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Lab Resource: Single Cell Line

Generation of a gene-corrected human isogenic iPSC line from an Alzheimer's disease iPSC line carrying the London mutation in APP (V717I)

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A B S T R A C T

We report the genome-editing of an existing iPSC line carrying the London mutation in APP (V717I) into an iPSC line in which the pathogenic mutation was corrected. The resulting isogenic iPSC line maintained pluripotent stem cell morphology, a normal karyotype, expression of pluripotency markers and the ability to differentiate into the three germ-layers *in vitro*.

Resource Table

Unique stem cell line identifier	UOMELBi002-A
Alternative name(s) of stem cell line	F16574c3_A_A1_G8
Institution	The University of Melbourne
Contact information of distributor	Dr. Damian Hernández, damian.hernandez@unimelb.edu.au
Type of cell line	iPSC
Origin	human
Additional origin info	Age: Blinded for publication due to risk of unblinding; available upon request Sex: Blinded for publication due to risk of unblinding; available upon request Ethnicity if known: Blinded for publication due to risk of unblinding; available upon request
Cell Source	iPSC
Clonality	Clonal
Method of reprogramming	Episomal for parental iPSC
Genetic Modification	YES
Type of Modification	Gene Correction and silent mutation in one single allele (Heterozygous mutation).
Associated disease	Alzheimer's disease
Gene/locus	APP London mutation (V717I, rs63750264) in exon 17 of *APP*. (GRCh38; Chr21:25891784 G > A)
Method of modification	CRISPR Cas9
Name of transgene or resistance	NA
Inducible/constitutive system	NA
Date archived/stock date	NA
Cell line repository/bank	NA
Ethical approval	All experimental work performed in this study was approved by the University of Melbourne (1545394) with the requirements of the National Health & Medical Research Council of Australia (NHMRC) and conformed with the Declaration of Helsinki.

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1. Resource utility

The amyloid beta precursor protein (APP) V717I London mutation is a dominant inherited mutation that causes early onset Alzheimer's disease. APP mutation carriers typically present with an onset at the age of 45 to 60 years (Bateman et al., 2011). Using isogenic cell pairs with and without the disease-causing mutation allows a controlled assessment of APP (V717I) in iPSCs and progeny.

