CRISPR/Cas9-mediated editing of COQ4 in induced pluripotent stem cells: A model for investigating COQ4-associated human coenzyme Q10 deficiency

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Lab Resource: Multiple Cell Lines

CRISPR/Cas9-mediated editing of *COQ4* in induced pluripotent stem cells: A model for investigating *COQ4*-associated human coenzyme Q_{10} deficiency

Sonja Herbrich [a], Haribaskar Ramachandran [b], Annette Seibt [a], Isabella Tolle [a], Annika Zink [a], Alessandro Prigione [a], Andrea Rossi [b,*,1], Felix Distelmaier [a,*,1]

[a] *Department of General Pediatrics, Neonatology, and Pediatric Cardiology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University, Düsseldorf 40225, Germany*
[b] *IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany*

A B S T R A C T

Pathogenic variants in the gene *COQ4* cause primary coenzyme Q_{10} deficiency, which is associated with symptoms ranging from early epileptic encephalopathy up to adult-onset ataxia-spasticity spectrum disease. We genetically modified commercially available wild-type iPS cells by using a CRISPR/Cas9 approach to create heterozygous and homozygous isogenic cell lines carrying the disease-causing *COQ4* variants c.458C > T, p.Ala153Val and c.437T > G, p.Phe146Cys, respectively. All iPSCs lines exhibited a normal cell morphology, expression of pluripotency markers, and the ability to differentiate into the three primary germ layers. The COQ4-deficient cell lines will provide a helpful tool to investigate the disease mechanism and to develop therapeutic strategies.

Resource Table

Unique stem cell line identifier	IUFi004-A-3 IUFi004-A-4 IUFi004-A-5 IUFi004-A-6 IUFi004-A-7 IUFi004-A-8 IUFi004-A-9
Alternative name(s) of stem cell line	DU319 DU320 DU321 DU329 DU330 DU331 DU332
Institution	IUF Leibniz Institut for Environmental Medicine UKD Universitätsklinikum Düsseldorf
Contact information of the reported cell line distributor	Felix Distelmaier, felix.distelmaier@uni-duesseldorf.de
Type of cell line	iPSC
Origin	human
Additional origin info *(applicable for human ESC or iPSC)*	Sex: female Age: 64
Cell Source	iPSC12-10 (IUFi004-A) Cell Aplications Inc, https://www.cellapplications.com/human-induced-pluripot nt-stem-cells-hipsc
Method of reprogramming	N/A
Clonality	Monoclonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
The cell culture system used	Cells were grown on Corning® Matrigel in mTeSR™1 medium
Type of the Genetic Modification	Generation of iPSC-based model carrying heterozygous and homozygous patient mutations in exon 5 of the *COQ4* gene
Associated disease	Primary coenzyme Q10 deficiency-7 (COQ10D7); OMIM # 616,276
Gene/locus modified in the reported transgenic line	COQ4 (Gene ID: 51117) 9q34.11 0c.458C > T, p.Ala153Val, c.437 T > G, p. Phe146Cys

* Corresponding authors.
E-mail addresses: andrea.rossi@iuf-duesseldorf.de (A. Rossi), distelmaier@uni-duesseldorf.de (F. Distelmaier).
[1] Equal contribution.

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(*continued*)

Method of modification / user-customisable nucleases (UCN) used, the resource used for design optimisation	CRISPR/Cas9 system Guide RNA design: CHOPCHOP v3
User-customisable nuclease (UCN) delivery method	Plasmid transfection
Analysis of the nuclease-targeted allele status	NGS of targeted allele
Cell line repository/bank	Registered in the hpscreg database
Ethical/GMO work approvals	The study was approved by the local ethics committee of the Heinrich Heine University, Düsseldorf, Germany, study number #5238. iPSCs were purchased from Cell Applications Inc for research use only.

1. Resource utility

Currently there are no effective therapies for primary CoQ_{10} deficiency caused by COQ4 defects as there is an insufficient response to orally administered CoQ_{10}. Our modified cell lines provide a tool for developing patient specific disease models and subsequently enable studies regarding disease mechanisms and potential therapeutic strategies.

