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Characterization of an induced pluripotent stem cell line (UMi040-A) bearing an auditory neuropathy spectrum disorder-associated variant in *TMEM43*

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Abstract

Hearing loss is one of the most common sensory disorders. *TMEM43* is expressed in cochlear glia-like supporting cells (GLSs) and is known to be associated with late-onset auditory neuropathy spectrum disorder (ANSD) and progressive hearing loss. Here, we describe the derivation of an induced pluripotent stem cell (iPSC) line from a patient lymphoblastoid cell line (LCL) carrying a single heterozygous nonsense variant (p.Arg372Ter (c.1114C > T)) in *TMEM43* that leads to a truncated protein lacking the 4th transmembrane domain. This cell line can serve as a tool for disease modelling and development of therapeutic approaches to restore inner ear function.

1. Resource Table

Unique stem cell line identifier	UMi040-A

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102758.

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Alternative name(s) of stem cell line	SB162–284 cl. 1
Institution	University of Miami
Contact information of distributor	Dr. Xue Zhong Liu
Type of cell line	Ipsc
Origin	Species: human
Additional origin info	Age: 43
	Sex: Male
	Ethnicity: Asian
Cell Source	Original cell type induced: lymphoblastoid cell line.
Clonality	Clonal
Method of reprogramming	Non-integrated, episomal (Oct4, Sox2, Lin28, Klf4, and L-Myc)
Genetic Modification	Yes
Type of Modification	Heredity
Associated disease	Auditory neuropathy spectrum disorder (ANSD)
Gene/locus	*TMEM43;* c.1114C > T; p.(Arg372Ter)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	Constitutive
Date archived/stock date	N/A
Cell line repository/bank	https://hpscreg.eu/cell-line/UMi040-A
Ethical approval	Our cell products are made from lymphoblastoid cells under FDA 21 CFR 1271 regulations by an approved tissue bank organization. Donor consent, eligibility determination, IRB approval and confidential information are maintained by the tissue collection agency and are protected under US HIPAA rules.

2. Resource utility

TMEM43 is associated with auditory neuropathy spectrum disorder (ANSD) (Jang et al., 2021). UMi040-A is an iPSC line that is heterozygous for the c.1114C > T variant (p.Arg372Ter). This cell line can be used to study the molecular mechanisms underlying the pathology and support the development of therapeutic strategies to treat ANSD.

3. Resource details

The iPSC line UMi040-A was derived from a lymphoblastoid cell line (LCL) generated from a patient with late-onset progressive hearing loss carrying a heterozygous point mutation c.1114C > T (p.Arg372Ter) in the *TMEM43* gene (Jang et al., 2021). Mutations in *TMEM43* gene are associated with several diseases, including auditory neuropathy spectrum disorder (ANSD; (Jang et al., 2021), arrhythmogenic cardiomyopathy (McNally et al., 1993), and Emry-Dreifuss muscular dystrophy (Heller et al., 2020). The *TMEM43* c.1114C > T variant was recently identified in two families with ANSD leading to progressive hearing loss (Jang et al., 2021). *TMEM43* is primarily expressed in cochlear glia-like supporting cells (GLS (Jang et al., 2021)). TMEM43 protein interacts with the KCNK3 protein to mediate the passive conductance current in GLS, which is critical for maintaining the homeostasis in inner ear (Jang et al., 2021).

UMi040-A cells were cultured in StemFlex™ medium (Thermo Fisher Scientific) on vitronectin (VTN; Thermo Fisher Scientific) -coated plates. This cell line formed colonies

