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Generation of four induced pluripotent stem cell lines (FHUi003-A, FHUi003-B, FHUi004-A and FHUi004-B) from two affected individuals of a familial neurohypophyseal diabetes insipidus family

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A B S T R A C T

Four disease-specific induced pluripotent stem cell (iPSC) lines were respectively derived from peripheral blood mononuclear cells of two affected individuals in a family affected by familial neurohypophyseal diabetes insipidus carrying the c.314G > C mutation. The expression of pluripotency markers (NANOG, OCT4, and SOX2), maintenance of a normal karyotype, absence of episomal vectors used for iPSC generation, and presence of the original pathogenic mutation were confirmed for each iPSC line. The ability to differentiate into three germ layers was confirmed by a teratoma formation assay. These iPSC lines can help in disease recapitulation *in vitro* using organoids and elucidation of disease mechanisms.

Resource Table

Unique stem cell lines identifier	FHUi003-A FHUi003-B FHUi004-A FHUi004-B
Alternative names of stem cell lines	FNDI-iPS-A1b, A1b2as1 (FHUi003-A) FNDI-iPS-A11b, A11b (FHUi003-B) FNDI-iPS-B3a, B3a (FHUi004-A) FNDI-iPS-B7a, B7as1 (FHUi004-B)
Institution	Fujita Health University School of Medicine
Contact information of distributor	Akifumi Matsuyama, akifumi-matsuyama@umin.ac.jp
Type of cell lines	iPSC
Origin	Human
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Transgene free (Episomal vectors)
Multiline rationale	Two lines from each of two patients (a male and a female) of the same family, harboring the same disease and genetic mutation
Gene modification	YES
Type of modification	Hereditary
Associated disease	Familial neurohypophyseal diabetes insipidus (FNDI)

Resource Table (*continued*)

Gene/locus	*AVP*/20p13
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	December 2019
Cell line repository/bank	https://hpscreg.eu/cell-line/FHUi003-A https://hpscreg.eu/cell-line/FHUi003-B https://hpscreg.eu/cell-line/FHUi004-A https://hpscreg.eu/cell-line/FHUi004-B
Ethical approval	Ethics Committee of Fujita Health University School of Medicine (approval number HG19-062). Ethical Committee of Nagoya University Hospital (approval number 2018-006-4). Ethical Committee of Mitoyo General Hospital (approval number 18CR01-067). All animal experiments were approved by the Animal Care and Use Committee of KATAGIRI VMD OFFICE (approval number FHU19-1).

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
FNDI-iPS-A1b, A1b2as1 (FHUi003-A)	FNDI_A1b	Male	71	Japanese	c.314G > C, heterozygous	Familial neurohypophyseal diabetes insipidus
FNDI-iPS-A11b, A11b (FHUi003-B)	FNDI_A11b	Male	71	Japanese	c.314G > C, heterozygous	Familial neurohypophyseal diabetes insipidus
FNDI-iPS-B3a, B3a (FHUi004-A)	FNDI_B3a	Female	45	Japanese	c.314G > C, heterozygous	Familial neurohypophyseal diabetes insipidus
FNDI-iPS-B7a, B7as1 (FHUi004-B)	FNDI_B7a	Female	45	Japanese	c.314G > C, heterozygous	Familial neurohypophyseal diabetes insipidus

1. Resource utility

The lack of good *in vitro* models of familial neurohypophyseal diabetes insipidus (FNDI) has made pathological analysis difficult. The generated FNDI-iPSCs can be useful for disease recapitulation *in vitro* using the hypothalamic-pituitary unit of organoids (Kasai et al., 2020; Suga, 2016), along with elucidation of the pathology and drug discovery Table 1.