2. Resource details

In the mitochondrial electron transport chain CoQ_{10} plays a vital role as an electron carrier, facilitating the transfer of electrons from complexes I and II to complex III. Furthermore, it is a cofactor in the metabolism of pyrimidines and fatty acids and a major antioxidant (Hargreaves et al. 2020). CoQ_{10} consists of a benzoquinone ring and a polyisoprenoid side chain. The benzoquinone ring undergoes several modifications during CoQ_{10} biosynthesis.

Pathogenic variants in genes encoding proteins directly involved in CoQ_{10} biosynthesis cause primary CoQ_{10} deficiencies (Laugwitz et al., 2022). One of those proteins is COQ4, which facilitates the decarboxylation of a precursor of CoQ_{10} and participates in the stabilisation of the COQ multienzyme complex (Nicoll et al. 2024, Laugwitz et al., 2022). COQ4 deficiency presents with various clinical features like cerebellar ataxia, epileptic encephalopathy and developmental disability (Laugwitz et al., 2022).

Here we modified commercially available human female iPSCs (Cell Applications Inc.) to carry the previously identified pathogenic variants c.458C > T, p.Ala153Val and c.437 T > G, p.Phe146Cys in exon 5 (Fig. 1 A). The wildtype iPSC12–10 cells (IUFi004-A) were genetically modified using CRISPR/Cas9 editing. To this end, guide RNAs (gRNAs) were designed and cloned into the GFP-coupled plasmid. Briefly, iPSCs were co-transfected with the plasmid and synthesized donor oligonucleotides and subsequently selected for GFP-positive cells using FACS sorting. Colonies grown from FACS sorted single cells where sequenced by Illumina MiSeq as described previously (Ramachandran et al. 2021).

Applying this approach, two homozygous and one heterozygous cell lines carrying the c.437T > G (IUFi004-A-3, IUFi004-A-4, IUFi004-A-5) mutation and two heterozygous and two homozygous cell lines carrying the c.458C > T mutation (IUFi004-A-6, IUFi004-A-7, IUFi004-A-8, IUFi004-A-9) were generated. To ensure that both alleles of *COQ4* were represented and to rule out allelic dropout, we amplified and sequenced approximately 1.3 kb of the targeted region using Nanopore sequencing. In the sequencing data from clones *DU319* and *DU320* (*COQ4*; T > G) and *DU331* and *DU332* (*COQ4*; C > T), we consistently observed a heterozygous SNP within intron 5–6, regardless of clonal differences. The presence of this endogenous heterozygous SNP in all clones confirms that both alleles of *COQ4* were successfully amplified and sequenced, supporting the conclusion that homozygous knock-in was achieved (Fig. 1A).

All cell lines showed typical iPSC morphology including compact colony formation with well-defined borders and a high cytoplasm to nucleus ratio (exemplary images Fig. 1 B, acquired with Zeiss Axio Vert. A1, scale bars 100 μM). Immunological staining confirmed the expression of pluripotency associated protein markers NANOG, OCT4, SOX2 and TRA1-60 (exemplary images Fig. 1 B, acquired with Zeiss Axio Observer 7, scale bars 100 μM). The expression of the pluripotency markers was further confirmed by quantitative real-time PCR of *OCT4*, *NANOG* and *SOX2*. The absent expression of the fibroblast marker vimentin (*VIM*) further confirmed pluripotency (Fig. 1 C). Commercially available fibroblasts (Lonza) were used as control. The potential for differentiation into the three germ layers was verified using the STEMdiff ™ Trilineage Differentiation Kit (Cat# 05230) as shown by endodermal, ectodermal and mesodermal markers (SOX17, PAX6 and Brachyury, counterstained with DAPI, exemplary images Fig. 1 D, scale bars 100 μM). STR analysis affirmed the derivation from the parental cell line and karyotyping showed no anomalies (Supplementary Fig. 1). Finally, a standard PCR based assay confirmed that no mycoplasma contamination occurred (Supplementary Fig. 2). All results are summarised in Table 1.