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with morphology similar to that seen with embryonic stem cells (i.e. tight cell aggregates with rounded borders) (Fig. 1A). The UMi040-A iPSC line was confirmed to have the c.1114C > T variant by Sanger sequencing (Fig. 1B). G-band karyotyping of this iPSC line show that there was no evidence for abnormal chromosomal structure (Fig. 1C). The UMi040-A cell line was characterized for the expression of markers associated with pluripotency using immunocytochemistry (ICC) and quantitative real-time PCR (qRT-PCR). This line stained positively for NANOG, SSEA4, and OCT3/4 (Fig. 1D) and showed comparable expression of *POU5F1*, *NANOG*, and *SOX2* compared to a healthy control hiPSC line, CW50038 (Coriell), when analyzed by qRT-PCR (Fig. 1E). UMi040-A was successfully differentiated into cells representing three primary germ layers using the STEM-diff™ Trilineage Differentiation Kit (StemCell Technologies). Specific markers for each germ layer were examined using ICC (Fig. 1F). Specifically, ectodermal, mesodermal, and endodermal derivatives were stained with PAX6 and SOX1, Brachyury and α-SMA, and SOX17 and FOXA2, respectively. Our data shows that the UMi040-A iPSC was episomal reprogramming vectors and EBV free by passage 28 as measured by PCR and gel electrophoresis (Supp. fig. 1A). Short Tandem Repeat (STR) analysis showed that the UMi040-A line was derived from the parental LCL. All data are provided in Table 1. Our data show the derivation and characterization of the iPSC line UMi040-A from patient-derived LCL carrying the TMEM43 nonsense variant p.Arg372Ter. This cell line will be a great resource for understanding the role of GLSs and the *TMEM43* gene in the development of ANSD and serve as a platform for drug discovery and development efforts.

4. Materials and methods

4.1. Reprogramming of patient lymphoblastoid cell line (LCL)

The SB162–284 LCL was generated in Dr. Byung Yoon Choi's laboratory. The LCL was maintained in RPMI 1640 medium containing 2 mM L-glutamine (Thermo Fisher Scientific) and 15% fetal bovine serum (Thermo Fisher Scientific) at 37 °C under 5% CO_2.

Lymphoblastoid cells were reprogrammed using the Epi5™ Episomal iPSC Reprogramming Kit (Thermo Fisher Scientific) following the manufacturer's instructions. Briefly, lymphoblastoid cells were transduced with episomal vectors through electroporation using the P3 primary cell kit (Lonza) and Lonza's 4D nucleofector system. The cells were plated onto VTN-coated six well plate in LCL medium to recover from the electroporation. From the second day post transduction, cells were maintained in N2B27 medium (see Epi5™ Episomal iPSC Reprogramming Kit manufacture's protocol). On day 10, the reprogrammed cells were transitioned to growth in StemFlex™ media. Selection of individual clonal lines was conducted manually.

4.2. UMi040-A cell culture

UMi040-A line was cultured in StemFlex™ medium on VTN-coated plates at 37 °C under 5% CO_2. The medium was changed daily, and removal of differentiated cells was done manually. Cells were passaged at 1:6 every 3–4 days or when needed.

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4.3. Immunocytochemistry

Cells were fixed in 4% paraformaldehyde (Electron Microscopy Science) for 30 min at room temperature (RT) on the rocker followed by three washes with PBS. Blocking was performed in 10% goat or horse serum and 0.01% Triton X-100 in PBS for 30 min at RT followed by incubation with primary antibodies (Table 2) for 1 hr at RT. Subsequently, the cells were washed three times with PBS prior incubation with secondary antibodies (Table 2) for 1 hr at RT. Cells were washed three times with PBS at RT and mounted using ProLong™ Gold antifade mounting media (Thermo Fisher Scientific). Images were captured using the BZ-X810 all-in-one fluorescence microscope (Keyence).

4.4. Sanger sequencing

Genomic DNA was isolated using Monarch® Genomic DNA Purification Kit (NEB). PCR amplification of the region including *TMEM43* c.1114C > T variant (Table 2) was done using Platinum™ Hot Start PCR Master Mix (Invitrogen). Purified PCR products using Monarch® PCR & DNA Cleanup Kit (NEB) were submitted to GeneWiz for Sanger sequencing.

4.5. Quantitative real-time PCR (qRT-PCR)

RNA was isolated using Monarch® Total RNA Miniprep kit (NEB). iScript Reverse Transcription kit (BioRad) was used for reverse transcription reactions. Taqman assays were performed using the Applied Biosystems™ 7500 real-time PCR system. GAPDH was used as the endogenous gene. The relative gene expression levels were compared to a control hiPSC line CW50038 (Fig. 1F).