2. Resource details

FNDI is a progressive form of diabetes insipidus caused by mutation in the *AVP* gene, which encodes a preproprotein including the antidiuretic hormone arginine vasopressin (AVP), AVP carrier protein neurophysin 2, and copeptin. The mutation leads to deficient AVP secretion from the pituitary gland, resulting in excessive and thin urine production accompanied by a polyuric symptom due to impaired water reabsorption in the kidneys (Arima and Oiso, 2010; Hagiwara et al., 2019). We generated disease-specific iPSCs from peripheral blood mononuclear cells (PBMCs) of two donor patients of an FNDI family, both of whom carry a heterozygous mutation (c.314G > C) in the neurophysin 2-coding region of *AVP*. We here report two iPSC lines derived from each of the two patients for a total of four cell lines. Characterization of the iPSC lines is summarized in Table 2. Each generated iPSC colony was picked and expanded. Each iPSC line shows the typical iPSC morphology with a high nucleus to cytoplasm ratio (Fig. 1A), and expression of pluripotent markers NANOG, OCT4, and SOX2 was confirmed by immunocytochemical analysis (Fig. 1B). Positive cells for the markers in at least three independent areas were detected for each iPSC line with an average positive ratio of > 95%, > 92%, and > 95% for NANOG, OCT4, and SOX2, respectively (Fig. 1D; Table 2). The ability of each iPSC line to differentiate into the three germ layers was confirmed by an *in vivo* teratoma formation assay; histological analysis of the developed teratoma revealed formation of the neural tissue (ectoderm), cartilage and muscle (mesoderm), and gut-like columnar epithelium (endoderm, Fig. 1C). Differentiation into pigment cells (ectoderm) and the adipose tissue (mesoderm) was also found in FNDI-A1b (FHUi003-A) and FNDI-A11b (FHUi003-B) (Fig. 1C). After more than a dozen passage cultures, none of the residual episomal vectors used for generating the iPSCs was detected by polymerase chain reaction (PCR) analysis targeting the DNA sequence of *OriP* and *EBNA1* in the vector (Fig. 1E). The original heterozygous mutation causing the disease was confirmed in the iPSCs derived from both patients by sequencing (Fig. 1F). G-banding analysis showed a normal karyotype of each iPSC line as 46XY (FNDI-A1b and A11b) or 46XX (FNDI-B3a and B7a; Supplementary Fig. 1A). No sign of *Mycoplasma* infection was detected by the MycoAlert™ assay (Supplementary Fig. 1B). The identity of each iPSC line was confirmed by STR analysis (submitted in archive with journal) with an evaluation value of 1.

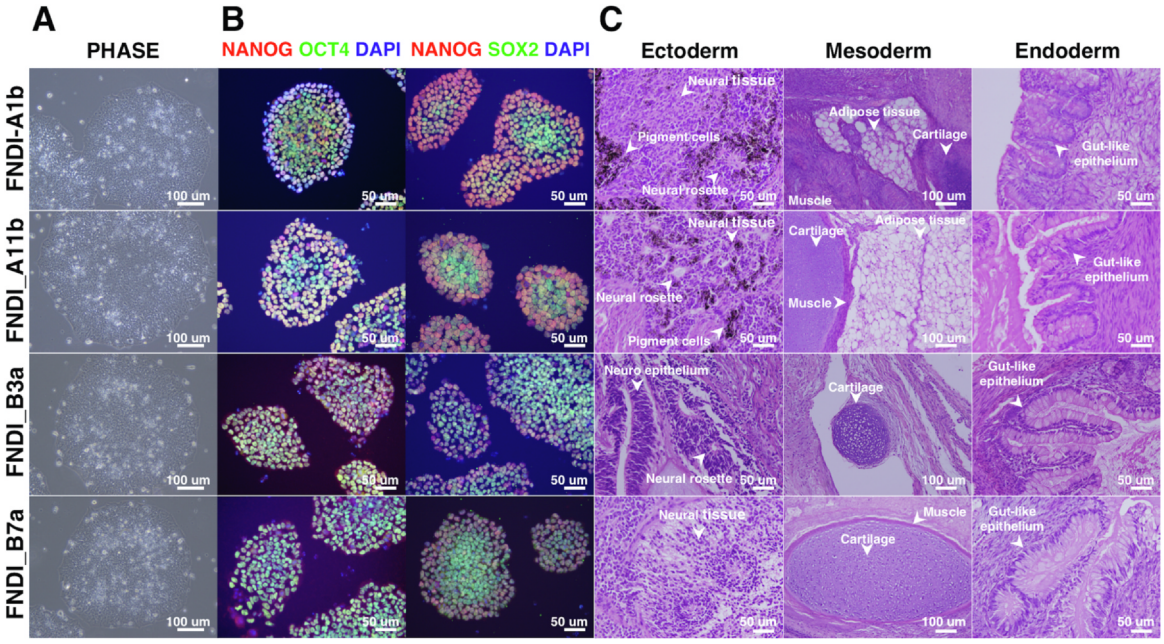
3. Materials and methods

3.1. Generation iPSCs

PBMCs were separated from whole blood of the patients using density-gradient centrifugation with Lympho Spin Medium (pluriSelect) and cultured for 5 days in PBMC medium (StemFit® AK02N medium (Ajinomoto) without C solution, supplemented with 50 ng/mL of IL-6, 50 ng/mL of SCF, 10 ng/mL of TPO, 20 ng/mL of Flt-3L, 20 ng/mL of IL-3, and 10 ng/mL of G-CSF). On day 5, to generate iPSCs from PBMCs, episomal vectors of transgenes for reprogramming (Epi5™ Episomal iPSC Reprogramming Kit, Thermo Fisher Scientific) were delivered into the cultured PBMCs by electroporation using 4D-Nucleofector™ System (Lonza) with the P3 Primary Cell 4D-Nucleofector™ X kit and the pre-installed program EO-117 according to the manufacturer's instructions. The Cells were plated with PBMC