3. Materials and Methods

3.1. Cell culture

IPSCs were cultured in 6-well plates coated with Corning® Matrigel in mTeSR™1 medium (Stem Cell Technologies Cat# 85850) with MycoZap at 37 °C with 5 % CO_2. Media changes were performed daily and passaging was done every 3–4 days with EDTA, afterwards Y-27632 was added.

3.2. Genome Engineering

Guide RNAs (gRNAs) were designed using CHOPCHOP v3 (https://chopchop.cbu.uib.no/) (Labun et al. 2019) and cloned into pSpCas9(BB)-2A-GFP (PX458, Addgene #48138). Guide RNAs were transfected into iPSCs using Lipofectamine Stem Reagent (Thermo Scientific) following the manufacturer's instructions. Three gRNAs were screened and the most efficient was selected for targeted knock-ins. Single-stranded donor oligonucleotides with the intended point mutations and short homology arms were synthesized, including a silent mutation to disrupt the PAM motif and enhance knock-in efficiency.

Female wild-type cells (IUFi004-A) were used for generating the

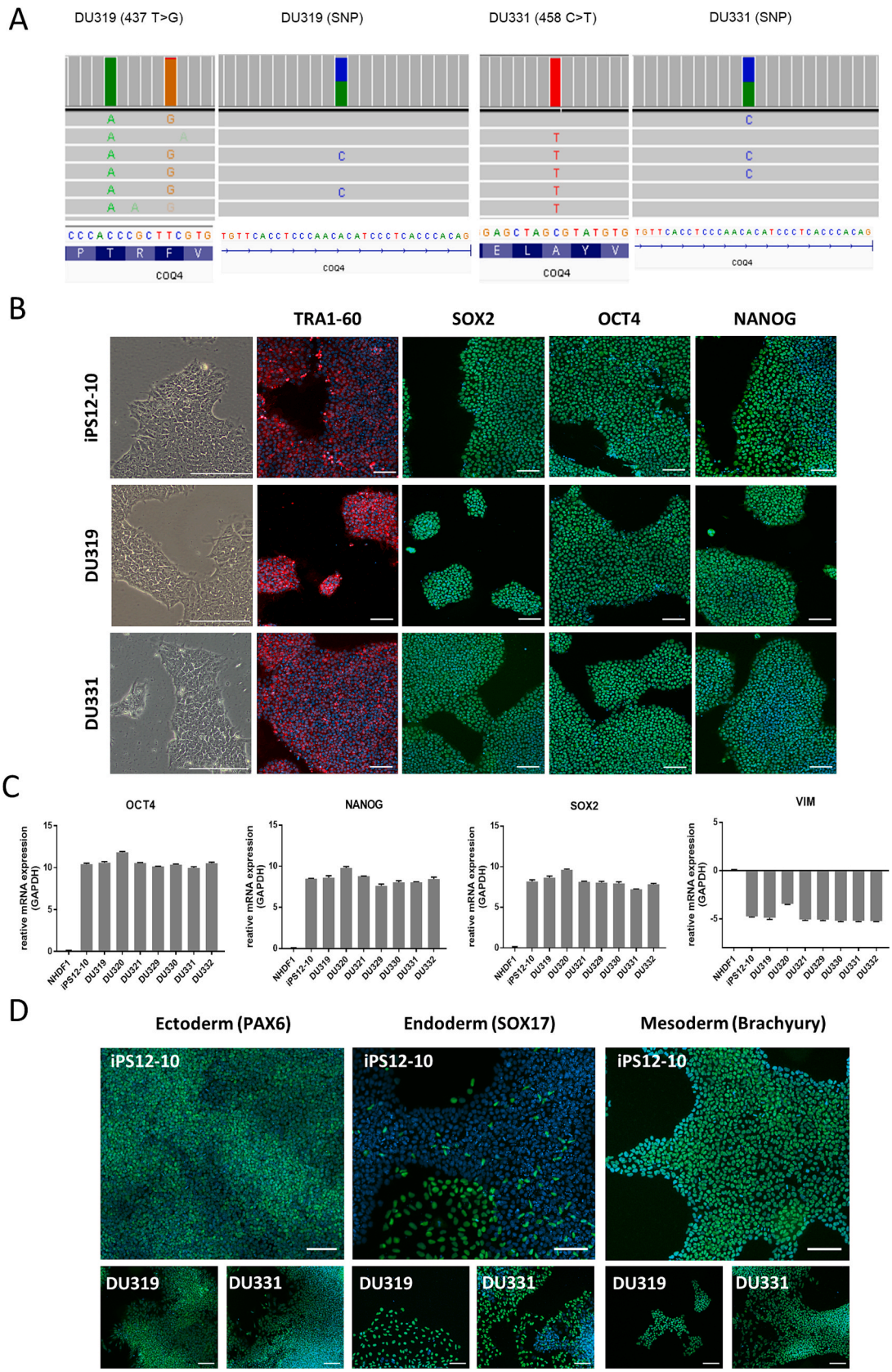

Fig. 1. Characterization of the DU319 and DU331 iPSC lines.

Table 1
Characterization and validation.

Classification	Output type	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel B
Pluripotency status evidence for the described cell line	Immunocytochemistry	Positive for OCT3/4, NANOG, TRA1-60 and SOX2	Fig. 1 panel B
	RT-qPCR	Positive for *OCT4*, *Nanog*, and *SOX2* and negative for *VIM*	Fig. 1 panel C
Karyotype	Karyotype	46XX	Supplementary Fig. 2
Genotyping for the desired genomic alteration/ allelic status of the gene of interest	PCR and nanopore sequencing	Homozygous/ heterozygous status confirmed	Fig. 1 panel A
Verification of the absence of random plasmid integration events	PCR	No PCR amplicon detected	Data not shown, available with the author
Parental and modified cell line genetic identity evidence	STR analysis	21 loci tested, all lines matched the parental fibroblasts	Submitted in the archive with journal
Mutagenesis / genetic modification outcome analysis	Sequencing	NGS MiSeq (Illumina)	Fig. 1 panel A
	Western Blot	Protein reduction	Data not shown, available with the author
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. 1
Multilineage differentiation potential	Directed differentiation	Immunostaining positive for SOX17 (Endoderm), Brachyury (Mesoderm) and PAX6 (Ectoderm) in all cells	Fig. 1 panel D

