Generation of a Bartter syndrome type 3 patient-derived induced pluripotent stem cell line ISRM-BS3-UM18-iPSC (HHUUKDi014-A)

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Lab Resource: Single Cell Line

Generation of a Bartter syndrome type 3 patient-derived induced pluripotent stem cell line ISRM-BS3-UM18-iPSC (HHUUKDi014-A)

Chantelle Thimm [a], Chutong Zhong [c], Wasco Wruck [a], Alessandra Grillo [c], Rosanne Mack [a], Martina Bohndorf [a], Nina Graffmann [a], Anson Tang [c], Viola D'Ambrosio [c], Elizabeth R Wan [c], Keith Siew [c], Rhys D Evans [c], Stephan B. Walsh [c,†], James Adjaye [a,b,*]

[a] Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich-Heine- University Düsseldorf 40225 Düsseldorf, Germany
[b] EGA Institute for Women's Health, Zayed Centre for Research into Rare Diseases in Children (ZCR), University College London (UCL), 20 Guilford Street, London WC1N 1DZ, UK
[c] Department of Renal Medicine, University College London, Royal Free Hospital, London, UK

A B S T R A C T

SIX2-positive urine-derived renal progenitor cells (UdRPCs) were isolated from an 18-year-old Bartter syndrome type 3 (BS3) patient within a homozygous CLCNKB gene deletion. Two episomal-based plasmids expressing OCT4, SOX2, NANOG, KLF4, c-MYC and LIN28 we were able to generate an integration-free induced pluripotent stem cell line (iPSC). Pluripotency was confirmed by fluorescence-activated cell sorting analysis and immunocytochemistry for the markers-OCT4, SOX2, NANOG, TRA-1-60, TRA-1-81 and SSEA4. Embryoid body-based differentiation into the three germ layers was the conducted and confirmed by immunocytochemistry. Pluritest analysis revealed a Pearson correlation of 0,93. Short tandem repeat DNA fingerprinting and karyotype analyses were performed.

Unique stem cell line identifier	**HHUUKDi014-A** https://hpscreg.eu/cell-line/HHUUKDi014-A
Alternative name(s) of stem cell line	ISRM-BS3-UM18-iPSC
Institution	Institute for Stem Cell Research and Regenerative Medicine
Contact information of distributor	James Adjaye, James.Adjaye@med.uni-duesseldorf.de
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 18 Sex: male Ethnicity if known: any other ethnic group
Cell Source	SIX2-positive urine derived renal progenitor cells (UdRPCs)
Clonality	Clonal
Method of reprogramming	Episomal expression of OCT4, SOX2, NANOG, LIN28, c-MYC, KLF-4
Genetic Modification	YES
Type of Genetic Modification	Hereditary.

(continued on next column)

(*continued*)

Unique stem cell line identifier	**HHUUKDi014-A** https://hpscreg.eu/cell-line/HHUUKDi014-A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	Bartter Syndrome type 3
Gene/locus	Homozygous gene deletion of CLCNKB
Date archived/stock date	30.01.2024
Cell line repository/bank	
Ethical approval	The study was approved under the ethical approval "Identification of genes involved in renal, electrolyte, and urinary tract disorders" (REC reference 05/Q0508/6). The ethic approval was provided by the ethical committee of university College London. The study was also approved under the ethical approval "Isolation, characterization and reprogramming of urine-derived stem cells" (Study number: 2017-2457_3. The ethic approval was provided by the ethical committee of the

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* Corresponding author at: Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich-Heine- University Düsseldorf, 40225 Düsseldorf, Germany.
E-mail address: james.adjaye@med.uni-duesseldorf.de (J. Adjaye).
† Deceased (RIP).

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(*continued*)

Unique stem cell line identifier	**HHUUKDi014-A** https://hpscreg.eu/cell-line/HHUUKDi014-A
	medical faculty of the Heinrich-Heine university Düsseldorf. All patients provided written informed consent.