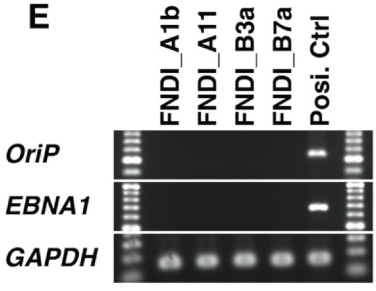
Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive staining for NANOG, OCT4, and SOX2	Fig. 1 panel B
	Quantitative analysis (Immunocytochemistry counting)	NANOG: > 95% OCT4: > 92% SOX2: > 95%	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	FNDI-A1b, A11b: 46XY FNDI-B3a, B7a: 46XX Resolution 300–500	Supplementary Fig. 1 panel A
Identity	Microsatellite PCR (mPCR) OR STR analysis	Not performed 16 STR loci tested; 16/16 matched	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous mutation, NM_000490:c.314G > C	Fig. 1 panel F
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary Fig. 1 panel B
Differentiation potential	Teratoma formation	Ectoderm: neural tissue, pigment cells Mesoderm: cartilage, muscle, adipose tissue Endoderm: Gut-like columnar epithelium	Fig. 1 panel C
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A



D

	Pluripotent marker positive rate (Average)					
	NANOG	STDEV	**OCT4**	STDEV	**SOX2**	STDEV
FNDI_A1b	95.56%	1.76	96.55%	1.32	95.73%	2.05
FNDI_A11b	96.59%	0.46	92.54%	1.10	95.71%	1.78
FNDI_B3a	98.77%	0.84	96.16%	1.83	97.81%	0.50
FNDI_B7a	96.19%	0.74	94.44%	2.55	96.94%	1.16



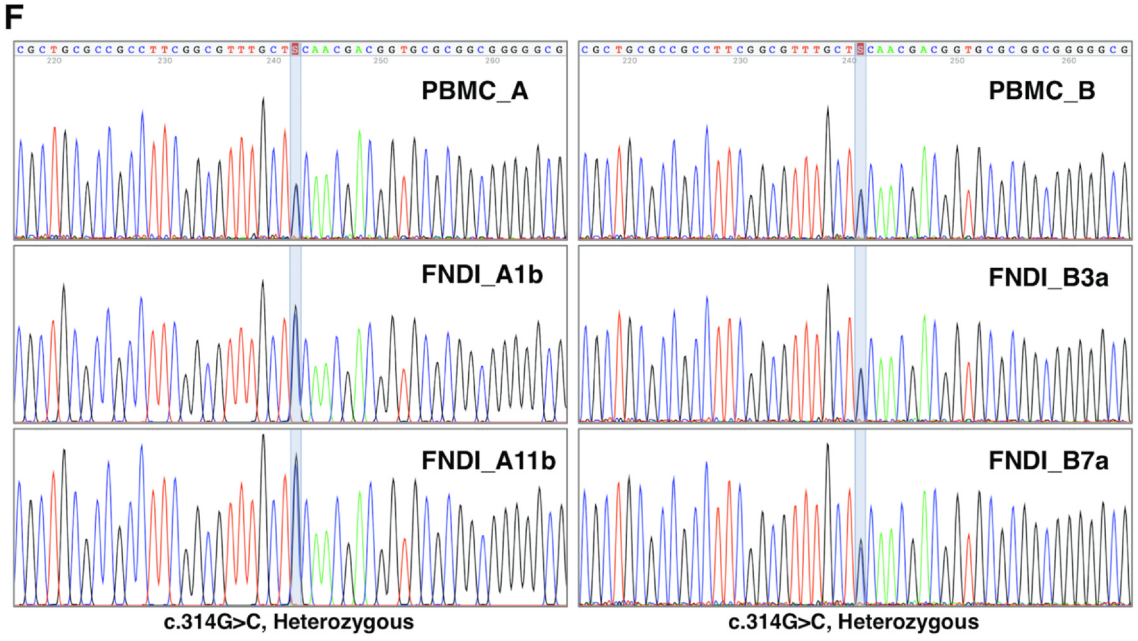


Fig. 1. Characterization of generated FNDI-iPSC lines.