COQ4 disease models. The iPSCs were co-transfected with the PX458 plasmid and the donor oligo. After 48 h GFP positive cells were sorted by FACS and plated as single cells in 96-well plates. Once colonies were established, plates were divided into maintenance and lysis plates. DNA from lysis plates was extracted, and initial PCR was performed with gene-specific primers, followed by a second barcoding PCR. The barcoded libraries were pooled and sequenced using the MiSeq (Illumina) benchtop sequencer as described previously (Ramachandran et al. 2021). Results were analyzed with Outknocker (Schmid-Burgk et al. 2014) and CRISPRnano (Nguyen et al. 2022).

To identify heterozygous SNPs near the targeted region, PCR primers were designed to amplify approximately 1.3 kb of the region of interest. Amplification was performed using Q5 High-Fidelity DNA Polymerase (NEB), and the products were verified on a 1 % agarose gel. Desired bands were excised and purified using the FastPure Gel DNA Extraction Mini Kit (Vazyme). Library preparation for Oxford Nanopore sequencing was conducted with the Native Barcoding Kit 24V14 (SQK-NBD114.24), following the manufacturer's instructions. Sequencing was performed on a MinION device using a Flongle flow cell and adapter. Oxford Nanopore data were processed using Dorado basecaller (Version dorado-1.0.2) with a super high accuracy model. BAM files were then loaded into IGV for visualization at the locus of interest.

3.3. Immunofluorescence

Cells were fixed in 4 % paraformaldehyd for 15 min, washed with PBS and blocked (1 % BSA, 1 % Triton-X, 0,1 % Tween in PBS) for 1 h at RT. Primary antibodies were incubated over night at 4°C. After washing with PBS, secondary antibodies and DAPI were incubated at room temperature for 30 min. Images were obtained using an Axio Observer 7 (Zeiss). Antibodies are listed in Table 2.

3.4. Trilineage differentiation

Trilineage differentiation was performed using the Stemdiff™ Trilineage Differentiation Kit (Cat# 05230) according to the manufacturer's instructions. Successful differentiation was visualised by immunological staining of the markers for endoderm (SOX17), ectoderm (PAX6) and mesoderm (Brachyury) (Fig. 1D).