4.6. Trilineage Differentiation

Three germ layer cells were differentiated using the STEMDiff™ Trilineage Differentiation Kit (StemCell Technologies) following the manufacturer's protocol. Selected gene markers (Table 2) were used to validate identities of differentiated cells.

4.7. STR, karyotype, and mycoplasma anlyses

G-band karyotyping, mycoplasma testing, and STR analysis were performed by WiCell.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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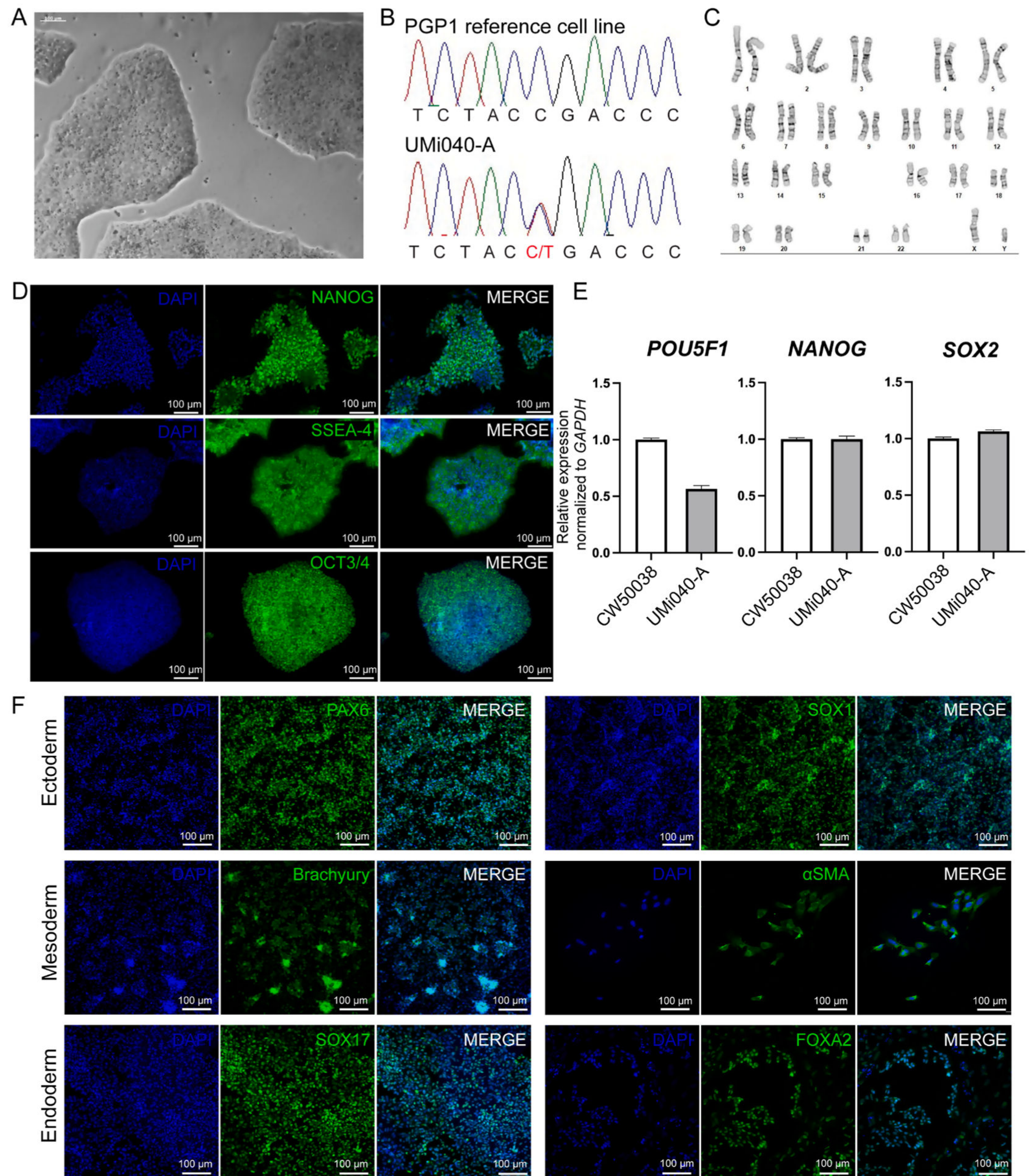

Fig. 1.