1. Resource utility

The kidneys of Bartter syndrome (BS) patients excrete excessive electrolytes such as potassium, sodium and chloride due to a congenital defect in the tubules. BS is a rare polygenic disease caused by homozygous or mixed heterozygous mutations in one of the genes: SLC12A1, KCNJ1, CLCNKB, BSND or CASR (Fulchiero and Seo-Mayer, 2019; Thimm and Adjaye, 2024). This iPSC line can be used for studying the etiology of Bartter syndrome at the cellular and molecular level. Table 1 displays the results of the characterization and validation.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	*Normal*	Fig. 1 *panel A*
Phenotype	*Immunocytochemistry*	*Expression of pluripotency markers: OCT4, NANOG, SOX2, Tra 1–60, SSEA-4, Tra-1–81.*	Fig. 1 *panel D*
	Flow cytometry	1. *Assess 98.3 % of OCT4 positive cells* 2. *Assess 99.6 % of SSEA-4 positive cells*	Fig. 1 *panel E*
Genotype	Karyotype (G-banding) and resolution	*BS3-UM18-iPSC, 46XX Resolution 450–500*	*panel I*
Identity	Microsatellite PCR (mPCR) OR	*Not performed*	*N/A*
	STR analysis	STR analysis by PCR of nine loci, matched.	*Supplementary file 2*
Mutation analysis (IF APPLICABLE)	PCR with Sanger Sequencing	*CLCNKB mutation was found, position: 16049-17081nt.*	Fig. 1 *panel G*
	Southern Blot OR WGS	*Not performed*	*N/A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by RT-PCR*	*Supplementary* Fig. 1*B*
Differentiation potential	Embryoid body formation	*Expression of germ layer specific proteins:* *Mesoderm – α-smooth muscle actin (α-SMA), BRACHYURY* *Ectoderm – PAX6, βIII-TUBULIN* *Endoderm – SOX17, AFP*	Fig. 1 *panel F*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*Not performed*	*N/A*
Genotype additional info (OPTIONAL)	Blood group genotyping	*Not performed*	*N/A*
	HLA tissue typing	*Not performed*	*N/A*

2. Resource details

In 1962, Bartter et al. identified a new syndrome and named it Bartter syndrome (BS). BS is one of the rare hereditary renal tubular disorders caused by impaired salt reabsorption in the thick ascending limb (TAL) of the loop of Henle. It is associated with several electrolyte abnormalities including low potassium and chloride, salt wasting, hypokalemia, and metabolic alkalosis with hyperaldosteronism with normal blood pressure and hyperplasia of the juxtaglomerular apparatus (JGA) (Bartter et al., 1962; Lee et al., 2012; da Silva and Pfefermann, 2018; Bokhari et al., 2023). BS is a polygenic disease caused by homozygous or mixed heterozygous mutations in one of the following genes: SLC12A1, KCNJ1, CLCNKB, BSND, or CASR (Fulchiero and Seo-Mayer, 2019; Thimm and Adjaye, 2024). We generated an iPSC line from a male Bartter syndrome type 3 patient (ISRM-BS3-UM18-iPSC). SIX2-positive urine-derived renal progenitor cells (UdRPCs) were first isolated from the patient and used to generate the described iPSC line. Reprogramming was carried out by nucleofection of the episomal plasmids pEP4EO2SCK2MEN2L and pEP4EO2SET2K (7F1) expressing OCT4, SOX2, NANOG, LIN28, c-MYC and KLF-4 (Supplementary Fig. 1A). The T- 13PC3 (ATCC) program and epithelial cells nucleofection kit were used. The resulting iPSC line has the typical iPSC morphology(Fig. 1A). The renal progenitor origin of UdRPC-BS3-UM18 were confirmed by immunocytochemistry for the renal stem cell marker SIX2 (Fig. 1B). The absence of exogenous OCT4 is shown in Fig. 1C. Pluripotency was confirmed by immunocytochemistry for the pluripotency-regulating transcription factors OCT4, NANOG, SOX2 and cell surface markers SSEA-4, TRA-1–60 and TRA-1–81 (Fig. 1D). Flow cytometry-based detection and quantification of OCT4 and SSEA4 expressing cells was confirmed (Fig. 1E). Pluripotency was also shown in vitro by embryoid body (EB)-based differentiation into the cell types of the three germ layers- endoderm (SOX17, AFP), ectoderm (PAX6, βIII-TUBULIN) and mesoderm (α-SMA –smooth muscle actin, BRACHYURY) (Fig. 1F). The deletion was confirmed by RT-PCR (Fig. 1G). Comparative transcriptome and cluster analysis of the ISRM-BS3-UM18-iPSC and the human embryonic stem cell line H9 revealed a Pearson correlation of 0,93. (Fig. 1H). Chromosomal content analysis counting 24 mitoses revealed a normal karyotype: ISRM-BS3-UM18-iPSC – 46, XY [22] (Fig. 1I). DNA fingerprinting confirmed the identity of ISRM-BS3-UM18-iPSC with the respective parental UdRPC (Supplementary file 2). A PCR-based Mycoplasma contamination test was negative (Supplementary Fig. 1B).