3.5. Quantitative real-time PCR (RT-qPCR)

RNA was isolated using the RNeasy Mini Kit (Cat. 74104, Qiagen) and 800 ng was transcribed into cDNA using the QuantiTect® Reverse Transcription Kit (Cat. 205311, Qiagen). For the RT-qPCR run the QuantiFast® SYBR® Green PCR Kit (Cat. 204054) and CFX96 Real-Time System (Bio-Rad) was used. Testing was carried out in triplicates and the relative gene expression normalized to housekeeping gene GAPDH was calculated according to the $2^{\Delta\Delta CT}$ method relative to a commercially purchased fibroblast line. Depicted are the log2 ratios ± SD (Fig. 1E).

3.6. Karyotyping and STR-Analysis

Life & Brain GmbH (Bonn, Germany) performed karyotyping using Illumina BeadArray and iScan and applied cnvPartition version 3.2 (Illumina) for copy number analysis. The Institut für Rechtsmedizin at Universitätsklinikum Düsseldorf carried out the STR analysis. 21 loci were evaluated using GeneMapper ID v3.2.1 (Applied Biosystems).

3.7. Mycoplasma

Mycoplasma contamination was tested using the EZ-PCR Mycoplasma Detection Kit (Sartorius, Cat# 20-700-20).

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Marker	Mouse IgM anti-TRA1-60	1:500	Abcam Cat# AB16288 RRID: AB_778563
Pluripotency Marker	Mouse IgG2b anti-OCT3/4	1:50	Santa Cruz Cat# sc-5279 RRID: AB_628051
Pluripotency Marker	Mouse anti-SOX2	1:100	Santa Cruz Cat# sc-365823 RRID: AB_10,842,165
Pluripotency Marker	Rabbit anti-NANOG	1:1000	Abcam Cat# AB21624 RRID: AB_355097
Differentiation Marker	Rabbit anti-PAX6	1:300	Abcam Cat# AB195045 RRID: AB_2750924
Differentiation Marker	Rabbit anti-SOX17	1:500	Abcam Cat# AB224637 RRID: AB_2801385
Differentiation Marker	Mouse IgG2b anti-Brachyury	1:100	Invitrogen Cat# 14–9770-82 RRID: AB_2573016
Secondary antibody	Alexa Fluor 488 Chicken anti-mouse	1:1000	Invitrogen Cat# A21200 RRID: AB_2535786
Secondary antibody	Alexa Fluor 594 Goat anti-mouse IgM	1:1000	Invitrogen Cat# SA5-10152 RRID: AB_2556732
Secondary antibody	Alexa Flour 488 Goat anti-mouse IgG2b	1:1000	Invitrogen Cat# A21141 RRID: AB_2535778
Secondary antibody	Alexa Fluor 488 Chicken anti-rabbit	1:1000	Invitrogen Cat# A21441 RRID: AB_2535859
Nuclear stain	DAPI	0.5 μg/mL	Calbiochem Cat# 268,298
Site-specific nuclease			
Nuclease information	Nuclease	pSpCas9(BB)-2A	
Delivery method	Lipofection	Lipofectamine Stem Reagent (Thermo Scientific)	
Selection	FACS		
Primers and Oligonucleotides used in this study			
	Target	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers	OCT4	GGAGAAGCTGGAGCAAAACC / TGGCTGAATACCTTCCCAA	
Pluripotency Markers	NANOG	CCTGTGATTTGTGGGCCTG / GACAGTCTCCGTGTGAGGCAT	
Pluripotency Markers	SOX2	GTATCAGGAGTTGTCAAGGCAGAG / TCCTAGTCTTAAAGAGGCAGCAAAC	
Pluripotency Markers	VIM	GGAGCTGCAGGAGCTGAATG / GACTTGCCTTGGCCCTTGAG	
House-Keeping Gene	GAPDH	AGGGCTGCTTTTAACTCT / CCCCACTTGATTTTGGAG	
Genotyping (desired allele)	0c.458C > T	TCCTGGATGTGAACAGGGTCTCCCCAGACACCCGAGCACCCAC**A**CGCTTCGTGGATGATGAGGAGCTAG**T**GTATGTGATTCAGCGGTACCGGGAGGTGCA	
Genotyping (desired allele)	c.437 T > G	TCCCCAGACACCCGAGCACCCAC**A**CGCT**G**CGTGGATGATGAGGAGCTAGCGTATGTGATT	
Verification of hetero-/ homozygous allele status	COQ4 incl. SNP	AGACAGACTGGCAAATCGGG/ TCATCCTCACAAAGGCCTCG	
Verification of the absence of random plasmid integration events	GFP	CGACTTCTTCAAGTCCGCCA/ GTCCATGCCGAGAGTGATCC	
Verification of the absence of random plasmid integration events	CAS9	CAGAGCTTCATCGAGCGGAT/ CGAACAGGTGGGCATAGGTT	
Targeted mutation analysis/sequencing	COQ4	GTCGCCAGAGTTTTCTAGTAGGT/ GTCAGGGCGCAGAGGAC	
gRNA	COQ4 guide RNA	CTCCTCATCATCCACGAAGC GGG	
Genomic target sequence(s)	Including PAM and other sequences likely to affect UCN activity	COQ4: NM_01635, 9q34.11 location: 131085022–131096351 PAM: CAC location: 131094460–131094462	
Bioinformatic gRNA on– and −off-target binding prediction tool used, specific sequence/outputs link(s)	CHOPCHOP v3	https://chopchop.cbu.uib.no/	

