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Generation of two human iPSC lines from fibroblasts of BPAN patients carrying pathogenic variants in the *WDR45* gene

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A B S T R A C T

Beta-propeller Protein-Associated Neurodegeneration (BPAN) is a rare X-linked dominant disorder (ORPHA:329284) characterized by brain iron accumulation, developmental delay, seizures, motor dysfunction, and progressive neurodegeneration. It results from pathogenic variants in *WDR45*, encoding WDR45/WIPI4, a key autophagy protein. No curative treatment exists; management is supportive. As BPAN pathogenesis remains unclear, research aims to elucidate its molecular mechanisms and develop targeted therapies. We generated and characterized two induced pluripotent stem cell (iPSC) lines from BPAN patient fibroblasts, providing essential models for studying disease mechanisms and developing effective therapeutic strategies.

1. Resource Table

Unique stem cell lines identifier	HMGUi005-A (https://hpscreg.eu/cell-line/HMGUi005-A) HMGUi006-A (https://hpscreg.eu/cell-line/HMGUi006-A)
Alternative name(s) of stem cell lines	Optional name from cell line HMGUi005-A: 150553 Optional name from cell line HMGUi006-A: 152350
Institution	Helmholtz Zentrum München, Germany
Contact information of distributor	Arcangela Iuso, arcangela.iuso@helmholtz-munich.de
Type of cell lines	iPSCs
Origin	Human
Additional origin info required	150553: Female 152350: Male
Cell Source	Patient derived skin fibroblasts
Clonality	Clonal
Method of reprogramming	mRNA
Genetic Modification	150553: NC_000023.10 (NM_001029896.2):c.726-2A>T 152350: NC_000023.11 (NM_001029896.2):c.749_751del
Type of Genetic Modification	Spontaneous mutation
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	n.a.
Associated disease	Beta-propeller Protein-Associated Neurodegeneration (BPAN)
Gene/locus	*WDR45*/Xp11.23
Date archived/stock date	December 2024
Cell line repository/bank	n.a.
Ethical approval	Ethical committee: Technical University of Munich (TUM) Approval number: 2025–301-S-NP Approval date: 09.07.2025

2. Resource utility

The two generated human induced pluripotent stem cell (hiPSC) lines serve as a valuable resource for deriving disease-relevant cell types, including neurons and astrocytes, enabling investigation of BPAN pathomechanisms and supporting the development of potential therapeutic strategies.Table 1

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3. Resource details

In this study, we established hiPSC lines (HMGUi005-A or 150553 and HMGUi006-A or 152350) from skin fibroblasts of two unrelated BPAN patients with a phenotype of brain iron accumulation, severe developmental delays/intellectual disability, seizures, motor dysfunction, and progressive neurodegeneration.

hiPSC line 150553 was derived from a 21-year-old female patient harbouring a de novo splice variant, *WDR45*- NM_001029896.2):c.726-2A>T. This variant is absent from gnomAD and predicted (https://spliceailookup.broadinstitute.org) to disrupt the canonical splicing acceptor site, leading to abnormal splicing. It is not listed in ClinVar, or HGMD as of today.

hiPSC line 152350 was derived from a 7-year-old male patient harbouring a de novo indel variant, *WDR45*- NM_001029896.2): c.749_751del, which results in an in-frame deletion of serine at position 250 (p.Ser250del). This variant has previously been reported as pathogenic (Ferrera, 2024) (Fig. 1A).

The reprogramming of patient derived fibroblasts into iPSCs was performed using a vector-free system consisting of mRNA for SOX2, KLF4, OCT4, LIN28, NANOG, and c-MYC (Yu, 2007). The established iPSC lines (150553 and 152350) exhibited normal morphology (Fig. 1B) and normal karyotype, as confirmed by G-banding, with 46, XX for 150553 and 46, XY for 152350 (Fig. 1C). The iPSCs were first assessed qualitatively for an undifferentiated hPSC state by immunocytochemical staining for Oct4, Sox2, Lin28, and Nanog, which showed positive staining for all four markers (Fig. 1D). The pluripotent population was then quantified by flow cytometry using the PSC Analysis Cocktail, revealing a high proportion (>98 %) of undifferentiated pluripotent cells (Fig. S1A). The differentiation potential of the 150553 and 152350 lines was demonstrated by direct trilineage differentiation followed by FACS sorting, showing expression of CD144 and CD140b for the mesoderm, CD184 and SOX17 for the endoderm, and SOX2 and PAX6 for the ectoderm (Fig. 1E). Luminometric tests confirmed the absence of mycoplasma contamination in both lines (Fig. S1B).