Characterization of induced pluripotent stem cell line carrying the heterozygous *TMEM43* c.1114C > T variant.

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Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast imaging	Visual record of the line: normal	Fig. 1A
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining for NANOG, SSEA-4, OCT3/4	Fig. 1D
	Quantitative analysis qRT-PCR	Comparable expression level of POU5F1, NANOG, and SOX2 to a control hiPSC line.	Fig. 1E
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 400–450	Fig. 1C
Identity	STR analysis	No contamination with other cell lines/types, same genetic identity as parental lines	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sanger sequencing	Heterozygous mutation in *TMEM43* c.1114C > T	Fig. 1B
Microbiology and virology	Mycoplasma	Band was not seen at 270 bp, indicating the absence of mycoplasma.	Supplemental Fig. 1B
Differentiation potential	Trilineage differentiation	Necessary markers were present for each of the three germ layers	Fig. 1F
List of recommended germ layer markers	Expression of these markers has been demonstrated at protein (ICC) levels	*Ectoderm:* PAX6 and SOX1. Endoderm: SOX17 and FOXA2. Mesoderm: BRACHYURY/TBXT and α-SMA	ICC with specific antibodies (Table 2)
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	NA
Genotype additional info (OPTIONAL)	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

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Table 2

Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency markers	Mouse anti-OCT3/4	1:50	Santa Cruz Cat#sc-5279, RRID:AB_628051
Pluripotency markers	Mouse anti-SSEA-4	1:100	STEMCELL Technologies Cat#60062, RRID:AB_2721031
Pluripotency markers	Rabbit anti-NANOG	1:100	Invitrogen Cat#PA1–097, RRID:AB_2539867
Secondary antibody	Goat anti-Rabbit IgG AlexaFlour488	1:500	Thermo Fisher Scientific Cat# A-11034, RRID:AB_2576217
Secondary antibody	Goat anti-Mouse IgG AlexaFlour488	1:500	Thermo Fisher Scientific Cat# A-11029, RRID:AB_2534088
Trilineage Differentiation	Mouse anti-PAX6	1:100	Abcam Cat# ab195045, RRID:AB_2750924
Trilineage Differentiation	Rabbit anti-SOX1	1:100	Abcam Cat# ab109290, RRID:AB_10858336
Trilineage Differentiation	Rabbit anti-Brachyury	1:200	Abcam Cat# ab20680, RRID:AB_727024
Trilineage Differentiation	Mouse anti-Asma	1 ug/mL	Novus Cat# NBP2–33006, RRID:AB_1726236
Trilineage Differentiation	Goat anti-SOX17	1:100	R&D Cat# AF1924, RRID:AB355060
Trilineage Differentiation	Mouse anti-FOXA2	1:200	Abcam Cat# ab60721, RRID:AB_941632
Primers			
	Target	Size of band	Forward/Reverse primer (5′–3′)
Genotyping	*TMEM43*	447 bp	GGTTTCCTGTTTTCCGAGAC/GTCAGCTTGCCATTCATGAG
Episomal plasmid	OriP	544 bp	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGAGACAAC
EBV	EBNA-1	666 bp	ATCGTCAAAGCTGCACACAG/CCCAGGAGTCCCAGTAGTCA
qRT-PCR	*POU5F1*	N/A	Hs04260367_gH
qRT-PCR	*SOX2*	N/A	Hs01053049_s1
qRT-PCR	*NANOG*	N/A	Hs4399610_g1
qRT-PCR	*DPPA5*	N/A	Hs00988349_g1
qRT-PCR	*GAPDH*	N/A	Hs99999905_m1