3. Materials and methods

3.1. Cell culture

UdRPCs (BS3-UM18) were isolated from urine samples and expanded as previously described (Bohndorf et al., 2017; Rahman et al., 2020). Cells were cultured on plastic plates in proliferation medium (PM) composed of 50 % DMEM high Glucose (Gibco®, life technologies, California) and 50 % Keratinocyte growth basal medium (Lonza, Basel, Switzerland) supplemented with 5 ng/ml bFGF. The DMEM high Glucose medium is supplemented with 10 % fetal bovine serum (Gibco®,), 0.1 % Non-Essential Amino Acid (Gibco®,), 0.5 % Glutamax (Gibco®,) and 1 % Penicillin and Streptomycin (Gibco®. Cells were cultured at 37 °C, 5 % CO_2 and 5 % O_2. ISRM-BS3-UM18 was cultured in StemMACS iPS-Brew XF Medium (Miltenyi Biotec, Bergisch Gladbach, Germany) with 1 % Penstrep on Matrigel at 37 °C and 5 % CO_2. Cells were split at a 1:6 ratio into new matrigel coated plates. The characterization of the cells was carried out at passage 19.

3.2. Derivation of iPSCs

SIX2-positive BS3-UM18 UdRPC were reprogrammed into iPSCs (Bohndorf et al., 2017; Yu et al., 2011; Ncube et al., 2023) using the Amaxa nucleofector (Lonza, Basel, Switzerland) program T-13PC3 (ATCC). The epithelial cells nucleofection kit from LONZA was used for nucleofecting approximately 3 μg each of the two plasmids pEP4EO2SCK2MEN2L and pEP4EO2SET2K (Supplementary Fig. 1A). In addition, 0.5 μm A83-01, 0.5 μM PD0325901, 10 μM Y-27632, and 3 μM CHIR99021 were supplemented to inhibit TGFß-, MEK-, and Rock-signalling and activate WNT-signalling pathway. Cells were cultured for 24 h in Proliferation medium supplemented with 10 μM Y-27632 on Matrigel® at 37 °C and 5 % CO_2. After 24 h the Medium was changed to StemMACS iPS-Brew XF Medium (Miltenyi Biotec, Bergisch Gladbach, Germany) with daily medium changes. Well-defined iPSC colonies were picked and detached using PBS lacking Mg^{2+} and Ca^{2+} and distributed at a 1:6 ratio into new matrigel coated plates.

3.3. PCR

Supernatants from dense cultures were collected, and PCR was run to confirm the absence of mycoplasma contamination. To confirm the vector dilution, genomic DNA was extracted using the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany). PCR was carried out using the GoTaq DNA Polymerase kit (Promega) with exogenous OCT4 primers (Table 1).