4. Materials and methods

4.1. iPSCs generation and culturing

hiPSC lines 150553 and 152350 were generated from fibroblasts using NM-RNA kit (Reprocell, #00-0076). Fibroblasts were cultured in NutriStem® XF (Sartorius, #05-100-1A). Four daily transfections were performed. On day 10, cells were switched to iPS-Brew XF (Miltenyi Biotec, #130-104-368). iPSCs were cultured on Matrigel-coated (Corning, #354234) culture-ware in mTeSR™ Plus (STEMCELL Technologies, #100-0276) at 37 °C and 5 % CO_2 and passaged as clumps using StemMACS™ Passaging Solution XF (Miltenyi Biotec, #130-104-388).

4.2. Immunocytochemistry

Cells were fixed with 4 % PFA (Thermo Fisher, #28906) for 10 min and permeabilized with 0.2 % Triton™X-100 (Merck, #X100-500ML) for 15 min at room temperature (RT). Antibodies, listed in Table 2, were diluted in 0.2 % Triton™X-100 with 10 % FBS (Cytiva, #SH30071.03IR25-40) and incubated overnight at 4 °C (primary) or 1 h at RT (secondary). Slides were mounted using ProLong™ Glass Antifade Mountant with NucBlue™ Stain (Thermo Fisher, #P36985) and imaged using a Zeiss Axio Imager M.2 with Colibri 7 and an EVOS™ M500 microscope.

4.3. Flow cytometry

Cells were harvested as single-cell suspensions and collected via centrifugation. The cells were fixed and permeabilized using the FoxP3 Staining Buffer Set (Miltenyi, #130-093-142). Primary antibodies were used following manufacturer recommendation for flow cytometry use. The samples were measured using BD FACS Symphony™ A3 Cell Analyzer. Data were analyzed with the FlowJo software.

4.4. Potency assay

Upon reaching 90 % confluence, iPSC clones were differentiated

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1B
Phenotype	Qualitative(Immunocytochemistry) and quantitative (FACS) analyses	All clones express the undifferentiated hPSC state markers OCT4, NANOG, SOX2 and LIN28. Passage number at which the cells were tested: 150553 P8; 152350 P12	Fig. 1D, Fig. S1A
Genotype	Karyotype (G-banding) and resolution	150553: Passage P20-22, 46XX, 450–450 bands per haploid set (bphs) [reference range: ≥ 400 bphs]. 152350: Passage P20-22, 46XY, 450–500 bphs [reference range: ≥ 400 bphs]	Fig. 1C
Identity	STR analysis	16 sites tested, all matching 16 sites tested, all matching	Submitted in archive with journal
Mutation analysis	Sequencing	150553: c.(726-2A>T); (=)152350: c.(749_751del); (0)	Fig. S1A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. All clones were negative. Passage number to which the cells were tested: 150553 P9; 152350 P11	Fig. S1B
Differentiation potential	Directed differentiation followed by FACS	CD144/CD140b: 94–97 % (mesoderm); CD184/SOX17: 70–80 % (endoderm); SOX2/PAX6: >97 % (ectoderm) Passage number to which the cells were tested: 150553 P12; 152350 P16	Fig. 1E
List of recommended germ layer markers	**Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer**	CD144/CD140b: 94–97 %; CD184/SOX17: 70–80 %; SOX2/PAX6: >97 % Passage number to which the cells were tested: 150553 P12; 152350 P16	Fig. 1E

