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Generation and characterization of six human induced pluripotent stem cell lines (iPSC) from three families with *AP4B1*-associated hereditary spastic paraplegia (SPG47)

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Abstract

Bi-allelic variants in the subunits of the adaptor protein complex 4 lead to childhood-onset, complex hereditary spastic paraplegia (AP-4-HSP): SPG47 (*AP4B1*), SPG50 (*AP4M1*), SPG51 (*AP4E1*), and SPG52 (*AP4S1*). Here, we describe the generation of induced pluripotent stem cells (iPSCs) from three AP-4-HSP patients with compound-heterozygous, loss-of-function variants in *AP4B1* and sex-matched parents. Fibroblasts were reprogrammed using non-integrating Sendai virus. iPSCs were characterized according to standard protocols including karyotyping, embryoid body formation, pluripotency marker expression and STR profiling. These first iPSC lines for SPG47 provide a valuable resource for studying this rare disease and related forms of hereditary spastic paraplegia.

Resource utility

These iPSC lines are the first human disease model of AP-4-HSP and provide a valuable resource to study adaptor protein complex 4 biology, disease mechanisms and therapeutic interventions.

Resource details

The hereditary spastic paraplegias are a group of > 80 neurodegenerative diseases and the most common cause of inherited spasticity and associated disability (Blackstone, 2018). Here, we focus on prototypical yet poorly understood forms of complex hereditary spastic paraplegia in children caused by bi-allelic variants in genes that encode subunits

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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.2019.101575.

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of the adaptor protein complex 4 (AP-4-HSP): SPG47 (*AP4B1*); SPG50 (*AP4M1*), SPG51 (*AP4E1*), and SPG52 (*AP4S1*) (Ebrahimi-Fakhari et al., 2018b). AP-4 is a heterotetrameric protein complex that selectively incorporates transmembrane cargo proteins into vesicles and mediates their intracellular transport. Recently, several groups identified the core autophagy protein ATG9A as the major cargo of AP-4 (Davies et al., 2018; De Pace et al., 2018), linking loss of AP-4 function to defective autophagy. To understand AP-4 deficiency in patient derived cells, we created iPSC lines from three well-characterized patients with *AP4B1*-associated AP-4-HSP (or SPG47) (Ebrahimi-Fakhari et al., 2018a) and sex-matched parents as controls. The *AP4B1* variants present are nonsense, missense, frameshift or canonical splice site mutations leading to no functional protein. Fibroblasts were obtained by standard punch biopsy and reprogrammed using non-integrating Sendai virus to overexpress OCT4, SOX2, KLF4 and hc-MYC. All iPSC lines recovered well after thawing (Fig. 1A, scale bar 400 μm). For each line, one clone was selected based on expression of pluripotency markers and differentiation potential. Pluripotency was assessed by immunofluorescence staining for OCT4, Nanog, SSEA4 and Tra-1–60 (Fig. 1B, scale bar 300 μm) as well as qRT-PCR of NANOG, OCT4, REX1, and SOX2 (Fig. 1D). All iPSC lines showed robust expression of pluripotency markers. To examine the potential to differentiate into all three germ layers, embryoid bodies from iPSC lines were tested for expression of ectodermal (EN1, MAP2 and NR2F2), mesodermal (SNAI2, RGS4 and HAND2) and endodermal (SST, KLF5 and AFP) markers using qRT-PCR (Fig. 1E). Karyotype analysis showed normal karyotypes and no clonal abnormalities (Fig. 1C). STR analysis for 16 short tandem repeat markers (Table 2) showed identical profiles for iPSC lines with their respective fibroblast line. To verify *AP4B1* variants in iPSC lines, Sanger sequencing was performed (Fig. 1F). Mycoplasma testing using a standard assay (MycoAlert™) was negative.

Materials and methods

Generation of iPSC

Skin punch biopsies (2–3 mm) were incubated in 0.5% Dispase Solution (STEMCELL Technologies) to remove epidermis. Samples were placed in gelatin-coated wells under growth conditions (37 °C, 5% CO2), and a coverslip was added to prevent lifting. Fibroblast media consisted of DMEM with 10% FBS and 1% Penicillin/Streptomycin (Thermo Fisher Scientific). The CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) was used to transduce cells (Tables 1 & 2). Eight days after transduction, iPSCs were re-plated on Geltrex™-coated dishes and maintained in StemFlex medium (Thermo Fisher Scientific). Emerging stem cell colonies were picked and re-plated on Geltrex™-coated dishes for expansion. Cells were passaged weekly using Gentle Cell Dissociation Reagent (STEMCELL Technologies).