3.4. DNA fingerprint analysis

STR analysis was performed using genomic DNA extracted using the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany). By using the GoTaq DNA Polymerase kit (Promega), the STR analysis was performed by PCR using the following primers: D16S539, D13S317 and D7S820. RPL0 served as a housekeeping gene for normalization. The PCR program is as followed:

Cycle 1 (x1): 94 °C for 3 min
Cycle 2 (x10): 94 °C for 30 s, 60 °C for 15 s, 70 °C for 45 s
Cycle 3 (x20): 94 °C for 30 s, 60 °C for 15 s, 70 °C for 45 s
Cycle 4 (x1): 60 °C for 30 min
Cycle 5: 4 °C end

Primary BS3-UM18 UdRPC cells was used for comparison·H_2O served as a negative control. The PCR amplicons were resolved by gel electrophoresis using 2.5 % agarose. Primer sequences are shown in Table 1.

3.5. Embryoid body (EB) formation

Embryoid bodies are three-dimensional aggregates that confirm the pluripotent properties of iPSCs. Based on this, EBs were also produced from the cultured iPSCs. First, the iPSCs were cultured on Matrigel-coated plates until they were subconfluent. Then a 6 mm Petri dish (greiner BIO-ONE, Kremsmünster, Austria) was coated with an anti-adherence rinsing solution (Stemcell technologies) for 10 min at 37 °C. The subconfluent wells were transferred 1:1 into a coated Petri dish. The Petri dish was cultivated for 24 h in a low oxygen incubator. As usual, the cells were cultivated in StemMACS iPS-Brew XF medium with 10 μM Y-27632. After 24 h, the cells were transferred to a normoxic incubator on a shaking platform. After approximately three days, the medium was changed to high-glucose DMEM, containing 1 % NEAA, to generate the EBs. After 7 days, the EBs are transferred to a 24-well plate coated with Matrigel and cultured till confluent.

3.6. Immunofluorescence

Cells were fixed with 4 % paraformaldehyde for 15 min at room temperature (RT). Cells were washed three times with PBS. Afterwards an incubation with 0.05 % Tween-20/PBS for 10 min. The cells were treated three times with PBS and continued blocking the cells with 3 % BSA/PBS. Thereafter they were incubated with the respective primary antibodies (Table 2) overnight at 4 °C. Followed by one washing step with Triton x-100/PBS and two washing steps with PBS. The cells were incubated for 2 h with Alexa488 or Alexa555-conjugated secondary antibodies and nuclear Hoechst at RT. Fluorescence images were captured by a LSM700 microscope (Carl Zeiss).

3.7. Flow-cytometry and immunofluorescence-based detection of pluripotency-associated proteins

Cells cultured in 6-well plates were washed twice with PBS. Afterwards they were replaced with 500 μl Triple-E (Gibco®, life technologies, California) for 10 min at 37 °C and 5 % CO_2. in the incubator. The reaction is stopped by 1 ml StemMACS iPS-Brew XF Medium (MiltenyiBiotec, Bergisch Gladbach, Germany). Cells from each 6-well was transferred into FACS-Tubes (Falcon,New York, USA) and centrifuged by 190 g, 5 min. Afterwards the cells were fixed with 4 % paraformaldehyde (polysciences, Illinois, USA) for 15 min at RT. Followed by three washings steps with PBS at 500 g 5 min. Blocking was carried out with 1 – 2 ml 3 % BSA (Sigma-Aldrich, St.Louis, USA) for 1 h at RT. The cells were separated with a 0.4 μm meshstainer (greiner BIO-ONE, Kremsmünster, Austria). Followed by a centrifugation step at 300 g, 10 min, RT. Cell pellet were resuspended with 98 μL FACS Buffer consisting of 50 ml PBS (−/-), 0.5 % BSA, 2 mM EDTA, which can be stored by 2–8 °C. 2 μl of antibody were added. Incubation was overnight at 4 °C. Cells were washed three times with PBS and diluted in 100 μl FACS Buffer, 0.2 ul of second antibody (Table 2) for 1 h at the shaking plate in the dark, RT. After three washing steps the cell pellet were resuspend in 200–300 μl FACS Buffer and stored at 4 °C.

3.8. Karyotype analysis

Karyotype analysis was performed and evaluated at the Institute of Human Genetics and Anthropology, Heinrich-Heine-University, Düsseldorf.

