit (Thermo Fisher). qRT-PCR was carried out using Luna Universal qPCR Mastermix (New England Biolabs) on the QuantStudio 5 real-time PCR system using the SYBR-Green protocol following manufacturer's instructions. The $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels of pluripotency-related genes including *NANOG*, *LIN28* and *OCT4*, normalised to GAPDH. RNA isolated from parental fibroblasts acted as a negative control. Primers used are listed in Table 2.

4.4. Karyotyping

The karyotype of each line at passage four was investigated using single nucleotide polymorphism (SNP) analysis. Genomic DNA samples
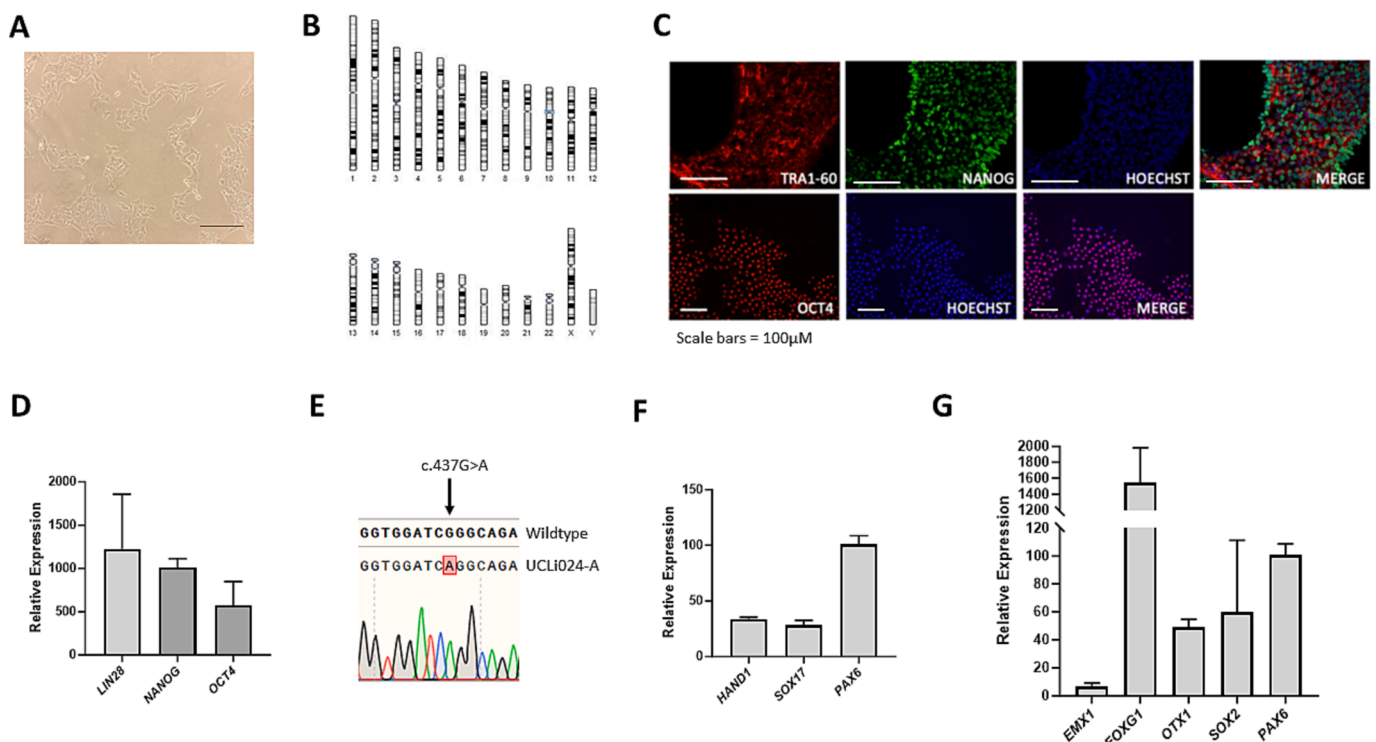

Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis: Immunofluorescent staining	Assess staining of pluripotency markers: OCT4, NANOG, TRA 1–60	Fig. 1C
	Quantitative analysis: RT-qPCR	Expression of endogenous pluripotency markers *OCT4*, *LIN28* and *NANOG*	Fig. 1D
Genotype	Karyotype (G-banding) and resolution	46XY, normal karyotype	Fig. 1B
Identity	Microsatellite PCR (mPCR) OR	Performed STR analysis	Submitted in archive with journal
	STR analysis	16 loci tested, matched genetic identity between hiPSC line and fibroblast	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Homozygous c.437G > A	Fig. 1E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence – negative.	Supplementary Fig. 1
Differentiation potential	Embryoid Body Formation	Trilineage differentiation –	Fig. 1F
	In vitro directed differentiation	*SOX17* (endoderm), *HAND1* (mesoderm) and *PAX6* (ectoderm) Directed differentiation. Expression of cortical neuron markers *FOXG1*, *OTX1*, *EMX1*, *SOX2* and *PAX6*	Fig. 1G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

were submitted to UCL Genomics before karyotyping analysis.