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2. Resource details

The iPSC line F16574 was previously generated from one individual with a mutation in V717I APP (London mutation) (Karch et al., 2018). These iPSCs were generated from fibroblasts reprogrammed by nucleofection of episomal vectors containing OCT4, SOX2, KLF4, L-MYC, LIN28, and shRNA against p53 with selection of multiple clones and subsequent characterisation of pluripotency and genomic integrity (Karch et al., 2018). Here, we used the parental iPSC line F16574 clone 3 for genome editing of the APP locus and correction of the disease-causing mutation V717I allowing the generation of isogenic iPSC lines for APP mutation (UOMELBi002-A) (Table 1, Fig. 1A). One clone was subsequently isolated, expanded and recharacterized as above. The CRISPR/Cas9- edited iPSC line showed the typical human pluripotent stem cell- morphology and expressed markers of pluripotency TRA-1-60 and OCT4 (Fig. 1A). Quantification by flow cytometry analysis demonstrated 81.3% and 78.3% of live cells were positive for TRA-1-60 and OCT4 respectively (Fig. 1B). Gene editing correction of the APP V717I mutation was confirmed by Sanger DNA sequencing in UOMELBi002-A by comparing to the parental FA1657 clone 3 line (Fig. 1C). A silent DNA mutation at the PAM sequence (codon TTA instead TTG both encoding for Leucine) was included to enhance homology directed repair efficiency, allow identification of targeted allele by Sanger sequencing and to prevent recutting of the edited allele. Genomic integrity was assessed by copy number variation analysis of the parental iPSCs and the CRISPR/Cas edited iPSCs, represented with log R ratio and B allele frequency (Fig. 1D). This analysis confirmed the absence of deletions, insertions and aneuploidies. Of note, balanced rearrangements cannot be detected by this method. Cell identity was confirmed by PCR-based fingerprinting system using short tandem repeat (STR) profiling of samples (data not shown). The iPSCs were also able to differentiate into the three germ layers, as demonstrated by positive immunostaining for endodermal (alpha-fetoprotein, AFP), mesodermal (smooth muscle actin, SMA) and ectodermal (NESTIN) markers following embryoid body formation (Fig. 1E).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast brightfield morphology pictures	*Normal*	Fig. 1 *panel A*
Phenotype	Qualitative analysis of pluripotency Immunochemistry	*Expression of TRA-1–60 and OCT4*	Fig. 1 *panel A*
	Quantitative analysis *(Flow cytometry]*	*TRA-1–60: 78.3%; OCT4: 81.3%*	Fig. 1 *panel B*
Genotype	CNV array Illumina HumanCore Beadchip array which contains over 300,000 informative SNPs with a median spacing of 5.8 kb.	*46, Resolution 450–500. Sex chromosomes were cropped to mantain blinding of gender identity (available upon request)*	Fig. 1 *panel D*
Identity	STR analysis	*10 sites tested, all sites matched between parental and isogenic cell lines*	*Submitted, in archive with journal*
Mutation analysis (IF APPLICABLE)	Sanger Sequencing	*Assessment of rs63750264 status confirmed (A/G) in parental and UOMELBi002-A cell line (G/G)*	Fig. 1 *panel C*
	Southern Blot OR WGS	*NA*	*NA*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by luminescence were Negative*	Reading B/A ratio; UOMELBi002-A = 0.59 F16574 clone 3 = 0.50 Positive control = 3.78 Negative control = 0.34
Differentiation potential	Direct differentiation, STEMdiff™ Trilineage Differentiation Kit (Stemcell technologies)	*Expression of AFP, SMA and NESTIN by immunostaining*	Fig. 1 *panel E*
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

3. Materials and methods

3.1. Ethics

All experimental work performed in this study was approved by the Human Research Ethics committees of the University of Melbourne (1545394) with the requirements of the National Health & Medical Research Council of Australia (NHMRC) and conformed with the Declaration of Helsinki (McCaughey et al., 2016).

3.2. iPSC culture

The iPSCs were maintained in StemFlex medium (Gibco) using 6-well plates pre-coated with vitronectin (Stemcell Technologies). Media was changed every second day and cells were passaged with ReleSR (Stemcell Technologies) on a weekly basis when colonies reached 80% confluency.

3.3. Generation of isogenic lines

Genome editing was performed with the CRISPR/Cas9 system in combination with a single-stranded DNA (ssDNA, Table 2) to guide the single nucleotide correction of the APP V717I mutation by homologous recombination. Single guide (sg) RNA sequence was designed as described by Zhang's laboratory (Ran et al., 2013) and selected based on the highest on-target and off-target score (Doench et al., 2016; Hsu et al., 2013). The APP SNP rs63750264 allele (A) of the parental iPSC F16574 clone 3 line was genetically modified to generate isogenic lines with a homozygous G/G nucleotide with the designed sgRNA and the ssDNA (Table 2). The ribonucleoprotein (RNP) complex consisting of Cas9 protein and sgRNA (containing a tracrRNA labelled with a red fluorophore ATTO 550) was assembled in Duplex buffer (all from Integrated DNA Technologies). RNP complex was subsequently transfected into dissociated iPSCs by electroporation (1200 V, 30 ms, 1 pulse) with the Neon transfection system (Invitrogen). After electroporation the cells were immediately plated onto vitronectin-plated 6 well plates containing Stemflex medium supplemented with 10 μM ROCK inhibitor (RevitaCell, Gibco). After 48 h, ATTO 550 positive / DAPI negative cells were sorted by flow cytometry (BD) (Fig. 1S) and plated at low density for clonal selection (1505 cells into 1 well of a six-well plate). Cells were screened for SNP editing by PCR and Sanger sequencing (Australian Genome Research Facility), from 96 clones that were analysed only one clone was re-plated at low density for sub-clonal selection. One colony out of ninety-six was subsequently dissociated for sub-clonal selection, as to obtain a pure edited population from one out of eight sub-clones analysed. The resultant isogenic lines were named UOMELBi002-A.