3.9. NGS analysis

For the NGS experiments, 50 ng RNA preparations were sequenced via 3′RNA-Seq on a Illumina NextSeq 2000 at the core facility Biomedizinisches Forschungszentrum (BMFZ)- Heinrich Heine University Düsseldorf. The reads from the resulting fastq files were aligned against the GRCh38 genome via the HISAT2 (version 2.1.0) software (Kim et al., 2015) and summarized per gene with the subread (1.6.1) featurecounts software (Liao et al., 2014) using the ENSEMBL annotation file Homo_sapiens.GRCh38.109.gtf as described in our previous publication (Martins et al., 2020). The summarized RNA-Seq counts were corrected for batch effects and normalized within the R/Bioconductor (Gentleman et al., 2004) environment using the batch effect removal and voom normalization from the package limma (Smyth, 2004). Dendrograms and correlations were calculated using Pearson correlation as a similarity measure.

3.10. Genotyping

Genotyping was performed by multiplex polymerase chain reaction and next generation sequencing of patient genomic DNA for a panel of genes associated with inherited tubular disease (inclusive of CLCNKB). The panel (R198) of genes associated with tubular disease currently tested can be found at https://panelapp.genomicsengland.co.uk/pan

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Characterization	*Mouse-SIX2*	*1:200*	*Abnova #H0010756-M01*	*AB_489974*
Flow Cytometry	*Rabbit anti-OCT4*	*1:50*	*Cell Signaling Technologies #2840S*	*AB_2167691*
Flow Cytometry	*Mouse anti-SSEA4*	*1:50*	*Cell Signaling #MC813*	*AB_4755*
Pluripotency Marker	*Rabbit anti-OCT4*	*1:200*	*Cell Signaling Technologies #2840S*	*AB_2167691*
Pluripotency Marker	*Rabbit anti-SOX2*	*1:200*	*Cell Signaling Technologies #3579S*	*AB_2195767*
Pluripotency Marker	*Rabbit anti-NANOG*	*1:200*	*Cell Signaling Technologies #4903S*	*AB_10559205*
Pluripotency Marker	*Mouse anti-Tra-1–60*	*1:200*	*Cell Signaling Technologies #4746S*	*AB_2119059*
Pluripotency Marker	*Mouse anti-Tra-1–81*	*1:200*	*Cell Signaling Technologies #4745S*	*AB_2119060*
Pluripotency Marker	*Mouse anti-SSEA4*	*1:200*	*Cell Signaling #MC813*	*AB_4755*
e.g. Differentiation Markers	*Rabbit anti-PAX6*	*1:200*	*Cell Signaling #D3A9V*	AB_60433
	Mouse anti-aSMA	*1:1000*	*Dako # M0851*	*AB_2223500*
	Mouse anti-IgG-SOX17	*1:50*	*R&D System #AF1924*	*AB_355060*
	Rabbit anti-BIII Tubulin	*1:1000*	*SySy # 302,304*	*AB_10805138*
	Rabbit anti-AFP	*1:200*	*Cell Signaling Technology # 2137S*	*AB_2209744*
	Rabbit anti-brachyury	*1:200*	*Abcam #ab209665*	*AB_270925*
Secondary antibodies Flow Cytometry	*anti-rabbit- Alexa488*	*1:50*	*Thermo Fisher Scientific # A27034*	*AB_2536097*
Secondary antibody	*anti-rabbit- Alexa488*	*1:500*	*Thermo Fisher Scientific # A27034*	*AB_2536097*
Secondary antibody	*anti-mouse- Alexa555*	*1:500*	*Thermo Fisher Scientific # A21424*	*AB_141780*
Nuclear Co– Staining	*Hoechst*	*1:5000*	*Thermo Fisher Scientific # H3569*	*AB_2651133*
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal plasmid (exogenous)	*OCT4*	*657 bp*	*AGTGAGAGGCAACCTGGAGA/ AGGAACTGCTTCCTTCACGA*	
House-Keeping Genes	*RPL0*	*42 bp*	*TCGACAATGGCAGCATCTAC/ ATCCGTCTCCACAGACAAGG*	
Mycoplasma ITW Reagents #A3744	*mycoplasma-specific 16S rRNA gene*	*270 bp*	Negative samples show a 270 bp band only	
		357 bp	Positive ones have two bands 270 bp & 357 bp	
STR analysis	*D16S539*		*GGGGGTCTAAGAGCTTGTAAAAAG GTTTGTGTGTGATCTGTAAGCATGTATC*	
	D13S317		*ACAGAAGTCTGGGATGTGGAGGA GGCAGCCCAAAAAGACAGA*	
	D7S820		*ATGTTGGTCAGGCTGACTATG GATTCCACATTTATCCTCATTGAC*	
	vWA		*CTAGTGGATGATAAGAATAATCAGTATGTG GGACAGATGATAAATACATAGGATGGATGG*	
	Th01		*ATTCAAAGGGTATCTGGGCTCTGG GTGGGCTGAAAAGCTCCCGATTAT*	
	Amelogenin		*ACCTCATCCTGGGCACCCTGGTT AGGCTTGAGGCCAACCATCAG*	
	D5S818		*GGTGATTTTCCTCTTTGGTATCC AGCCACAGTTTACAACATTTGTATCT*	
	CSF1		*AACCTGAGTCTGCCAAGGACTAGC TTCCACACACCACTGGCCATCTTC*	
	TPOX		*ACTGGCACAGAACAGGCACTTAGG GGAGGAACTGGGAACCACACAGGT*	
Genotyping	*CLCNKB 4 Position:15481-16028nt*	*568 bp*	*GAAGATAAGGCGGGGTTGGGG CTTGGGTGAGCAGTGGGGTCC*	
	CLCNKB 5 Position: 16049-16537nt	*507 bp*	*AAATAGCCTTCAGTGTTCAT TATGGGGCTATTGTGACTA*	
	CLCNKB 6 Position: 16537-17081nt	*562 bp*	*TAGTCACAATAGCCCCATAG AGGAAGCAGCATAAGACG*	