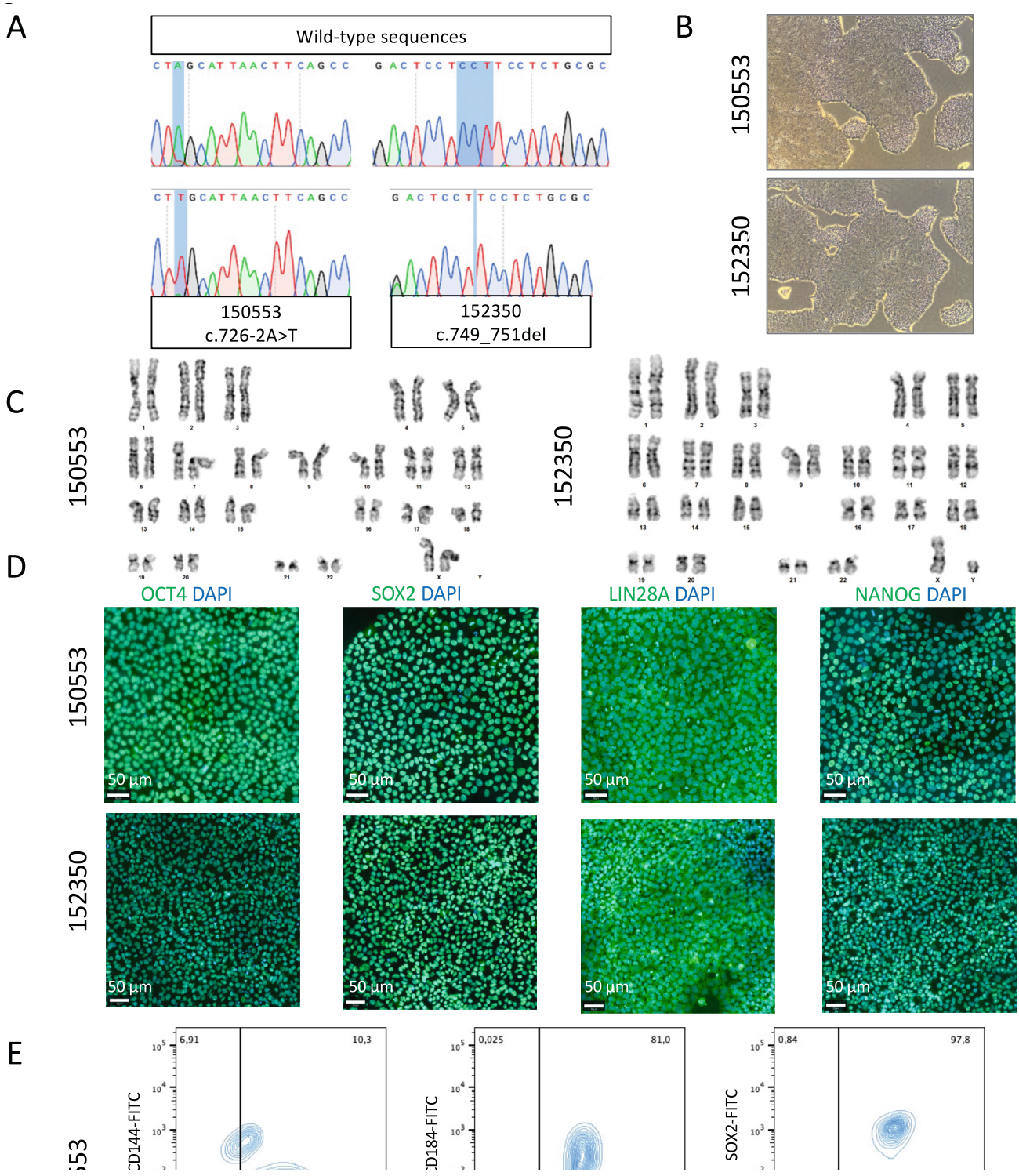

Fig. 1. A) Sanger sequencing of the 150553 and 152350 iPSC lines. B) Brightfield morphology of iPSC colonies. C) Karyotype analysis by G-banding. D) Immunofluorescence staining for undifferentiated hPSC state markers Oct4, Sox2, Lin28, and Nanog. E) FACS analysis of cells positive for lineage-specific markers: CD144 and CD140b (mesodeerm), CD184 and SOX17 (endoderm), and SOX2 and PAX6 (ectoderm).

using the StemMACS™ Trilineage Differentiation Kit (Miltenyi, #130–115-660) for 5–7 days following the manufacturer's instructions. Cultures were washed three times with DPBS (Thermo Fisher, #14190144) and harvested using TrypLE™ Express Enzyme Thermo Fisher, #12605010). Previously published ISFi001-A hiPSC line was used as a positive control (Kunze, 2018).

4.5. Sequencing

DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, #69504). The region of interested was amplified via PCR using the Thermo-Start Taq DNA Polymerase (Thermo Fisher, #AB-1057/B), applying the following thermocycling parameters: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation: 95 °C, 30 s, annealing:

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Undifferentiated hPSC state, immunocytochemistry	Rabbit anti-OCT-4a	1:400	CST #2840S	RRID: AB_2167691
Undifferentiated hPSC state, immunocytochemistry	Rabbit anti-SOX2	1:200	CST #2748S	RRID:AB_823640
Undifferentiated hPSC state, immunocytochemistry	Rabbit anti-NANOG	1:200	CST #4903S	RRID:AB_10559205
Undifferentiated hPSC state, immunocytochemistry	Rabbit anti-LIN28A	1:800	CST #3978S	RRID: AB_2297060
Undifferentiated hPSC state, FACS	PSC Analysis Cocktail, anti-human including:CD15, anti-human, VioBlue® (clone: VIMC6)SSEA-4, anti-human, VioGreen™ (clone: REA101) TRA-1–60, anti-human, PE (clone: REA157) Oct3/4, anti-human /mouse, APC (clone: REA622)	1:10	130–136-292	RRID: AB_2733931 RRID: AB_2877031 RRID: AB_2801969 RRID: AB_2819457
Undifferentiated hPSC state, FACS	PSC Control Cocktail, anti-human includingIsotype Control mouse IgMκ, VioBlue (clone: IS5-20C4)REA Control, human IgG1, VioGreen, REAfinity™ (clone: REA293)REA Control, human IgG1, PE, REAfinity (clone: REA293)REA Control, human IgG1, APC, REAfinity (clone: REA293)	1:10	130–136-292	RRID: AB_2727473 RRID: AB_2734113 RRID: AB_2733892 RRID: AB_2733446
Secondary antibodies, immunocytochemistry	Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed, Alexa Fluor 488	1:1000	Thermo Fisher # A-11034	RRID: AB_2576217
Nuclear stain	ProLong Glass Antifade Mountant with NucBlue Stain (Hoechst 33342)	N/A	Thermo Fisher # P36985	
FACS	CD144 (VE-Cadherin)-FITC	1:10	Miltenyi Biotec	RRID: AB_2655150
FACS	CD140b-APC	1:10	Miltenyi Biotec	RRID: AB_2655084
FACS	CD184-FITC	1:10	Miltenyi Biotec	RRID: AB_2734060
FACS	SOX17-APC	1:50	Miltenyi Biotec	RRID: AB_2653495
FACS	SOX2-FITC	1:10	Miltenyi Biotec	RRID: AB_2653499
FACS	PAX6-APC	1:10	Miltenyi Biotec	RRID: AB_2653168
	Primers **Target**	**Size of band**	**Forward/Reverse primer (5′-3′)**	
WDR45 (Sanger sequencing)	WDR45 mutations c.(726-2A>T) and c.(749_751delCCT	300 bp	AGGGTATGCATGGTGGGCTGGT/ GCCAGGCTCCACTGAGAGTCCA	

62 °C, 30 s and extension: 72 °C, 30 s, followed by a final extension at 72 °C, 7 min and sequenced via Sanger sequencing (Eurofins Genomics) with primers (Eurofins Genomics) listed in Table 2.

4.6. Karyotyping

Karyotyping was performed by conventional GTG-banding on cultured iPSCs by the accredited cytogenetics laboratory at the Institute of Human Genetics of the Technical University of Munich. Briefly, metaphase chromosomes were prepared after colcemid treatment, hypotonic swelling, and methanol–acetic acid fixation, followed by trypsin–Giemsa (GTG) banding. A minimum of 20 metaphases per line were analyzed at 450–500 bands per haploid set (bphs), meeting the recommended resolution of at least 400 bphs.

4.7. Mycoplasma testing

Mycoplasma was measured using the MycoAlert® Mycoplasma detection kit (Lonza, #LT07-118).

5. STR analysis

Genomic DNA was extracted from iPSCs and original fibroblasts. Genetic characteristics were determined by PCR-single-locus-technology. 16 independent PCR-systems D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, AMEL, D5S818, FGA, D19S433, vWA, TPOX and D18S51 were investigated. In parallel, positive and negative controls were carried out yielding correct results. The analysis confirmed a 100 % identity between established iPSCs and donors' fibroblasts (submitted in archive with journal).

CRediT authorship contribution statement

Gemma Gasparini: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Carolin Kraus:** Writing – original draft, Investigation, Data curation. **Ejona Rusha:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Tanja Orschmann:** Methodology. **Saskia B. Wortmann:** Writing – review & editing, Resources. **Johannes H. Mayr:** Resources. **Anna Ardissone:** Resources. **Arcangela Iuso:** Writing – review &

editing, Supervision, Resources, Funding acquisition, Data curation, 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.2025.103892.

Data availability

We confirm that all data underlying the findings described in this manuscript are fully available within the article and/or its supplementary materials.

References

Ferrera, G., et al., 2024. WDR45-related encephalopathy mimicking Leigh syndrome associated with complex I deficiency: a case report. Eur. J. Hum. Genet. EJHG. https://doi.org/10.1038/s41431-024-01745-1.

Yu, J., et al., 2007. Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917–1920.

Kunze, C., et al., 2018. Synthetic AAV/CRISPR vectors for blocking HIV-1 expression in persistently infected astrocytes. Glia 66, 413–427.