4.5. Short tandem repeat (STR) analysis

STR analysis was carried out on iPSCs at passage four using the PowerPlex® 16 HS System containing 16 polymorphic markers (15 STR loci and Amelogenin) including loci on 13 different autosomal chromosomes and the sex chromosomes. The analysis was conducted in the Genetic laboratory, Great Ormond Street Hospital for Children on the iPSC and parental fibroblast lines.

4.6. Tri-lineage differentiation

Tri-lineage differentiation was assessed by embryoid body (EB) formation for 14–20 days, using Essential 6 media (Thermo Fisher), with subsequent harvesting of RNA and assessment of endoderm, ectoderm and mesoderm markers via RT-qPCR (Table 2) (Fig. 1F).

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	OCT4A NANOG TRA-1-60	0.181	Cell Signaling Technology Cat# 9656	RRID: AB_1658242
Secondary Antibodies	Goat anti-rabbit IgG 488	0.736	A11034	RRID: AB_2576217
	Goat anti-rabbit IgG 568	0.736	A11036	RRID: AB_10563566
	Goat anti-mouse IgG 488	0.736	A11029	RRID: AB_2534088
	Goat anti-mouse IgG 568	0.736	A11031	RRID: AB_144696
	Primers Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers (qRT-PCR)	*LIN28-F*		CCAGTGGATGTCTTTGTGCACC	
	LIN28-R		GTGACACGGATGGATTCCAGAC	
	NANOG-F		CTCCAACATCCTGAACCTCAGC	
	NANOG-R		CGTCACACCATTGCTATTCTTCG	
	OCT4-F		CCTGAAGCAGAAGAGGATCACC	
	OCT4-R		AAAGCGGCAGATGGTCGTTTGG	
Embryoid body and Directed differentiation markers (qRT-PCR)	*HAND1-F*		CAAGGATGCACAGTCTGGCGAT	
	HAND1-R		GCAGGAGGAAAACCTTCGTGCT	
	SOX17-F		ACGCTTTCATGGTGTGGGCTAAG	
	SOX17-R		GTCAGCGCCTTCCACGACTTG	
	FOXG1-F		CGTTCAGCTACAACGCGCTCAT	
	FOXG1-R		CAGATTGTGGCGGATGGAGTTC	
	OTX1-F		CTACCCTGACATCTTCATGCGG	
	OTX1-R		GGAGAGGACTTCTTCTTGGCTG	
	EMX1-F		GCCTTCGAGAAGAACCACTACG	
	EMX1-R		CGGTTCTGGAACCACACCTTCA	
	SOX2-F		GCTACAGCATGATGCAGGACCA	
	SOX2-R		TCTGCGAGCTGGTCATGGAGTT	
	PAX6-F		CTGAGGAATCAGAGAAGACAGGC	
	PAX6-R		ATGGAGCCAGATGTGAAGGAGG	
House-Keeping Genes (qRT-PCR)	*GAPDH-F*		GTCTCCTCTGACTTCAACAGCG	
	GAPDH-R		ACCACCCTGTTGCTGTAGCCAA	

4.7. In vitro differentiation

iPSCs were differentiated into cortical neurons as previously outlined by Shi et al. (2012). After 40 days in culture, RNA was obtained from the cells and gene expression analysis of cortical neurons was undertaken using qRT-PCR.

4.8. Mycoplasma detection

Culture medium was collected from cells at passage 16 and assessed for Mycoplasma using the MycoAlert™ Mycoplasma Detection Kit (Lonza) following the manufacturer's instructions. Both a positive and negative control were included, with luminescence ratio calculated by Reading B/Reading A and compared to the positive and negative control readings. Readings below one indicated that the sample was mycoplasma free.

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CRediT authorship contribution statement

Claire Duff: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Madeha Islam:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Onelia Gagliano:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Hema Pramod:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Hassan Rashidi:** Writing – review & editing, Validation, Investigation, Formal analysis. **Manju Kurian:** Writing – review & editing, Resources, Methodology. **Paul Gissen:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Julien Baruteau:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2024.103365.

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