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Lab Resource: Multiple Cell Lines

Generation of two iPSC lines (UGENTi003 and UGENTi004) from patients with intermediate rod-cone dystrophy carrying the c.[-123C>T;701G>A];[806_810del] variants in the *RDH12* gene

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ABSTRACT

Biallelic variants in *RDH12* are associated with early-onset retinal dystrophy and Leber congenital amaurosis. RDH12 plays a role in the phototransduction cascade by converting all-*trans* retinal into all-*trans* retinol in the photoreceptor inner segments. Induced pluripotent stem cells (iPSCs) were generated and fully characterized from two patients that are compound heterozygous for the *RDH12* c.[-123C>T;701G>A];[806_810del], p.[(?;Arg234His)];[(Ala269Glyfs*2)] variants. These iPSC lines can be used for differentiation towards retinal models to evaluate disease mechanisms and novel therapies.

1. Resource table

Unique stem cell lines identifier	*UGENTi003-A, UGENTi003-B, UGENTi003-C, UGENTi004-A, UGENTi004-B and UGENTi004-C*
Alternative name(s) of stem cell lines	*UGENTi003-A = EMC379i/c1* *UGENTi003-B = EMC379i/c3* *UGENTi003-C = EMC379i/c4* *UGENTi004-A = EMC380i/c1* *UGENTi004-B = EMC380i/c6* *UGENTi004-C = EMC380i/c7*
Institution	*Ghent University*
Contact information of distributor	*Prof. Dr. Frauke Coppieters (Frauke.Coppieters@UGent.be)*
Type of cell lines	*iPSC*
Origin	*Human*
Additional origin info required *for human ESC or iPSC*	*UGENTi003:* • *Gender: female* • *Age: 38 years* • *Ethnicity: Caucasian* *UGENTi004:* • *Gender: male* • *Age: 20 years* • *Ethnicity: Caucasian*
Cell Source	*Human erythroid progenitor cells derived from blood*
Clonality	*Clonal*
Method of reprogramming	*Non-integrating Sendai viral reprogramming (OCT3/4, KLF4, SOX2 and c-MYC)*
Genetic Modification	*Yes*
Type of Genetic Modification	*Hereditary*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*RT-PCR and immunocytochemistry*
Associated disease	*Intermediate rod-cone dystrophy with macular atrophy (patients P5 and P7 in Zaeytijd et al., 2021)*
Gene/locus	*RDH12 (NM_152443.3): c.[-123C>T;701G>A];[806_810del], p.[(?;Arg234His)];[(Ala269Glyfs*2)]*

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(*continued*)

Date archived/stock date	*02/10/2023*
Cell line repository/bank	*UGENTi003-A, hPSCreg (https://hpscreg.eu/cell-line/UGENTi003-A)* *UGENTi003-B, hPSCreg (https://hpscreg.eu/cell-line/UGENTi003-B)* *UGENTi003-C, hPSCreg (https://hpscreg.eu/cell-line/UGENTi003-C)* *UGENTi004-A, hPSCreg (https://hpscreg.eu/cell-line/UGENTi004-A)* *UGENTi004-B, hPSCreg (https://hpscreg.eu/cell-line/UGENTi004-B)* *UGENTi004-C, hPSCreg (https://hpscreg.eu/cell-line/UGENTi004-C)*
Ethical approval	*This study was approved by the Medical Ethics Committee of Ghent University Hospital (B6702021000313).*

2. Resource utility

Biallelic *RDH12* variants underly 4–7 % of patients with Leber congenital amaurosis or early-onset retinal dystrophy (De Zaeytijd et al., 2021). The patient-derived iPSC lines enable differentiation to retinal cultures, providing a platform to study *RDH12* disease mechanisms and evaluate therapies.