Immunocytochemistry for markers of pluripotency

For immunocytochemistry, iPSC were grown on coverslips. After washing with PBS, cells were fixed in 4% PFA at room temperature for 20 min. After three washes with PBS containing 0.05% Tween 20 (PBST), cells were permeabilized with PBS containing 0.1% Triton X-100 for 15 min and subsequently washed with PBST. Blocking was performed overnight at 4 °C with 4% donkey serum in PBS. After washing with PBS, cells were

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incubated with primary antibodies (Table 3) for 1 h at room temperature, washed with PBST and incubated with secondary antibodies for 1 h at room temperature and stained with DAPI. Imaging was performed using the Olympus IX71 Inverted Microscope.

RNA isolation and qRT-PCR

Total RNA isolation was done using RNeasy Mini Kit (Qiagen), and RNA quantification was done using the qScript® cDNA Synthesis Kit (Quanta Bio). qRT-PCR cycles were performed using the QuantStudio 12 K Flex System (Thermo Fisher Scientific).

Embryoid body formation

To assess their ability to form three germ layers, iPSCs were lifted in clumps to allow for spontanious EB formation in suspension. iPSC cultures were washed with DPBS and incubated with Accutase (STEMCELL Technologies) for 3 min at 37 °C. Cell clumps were lifted with a cell scraper in EB formation medium, consisting of DMEM, 5% KnockOut™ Serum Replacement and 0.5% Penicillin/Streptomycin (Thermo Fisher Scientific). Clumps were added to a 15 ml tube and settled for 10 min before removing the supernatant. EB formation medium with ROCK inhibitor (STEMCELL Technologies) was added, and cell clumps were plated on ultra-low attachment plates (Corning Costar) for 24 h. EB formation medium was replaced every other day until replating to Gelatin-coated-plates on day 8. Cells were then cultured until day 15 in DMEM with 10% FBS. RNA isolation and qRT-PCR was performed, as described above.

Karyotyping

Karyotyping was performed at WiCell.

Mycoplasma detection

Testing for mycoplasma contamination was done using the MycoAlert™ Detection Kit (Lonza).

STR profiling

Genomic DNA was isolated from fibroblasts and iPSCs using the DNeasy Blood & Tissue Kit (Qiagen). STR analysis was performed at Genetica DNA Laboratories. Sixteen loci and an additional mouse marker for the detection of mouse DNA contamination were analyzed using the PowerPlex® 16 HS System (Promega).

Mutation verification

To verify variants in *AP4B1*, PCR was performed on gDNA samples using Platinum PCR SuperMix High Fidelity and the SimpliAmp Thermal Cycler (Thermo Fisher Scientific). PCR products were verified on 1% agarose gel before purification using DNA Clean & Concentrator (Zymo Research) and submitted to Eton Bioscience and Genewiz for Sanger Sequencing.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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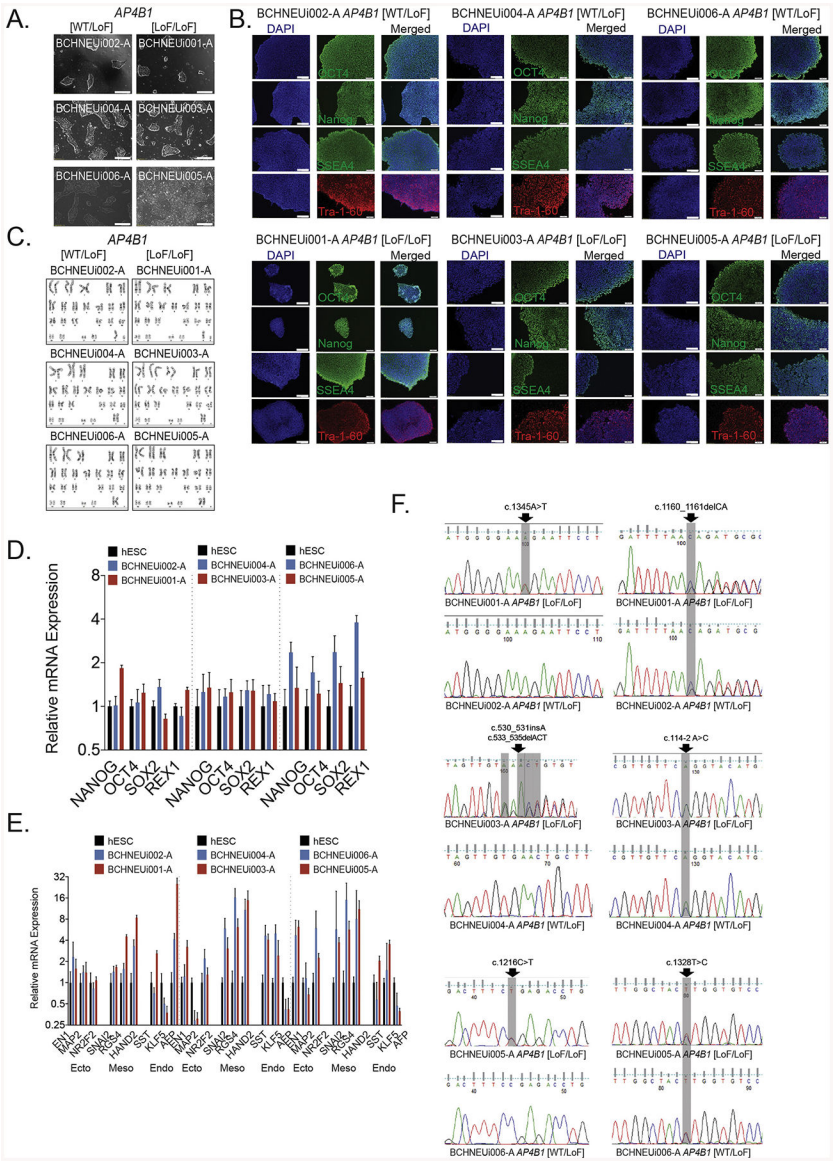