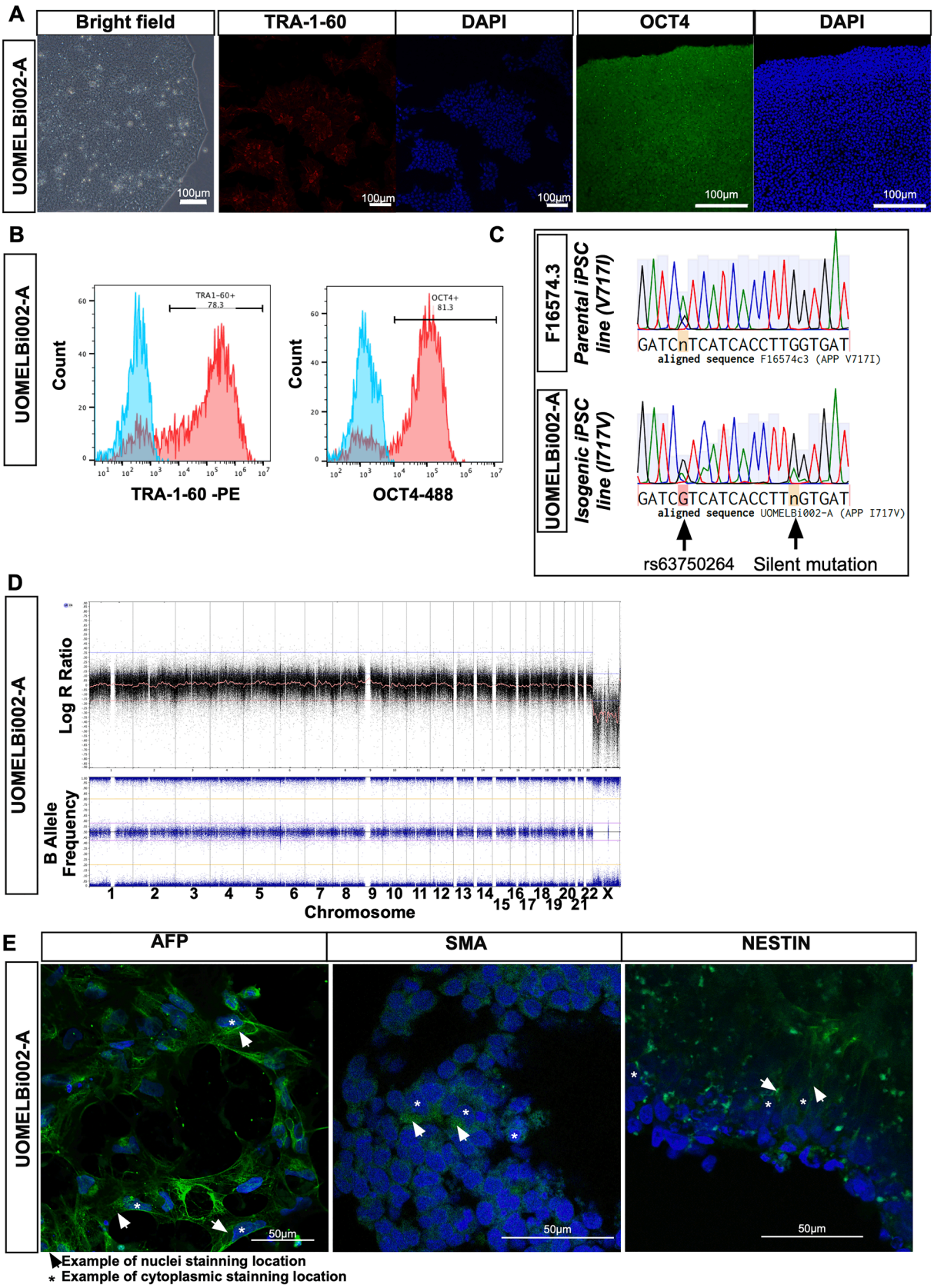

Fig. 1. Characterization of UOMELBi002-A.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	mouse anti-TRA-1-60	1:200	Invitrogen Cat#MA1-023-PE, RRID:AB_2536704
Pluripotency Markers	mouse anti-OCT3/4	1:80	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051
Differentiation Markers	mouse anti-SMA	1:500	R and D Systems Cat# MAB1420, RRID:AB_262054
Differentiation Markers	mouse anti-NESTIN	1:1000	Abcam Cat# ab22035, RRID:AB_446723
Differentiation Markers	mouse anti-AFP	1:1000	Millipore Cat# ST1673-100UG, RRID:AB_10697987
Secondary antibodies	Alexa Fluor 568 Goat Anti-Mouse IgG	1:1000	Thermo Fisher Scientific Cat# A-11031, RRID:AB_144696
Secondary antibodies	Alexa Fluor 488 Goat Anti-Mouse IgG	1:1000	Thermo Fisher Scientific Cat# A-11029, RRID:AB_2534088
Primers			
	Target		**Forward/Reverse primer (5′-3′)**
Targeted mutation	sgRNA, *rs63750264*		TGGTGATGCTGAAGAAGAAA
Targeted mutation	ssDNA, *rs63750264*		GTGCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGACAGTGATCgTCATCACCTTaGTGATGCTGAAGAAGAAACAGTACACATCCATTCATCATGGTGTGGTGGA
Genotyping	PCR, *rs63750264*		Forward (GTCACACATCAGGGCTCAGAGT)
Genotyping	PCR, *rs63750264*		Reverse (AACCCAAGCATCATGGAAGCAC)
Genotyping	Sequencing, *rs63750264*		Forward (CCTCATCCAAATGTCCCCTGCA)

3.4. Virtual karyotype

Copy number variation analysis of isogenic iPSCs was performed using Illumina Infinium CoreExome-24 v1.1, performed by the Victorian Clinical Genetics Services (VCGS, Melbourne, Australia).

3.5. Cell identity

Short tandem repeat (STR) profiling of samples was performed by PCR-based fingerprinting using Promega GenePrint 10 system, and performed by the Australian Genome Research Facility (AGRF, Melbourne, Australia).

3.6. Differentiation to the three-germ layer