3. Resource details

Retinol dehydrogenase 12 (RDH12) functions in the visual cycle through the conversion of all-*trans* retinal (AT-RAL) into all-*trans* retinol in the photoreceptor inner segments (Daich Varela and Michaelides, 2022; Sarkar and Moosajee, 2019). Previous studies suggested that RDH12 may play a protective role against excessive illumination by reducing AT-RAL and thereby preventing oxidative stress, apoptosis and accumulation of toxic byproducts (Chrispell et al., 2009; Maeda et al., 2007; Marchette et al., 2010; Sarkar et al., 2021). Biallelic loss-of-function variants in *RDH12* are associated with Leber congenital amaurosis, early-onset severe retinal dystrophy, retinitis pigmentosa and cone-rod dystrophy, presenting vision loss in the first decades of life followed by progressive retinal degeneration (Daich Varela and Michaelides, 2022; Sarkar and Moosajee, 2019). Heterozygous *RDH12* variants (gain-of-function/dominant-negative effect) are associated with autosomal dominant late-onset retinitis pigmentosa (Méjécase et al., 2024; Sarkar and Moosajee, 2019). As RDH12 mouse models fail to fully replicate the human phenotype, patient-derived retinal organoids offer a promising model to study disease mechanisms and evaluate novel therapeutic approaches (Méjécase et al., 2024; Sarkar and Moosajee, 2019).

We recently identified 11 patients carrying the *RDH12* c.[-123C>T;701G>A], p.[(?;Arg234His)] complex allele that is associated with a milder phenotype (Dueñas Rey et al., 2024; De Zaeytijd et al., 2021). We demonstrated that the *RDH12*:c.-123C>T variant results in reduced translation efficiency (Dueñas Rey et al., 2024), while the *RDH12*:c.701G>A variant was previously described as a hypomorphic allele (Thompson et al., 2005). Here, we generated iPSC lines from two siblings (38-year-old UGENTi003 and 20-year-old UGENTi004) who are biallelic for this complex allele and the *RDH12*:c.806_810del variant (Table 1). These lines can be used to further elucidate the disease mechanism and evaluate novel therapeutics.

Written consent and ethical approval for iPSC reprogramming were obtained by the UZ Ghent Medical Ethics Committee. The Erasmus MC iPS core facility (the Netherlands) isolated and enriched human erythroid progenitor (HEP) cells from peripheral blood and subsequently reprogrammed them using non-integrating Sendai viral vectors. Three clones (A, B, C) were expanded per iPSC line and further characterized. The patients' variants were confirmed by Sanger sequencing (Fig. 1A, reference sequence: NG_008321). Genome integrity was evaluated using shallow whole genome sequencing to detect copy number variations (CNV-Seq) (Fig. 1F) (Mus et al., 2021), revealing a deletion in UGENTi004-A (2p16.1p16.1) which is absent in healthy individuals (Database of Genomic Variants, DGV) and the original HEP cell line. This region includes the *BCL11A* gene linked to Dias-Logan Syndrome (OMIM #617101). Additionally, two other deletions, 12q13.13q13.13 (UGENTi004-A) and 16p11.2p11.1 (UGENTi003-A), were identified. Both are present in DGV (MacDonald et al., 2014). Brightfield imaging confirmed normal iPSC morphology (Fig. 1B, consistent across other clones). Stem cell markers were verified by RT-qPCR (Fig. 1C, relative to a human ESC line (HuES9) as positive control) and immunocytochemistry (ICC) (Fig. 1D and Suppl. Fig. 1A). Pluripotency potential was validated through trilineage differentiation followed by RT-qPCR (Fig. 1C, relative to HuES9) and ICC (Fig. 1E). Viral vector clearance was evaluated by RT-PCR and ICC (Suppl. Fig. 1B-C). All clones tested negative for mycoplasma (Suppl. Fig. 1D).

4. Materials and methods

4.1. Cell collection and culture of HEP cells

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly collected peripheral blood. Briefly, the buffy coat was collected, diluted in PBS, and processed using a Ficoll density gradient. PBMCs were cultured in StemSpan SFEM medium (STEMCELL Technologies) supplemented with 2 mM Ultraglutamine (Lonza), 1 % Nonessential

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field image	Normal	Fig. 1*B*
Phenotype	Qualitative analysis (immunocytochemistry)	Expression of *SSEA-4, NANOG, TRA-1–81* and *OCT-4*	Fig. 1*D* *Supplementary Fig. 1A*
	Quantitative analysis (RT-qPCR)	Expression of *OCT3/4, SOX2* and *NANOG*	Fig. 1*C*
Genotype	CNV-Seq	UGENTi003: 46XX UGENTi004: 46XY	Fig. 1*F*
Identity	STR analysis	STR analysis of 16 loci	*submitted in archive with journal*
Mutation analysis	Sanger sequencing	*RDH12* (NM_152443.3): c.[-123C > T;701G > A];[806_810del], p.[(?;Arg234His)];[(Ala269Glyfs*2)]	Fig. 1*A*
	Southern Blot OR WGS	NA	*NA*
Microbiology and virology	Mycoplasma	Luminescence negative	*Supplementary Fig. 1C*
Differentiation potential	Directed differentiation (RT-qPCR and immunocytochemistry)	Expression of *BRACHYURY, KDR, SOX17, FOXA2, SOX1* and *PAX6* (RT-qPCR) Expression of SOX17, NCAM and β-tubulin (immunocytochemistry)	Fig. 1*C and E*
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	NA
Genotype additional info	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

