Understanding neurodegenerative diseases in human iPS cell models by genome editing with CRISPR-Cas9

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Declaration

In accordance with the policies and guidelines of the University of Cambridge, the following declarations are made:

I hereby declare that this thesis, conducted at the Wellcome Trust Sanger Institute from January to September 2016, is my own work on the research conducted by me and contains no material which has been submitted for the award of any other degree at any university or equivalent institution. This thesis, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference and acknowledgement is made in the text.

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Abbreviations

AAAS	Achalasia-Addisonianism-Alacrima syndrome
ALADIN	ALacrima-Achalasia-aDrenal Insufficiency Neurologic disorder
amp	ampicillin
BCA	bicinchoninic acid
BSA	bovin serum albumin
Cas9-RNP	Cas9-ribonucleoprotein
CRISPR	clustered regularly interspaced short palindromic repeats
crRNA	CRISPR RNA
DMSO	dimethyl sulfoxide
DNTP	deoxynucleoside triphosphate
DSB	double-stranded break
EDTA	ethylene diamine tetraacetic acid
FTH1	ferritin heavy chain
gRNA	guide RNA
HD	Huntington's disease
HDR	homology-directed repair
hESC	human embryonic stem cells
hiPSC	human induced pluripotent stem cells
HR	homologous recombination
HTT	huntingtin
kan	kanamycin
KSR	knock-out serum replacement
NHEJ	non-homologous end joining
NII	neuronal intranuclear inclusions
NPC	nuclear pore complex
OTS	off-target site
PAM	protospacer adjacent motif
PBS	phosphate buffered saline
pheS	phenylalanyl tRNA synthetase
polyQ	poly-glutamine
puro	puromycin
Ri	ROCK inhibitor
ROS	reactive oxygen species
RT	room temperature
SDS	sodium dodecyl sulphate
ssODN	single-stranded oligodeoxynucleotide
TALEN	transcription activator-like effector nucleases
TBS	tris buffered saline
TBS-T	TBS-Tween
tet	tetracycline
TE	tris-EDTA buffer
TENS	tris-EDTA-NaCl-SDS buffer
tracrRNA	trans-activating crRNA
WD-repeat	tryptophan-aspartic acid repeat
zeo	zeocin
ZFN	zinc finger nuclease

Abstract

Genetic neurodegenerative disorders are inherited diseases of the brain and nervous system, many of which are poorly understood. Human induced pluripotent stem (hiPS) cells, upon introduction of disease relevant mutations by genome editing, can be used to model diseases, gain insights into the pathophysiology of such diseases, and develop therapies. In this study, CRISPR-Cas9 system was used to edit the hiPS cell genome to generate models for two diseases – Achalasia-Addisonianism-Alacrima syndrome (AAAS) and Huntington's disease.

AAAS is a progressive neurodegenerative disorder mainly characterized by oesophageal muscle disorders, adrenal insufficiency, and tear production failure. Multiple mutations in the *AAAS* gene encoding the ubiquitously expressed ALADIN (ALacrima-Achalasia-aDrenal Insufficiency Neurologic) protein are responsible for this autosomal recessive disorder. Using two different strategies, I generated human iPS cells homozygous for a common allele of *AAAS*, a C>A point mutation in exon 1 that creates a novel splice-donor site, and for comparison, a biallelic knockout of *AAAS* exon 2. The biallelic knockout relied on replacing one allele with a puromycin selection cassette by homologous recombination (HR) and simultaneously damaging the other allele by non-homologous end-joining (NHEJ). Sequencing the non-targeted allele demonstrated frameshift indels with a biallelic targeting efficiency of 3%. The homozygous point mutation was generated by homology-directed repair (HDR) with a single-stranded oligonucleotide template. Clones homozygous for the point mutation were generated in two rounds of genome editing. A complete loss of ALADIN protein in undifferentiated human iPS cells was observed in both knockout and point mutant cells, suggesting that the disease is caused by null mutations in the *AAAS* gene. These models lay the foundation of future detailed phenotype analysis and understanding of disease manifestations by differentiating them to the disease-relevant cell types, particularly cortical neurons and adrenocortical cells.

Huntington's disease is a progressive neurodegenerative genetic disorder that mainly causes motor, behavioral, and cognitive abnormalities in an individual. This disease occurs due to an autosomal dominant expansion of CAG-repeats in the huntingtin (HTT) gene. I attempted to insert 67 Q-repeats into HTT by Cas9-assisted homologous recombination (HR) with a donor plasmid containing the extended repeat fragment. From a screen of 192 unselected clones, I was unable to recover any clones containing the expanded repeats. Presumably the rate of homologous recombination was too low in unselected clones and future experiments will require drug selection to ensure retention of the expanded Q-repeats.