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:500	Abcam Cat# ab19857, RRID: AB_445175
	Rabbit anti-SOX2	1:1000	Wako Cat# 012-27541, RRID: N/A
	Mouse anti-NANOG	1:1000	Thermo Fisher Scientific Cat# MA1-017, RRID: AB_2536677
Secondary antibodies	Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H + L)	1:100	Jackson ImmunoResearch Labs Cat# 711-545-152, RRID:AB_2313584
	Cy3-AffiniPure Donkey Anti-Mouse IgG (H + L)	1:100	Jackson ImmunoResearch Labs Cat# 715-165-151, RRID:AB_2315777
Primers			
	Target	Forward/Reverse primer (5′-3′)	
Episomal Plasmids (PCR)	*OriP*	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGAGACAAC	
	EBNA1	TGGACGTGGAGAAAAGAGGC/CAAAGCTGCACACAGTCACC	
Internal Control	*GAPDH*	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT	
Targeted mutation analysis/ sequencing	*AVP*	TGTAAAACGACGGCCAGTTCCCCTCCAACCCCTCGACTCCCG/ CAGGAAACAGCTATGACTCGGGCTCGGTCACGCAGCTCTCTGC	
	M13_-20	CGACGTTGTAAAACGACGGCCAGT	

medium onto a multi-well plate coated with iMatrix-511 (Nippi) and the half volume of complete StemFit® medium were added on day 3, 5, and 7. After day 9, medium change with fresh StemFit® medium was performed every 2–3 days until the iPSCs colonies grew enough for picking. Obtained iPSC colonies were individually picked and transferred onto new multi-well plates coated with iMatrix-511, followed by culture in StemFit® for further expansion. For subculture, the cultured iPSCs were dissociated using Accutase (Innovative Cell Technologies) and plated onto an iMatrix-511-coated plate once a week. During dissociation and plating, 10 μM of the ROCK-inhibitor Y-27632 (Cayman Chemical) was added to the media and was removed after culture day 1. Cells were maintained at 37 °C in a humidified 5% CO2 incubator. To confirm the absence of the reprograming vectors in the iPSCs, total DNA isolated from the established iPSCs at passage (P14–P17) using QIAamp® DNA mini kit (QIAGEN) was subjected to PCR analysis using the specific primer pairs listed in Table 3. DNA from cells at P1 carrying the episomal vector was used as a positive control.

3.2. Immunocytochemistry

The expression of pluripotent markers was examined by fluorescent immunocytochemistry. iPSCs were fixed with 4% paraformaldehyde for 10 min. The blocking reaction and dilution of antibodies (Table 3) were performed using 5% normal donkey serum (Jackson Immunoresearch) in phosphate-buffered saline with 0.01% Triton-X. The fixed cells were incubated with the primary antibody overnight at 4 °C, followed by reaction with the secondary antibodies for 1 h at room temperature. The fluorescent signal was visualized using a BX-51 fluorescent microscope and DP71 digital camera (Olympus).

3.3. Teratoma formation assay

A teratoma formation assay was performed to assess the differentiation ability of the iPSCs. A total of 5×10^5 iPSCs (P21–P24) were suspended in Matrigel (Corning) diluted in StemFit® AK02N at a 1:1 ratio and injected into the testes of 7-week-old NOD.CB17-Prkdc^{scid}/Jcl mice (CLEA Japan). Fourteen weeks after injection, the animals were sacrificed and the developed teratoma was excised. Paraffin-embedded sections prepared from the excised tissue were stained with hematoxylin and eosin for histological analysis. All animal experiments were conducted by KATAGIRI-OFFICE.

3.4. Direct sequencing

Direct sequencing was performed to detect the pathogenic mutation in the established iPSCs. The target region of the isolated genomic DNA was amplified by PCR using Blend Taq® Plus (TOYOBO) and the primer pair listed in Table 3. PCR products purified using LaboPass™ PCR (COSMO GENETECH) were subjected to cycle sequencing by Eurofin Genomics using the primer shown in Table 3.

3.5. Karyotype analysis

The karyotype of 20 cells (P25–P28) in metaphase was examined for each iPSC line by G-banding at Nihon Gene Research Laboratories.

3.6. STR analysis

The identity of each established iPSC line was confirmed by STR analysis using the PowerPlex® 16 System (Promega) at BEX Co., Ltd.

3.7. Mycoplasma test

Mycoplasma contamination was tested using MycoAlert™ Mycoplasma Detection Kit (Lonza) according to the manufacturer's instructions. The test was performed at the same time as the test for MEN1-iPSC lines, which previously we reported (Yoshida et al., 2020), and the same negative/positive control value was shown (Supplementary Fig. 1B).

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.2020.101960.

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