els/292/ (Hureaux et al., 2019; Ashton et al., 2018). In addition the deletion within the CLCNKB gene of the BS3-UM18 UdRPC and the derived iPSC was confirmed by PCR and Sanger sequenced at the core facility- Biomedizinisches Forschungszentrum (BMFZ)-Heinrich Heine University Düsseldorf.

3.11. Patient recruitment

Patients were recruited from the University College London Department of Renal Medicine specialist clinics for renal tubular disorders based at the Royal Free Hospital and Great Ormond Street Hospital. Patients are referred to this clinic with confirmed or possible inherited disorders of tubular function. From our cohort, patients with genotyped Bartter's Syndrome Type 3 [OMIM: 607,364 | GENE: CLCNKB] were selected for isolation of urine-derived cells.

3.12. Ethics

The study was approved under the ethical approval "Identification of genes involved in renal, electrolyte, and urinary tract disorders" (REC reference 05/Q0508/6). The study was also approved under the ethical approval "Isolation, characterization and reprogramming of urine-derived stem cells" (Study number: 2017-2457_3. The ethic approval was provided by the ethical committee of the medical faculty of the

Heinrich Heine university Düsseldorf.

All patients provided written informed consent

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CRediT authorship contribution statement

Chantelle Thimm: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Chutong Zhong:** Resources, Methodology, Investigation. **Wasco Wruck:** Software, Formal analysis. **Alessandra Grillo:** Resources, Methodology. **Rosanne Mack:** Methodology. **Martina Bohndorf:** Methodology. **Nina Graffmann:** Methodology. **Anson Tang:** Resources. **Viola D'Ambrosio:** Resources. **Elizabeth R Wan:** Resources. **Keith Siew:** Supervision, Funding acquisition, Conceptualization. **Rhys D Evans:** Resources. **Stephan B. Walsh:** Supervision, Funding acquisition, Conceptualization. **James Adjaye:** Supervision, Project administration, 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.

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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.2025.103760.

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