CRediT authorship contribution statement

Sonja Herbrich: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Haribaskar Ramachandran:** Writing – review & editing, Resources, Formal analysis, Data curation. **Annette Seibt:** Writing – review & editing, Formal analysis, Data curation. **Isabella Tolle:** Writing – review & editing, Data curation. **Annika Zink:** Writing – review & editing, Validation, Supervision, Methodology. **Alessandro Prigione:** Writing – review & editing, Supervision, Resources, Methodology. **Andrea Rossi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Felix Distelmaier:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Felix Distelmaier reports financial support was provided by German Research Foundation. Felix Distelmaier reports financial support was provided by Elterninitiative Kinderkrebsklinik e.V. Düsseldorf. 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.].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103825.

Data availability

Data will be made available on request.

References

Hargreaves, I., Heaton, R.A., Mantle, D., 2020. Disorders of Human Coenzyme Q10 Metabolism: an Overview. Int. J. Mol. Sci. 21 (18).
Labun, K., Montague, T.G., Krause, M., Torres Cleuren, Y.N., Tjeldnes, H., Valen, E., 2019. CHOPCHOP v3: expanding the CRISPR web toolbox beyond genome editing. Nucl. Acids Res. 47 (W1), W171–W174.
Laugwitz, L., Seibt, A., Herebian, D., Peralta, S., Kienzle, I., Buchert, R., et al., 2022. Human COQ4 deficiency: delineating the clinical, metabolic and neuroimaging phenotypes. J. Med. Genet. 59 (9), 878–887.
Nguyen, T., Ramachandran, H., Martins, S., Krutmann, J., Rossi, A., 2022. Identification of genome edited cells using CRISPRnano. Nucl. Acids Res. 50 (W1), W199–W203.
Nicoll, C.R., Alvigini, L., Gottinger, A., Cecchini, D., Mannucci, B., Corana, F., et al., 2024. In vitro construction of the COQ metabolon unveils the molecular determinants of coenzyme Q biosynthesis. Nat. Catal. 7 (2), 148–160.
Ramachandran, H., Martins, S., Kontarakis, Z., Krutmann, J., Rossi, A., 2021. Fast but not furious: a streamlined selection method for genome-edited cells. Life Sci. Alliance 4 (6).
Schmid-Burgk, J.L., Schmidt, T., Gaidt, M.M., Pelka, K., Latz, E., Ebert, T.S., Hornung, V., 2014. OutKnocker: a web tool for rapid and simple genotyping of designer nuclease edited cell lines. Genome Res. 24 (10), 1719–1723.