Fig. 1. Figure 1

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Table 1

Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
BCHNEUi001-A	AP4B1 [LoF/LoF]	Male	2 years	Mixed	c.1345A > T/c.1160_1161delCA	SPG47
BCHNEUi002-A	AP4B1 [WT/LoF]	Male	38 years	Mixed	c.1160_1161delCA	Unaffected control
BCHNEU003-A	AP4B1 [LoF/LoF]	Female	3 years	Caucasian	c.530_531insA & c.533_535delACT/c.114-2A > C	SPG47
BCHNEUi004-A	AP4B1 [WT/LoF]	Female	33 years	Caucasian	c.114-2A > C	Unaffected control
BCHNEUi005-A	AP4B1 [LoF/LoF]	Female	3 years 9 months	Caucasian	c.1216C > T/c.1328 T > C	SPG47
BCHNEUi006-A	AP4B1 [WT/LoF]	Female	39 years	Caucasian	c.1328 T > C	Unaffected control

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Table 2

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*Normal*	Fig. 1 *panel A*
Phenotype	Qualitative analysis by immunocytochemistry	*Immunocytochemistry for pluripotency markers OCT4, Nanog, SSEA4 and Tra-1–60*	Fig. 1 *panel B*
	Quantitative analysis by RT-qPCR	*qRT-PCR for expression of Nanog OCT4, REX1, SOX, HTERT and DNMT3B*	Fig. 1 *panel B*
Genotype	Karyotype (G-banding) and resolution	*BCHNEUi001-A: 46,XY*	Fig. 1 *panel E*
		Band Resolution: 425–500	
		BCHNEUi002-A: 46,XY	
		Band Resolution: 400–425	
		BCHNEUi004-A: 46,XX	
		Band Resolution: 375–475	
		BCHNEUi005-A: 46,XX	
		Band Resolution: 425–475	
		BCHNEUi005-A: 46,XX	
		Band Resolution: 425–500	
		BCHNEUi006-A 46,XX	
		Band Resolution: 450–500	
Identity	STR analysis	*Performed*	*Archived with the journal*
		16 loci tested, all matched (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX, FGA, Amelogenin)	*Archived with the journal*
Mutation analysis	Sanger sequencing	*Confirmed variants listed in* Table 1.	Fig. 1 *panel F*
	Southern Blot OR WGS	*N/A*	*N/A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by luminescence (MycoAlert™): Negative*	*Archived with the journal*
Differentiation potential	Embryoid body formation	*Expression of ectodermal (EN1, MAP2 and NR2F2), mesodermal (SNA1L2, RGS4 and HAND2) and endodermal markers (SST, KLF5 and AFP)*	Fig. 1 *panel D*
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*