Embryoid bodies were generated using a tri-lineage differentiation kit (Stem Cell Technologies). Germ layer differentiation was assessed by immunochemistry.

3.7. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X (Sigma). Immunocytochemistry was performed using the following primary antibodies: mouse anti-TRA-1-60-PE (Invitrogen), mouse anti-OCT3/4 (Santa Cruz Biotechnology), smooth muscle actin (R&D Systems), mouse anti-NESTIN (Abcam) or rabbit anti-alpha-fetoprotein (Sigma-Aldrich) (Table 2). Cells were then immunostained with isotype-specific secondary antibodies (Alexa Fluor 568 or 488, Life Technologies, Table 2). Nuclei were counterstained using DAPI (Sigma-Aldrich) and mounted in Vectashield (Vector Labs). Specificity of the staining was verified by the absence of staining in negative controls consisting of the appropriate negative control immunoglobulin fraction (Dako). Images were acquired on a Zeiss AxioImager M2 fluorescent microscope or LMS 880 confocal microscope using ZEN software (Zeiss).

3.8. Flow cytometry analysis

Cells were dissociated into single cells with ReleSR and incubated with a fixable viability dye (Miltenyi). Then cells were fixed and permeabilized with the Inside stain Kit (Miltenyi). Cells were incubated with primary antibodies mouse anti-TRA-1-60-PE (Invitrogen) and mouse anti-OCT3/4 (Table 2), following incubation with isotype-specific secondary antibody (Alexa Fluor 488, Life Technologies, Table 2). Unstained cells were used as negative controls. Cells were analysed by flow cytometry analysis (Cytoflex S, Beckman Coulter).

3.9. Mycoplasma testing

Mycoplasma test was performed using the MycoAlert kit (Lonza) following the manufacturer's instruction.

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.2021.102373.

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