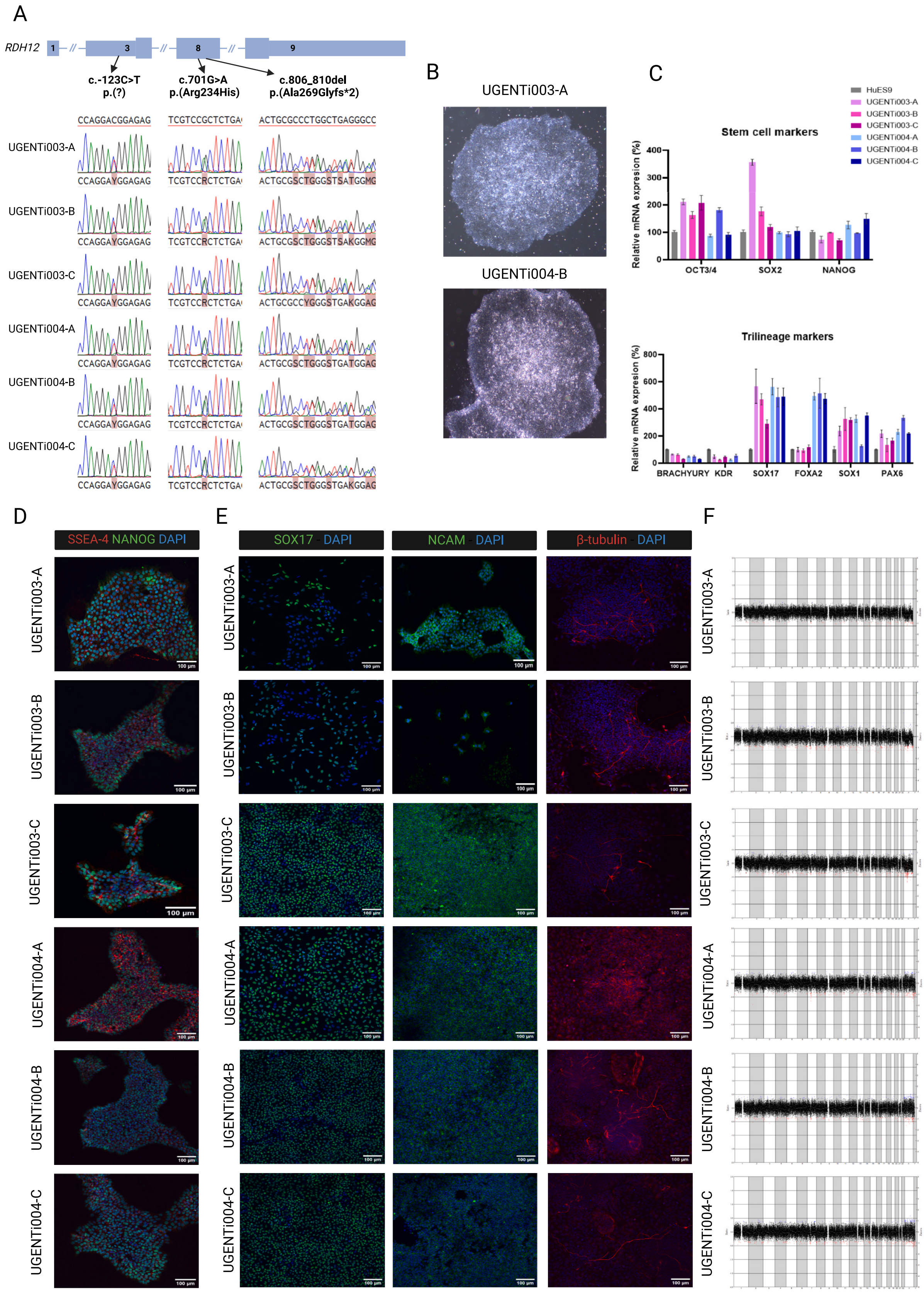

Fig. 1. Characterization of patient-derived iPSC lines (UGENTi003 and UGENTi004).