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Table 3

Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency markers	Rabbit anti-OCT4	1:100	Abcam Cat# ab19857, RRID:AB_445175
	Rabbit anti-NANOG	1:50	Abcam Cat# ab21624, RRID:AB_446437
	Rat anti-SSEA3	1:200	MiUipore Cat# MAB 4303, RRID:AB_177628
	Mouse anti-SSEA4	1:200	MiUipore Cat# MAB 4304, RRID:AB_177629
	Mouse anti-TRA-1–60	1:200	MiUipore Cat# MAB 4360, RRID:AB_10917470
Secondary antibodies	AlexaFluor 488 Donkey Anti-Rabbit IgG	1:500	Thermo Fisher Scientific Cat# A-21206, RRID:AB_2535792
	AlexaFluor 488 Donkey Anti-Mouse IgG	1:500	Thermo Fisher Scientific Cat# A-21202, RRID:AB_141607
	AlexaFluor 555 Goat Anti-Mouse IgM	1:500	Thermo Fisher Scientific Cat# A-21426, RRID:AB_2535847
Primers			
	Target		**Forward/Reverse primer (5’−3’)**
Pluripotency markers (qPCR)	NANOG		CAGTCTGGACACTGGCTGAA/CTCGCTGATTAGGCTCCAAC
	OCT4		TGTACTCCTCGGTCCCTTTC/TCCAGGTTTTCTTTCCCTAGC
	SOX2		GCTAGTCTCCAAGCGACGAA/GCAAGAAGCCTCTCCTTGAA
	DNMT3B		ATAAGTCGAAGGTGCGTCGT/GGCAACATCTGAAGCCATTT
	HTERT		TGTGCACCAACATCTACAAG/GCGTTCTTGGCTTTCAGGAT
	REX1		TGGACACGTCTGTGCTCTTC/GTCTTGGCGTCTTCTCGAAC
House-keeping genes (qPCR)	ACTB		GGACTTCGAGCAAGAGATGG/AGCACTGTGTTGGCGTACAG
Targeted sequencing of AP4B1	AP4B1		BCHNEUi001-A & BCHNEUi002-A:
			1) GTCAAGTGTCCCCCACAAAA/AAAGGCAGGCATTACCTGTG
			2) 2) ACACCTTTTCTGCTGGCACT/GCAGTGAGCAGCTCCATCTT BCHNEUi003-A & BCHNEUi004-A:
			1) AATCCTGGCTGCTACCCTCT/GTATTGATGGCCAGGAGAGC
			2) CTTTAGTGTGGCCTTTGTCATT/TGAACCCAGGAGGCGGAGGT BCHNEUi005-A & BCHNEUi006-A:
			1) TTGACCACACCTCCAAAACC/CAGGGCCTGACATACAGCTT
			2) ACACCTTTTCTGCTGGCACT/GCAGTGAGCAGCTCCATCTT

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Key resource table

Unique stem cell lines identifier	BCHNEUi001-A
	BCHNEUi002-A
	BCHNEUi003-A
	BCHNEUi004-A
	BCHNEUi005-A
	BCHNEUi006-A
Alternative names of stem cell lines	HNDS_0052–01
	HNDS_0052–03
	HNDS_0054–01
	HNDS_0054–02
	HNDS_0058–01
	HNDS_0058–02
Institution	Boston Children's Hospital, Harvard Stem Cell Institute
Contact information of distributor	Darius Ebrahimi-Fakhari darius.ebrahimi-fakhari@childrens.harvard.edu
Type of cell lines	iPSC
Origin	Human
Cell Source	Fibroblasts
Clonality	Clonal cell lines
Method of reprogramming	Sendai Virus, non-integrating (OCT4, SOX2, KLF4 and hc-MYC)
Multiline rationale	Three lines from patients with AP-4-HSP due to compound-heterozygous variants in *AP4B1* and control lines from sex-matched parents who are clinically unaffected heterozygous carriers.
Gene modification	3 cell lines with homozygous variants
	3 cell lines with heterozygous variants
Type of modification	N/A
Associated disease	*AP4B1* , Hereditary Spastic Paraplegia type 47, SPG47
Gene/locus	*AP4B1*; Reference sequences: NM_001253852.1
	BCHNEUi001-A: c.1345A > T/c.1160_1161delCA
	BCHNEUi002-A: c.1160_1161delCA
	BCHNEUi003-A: c.530_531insA/c.114-2A > C
	BCHNEUi004-A: c.114-2A > C
	BCHNEUi005-A: c.1216C > T/c.1328T > C
	BCHNEUi006-A: c.1328T > C
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	May 3rd 2018
Cell line repository/bank	N/A
Ethical approval	This study was approved by the Institutional Review Board at Boston Children's Hospital (IRB#: P00016119). Written informed consent was obtained.