Amino Acids, 1 % penicillin/streptomycin, 50 ng/ml Stem Cell Factor, 2 U/ml Erythropoietin, 1 μM Dexamethasone (Sigma), 10 ng/ml Interleukin-3 (R&D Systems), 10 ng/ml Interleukin-6 (R&D Systems), 40 ng/ml IGF-1 (R&D Systems), and 50 μg/ml Ascorbic Acid (Merck). Cells were cultured for 5 to 7 days, with half of the medium replaced daily from day 2. Erythroblasts were isolated at 60–70 % of the total cell population using a Percoll gradient (GE Healthcare) at room temperature.

4.2. iPSC reprogramming and culture conditions

A total of 1.5 × 10^5 erythroblasts were transduced using the CytoTune-iPSC 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific) according to manufacturer's instructions. Colonies were picked and expanded on Matrigel-coated plates (Corning) and cultured in mTeSR-plus medium (STEMCELL Technologies) at 37 °C and 5 % CO_2. Sendai virus clearance was confirmed at passage 8 by RT-PCR using specific primers from the CytoTune-iPSC 2.0 Sendai Reprogramming Kit (Table 2). iPSCs were passaged every 4–5 days as clamps using ReLeSR (STEMCELL Technologies) and cryopreserved with Bambanker (Nippon) or mFreSR (STEMCELL Technologies).

4.3. DNA extraction, mutation analysis and CNV-Seq

Genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega) according to manufacturer's instruction. Variants were validated by PCR followed by Sanger sequencing using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems). For CNV-Seq, DNA was extracted with the QIAamp DNA Blood Mini kit using the QIAcube. Libraries were prepared using the DNA PCR-Free Library Prep (Illumina) and paired-end sequenced (2x50 cycles) on a NovaSeq6000 (Illumina) at a sequencing depth of 15 M reads. Data analysis was performed using Vivar (Sante et al., 2014).

4.4. Trilineage differentiation

Trilineage differentiation was performed with the STEMdiff trilineage differentiation kit (STEMCELL Technologies) following

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	Dilution	Company Cat #	RRID
Episomal plasmid	*SEV*	*1:200*	*MBL Cat# PD029*	*RRID: AB_10597564*
Stem cell Markers	*SSEA*	*1:75*	*Invitrogen Cat# 41–4000*	*RRID: AB_2533506*
	NANOG	*1:100*	*Abcam Cat# AB21624*	*RRID: AB_446437*
	TRA-1–81	*1:150*	*Abcam Cat# AB16289*	*RRID: AB_2165986*
	OCT4	*1:300*	*Abcam Cat# AB19857*	*RRID: AB_445175*
Trilineage Markers	*SOX17*	*1:100*	*R&D Cat# AF1924-SP*	*RRID: AB_355060*
	NCAM	*1:100*	*R&D Cat# AF2408*	*RRID: AB_442152*
	β-tubulin	*1:1000*	*Sigma Cat# T8660*	*RRID: AB_477590*
Secondary antibodies	*Alexa Fluor 488 Goat anti-rabbit IgG*	*1:500*	*Invitrogen Cat# A11008*	*RRID: AB_143165*
	Alexa Fluor 546 Goat anti-mouse IgG	*1:500*	*Invitrogen Cat# A11003*	*RRID: AB_2534071*
	Alexa Fluor 488 Donkey anti-goat	*1:500*	*Invitrogen Cat# A11055*	*RRID: AB_2534102*
Nuclear staining	*DAPI*	*NA*	*Vector Laboratories Cat# H-1200*	*RRID: AB_2336790*

Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Episomal plasmid (CytoTune™-iPSC 2.0 Sendai Reprogramming Kit)	*c-MYC*	*532 bp*	*TAACTGACTAGCAGGCTTGTCG (F)* *TCCACATACAGTCCTGGATGATGATG (R)*
	KLF4	*410 bp*	*TTCCTGCATGCCAGAGGAGCCC (F)* *AATGTATCGAAGGTGCTCAA (R)*
	KOS	*528 bp*	*ATGCACCGCTACGACGTGAGCGC (F)* *ACCTTGACAATCCTGATGTGG (R)*
	SeV	*181 bp*	*GGATCACTAGGTGATATCGAGC (F)* *ACCAGACAAGAGTTTAAGAGATATGTATC (R)*
Stem cell Markers (RT-qPCR)	*SOX2*	*151 bp*	*GGGAAATGGGAGGGGTGCAAAAGAGG (F)* *TTGCGTGAGTGTGGATGGGATTGGTG (R)*
	NANOG	*391 bp*	*CAGCCCTGATTCTTCCACCAGTCCC (F)* *TGGAAGGTTCCCAGTCGGGTTCACC (R)*
	OCT3/4	*144 bp*	*GACAGGGGGAGGGGAGGAGCTAGG (F)* *CTTCCCTCCAACCAGTTGCCCCAAAC (R)*
Trilineage Markers (RT-qPCR)	*Brachyury*	*208 bp*	*GGATGAAGGCTCCCGTCTC (F)* *GCTGTGATCTCCTCGTTCTGATA (R)*
	KDR	*790 bp*	*CTGGCATGGTCTTCTGTGAAGCA (F)* *AATACCAGTGGATGTGATGCGG (R)*
	SOX17	*105 bp*	*GCTTTCATGGTGTGGGCTAA (F)* *CGCCTTCCACGACTTGC (R)*
	FOXA2	*216 bp*	*TACAGGCGCAGCTACACGCACGCAAAG (F)* *GCGGGGCACCTTCAGGAAACAGTCGT (R)*
	SOX1	*249 bp*	*GGTCAAACGGCCCATGAACGC (F)* *TCCTTCTTGAGCAGCGTCTTGGTCTT (R)*
	PAX6	*83 bp*	*TTTGCCCGAGAAAGACTAGC (F)* *CATTTGGCCCTTCGATTAGA (R)*
House-Keeping Genes (RT-qPCR)	*GAPDH*	*117 bp*	*GCCAAAAGGGTCATCATCTC (F)* *GGTGGTGCAGGAGGCATT (R)*
Targeted mutation analysis (Sanger sequencing on gDNA)	*RDH12_UTR*	389 bp	*TGCCAGTAAACCTAAGTAAGC (F)* *AGTGCAGGTCTTTGTAAACG (R)*
	RDH12_ex8	376 bp	*CTGCAGGAGATAAGCTGTTT (F)* *CCAAACCTGGATTGCATCAT (R)*

manufacturer's instructions, directing iPSCs into ectoderm, mesoderm and endoderm.

4.5. RT-qPCR

Total RNA was extracted using the ReliaPrep RNA Cell Miniprep System and reverse-transcribed into cDNA using SuperScript II RT (Invitrogen). qPCR was performed on the CFX96 C1000 Thermal Cycler (Bio-rad) using SYBR Green (Sigma), Platinum Taq polymerase (Invitrogen) and primers (Table 2). mRNA expression was normalized to *GAPDH*.

4.6. Immunocytochemistry

Cells were fixed with 4 % paraformaldehyde for 15 min at room temperature, then blocked for 1 h in fresh blocking buffer (1 % BSA (Sigma), 0.1 % Triton X-100 (Sigma) in PBS sterilized by filter). Primary and secondary antibodies (Table 2) were diluted in 1 % BSA with 0.05 % Tween 20 (Sigma) in PBS, incubated overnight at 4 °C or for 1 h at room temperature, respectively. Imaging was performed using a Leica Stellaris 5 LIA microscope.

4.7. STR profiling, mycoplasma testing and sterility

STR profiling was performed with the PowerPlex 16 System (Promega). Mycoplasma contamination was assessed using the MycoAlert Mycoplasma Detection Kit (Lonza). Sterility testing was conducted by Erasmus MC's microbiology department using blood agar and Sabouraud agar on supernatant samples.

CRediT authorship contribution statement

M. Bouckaert: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **F.Van Den Broeck:** Resources, Methodology. **M. Ghazvini:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **A. Dueñas Rey:** Resources, Investigation. **H. Lenaerts:** Methodology, Investigation. **A. Dheedene:** Methodology, Investigation. **K. Claes:** Methodology, Investigation. **E. De Baere:** Supervision, Investigation. **B.P. Leroy:** Supervision, Resources. **J. De Zaeytijd:** Supervision, Resources. **F. Coppieters:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: F. C., M.B. and A.D.R. have a patent pending. If there are other authors, they 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.2025.103739.

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